Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

HUMAN POLYOMAVIRUSES

HUMAN POLYOMAVIRUSES

MOLECULAR AND CLINICAL PERSPECTIVES

Edited by

Kamel Khalili
Director and Professor
Center for Neurovirology and Cancer Biology
Temple University

Gerald L. Stoner
Chief, Neurotoxicology Section
National Institute of Neurological Disorders and Stroke
National Institutes of Health

WILEY-LISS
A JOHN WILEY & SONS, INC., PUBLICATION

ISBN 0-471-22194-5

This title is also available in print as ISBN 0-471-39009-7.

For more information about Wiley products, visit our web site at www.Wiley.com.

This book is dedicated to George Khoury (1944–1987) and Norman P. Salzman (1926–1997) for their pioneering studies on the molecular virology of the polyomaviruses.

CONTENTS

PREFACE

It has been 15 years since the late Norman P. Salzman edited the *The Papovaviridae*, Volume 1, *The Polyomaviruses*. Then single chapters covered JC virus (JCV) and BK virus (BKV), and the chapter on BKV was shared with monkey lymphotropic papovavirus. The explosion of knowledge in the last 15 years warrants an update on these viruses focusing on those of immediate consequence to humans. Two of them, BKV and JCV, were discovered in humans in 1971. The third, simian virus 40 (SV40), was discovered in monkey kidney cells 10 years earlier, but is now viewed as a potential human pathogen for reasons described herein.

We begin with some historical background, recognizing the generation of virologists and neuropathologists who first worked in this field and having them tell of the excitement of discovery in their own words, where possible. Dr. Hilleman, who discovered SV40, recently described that achievement (Hilleman, 1998). Later chapters cover all aspects of the human polyomaviruses, including the clinical issues involved in diagnosis and treatment of these difficult, persistent infections.

This book will be of interest to graduate students, medical students, and advanced undergraduates and to anyone engaged in the study of DNA viruses, their molecular biology, evolution, epidemiology, and pathologic potential. The latter includes their oncogenic properties and their roles in nephritis in renal allografts and in the fatal viral CNS demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML was discovered in patients with leukemia and lymphoma, but is now primarily a disease of patients with AIDS. We have encouraged contributors set out areas of conflict, where these exist, which future research must resolve.

The polyomaviruses were originally considered to be a genus within the papovavirus family (the other genus including papillomaviruses), and sometimes the word *papovavirus* was applied to BKV, JCV, and SV40. The classification ''papovavirus'' has now been dropped by the International Committee on the Taxonomy of Viruses (ICTV). The polyomaviruses and the papilloma-

viruses are both small DNA viruses, but they do not share genomic organization or show homology of DNA sequence and are no longer placed in the same family. We have dropped the usage of *papovavirus* in this book in favor of *polyomavirus* except in its appropriate historical context. The mouse polyoma virus, which gave its name to this group of viruses, should be distinguished by the use of ''polyoma virus'' as two words.

We thank each of the contributors, without whom this book would not have been possible. The polyomaviruses are of international concern, and an international effort is underway to understand and defeat them. We thank our editor at Wiley, Luna Han and Danielle Lacourciere, for their expert assistance and dedication.

KAMEL KHALILI, PH.D.
Philadelphia, Pennsylvania

GERALD L. STONER, PH.D.
Bethesda, Maryland

REFERENCE

Hilleman MR (1998): Discovery of simian virus 40 (SV_{40}) and its relationship to poliomyelitis virus vaccines. Dev Biol Stand 94:183–190.

CONTRIBUTORS

HANSJÜRGEN T. AGOSTINI, M.D., Department of Ophthalmology, University of Freiburg, Freiburg, Germany

AMY S. ARRINGTON, B.A., Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

KARL E. ÅSTRÖM, M.D., Karolinska Institute (retired Professor), Stockholm, Sweden

WALTER J. ATWOOD, Ph.D., Department of Molecular Microbiology and Immunology, Brown University, Providence, RI

GIUSEPPE BARBANTI-BRODANO, Ph.D., Department of Experimental and Diagnostic Medicine, Section of Microbiology, and Center for Biotechnology, University of Ferrara, Ferrara, Italy

JOSEPH R. BERGER, M.D., Department of Neurology, University of Kentucky College of Medicine, Lexington, KY

JANET S. BUTEL, Ph.D., Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houson, TX

ALFREDO CORALLINI, Ph.D., Department of Experimental and Diagnostic Medicine, Section of Microbiology, and Center for Biotechnology, University of Ferrara, Ferrara, Italy

LUIS DEL VALLE, M.D., Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, Philadelphia, PA

KRISTINA DÖRRIES, M.D., Institute für Virologie und Immunbiologie, Julius-Maximilians-Universität Würzburg, Germany

PASQUALE FERRANTE, M.D., Laboratory of Biology, Don C. Gnocchi Foundation, Milan, Italy

RICHARD J. FRISQUE, Ph.D., Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA

JENNIFER GORDON, Ph.D., Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, Philadelphia, PA

JOHN W. HENSON, M.D., Brain Tumor Center and Molecular Neuro-Oncology Laboratory, Massachusetts General Hospital, Boston, MA

ROLAND HÜBNER, Ph.D., Laboratory of Pathology, University of Antwerpen, Wilrijk, Belgium

MICHAEL J. IMPERIALE, Ph.D., Department of Microbiology and Immunology, Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI

DAVID V. JOBES, Ph.D., Neurotoxicology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

KAMEL KHALILI, Ph.D., Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, Philadelphia, PA

HEE-SUN KIM, Ph.D., Molecular Neuro-Oncology Laboratory, Massachusetts General Hospital, Charleston, MA

WENDY A. KNOWLES, Ph.D., Enteric and Respiratory Virus Laboratory, Virus Reference Division, Central Public Health Laboratory, London, UK

DANA E. M. ROLLISON, Sc.M., Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

MÁRIA MÁZLÓ, M.D., Ph.D., University of Pécs, Faculty of Medicine, Central Electron Microscopy Laboratory, Pécs, Hungary

UGO MOENS, Ph.D., University of Tromsø, Institute of Medical Biology, Department of Molecular Genetics, Tromsø, Norway

KAZUO NAGASHIMA, M.D., Ph.D., Department of Molecular and Cellular Pathology, Hokkaido University School of Medicine, Sapporo, Japan

AVINDRA NATH, M.D., Department of Neurology and Department of Microbiology and Immunology, University of Kentucky, Lexington, KY

MASSIMO NEGRINI, Ph.D., Department of Experimental and Diagnostic Medicine, Section of Microbiology, and Center for Biotechnology, University of Ferrara, Ferrara, Italy

OLE PETTER REKVIG, M.D., Ph.D., University of Tromsø, Institute of Medical Biology, Department of Molecular Genetics, Tromsø, Norway

HOLLY G. RESSETAR, Ph.D., Department of Neurobiology and Anatomy, West Virginia University School of Medicine, Morgantown, WV

TODD D. SCHELL, Ph.D., Department of Microbiology and Immunology, Pennsylvania State University College of Medicine, Hershey, PA

KEERTI V. SHAH, M.D., Dr. P.H., Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

YUKIKO SHISHIDO-HARA, M.D., Ph.D., Laboratory of Molecular Neurobiology, Human Gene Sciences Center, Tokyo Medical and Dental University, Tokyo, Japan

GERALD L. STONER, Ph.D., Neurotoxicology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

CHIE SUGIMOTO, Ph.D., Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

SATVIR S. TEVETHIA, Ph.D., Department of Microbiology and Immunology, Pennsylvania State University College of Medicine, Hershey, PA

MAURO TOGNON, Ph.D., Department of Morphology and Embryology, Section of Histology and Embryology, and Center for Biotechnology, University of Ferrara, Ferrara, Italy

DUARD L. WALKER, Ph.D., Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, Madison, WI

THOMAS WEBER, Dr. MED., Neurologische Klinik, Kath. Marienkrankenhaus GmbH, Hamburg, Germany

YOSHIAKI YOGO, Ph.D., Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

GABRIELE M. ZU RHEIN, M.D., Department of Pathology, University of Wisconsin Medical School, Madison, WI

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

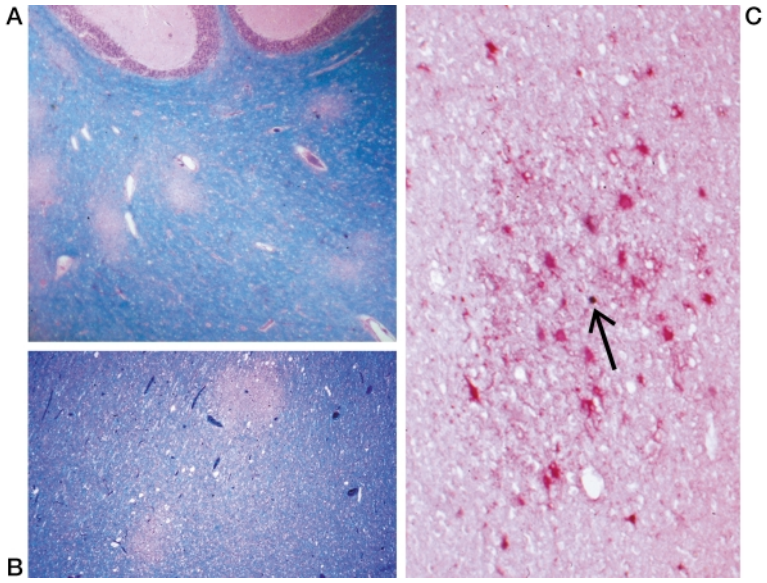


Figure 12.2. Early demyelinated lesions in cerebellar white matter. Luxol fast blue/H&E stain. For full caption, see page 260.

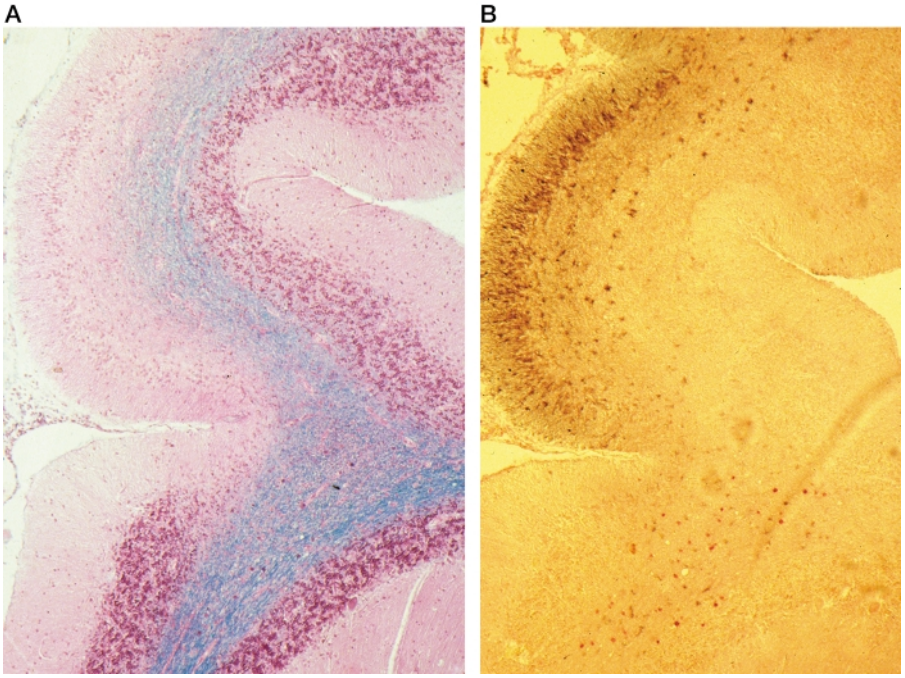


Figure 12.3. Cerebellar folia: Granule cell loss with underlying demyelination. For full caption, see page 261.

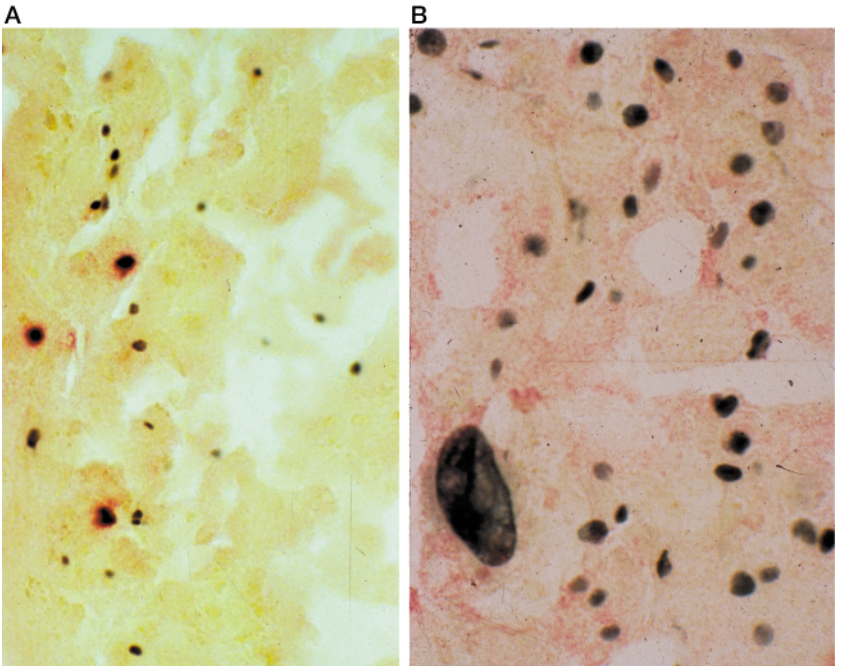


Figure 12.6. JCV protein expression revealed with double-label immunocytochemical staining for early (T antigen) and late (capsid) proteins. For full caption, see page 269.

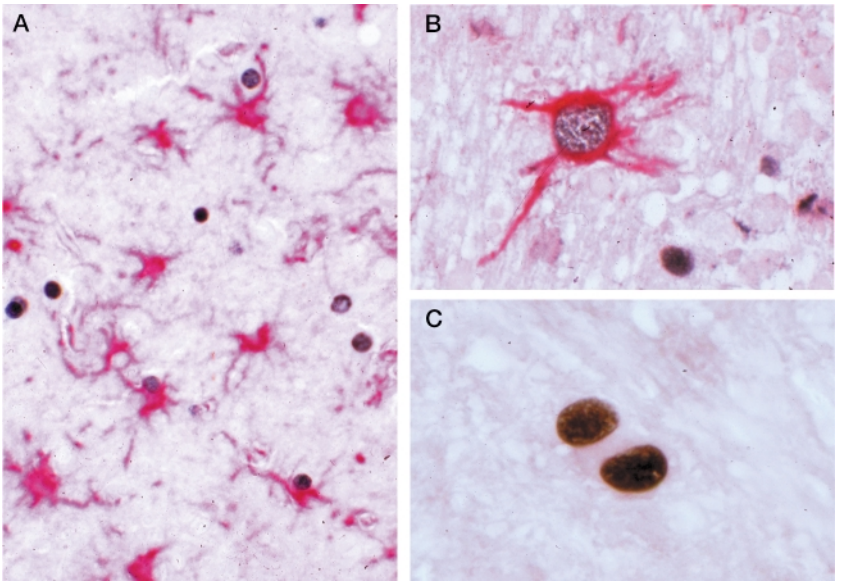


Figure 12.7. JCV protein expression revealed with double-label immunocytochemical staining for late (capsid) proteins and glial fibrillary acidic protein (GFAP). For full caption, see page 271.

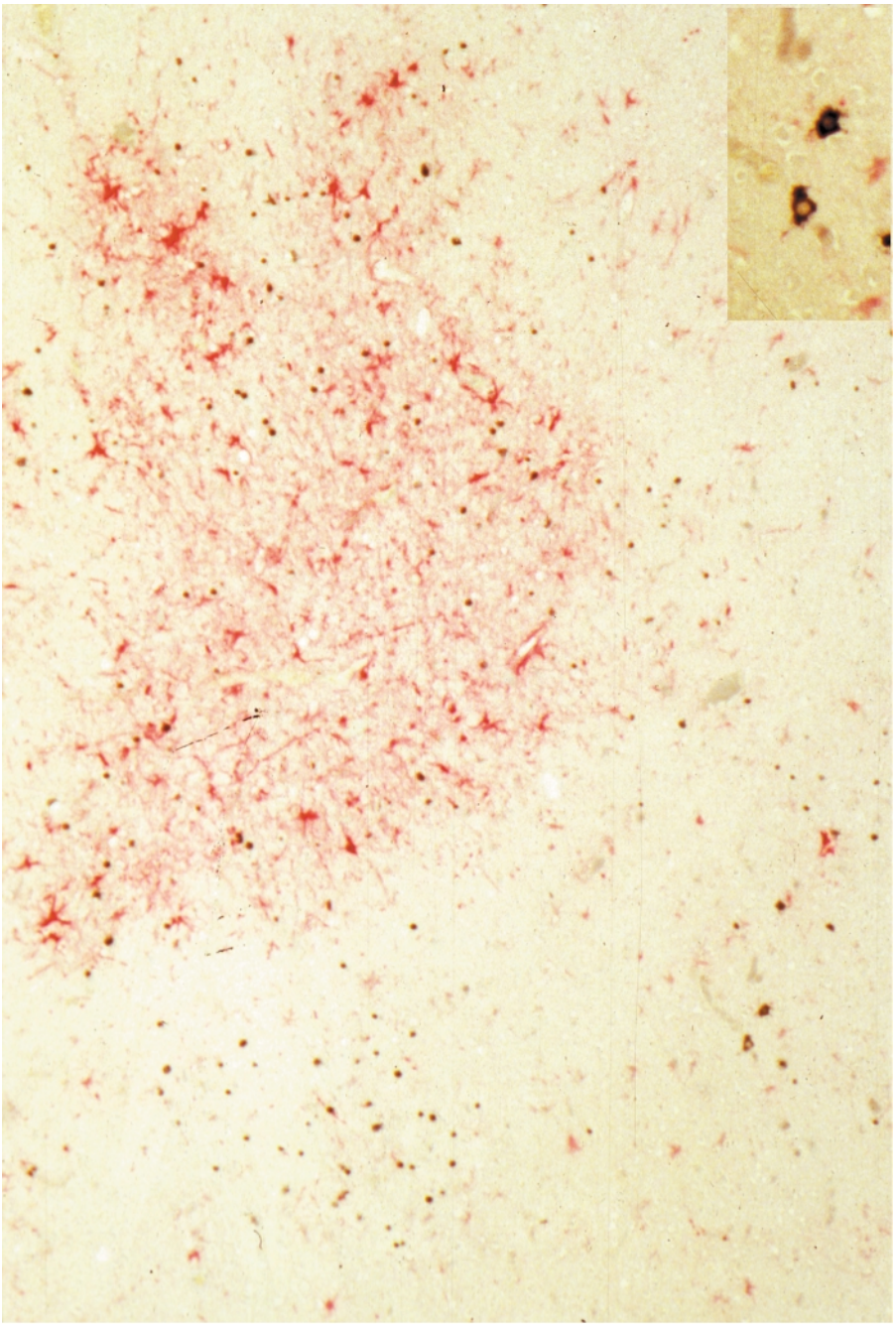


Figure 12.8. Double immunocytochemical label for GFAP and JCV capsid proteins (no counterstain). JCV-containing cells occur in the margins of the mature, gliotic demyelinated lesion and in the incipient lesion below the gliotic lesion with minimal astrocyte reactivity, but with numerous JCV-infected cells, including some with astrocyte morphology. Astrocytes with capsid located cytoplasmically are seen in the lower right corner and are enlarged in the inset.

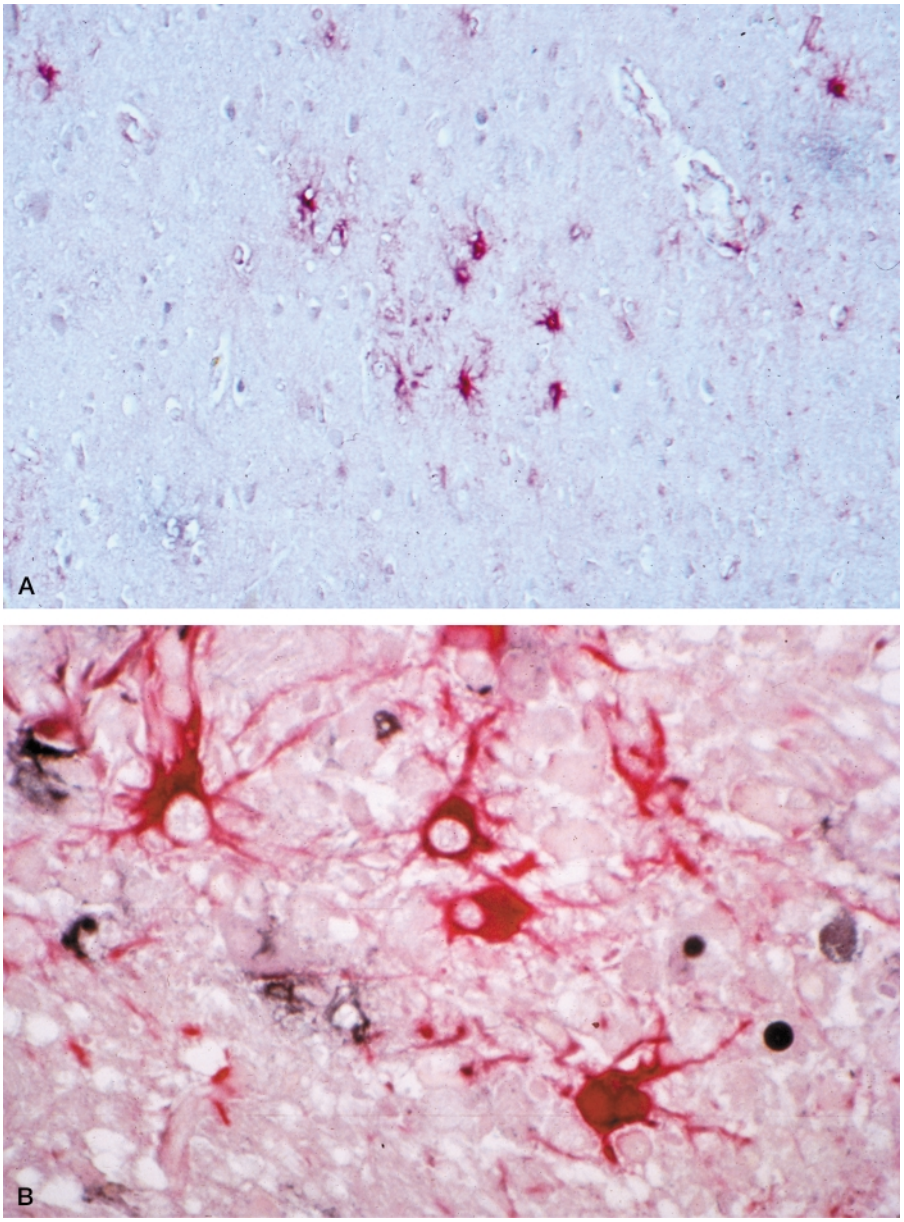


Figure 12.22. Astrocytes and PML pathogenesis. (**A**) Small cluster of enlarged, reactive astrocytes in the subcortical white matter of an early PML case discovered at autopsy (Åström and Stoner, 1994). Cells display GFAP reactivity (red), but no JCV-infected oligodendrocytes with capsid proteins (black) were evident. Method same as in Figure 12.2C. An adjacent section stained with LFB/H&E showed no evidence of myelin destruction (not shown). These astrogliotic clusters were the earliest discernible PML lesions. No counterstain. Medium magnification. Taken with permission from Åström and Stoner, 1994. (**B**) Established lesion in the basal ganglia with reactive astrocytes (red), including one with apparent perinuclear JCV capsid proteins in the cytoplasm (lower right). This cell extends a process to an infected oligodendrocyte with JCV capsid proteins in the nucleus (black). No counterstain. High magnification.

Figure 15.3. Induction of neural origin tumors by JCV T antigen in experimental animals. (**A**) JCV T antigen transgenic mice develop peripheral neuroblastoma, which can present as a solid, well-circumscribed mass, in the abdominal cavity (arrow). (**B**) Neuroblastomas are histologically characterized by densely cellular neoplasms with a high nuclear to cytoplasmic ratio. (**C**) The majority of the nuclei show immunoreactivity to the viral protein T antigen. (**D**) Transgenic mice generated with sequences for T antigen under the control of the JCV archetype promoter develop cerebellar medulloblastomas within the foliæ (arrow). (**E**) Histologically, the tumors appear similar to neuroblastoma. (**F**) JCV T antigen can be detected by immunohistochemistry in tumor cell nuclei. (**G**) T antigen transgenic mice may also develop pituitary tumors, which appear as large masses at the base of the skull (arrow). (**H**) Histology demonstrates a highly pleomorphic tumor (left) adjacent to normal pituitary (right). (**I**) Tumor cells show nuclear staining for JCV T antigen. (**J**) JCV-transformed HJC cells transplanted into the brain of syngeneic Syrian hamsters form an intracranial mass that can protrude through the superior aspect of the skull (arrows). (**K**) A clear line of demarcation is present between the highly cellular pleomorphic cells (left) and the surrounding normal brain parenchyma (right). (**L**) The majority of the tumor cells express nuclear T antigen. A, G, J, original magnification, $\times$4; D, original magnification, $\times$10; B, E, H, K, hematoxylin and eosin, original magnification, $\times$100; C, hematoxylin counterstain, original magnification $\times$100; F, L, hematoxylin counterstain, original magnification $\times$200; I, hematoxylin counterstain, original magnification $\times$400; D. Taken with permission from Krynska et al., 1999.

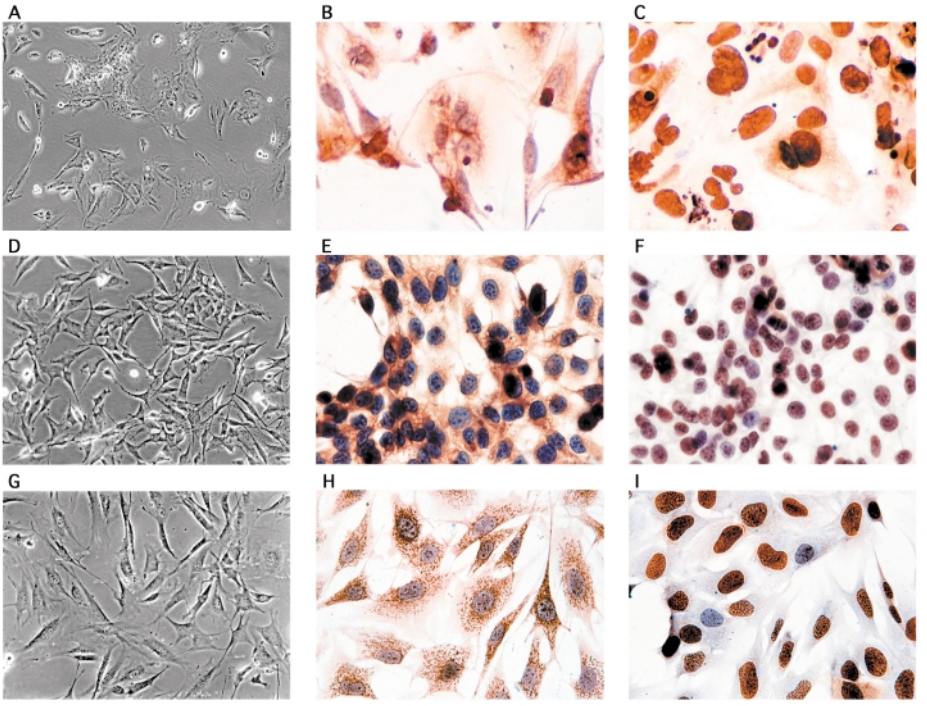


Figure 15.4. Morphologic features of cells derived from JCV-induced tumors in various experimental animals. (**A**) Phase microscopy of the glioblastoma cell line Owl 586 generated upon intracerebral inoculation of an owl monkey with JC virus. (**B**) Immunostaining with the cellular marker GFAP demonstrates that the tumor is of glial origin. (**C**) Immunostaining for JCV T antigen shows that the majority of the cells express T antigen in the nucleus. (**D**) HJC cells cultured from a tumor induced upon intracerebral injection of JCV into newborn Syrian hamsters shown by phase microscopy. (**E**) HJC glial cells are positive for GFAP. (**F**) Nearly all of the cells express JCV T antigen. (**G**) Phase contrast of BS-1 B8 cells derived from JCV T antigen–induced mouse medulloblastoma. (**H**) Synaptophysin staining of BS-1 B8 cells demonstrate the neuronal origin of the tumor cells. (**I**) The majority of the tumor cells show nuclear immunoreactivity for JCV T antigen.

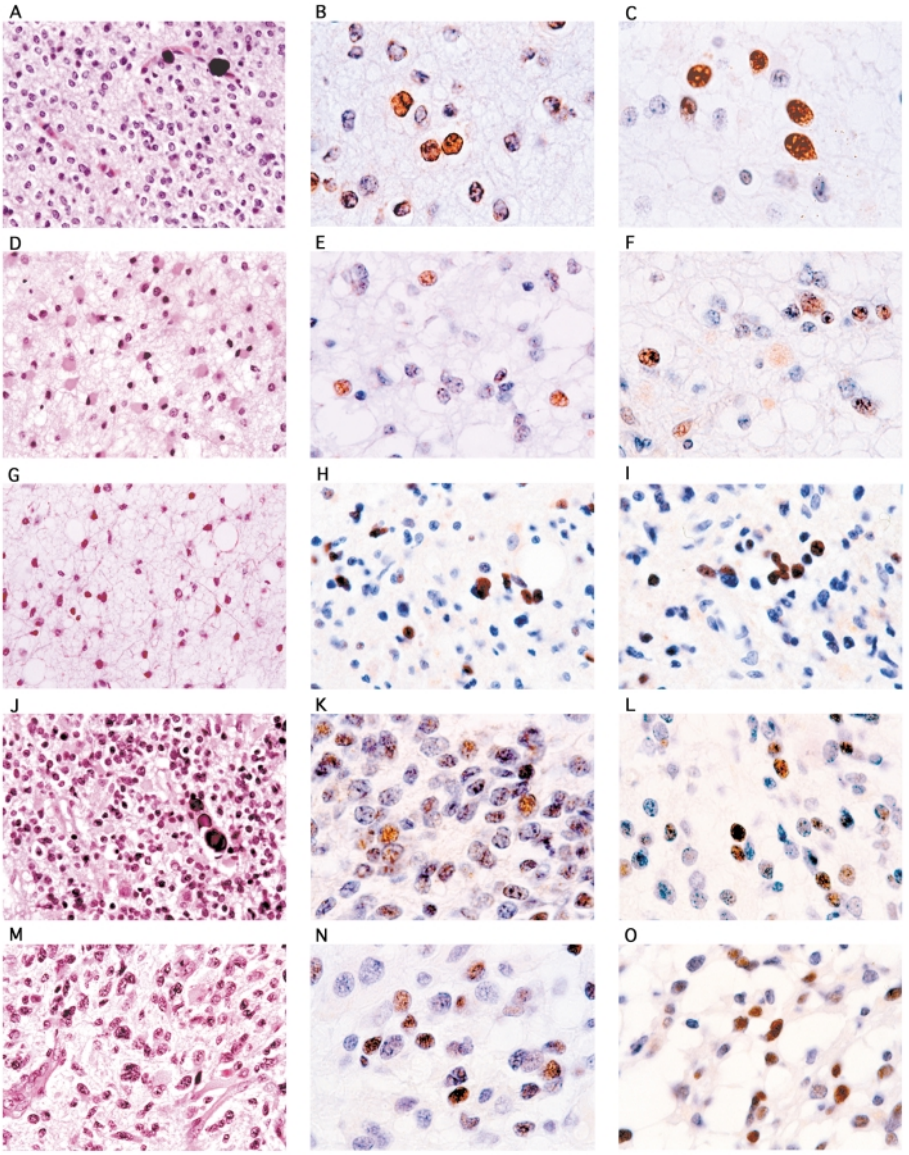


Figure 15.5. Detection of JCV T antigen and the cellular protein p53 in human glial origin tumors. Histologic evaluation and immunohistochemistry for JCV T antigen and the cellular tumor suppressor protein p53 in a number of human glial tumors is shown: oligodendroglioma (**A**, hematoxylin and eosin staining; **B**, immunohistochemical staining for T antigen; **C**, immunostaining for p53); gemistocytic astrocytoma (**D**–**F**); fibrillary astrocytoma (**G**–**I**); anaplastic oligodendroglioma (**J**–**L**); anaplastic astrocytoma (**M**–**O**). A, D, G, J, and M, hematoxylin and eosin, original magnification, ×400; B, C, E, F, H, I, K, L, N, O, hematoxylin counterstain, original magnification, ×1000.

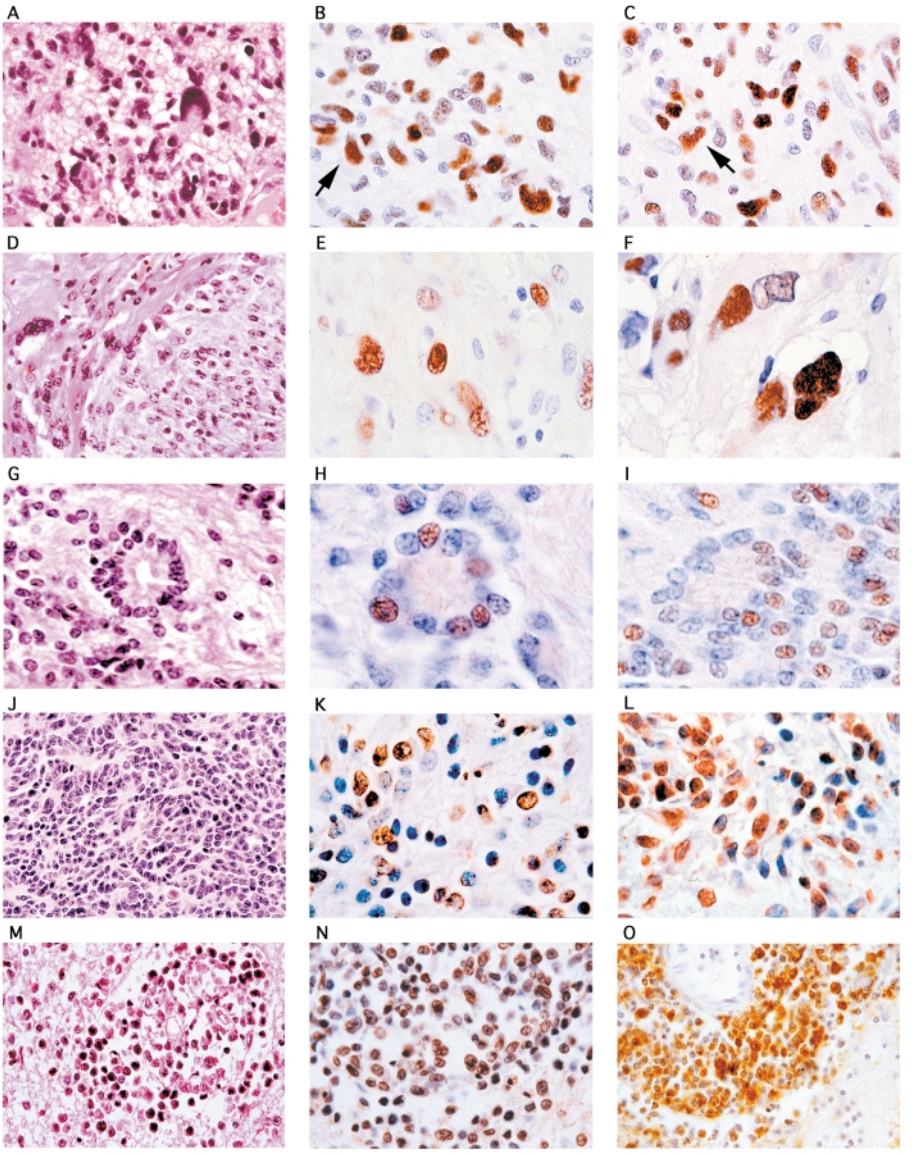


Figure 15.6. Immunohistochemical staining of various human tumors for T antigen expression. JCV T antigen has been detected in a variety of human tumors. Histology and immunohistochemistry for T antigen and cellular proteins are shown for the following tumors: glioblastoma multiforme (**A**, hematoxylin and eosin staining; **B**, immunohistochemical staining for T antigen; **C**, immunostaining for p53); gliosarcoma (**D**–**F**), ependymoma (**G**–**I**), medulloblastoma (**J**–**L**), B-cell lymphoma (**M**, hematoxylin and eosin staining; **N**, immunohistochemical staining for T antigen; **O**, immunostaining for the EBV protein latent membrane protein [LMP]). A, D, G, J, and M, hematoxylin and eosin staining, original magnification, ×400; B, C, E, F, K, L, N, and O, hematoxylin counterstain, original magnification, ×400; H and I, hematoxylin counterstain, original magnification ×1000.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

2

PAPOVA VIRIONS IN PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY: A DISCOVERY AT THE INTERFACE OF NEUROPATHOLOGY, VIROLOGY, AND ONCOLOGY

GABRIELE M. ZU RHEIN, M.D.

1. INTRODUCTION

Personal memory has a dubious reputation, and great caution is indicated when bringing recollections to paper. This report, however, has been chiefly synthesized from work records, from publications, from professional documents, from my correspondence, and from my personal diaries. Friends, such as Dr. Sam Chou and Dr. Richard Johnson, were kind enough to contribute details.

2. FROM PATHOLOGIST TO NEUROPATHOLOGIST—A PROFESSIONAL JOURNEY

Before starting my academic career in the United States in 1954, I lived in Munich, Germany, and graduated there from the Medical School of the Ludwig-Maximilian University in 1945. Already as a student my chief attention was devoted to pathology. As an old Sherlock Holmes addict, I enjoyed the required detective work, and, as a visually oriented person, I enjoyed the pleasures of microscopy, offering such a richness of colors and patterns. With little effort I was able to convince my father to buy me a microscope so that I could extend my slide studies into the weekends.

My favorite pathology professor, Dr. Ludwig Singer, hired me right after graduation. A few weeks later, however, units of the 6th U.S. Army marched into Munich and occupied the undamaged Municipal Hospital, where our laboratory was located. The new Chief of Laboratory of the 98th General Hospital, Dr. Maurice Lev, invited me to join his group, and this affiliation, as a pathologist, persisted for 8 years. My position was one of many given to German physicians, by the German government, to assist U.S. Medical Units. I was assigned to do autopsies and surgicals and to give clinicopathologic conferences. My training proceeded ''on the go.'' I was extremely fortunate that many of the drafted pathologists, back home, had been university professors or directors of laboratories, and I received from them personal instruction and guidance. The medical library contained the latest editions of textbooks and a fine selection of journals.

Just outside the walls of the 98th General Hospital was located the Max Planck Institute of Psychiatry, where Professor Willibald Scholz was the Director of the Neuropathology Division. A person of international renown, he conducted biannually an introductory course in neuropathology for army officers, to which I was also invited. Untold times in later years did I think back in gratitude to this exceptional experience, which gave me my first understanding of the complexities of nervous system diseases. An additional contact with neuropathology was offered to me by Dr. E. Manuelidis, from Yale University, who worked on porcine encephalomyelitis (Teschen's disease). He was a member of an epidemiology team, led by Drs. John Paul and Dorothy Horstmann, that was hosted—including their well hidden pigs!—by our laboratory.

Dr. Alfred Evans, of the Yale University team, a strong advocate for my emigration to the United States, became Professor of Epidemiology at the University of Wisconsin, in Madison, after his return to civilian life. He scouted out for me a position in the Department of Pathology, and I gladly accepted the challenge. In late December 1953, I sailed in the company of my newly acquired Leitz Ortholux microscope, the best on the German market, purchased with the shares of my mother's inheritance.

Dr. D. Murray Angevine ran a department well known for its experimental pathology. Diagnostic human pathology was of secondary interest because not much scientific progress was expected from it. Whoever did not work with rats,

guinea pigs, or dogs was a member of a lower caste. I was assigned to the autopsy service and to teaching medical students. A few months after my arrival, a professor, who had cut the brains, departed. With this came my greatest challenge. Very casually, Dr. Angevine said to me ''The Germans have a good reputation for neuropathology; why don't you do it? The others know much less than you.'' He threw me into the water, and I had to swim. My anguish about this situation was communicated in a May letter to Professor Scholz (in translation): ''My feelings are very mixed. On one hand I am glad that I can now put to use what I learned from you, but on the other hand it is difficult to find the courage to start as an embryo in this field while having to give lectures to medical students already in the fall.''

Neuropathology as a subspecialty of pathology was still a novelty in 1954. Where would I find appropriate texts to read? In English, there was one small volume by Dr. Ben Lichtenstein, published in 1949. A German book on inflammatory diseases of the nervous system, of 1942, had been in my immigration luggage. Eventually, my ''life jacket'' proved to be the seven volumes on diseases of the nervous system edited by Professor Scholz, and published by Springer Verlag from 1955 to 1958, with 95% of the contributions written in German. Our medical school librarian permitted me to keep these on my desk until another reader would request them. This never happened. It was only in 1958, and 1959, respectively, that the first comprehensive textbooks of neuropathology written in English became available: Greenfield's *Neuropathology* and Russell and Rubinstein's *Pathology of Tumours of the Nervous System*.

A benefactor, without whose support and friendship I could have hardly succeeded, was Dr. Hans Reese, Chairman of Neurology and Psychiatry at the University of Wisconsin. A German by birth, he was sympathetic to my plight. He supplied me with helpful books and journals, made his tissue technician available to me, and arranged for personal contacts with neurologists and pathologists at national and international meetings and congresses. Later, I became a member of the Neurology Department, in a double appointment, conducting clinicopathologic conferences and teaching residents on a neuropathology rotation.

3. AN INTEREST IN VIRAL DISEASES SHARED WITH DR. CHOU; ARRIVAL OF PML CASES AND AN ELECTRON MICROSCOPE

Another interdisciplinary contact, which became of considerable importance later on, developed with Veterinary Science. In this Department, Dr. Carl Olson had created a research unit for the study of papilloma viruses, especially of bovine and canine types. A viral oncologist who appreciated the contributions of morphology, he early had acquired an electron microscope. My affiliation with Veterinary Science began in 1957. All such students had to do course work in pathology, and I became their instructor in neuropathology. Over the years I served on at least nine examination committees for M.S. or Ph.D.

candidates, most of them from Dr. Olson's group and others from Dr. Robert Hanson's group which focused on scrapie and transmissible mink encephalopathy.

Meanwhile, in clinical autopsy work, one disease that attracted my particular interest was an acute necrotizing encephalitis with intranuclear inclusion bodies. We observed three such cases from 1957 to 1960 and published the data in 1962. The first case had occurred after head trauma, and I had difficulties with the diagnosis. However, Dr. Stanley Inhorn, a resident engaged in virus research, exhorted me to persist in looking for inclusion bodies. The slow and tedious work was eventually successful, and a herpetic infection could be suggested. With trained eyes the search became much easier in the two following cases. This was a time when immunocytochemistry was not yet part of the diagnostic arsenal.

In 1959, Dr. Sam (Shi-Ming) Chou joined the Department of Pathology as a postdoctoral student with the aim of completing a Ph.D. program in Zoology and Pathology. Supported by the National Multiple Sclerosis Society, he engaged in research in neurolathyrism. By 1962, he had decided that he would choose neuropathology as his career. He opted to take the course that I gave for the neurology residents.

In the fall of 1962, a particularly stimulating consultation case was presented to me by the pathologist of a downtown Madison hospital. The patient, a 33-year-old woman with lupus erythematosus, had died after several weeks of progressive cerebellar disease. The slides showed a multifocal demyelinating disease with a most striking combination of giant tumor-like astrocytes and large numbers of oligodendrocytes with greatly enlarged nuclei deeply stained with hematoxylin. There were no distinct inclusion bodies as one sees with herpes viruses. I was fascinated and knew I had never seen this disease before. I showed the slides to a visiting neuropathologist and he, too, was at a loss. At that time I was in the midst of a very time-consuming experiment with a group of sophomore students. It involved the induction of brain tumors in chicken with Rous sarcoma virus. There was no time for a library search. However, I did show the slides to Dr. Chou, and to my utter surprise, and delight, he brought from his desk a folder with reprints on demyelinating diseases from which he extracted the paper entitled ''Progressive Multifocal Leukoencephalopathy'' by Åström, Mancall, and Richardson, Jr. (1958) and Richardson's follow-up paper of 1961. We had no doubt that our consultation case was one of the less than 30 cases of this disease known at that time. Only 2 months later, a 67-year-old woman came to autopsy in our department (A 62-393). She had suffered from chronic sinusitis and bronchitis and had developed a left hemiparesis and mental changes during a 7 month period. The clinical diagnosis was multiple infarcts. In the formalin-fixed brain I noticed extensive myelin destruction, and the cytopathology, without doubt, was that of progressive multifocal leukoencephalopathy (PML). We used our sudden wealth of two PML cases for local conferences and teaching exercises.

In 1963, the Pathology Department faculty insisted on the acquisition of an electron microscope to aid various research projects. Dr. Chou, who had learned the technique from Dr. Hans Ris in Zoology, became one of the first users of our RCA EMU 3G instrument (Fig. 2.1). I realized my chance to do some acceptable research at a raised level of morphology. Dr. Angevine had agreed to a sabbatical leave, and Dr. Reese had secured for me a position in the Neuropathology Laboratory of Dr. Harry Zimmerman, at Montefiore Hospital, Bronx, New York. Before thinking of leaving, however, I had to find a colleague who would pitch in for me at home. Dr. Chou graciously consented to help. Thus, in a reciprocal arrangement, I taught him more diagnostic neuropathology and he taught me how to run the electron microscope.

In May 1964, another of Dr. Olson's students took his Ph.D. examination, and I was a thesis reader. Among the illustrations for ''The Cytology of Canine Oral Papilloma'' were electron micrographs of cell nuclei with dispersed or aggregated virions. Listed among the references was Dr. Melnick's paper in *Science* (1962) entitled ''Papova Virus Group.'' In it, he combined the papilloma and polyoma viruses into one group of oncogenic DNA viruses, capable of producing latent infections. The morphology of virions of this group, in thin sections, had been characterized just within the last few years.

Figure 2.1. The electron microscope, an RCA EMU 3G, in which we first saw the intranuclear virions in brain tissue with progressive multifocal leukoencephalopathy (PML).

In August, one of our autopsy cases showed an extensive esophagitis with distinct intranuclear inclusion bodies. I planned to make up for my lost chance with the encephalitis cases and to search for herpes group virions.

4. A LUCKY DAY FOR TWO NEUROPATHOLOGISTS AND MORE LUCK ON A SABBATICAL

In discussions with Dr. Chou, we decided to give another project priority, namely, the clarification of the mystery of the abnormal oligodendrocytes in PML. We felt unencumbered by the strict rules for optimal ultrastructural images as worked out by cell biologists. The structural proteins of viruses were perhaps still preserved despite postmortem delays in fixation and immersion in 10% formalin for 2 years. What was there to lose in trying? On August 7, 1964, Dr. Chou and myself attended a celebration for a student friend who had passed her preliminary examination. My diary shows the following entry, in mixed English and German: ''Pink Bacardi mit cherry!! Dann erste EM session mit Sam, 62-393. CRYSTAL INTRANUCLEAR VIRUS!!!! POLYOMA?? Bis 5 h.'' We found the crystalloid aggregate (Fig. 2.2) in the first ultrathin section,

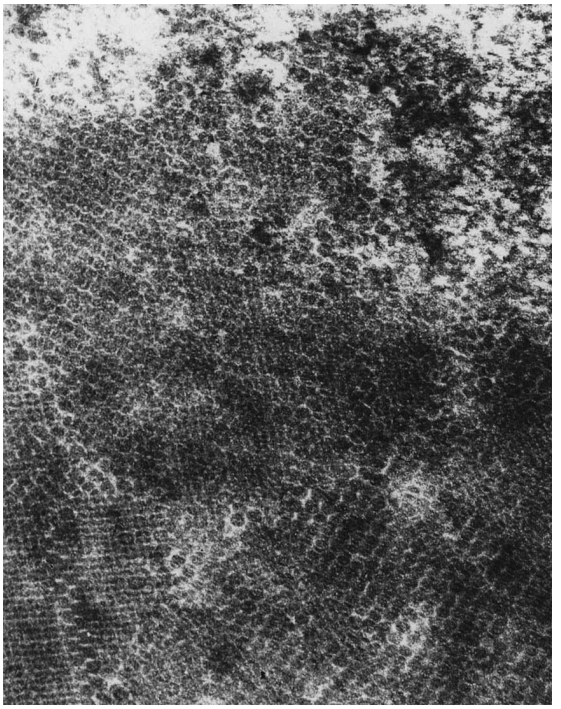


Figure 2.2. Electron micrograph obtained on the day of the discovery of the papova virions, here arranged in a crystalloid pattern. ×65,000 orig. mag.

and we could not believe our luck and kidded about the influence of the Bacardi. After Dr. Chou developed the first photographic plates that also revealed scattered virions and filamentous forms, we hugged in the dark room; what an exciting day in our lives it was. Whereas the tissue in general showed moderate autolysis, the virions were so well preserved that their images compared favorably with those in the literature. The virions belonged to an identifiable group, with oncogenic potential, perhaps explaining the bizarre shapes of astrocytes, and this virus had penetrated deep into the brain, unlike papilloma viruses. Yet no human polyoma virus was known at that time. When we showed the plates to Dr. Angevine, his dry comment was: ''This is the way discoveries are being made.''

In early September, I arrived at Montefiore Hospital equipped with Epon blocks of the esophagitis case and PML case 62-393, with the first prints of the latter and with Ludwik Gross's book titled *Oncogenic Viruses* (Gross, 1961), which became my ''bible.'' When Dr. Zimmerman saw the prints, he found them exciting enough to add a presentation, belatedly, to the program of the forthcoming ARNMD (Association for Research in Nervous and Mental Diseases) symposium entitled ''Infections of the Nervous System.'' Dr. Zimmerman, at that time, was President of the ARNMD and the program coordinator.

In addition to continuing electron microscopy now also on our second case of PML, I was forced to rapidly pursue library studies. Which journals and books was I to read in a field at the crossroads of virus and cancer research and cell biology? It required a fast reorientation for a diagnostic neuropathologist. In September, I was able to resolve the nature of the nuclear inclusion bodies of the esophagitis case: They consisted of herpes-type virions, well preserved in this autopsy tissue. In mid-November, I got a phone call from Dr. Lucien Rubinstein, of Stanford University. He had just received the ARNMD program with the listing of ''Papova Virus in Progressive Multifocal Leukoencephalopathy'' by Zu Rhein and Chou and wanted to inform me that he and Dr. Silverman had found similar virus particles in a recent case of PML (Silverman and Rubinstein, 1965). He offered to back us up in the meeting, with Dr. Zimmerman's consent.

5. THE "COMING-OUT PARTY"

On December 5, 1964, with several illustrious virologists presiding on a dais and Dr. Chou watching intently from the balcony, I presented our two cases, followed by Dr. Rubinstein. His case had never been in formalin. At once Dr. Sabin took over and tore into us with vigor. ''This is deplorable, everybody thinks everything is a virus'' (quote from my diary), and ''One cannot say one has morphologic evidence of a virus,'' and, with regard to the electron micrographs, ''That is a good way not to get a virologist interested'' (Sabin, 1968). Dr. Rubinstein countered that all electron microscopes should be thrown into

a garbage can if Dr. Sabin's attitude were correct. None of the panel members commented in our favor. I remember wishing that a helicopter would come and lift me out of there, as I had read in the papers they did with beleaguered white people in the jungles of the war-torn Congo. After the session, Dr. Richard Johnson (Cleveland) gave me a big hug and consoled me with the idea that this attack might bring some prestige later on. Dr. Sabin had said to him in disdain: ''She thinks there are warts in the brain!'' At lunch, I ended up next to Dr. Sabin at the table in the Rough Rider room of the Roosevelt Hotel. He leaned over and gave this parable: ''You know, Doctor, if you take a piece of wood, and you carve it, and you paint it, and you polish it, it will eventually look like an apple.''

6. THE "SELLING OF THE APPLES": ACCEPTANCE, COLLABORATIONS, AND INVITATIONS

Well, our ''apples'' sold fast and widely. *Science* published our first paper (Zu Rhein and Chou, 1965). Dr. Zimmerman had agreed that we should not wait for the publication of the ARNMD volume, which indeed became delayed for 4 years. A neighbor in the Bronx was Dr. Ludwik Gross, Director of the Cancer Research Unit of the VA Medical Center. A pioneer in virus research, he had stood at the cradle of the polyoma virus. Some of his work had also been met with disbelief early on. He was a virologist well versed in viral morphology. When he reviewed our PML electron micrographs, he shared our interpretation with enthusiasm. He invited me subsequently to join him in a research seminar on ''Viruses in Disease.'' We stayed in close contact for the later editions of the *Oncogenic Viruses*. Dr. Richard Shope (Rockefeller University), discoverer of the rabbit papilloma virus, also became an immediate supporter during a laboratory visit. ''You are in business'' he told me with confidence and true joy.

The first international support, early in 1965, came from Dr. Allan F. Howatson, of the Ontario Cancer Institute, who had extensive experience with the ultrastructure of wart and polyoma viruses. He requested PML tissue in order to apply the negative staining method developed in 1959 by Brenner and Horne for the visualization of viral capsid details in spray preparations. Despite the original formalin fixation of the tissues, it was possible to classify the PML virions as polyoma rather than papilloma virions (Howatson, et al., 1965). Previously, it had been the smaller particle size alone that made us favor polyoma virions. In 1966, Dr. Howatson collaborated with Dr. Chou and myself in a poster exhibit entitled ''Polyoma-like Virions in a Human Demyelinating Disease,'' which was shown during the annual meetings of the American Academy of Neurology (Fig. 2.3) and the Electron Microscopy Society of America. In it, the PML virions were compared with polyoma virions in infected mouse kidney.

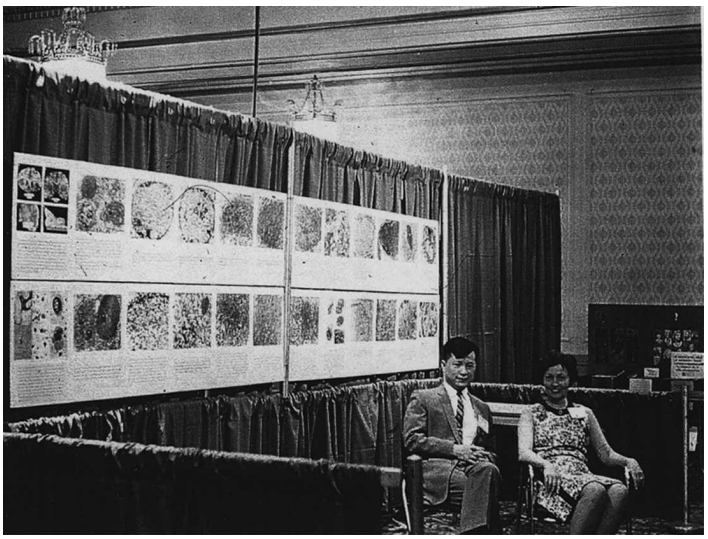


Figure 2.3. Dr. Sam Chou and myself with our poster exhibit "Polyoma-like Virions in a Human Demyelinating Disease" at the annual meeting of the American Academy of Neurology, in Philadelphia, in 1966.

The first European recognition came from Dr. W. Bernhard, Director of the Cancer Research Institute in Villejuif, France. He was a pioneer of viral research and electron microscopy with a special interest in murine polyoma virus and SV40. Following earlier correspondence, he invited me in the summer of 1965 to visit his laboratory—and also to enjoy French cuisine! He was convinced that the glial nuclei in PML were infected by a polyoma group virus.

The acceptance of our work by Dr. Joseph L. Melnick (Baylor College, Houston, Texas) was especially gratifying. As the Series Editor of *Progress in Medical Virology*, he solicited from me a review article (Zu Rhein, 1969) for which I could collect 27 cases of PML with ultrastructurally proven papova virions. Of these, 19 were confirmatory studies by other investigators. No negative findings came to my attention. Data on biologic studies were scarce chiefly due to the rarity of PML cases before the advent of AIDS. The results obtained by four laboratories, some related as personal communications, were negative in their in vivo and in vitro aspects. A variety of routinely used cell lines had been employed in these attempts.

Invitations to symposia, workshops, and meetings were gladly accepted to spread the knowledge of PML to a wider range of physicians in the hope for eventual tissue retrieval. In 1966, the National Multiple Sclerosis Society assembled a workshop at the USPHS Rocky Mountain Laboratory, in Hamilton, Montana (Fig. 2.4). Representatives of virology, pathology, epidemiology, and neurology discussed "slow virus" diseases such as subacute sclerosing panencephalitis, PML, scrapie, and Kuru at a time when the concept of "neurovi-

Figure 2.4. Workshop at the USPHS Rocky Mountain Laboratory, in 1966, dealing with "slow virus" diseases. Clockwise from left: Carl M. Eklund, Hamilton, Montana; John Seal, NIAID; Gabriele Zu Rhein, Madison, Wisconsin; Ellsworth C. Alvord, Jr., Seattle, Washington; Richard T. Johnson, Cleveland, Ohio; Jacob A. Brody, NINDB, Bethesda, Maryland; Hilary Koprowski, Philadelphia, Pennsylvania; William J. Hadlow, Hamilton, Montana; John Hotchin, Albany, New York; Clarence J. Gibbs, Jr., and D. Carleton Gajdusek, NINDB, Bethesda, Maryland.

rology'' had not yet been developed. In 1967, an international symposium on the ''Pathogenesis and Etiology of Demyelinating Diseases'' was convened in Locarno, Switzerland, with the support of a German multiple sclerosis foundation. It brought to light several Japanese cases of PML that were collected and studied, with the demonstration of virions, by Dr. F. Ikuta at Niigata University.

7. A NEW CULTURE SYSTEM FOR VIRUSES PRESENTED AT A NEUROPATHOLOGY MEETING

A remarkable, lucky coincidence in viral research happened in June 1965, when, during the annual meeting of the American Association of Neuropathologists, in Atlantic City, Dr. Harvey Shein (Boston) presented a paper entitled ''Interaction of a Tumor Virus (Simian Virus 40) with Human Fetal Spongioblasts and Astrocytes in Dispersed Cell Cultures.'' In this study, Shein, for the first time, had put to use a culture system that he had recently developed.

I was fascinated by the dual cytopathic effects of necrosis of spongioblasts and transformation of astrocytes so similar to the lysis of oligodendrocytes and ''transformation'' of astrocytes in PML. In a discussion of Shein's paper (Zu Rhein, 1966), I proposed the use of this culture system for the isolation of the postulated PML polyoma virus. Dr. Shein expressed to me personally, and in later letters, a strong desire for collaboration if fresh PML tissue became available.

8. HARVESTS OF FRESH PML TISSUES AND THE BIRTH OF JC VIRUS

A first ray of hope came from a resident in hematology at the VA Hospital in East Orange, New Jersey, in March 1967. In her letter, Dr. Aurea R. del Rosario referred to our paper in *Science* and asked for diagnostic help for a patient with Hodgkin's disease who had developed subacute neurologic symptoms consistent with PML. I offered to read a biopsy specimen. However, the patient declined the procedure. It became a long wait. Dr. del Rosario was able to obtain from the patient's mother an autopsy permit limited to the brain, with my name given as the recipient. The patient died on January 21, 1968, a Sunday, which I had spent with friends. As I learned the following day, Dr. Sidney Trubowitz, Chief of Hematology, who was anxious for a diagnosis but was unable to reach me, had already contacted another pathologist for help. After settling the issue amiably with this colleague in New York, I flew to East Orange, cut the brain, found the lesions compatible with PML, sampled for virology and pathology, and happily arrived with my harvest back in Madison around midnight, to find Dr. Duard Walker waiting with open arms in the autopsy room.

Dr. Walker, a Professor of Virology in the Department of Medical Microbiology, had been known to me since 1956, when he had invited me to participate in a paper—my very first one—on Coxsackie virus infection. He later followed the PML story with great interest and always received news from me, including that of Shein's culture system. With the tissue in hand, soon proved to contain the familiar virions, he wanted to have a first try at isolation before sharing material with Dr. Shein.

In June 1970, I received the histologic slide of a brain biopsy of John F. Cunningham, a patient with Hodgkin's disease and rather rapidly progressing neurologic deficits. The patient was under the care of Dr. Bertram Dessel, Chief of Hematology at the VA Hospital in Wood, Wisconsin. Dr. Dessel had known since 1967 of my interest in PML, when I had done ultrastructural studies on autopsy tissue of a previous PML case at his hospital. When Dr. Dessel conveyed my diagnosis of PML to Mr. Cunningham, he expressed the wish that his brain should aid research into this fatal disease. Dr. Dessel's call came on July 12, again on a Sunday. He was able to reach me in the laboratory. The alarm went promptly to Dr. Walker, and we joined forces for tissue retrieval

in the Wood VA Hospital autopsy room. It was Mr. Cunningham's brain out of which the new human polyoma virus, named JC virus after him, was born, thanks to Dr. Walker and his research associate Dr. Billie Padgett, and to Dr. Harvey Shein. The ''birthday party'' took place on March 24, 1971. We rejoiced and thought of Dr. Sabin.

9. A POST-ISOLATION LIFE WITH HAMSTERS AND—AGAIN—THE ELECTRON MICROSCOPE

In the following months and years many strategies were designed to determine the characteristics, and the biologic effects, of the new virus. My share of these investigations consisted of ultrastructural studies of the virus in spray preparations, in fetal glial cell cultures, and in immune reactions with sera of other papova viruses and, predominantly, of experiments with Syrian hamsters. My collaborators during a more than 10 year period were Robert J. Eckroade, D.V.M.; Albertina E. Albert, Ph.D.; John N. Varakis, M.D.; and Takeo Ohashi, M.D. Our support came initially from an NIH grant instigated by Dr. Walker and later from a grant in my name.

The first sick hamster was autopsied 4 months after subcutaneous and intracerebral inoculation. A large cerebellar medulloblastoma (Fig. 2.5) demonstrated not only the oncogenic potential of JC virus but also a difference in tumor phenotype from neoplasms induced in the same host by mouse polyoma virus and SV40. Overall, in numerous experiments, medulloblastomas remained the most frequent tumor type. However, JC virus behaved uniquely polyoncogenic for the nervous system (Zu Rhein, 1983), a joy and challenge for a neuropathologist. Tumors of the pineal gland had never before been experi-

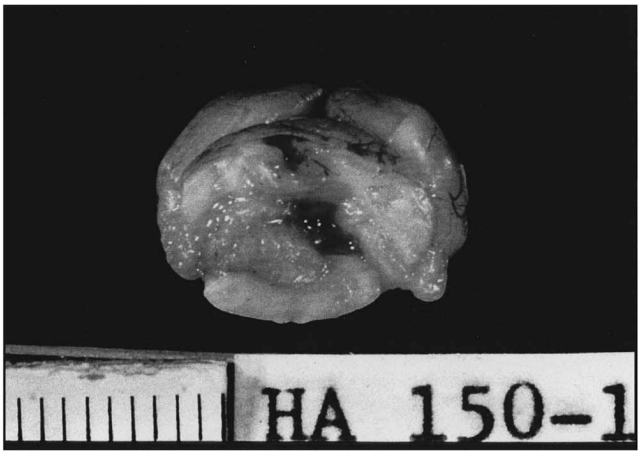


Figure 2.5. The first grossly noted JCV-induced hamster tumor, 4 months after inoculation: a medulloblastoma that has subtotally destroyed the cerebellum.

mentally induced. JC virus also became the first human virus to induce solid tumors, namely, glioblastomas, in primates. Neither in Syrian hamsters nor in owl monkeys did we observe a PML-like demyelinating disease. My sincerest wish is that JC virus would be found to be a causative factor in certain human brain tumors and that specific methods could be designed to prevent or treat them.

REFERENCES

Gross L (1961): Oncogenic Viruses. Pergamon Press: New York.

Howatson AF, Nagai M, Zu Rhein GM (1965): Polyoma-like virions in human demyelinating brain disease. Can Med Assoc J 93:379–386.

Sabin AB (1968): Discussion following presentation by G.M. Zu Rhein and S.M. Chou of ''Papova virus in progressive multifocal leukoencephalopathy.'' Res Publ Assoc Nerv Ment Dis 44:362.

Silverman L, Rubinstein LJ (1965): Electron microscopic observations on a case of progressive multifocal leukocencephalopathy. Acta Neuropathol (Berl) 5:215–224.

Zu Rhein GM (1966): Discussion following presentation by H.M. Shein of ''Interaction of a tumor virus (simian virus 40) with human fetal spongioblasts and astrocytes in dispersed cell cultures.'' J Neuropathol Exp Neurol 25:160.

Zu Rhein GM (1969): Association of papova-virions with a human demyelinating disease (progressive multifocal leukoencephalopathy). Prog Med Virol 11:185–247.

Zu Rhein GM (1983): Studies of JC virus–induced nervous system tumors in the Syrian hamster. Prog Clin Biol Res 105:205–221.

Zu Rhein GM, Chou SM (1965): Particles resembling papova viruses in human cerebral demyelinating disease. Science 148:1477–1479.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

3

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY: CULTIVATION AND CHARACTERIZATION OF THE ETIOLOGIC AGENT

DUARD L. WALKER, M.D.

1. INTRODUCTION

Following the 1965 demonstration by Gabriele Zu Rhein and Shi-Ming Chou (Zu Rhein and Chou, 1965) and independently by Lloyd Silverman and Lucien Rubenstein that oligodendrocytes in the brain lesions of patients with progressive multifocal leukoencephalopathy (PML) contained numerous particles that looked like papovavirus virions, it was inevitable that many virologists would try to isolate and cultivate the virus in cell cultures or experimental animals. In electron micrographs the particles looked very much like the virions of the polyomavirus genus of the papovavirus family of viruses. But in 1965 no polyomavirus was known to infect people. The particles were smaller than virions of papillomaviruses, which make up the other genus of the papovavirus family. However, some of the papillomaviruses were known to infect people, producing skin and mucous membrane tumors, so they had to be considered. The two major polyomaviruses were simian virus 40 (SV40), a virus found in wild

rhesus monkeys, and mouse polyoma virus found in mice. Those two viruses had been studied extensively and were not considered difficult to cultivate. Mouse polyoma virus is easily cultivated in many mouse cell types, but particularly in mouse kidney cells, and SV40 grows very well in primary monkey kidney cell cultures or kidney cell lines of several monkey species. Thus it was quite reasonable to expect that if the virus in PML was a polyomavirus, the cultural techniques that work with mouse polyoma virus and SV40 would work well with this human polyoma-like virus. If the virus should happen to be an unusual papillomavirus, then the chances of successful cultivation were poor because no one had succeeded in cultivating human papillomaviruses in cell cultures despite many attempts.

The simple way to start would be to put the virus into human fetal kidney cell cultures. I am quite certain that many virologists had that plan in mind—provided that they could get virus-containing tissue from a case of PML. That matter of obtaining diseased tissue for an inoculum was going to be a limiting factor. I do not know how many virologists were successful in obtaining appropriate tissue, but there were a few. There may have been others who did not report their trials, but a few did so. By mid-1966 we learned through a publication that Lucien Rubinstein, who had reported his electron microscopic observations of PML, had provided unfixed tissue to his Stanford virologist colleagues, P.R. Schwerdt and C.E. Schwerdt. The Schwerdts did some concentration, purification, and physical characterizations of the virus and concluded that it was a papovavirus and one that had the characteristics of the polyomavirus genus. They inoculated cultures of human diploid cell lines of embryonic lung, embryonic skin and muscle, and an established line of human glial cells but found no evidence of viral multiplication even after multiple subcultures. They inoculated newborn hamsters subcutaneously with brain tissue extract but found no evidence of infection or tumors during 10 months of observation.

In 1967, C.L. Dolman, in Vancouver, British Columbia, reported extensive work that obviously had been in progress for quite a long time. Dolman worked with tissue from two cases of PML and inoculated cultures of multiple human, monkey, and hamster cell types and made blind passages but saw no evidence of cytopathic effect or cell transformation. Two monkeys, newborn mice, and adult mice were inoculated intracerebrally and intraperitoneally, but no disease or tumor developed in 6 to 11 months of observation. In addition, Dolman sent tissue from one case to John Enders in Boston. There is no information available as to what Enders did with the tissue. However, Enders' main interest for years had been the propagation of viruses in cell culture, so it seems safe to assume that he inoculated cell cultures of some kind. His results were reported to Dolman simply as ''negative.''

Thus, by the end of 1966 it was beginning to appear that this virus that looked so abundant and enticing in Gabriele Zu Rhein's photographs was not going to be an easy one to cultivate. Little did we suspect at that time just how hard the task was going to be.

2. THE START OF A COLLABORATION

Gabriele Zu Rhein clearly wanted to see this virus cultivated and characterized. She had demonstrated its presence in oligodendrocytes in PML brain tissue. The logical next step was to cultivate and identify it in order to study its role in nervous system disease. She wanted to have a part in that process, but she did not have the virology laboratory or virology background to do this, so she began prodding me to take it on in a collaborative project. I had a virology laboratory in the Department of Medical Microbiology in the same building but one floor below the Department of Pathology and Gabriele's electron microscope suite. Gabriele and I had co-authored a paper in 1956 concerned with a virus infection, but had not worked together in the interim. I had been studying persistent, chronic viral infections in cell cultures and animals and also was in the middle of a study of myxoma and fibroma virus infections. I had no experience with polyomaviruses, but I had some experience with papillomaviruses, the other genus of the papovavirus family. In that project, like many other virologists, I had tried and failed to cultivate human papillomavirus in cell cultures, so I had experienced the frustrations of trying to cultivate a very fastidious and difficult virus.

In my consideration of an effort to try to cultivate the virus of PML, an important person was Billie Padgett (Fig. 3.1). Billie was an experienced virologist who had done her Ph.D. thesis research in my laboratory working with influenza virus. She continued in my laboratory as a postdoctoral fellow studying myxoma and fibroma viruses in both cell cultures and rabbits before going

Figure 3.1. Billie Padgett, Duard Walker and Gabriele Zu Rhein at Dr. Walker's retirement party in 1988 (Left to right). Courtesy of Gabriele Zu Rhein.

to Canberra, Australia, to work with Frank Fenner for 2 years. She returned to Madison, Wisconsin, in January 1967 to continue work with myxoma and fibroma viruses. Fortunately, she was willing to make a switch to work on PML, and, as the project developed, it was her patience and tenacity in learning how to get good cultures of fetal spongioblasts that led to the successful cultivation of JC virus. Since neither Billie nor I had experience with polyomaviruses, we were starting out as novices in that area. It would have been easy to obtain mouse polyoma virus and SV40 to study their cultivation, but we did not want to contaminate our laboratory with them. These are hardy viruses, and SV40, in particular, is notorious for contaminating a laboratory and persisting and appearing in subsequent experiments. Although our lack of experience with polyomaviruses might seem to have been a disadvantage, we did not view it as a serious problem, and we knew we would have one major advantage in our work. That was because this was to be a collaborative project with Gabriele, who would provide essential expertise in neuropathology and electron microscopy, and her prominence in the PML field would make it reasonably likely that we could obtain needed virus-containing tissue. Certainly, a serious problem facing anyone setting out to isolate the virus of PML was the paucity of virus-containing tissue available for study. PML was an uncommon disease, and locating terminal patients under circumstances where an autopsy could be performed and fresh or frozen tissue obtained was not likely to be achieved easily or quickly. As it turned out, Gabriele's recognition among pathologists and neuropathologists was essential to our obtaining good tissue.

Gabriele alerted pathologists and neurologists about our need for virus-containing tissue from cases of PML in sufficient quantity to allow significant work. We expected to have to wait a while for tissue, but we certainly hoped that the wait would not be as long as it turned out to be. It was nearly a year before a case appeared.

On March 9, 1967, Gabriele received a letter from Dr. Aurea del Rosario, a hematology resident at the Veterans Administration Hospital in East Orange, NJ. There was a patient in the VA Hospital who had Hodgkin's disease and the clinical features of PML. She had read Gabriele's paper on polyoma-like particles in PML. She was seeking help in confirming the diagnosis, but also offered any specimens that could be useful to Gabriele's research. Gabriele kept in very close touch with Dr. del Rosario by mail and telephone during the surprisingly long course of this patient's terminal illness. It was not until January 21, 1968, that Gabriele received word that the patient had died and that an autopsy would be performed the next day. Gabriele gathered appropriate containers, dry ice, and tissue culture medium and flew to New Jersey on the morning of January 22. She and the pathology resident removed the brain and sliced it immediately. Characteristic lesions were found in both hemispheres. Tissue was selected to go into culture medium, some was frozen, and some went into appropriate fixatives. Gabriele was soon on her way back to the airport and she was back in Madison very late that evening.

There is a later interesting addendum to this tissue procurement expedition. Three or four years later I was asked to discuss JC virus at a conference in New Jersey. During the discussion I mentioned, rather casually, that our first supply of fresh PML tissue had come from New Jersey. The Director of Public Health for the State of New Jersey happened to be in attendance and became quite agitated and incensed that he had not been informed about the case and that the tissue had been allowed to leave New Jersey rather than going to his laboratory. He had wanted to try cultivating the virus.

After obtaining the New Jersey tissue, we had enough virus-containing tissue to begin serious work. We began what turned into a long and frustrating series of efforts to cultivate the virus from the diseased tissue. We tried explants of the fresh brain tissue, but after many weeks of coaxing and coddling only a few fibroblasts and astrocytes grew. We made 10% extracts of diseased brain tissue and clarified it by centrifugation and then used the supernatant fluid to inoculate cell cultures. A similar extract of normal human brain tissue was used on control cultures. Monolayer cultures of primary and secondary human embryonic kidney cells, Hep-2 cells, and human fibroblasts were inoculated. None of these cell cultures showed any evidence of being infected even after weeks of cultivation and medium changes and subculturing. Mice, newborn mice, and hamsters were inoculated by the intracerebral and peritoneal routes and were observed for many months without showing signs of disease or tumor. Guinea pigs and rabbits were inoculated, guinea pigs intraperitoneally and rabbits intravenously to raise antibody against the virus. In this last effort we had a small measure of success. Using frozen sections of New Jersey brain tissue and the indirect method of immunofluorescent staining we found some guinea pig and rabbit sera that reacted at a low level with cell nuclei in the PML tissue. However, antibodies against human tissue were also present, and these interfered greatly. We had to absorb the antihuman antibodies out of the serum with dried human brain powder. This helped, but the antisera were very weak.

Billie began trying to cultivate primary human fetal glial (PHFG) cells. At the 1965 meeting of the American Association of Neuropathologists Gabriele had heard H.M. Shein describe a method for cultivating human fetal astrocytes and spongioblasts in dispersed cell cultures. He also described his use of these cultures to study the effects of SV40 infection on astrocytes and spongioblasts. Such cultures sounded very promising, but there were serious problems in producing them. Human fetal brain tissue was not easily obtained, and good cell cultures derived from the tissue were not easily produced. There was no commercial source, so they had to be produced in our laboratory from tissue obtained from local hospitals. We had gotten our fetal tissue supply problems worked out and Billie was making progress, but the cultures were still mainly what Shein considered to be astrocytes. Although we inoculated PHFG cell cultures with New Jersey brain tissue extract, they gave no indication of infection that we could recognize at that time.

Ten percent extracts of degenerating PML brain tissue tended to have a toxic effect on most cell cultures, and diluting away from that toxic effect obviously

reduced the inoculum size. Therefore we began trying to purify and concentrate the virus to escape the toxicity and to obtain antigen of sufficient potency to develop antisera in rabbits and guinea pigs. The lipids in white matter presented a real challenge. How could we free the virus from that mass of myelin lipid and cell membranes? It became a matter of trial and error and using any clues available from the work of other investigators. Many techniques were tried, including genetron extraction and centrifuging to a pellet through 5–20% sucrose, but we eventually settled on one that started with homogenizing the tissue in a mortar or a blender. It was then sonicated and treated with sodium deoxycholate and trypsin and subjected to differential centrifugation. Supernatant fluid from a final low-speed centrifugation was diluted to the equivalent of a 10% tissue extract in buffered saline. Extracts of diseased tissue and normal human brain tissue prepared in this way were used in subsequent animal inoculations and for inoculating cell cultures. We referred to these extracts as semipurified because the virus certainly was far from really purified.

During the early phases of our PML project our financial support was from two National Institutes of Health (NIH) grants that were for research on persistent viral infections but were not directly aimed at PML. We needed additional funds and ones designated for research on the viral agent of PML. Fortunately, at about this time the concept of ''slow virus infections'' and particularly ''slow virus infections of the central nervous system'' was attracting attention. The Icelandic virologist Björn Sigurdsson had introduced the terms in his research on rida, scrapie, visna, and maedi of sheep because of the long incubation periods and slow progression of these diseases. Carleton Gajdusek used the terms in his work on kuru and Creutzfeldt-Jakob disease, and Gajdusek and others began including PML among the slow virus infections of the central nervous system. This gave PML some prominence and ''pizzazz'' and made it more likely that we could gain support.

However, in 1968 we were already beyond those halcyon days of NIH when research funds were easily obtained. Funding had already tightened up, so it seemed rather unlikely that a proposal to study just the viral etiology of PML would be well received, particularly because we did not yet have even a suggestion of success in cultivating the PML virus. NIH study sections had developed a preference for supporting ''a sure thing,'' that is, a project already nearly accomplished, rather than risking money on an uncertainty. And we were a real uncertainty.

3. WIDENING OUR COLLABORATION

We thought that broadening our proposal might improve our chances, so we turned to colleagues in the Department of Veterinary Science. The Departments of Medical Microbiology and Pathology in the Medical School and the Department of Veterinary Science in the College of Agriculture (this was before the University had a School of Veterinary Medicine) had for many years main-

tained a cordial working relationship in both research and graduate teaching. Within the Department of Veterinary Science there were some very good virologists who were already working in the area of slow virus infections. One of these, Robert Hanson, was a prominent virologist whose eminence was based mainly on his extensive studies of Newcastle disease in poultry, but Bob had also maintained a smaller program studying transmissible mink encephalopathy (TME) and scrapie. Although the TME program had remained small with usually one or two graduate students working on it, enough work had been done on the epidemiology and pathology to provide a strong suggestion that the disease in mink was due to the same agent that caused scrapie in sheep. The pathology of TME in mink was very similar to that of scrapie in sheep. It appeared quite likely that in their pursuit of inexpensive high protein food mink ranchers were sometimes feeding meat of scrapie-infected sheep to their mink, thereby causing outbreaks of TME.

Gabriele had been working with Hanson's group on the neuropathology of TME and was currently mentoring Robert Eckroade, a graduate student who was working toward a Ph.D. with a double major in Veterinary Science and Pathology. Bob Hanson's program on TME had been supported by a mink grower's organization but was making enough progress to justify expansion, so Bob was interested in joining a proposal to NIH. In addition, June Osborn, who was a virologist in the Department of Medical Microbiology, had been developing a project to study subacute sclerosing panencephalitis. With her participation we had a three-pronged attack on slow virus infections of the central nervous system, and with this group approach we applied to NIH for support of the three laboratories. Today's NIH study sections would probably disapprove of our forming such a group and would tell us we were ''not focused,'' but in 1968 NIH looked favorably on such programs as a way to support more research on less money.

Meanwhile, we began meeting as a slow virus disease group in 1968 to plan, analyze, and discuss experiments and to discuss reports from other laboratories. Originally the group consisted of Bob Hanson and Robert Eckroade from Veterinary Science, Gabriele Zu Rhein from Pathology, and Billie Padgett, June Osborn, and Duard Walker from Medical Microbiology, but as time went on many others participated. Roland Rueckert from Biochemistry joined in efforts to purify the agent of TME. Cornelius Hopper from Neurology participated. An important addition was Richard Marsh, who returned to Madison from NIH where he had been fulfilling his military obligation working in Carleton Gajdusek's laboratory as a Public Health Service officer. The doctor's draft was still in force and Dick who had a D.V.M. was therefore draftable. Before going to NIH Dick had obtained his Ph.D. in Veterinary Science working on TME. Both Dick and Bob Eckroade were veterinarians before obtaining a Ph.D., and both became major contributors to the TME and the PML projects.

Our slow virus disease group met every second Friday morning for about 2 hours. I usually prepared an agenda, but the discussions ranged far and wide. Because we were working with pathogenic agents of uncertain risk and poten-

tial, it was important that graduate students, technicians, and all associated persons be fully aware of what we were doing, so they attended and participated as well. The discussions were often intense. In my view, these meetings were very valuable. Each subgroup would plan experiments and then present them to the group where they were likely to be taken apart and reassembled, often in altered form. We were all ''slow virologists'' planning experiments to be measured in months and years rather than minutes, hours, or days. We had to think everything through very carefully, particularly with animal experiments, because once we started an experiment we were committed for a long time, often for several years. These meetings were of such value that we kept them going for about 10 years.

Our NIH grant application for the ''Study of Chronic Viral Infections of the Nervous System'' was approved and funded for 5 years to begin January 1, 1969. Although, of course, the grant was not funded for all the money we had applied for and thought we really needed, it did provide money for one very expensive piece of equipment, a new Philips electron microscope. All of Gabriele's electron microscopy work had been done using an RCA EMV 3D microscope, which by 1968 was considered obsolete by most microscopists. Unfortunately, due to one delay after another, the new microscope was not installed until October 1971.

The year 1968–1969 was disappointing for those of us working on PML. Although we had obtained the New Jersey tissue, we had not yet had success in cultivating virus. However, we had many inoculated animals to observe, and we remained optimistic. In November 1968 we received a very small piece of frozen diseased tissue from a PML case in West Virginia. The tissue was sent by Sam Chou. This was the Shi-Ming Chou (usually known as Sam) who had worked with Gabriele and was co-author of the 1965 paper describing the virions in PML lesions. Sam had moved to West Virginia University and autopsied a PML patient in Morgantown. He gave us only 1 g of tissue, though, and this was so small that we did not use it in our trials at that time. Later, after we had succeeded in isolating and cultivating JC virus, we got it out of our freezer and had no difficulty isolating JC virus from it.

In 1968–1969 our colleagues working on TME were making great progress infecting multiple species, including monkeys, with TME and scrapie agents and comparing the neuropathology in these species. The possibility that Creutzfeldt-Jakob disease in people is caused by the agent of scrapie or a related agent that is transmitted from food animals was viewed as a distinct possibility as early as 1968–1969, and Hanson, Marsh, and Eckroade were pursuing this possibility.

However, progress was slow with PML. One problem was that we were not receiving new PML tissue. In November 1969, George Ellison at Yale offered us some tissue from a patient who had died in 1965. The tissue had been stored in 50% glycerine-saline at 4°C since 1965. We certainly accepted it, but the specimen consisted only of two very small pieces, and, considering its storage history, we did not view it as first-rate material. We moved it to −70°C storage and continued working with our New Jersey tissue. Much later, after we had

worked out our production of PHFG cultures and improved isolation techniques, we tried cultivating virus from this glycerin-stored tissue and succeeded in isolating JC virus from it. Storage of tissue containing hardy viruses in 50% glycerine-saline at 4°C was a technique used by virologists before freezers were available. Richard Shope stored his rabbit papillomas and fibroma tumors this way. Because JC virus is a relatively hardy virus, it remained viable during multiple years of storage by this method. After receiving this tissue from Ellison we learned that Ellison had also sent some of the tissue to Gajdusek, but we never learned its fate.

On May 20, 1970, our quest for tissue suddenly improved. Gabriele received word from Dr. Bert Dessel, Chief of Hematology at the VA Hospital in Wood, Wisconsin, a suburb of Milwaukee, that he had a patient with an 8 year history of Hodgkin's disease and neurologic signs strongly suggestive of PML. A brain biopsy had been done on May 20. Dessel sent the biopsy slides to Gabriele, who confirmed the diagnosis of PML. The patient, whose initials were J.C., died at 4:00 AM on July 12, and a brain autopsy was permitted.

Gabriele and I drove the 70 miles from Madison to Milwaukee armed with tubes of tissue culture medium, dry ice, and containers suitable for transporting tissue. When the brain was removed Gabriele sliced it immediately. Demyelination was found in all lobes. Extensive disease with cavitation was present in the right temporal and parietal lobes and the left occipital lobe. Many small samples were taken and placed in tissue culture medium in the hope that some brain cells would grow. Many areas of early lesions and the borders of large lesions were collected and frozen for later extraction of virus. The VA Hospital pathologists and hematologists made this very easy for us, and Gabriele and I were back in Madison in a few hours with a much improved reservoir of virus-containing tissue.

We did not, however, get all of the tissue destined for laboratory study. Dr. Dessel told us that Carleton Gajdusek had also been advertising his interest in PML tissue, so Dessel collected several grams to be sent frozen to Gajdusek. We never learned to what use this tissue was put or the outcome of its use, but it is very likely that it was used to inoculate one or more primates.

The tissue that we got from patient J.C. was much larger in quantity and proved to be much richer in virus than that from the New Jersey case. We renewed our efforts to concentrate and purify virus for use in inoculating cell cultures and animals and in producing antiserum. The only way to follow the virus through various purification procedures was to use negatively stained preparations examined by electron microscopy. Bob Eckroade did most of this microscopy.

In our previous efforts to concentrate and purify virus from brain tissue of the New Jersey case all our extractions and differential centrifugations had not seemed to accomplish much. However, when brain tissue from patient J.C. was treated in the same way, the semipurified extract contained virions in sufficient numbers that they could be found by electron microscopy in negatively stained preparations. This was probably because we started from a much richer source of virus. The New Jersey patient had a very slowly progressive clinical course

of PML. From the first signs of PML to death was about 1 year, whereas patient J.C. had rapidly progressive disease that led to death in 2 months and had very extensive brain involvement.

Groups of animals were inoculated by the cerebral and peritoneal routes. These included mice, newborn mice, guinea pigs, rabbits, hamsters, ferrets, and mink. The mink were used because they were available from the associated TME project and because they were a species not likely to have been tested or to be tested by other investigators. Most of the inoculation of animals and much of the long-term observation of the animals was done by Dick Marsh and Bob Eckroade. The animals were housed in animal quarters belonging to and managed by the Department of Veterinary Science. They had the only animal quarters with what could be considered isolation rooms. These animal quarters were located on a university-owned experimental farm (Charmany Farm) on the outskirts of Madison. Although they were primitive by today's standards, the Charmany Farm quarters were considered adequate for that time, and we were very fortunate to have them available.

In an effort to develop antisera against the virus, rabbits and guinea pigs were injected by various routes, including intravenous, intramuscular, and intramuscular injection of extract mixed with adjuvant. These animals were bled for their serum over a period of 10 days to 6 weeks.

Inoculated animals were observed for many months, often for the lifetime of the smaller animals. We were able to hold animals for long periods only because the Department of Veterinary Science bore most of the cost. We contributed only the salary of one animal caretaker each year. Our work was done before the requirements for animal quarters and the costs for animal care escalated to levels that would have been prohibitive for us.

Even though the animals were observed for long periods and Gabriele autopsied every animal, we found no indication of disease attributable to inoculation with brain tissue extracts.

At the time all these animals were being inoculated and observed, work with cell cultures was also progressing—slowly. Many cell types were planted in Leighton tubes on top of cover glasses. The cell types included primary human embryonic kidney cells, human embryonic fibroblasts, Vero cells (a line of African green monkey kidney cells), primary African green monkey kidney cells, Hep-2, WI-38, mouse embryo cells, and PHFG cells. Each tube was inoculated with 0.1 ml of J.C. brain extract or a normal control extract. These cultures were incubated and observed for cytopathic effects for several weeks. Periodically the cover glasses were removed, and the cells were fixed and stained with hematoxylin and eosin (H&E) and searched microscopically for cells containing inclusion bodies. Cells were also removed from cover glasses, centrifuged into a pellet, and frozen and thawed to disrupt them. The cell debris was negatively stained and searched for virions in the electron microscope.

In general, no cytopathic effect or other evidence of infection was found in these cultures, even after several subcultures. However, we began to think something interesting was happening in some of the inoculated cultures of

PHFG cells. Subtle changes were slowly developing that were not found in control cultures.

4. FINALLY, SOME GLIMMERING OF SUCCESS

Billie had been working hard for months trying to improve cultures of PHFG cells. It was slow going. Initially the cultures consisted of astrocytes that would form a monolayer, but spongioblasts were either sparse or absent. We thought it important to have good cultures of spongioblasts because neurobiologists considered spongioblasts to be precursors of oligodendrocytes, the cells in which the virus was found in human brain.

Through trial and error over many months, Billie developed techniques that produced cultures with increasing numbers of spongioblasts. The spongioblasts were usually in clumps sitting on top of a layer of astrocytes. The procedure was complicated and required multiple medium changes and manipulations. It required periodic adjustments in fetal calf serum and high levels of glucose. In particular, it required much patience and tenacity. Fortunately, Billie had those attributes, and it was beginning to look like this effort was paying off.

Three or four weeks after inoculation with J.C. brain extract, PHFG cell cultures stained with H&E were found to contain a scattering of enlarged cells with dark-staining, bizarre nuclei. We judged these cells to be enlarged astrocytes. This effect could be transmitted to new cultures with frozen, thawed, and sonicated cell debris from affected cultures. The smaller number of spongioblasts that had been in the cultures were usually gone by the time we recognized enlarged astrocytes. Later, when we had cultures richer in spongioblasts, we came to realize that the spongioblasts had already been released from the astrocyte layer and were floating in the medium by the time the astrocytes showed abnormalities.

In addition to staining cells with H&E, we were periodically removing cells from tubes and freezing, thawing, sonicating, and negatively staining them for examination in the electron microscope. Bob Eckroade did the negative staining and electron microscopy in an electron microscopy suite that was one floor above my office. On the afternoon of March 24, 1971, Bob had been examining material from inoculated PHFG cell cultures when he came to my door with a big grin on his face and said, ''Come see what we have.'' I ran back up the stairs with him and looked at the microscope screen. There was no question about what was there. Polyoma-like virions were evident in abundance, many times what could be carried over from the inoculum. This had to be a successful culture. Bob and I looked for Billie and Gabriele. Gabriele was away that afternoon, but Billie and the technicians got to see what they had been working toward for more than 3 years. Bob took pictures of the virions, and the next morning we showed them to Gabriele. That day the laboratory crew had a little champagne to mark the occasion. Gabriele chose to call this occasion the birthday party for JC virus.

We then began doing all the things necessary to identify and characterize the virus and to confirm our ability to cultivate it. We also began tightening our safety precautions. Up to this time we had been using techniques that were standard in microbiology laboratories working with pathogens. Our cell culture work and inoculations were done in cubicles under negative pressure, but without additional protection for the worker other than good aseptic technique. However, we now believed that we had the etiologic agent of PML in hand and in quantity. This caused some nervousness because at that time we knew nothing about its epidemiology or anything else about it except that when it caused disease it was lethal.

The concept of and term *biohazard* had fairly recently been introduced, popularized, and discussed. This had led to increasing pressure on laboratories and NIH to improve safety. Commercial companies responded with production of biosafety cabinets. We received the first one in the department and added others as they became available. All future handling of the virus was done in biosafety cabinets.

To demonstrate our ability to cultivate the virus, it was isolated twice from each of two extracts of J.C. brain tissue. By electron microscopy the isolated virions had the same size and shape as those in brain tissue. As PHFG cell cultures richer in spongioblasts became available we could demonstrate a 1000-fold increase in infectious virus in PHFG cell cultures during a 3 week period. Wallace Rowe, Werner Henle, Hilary Koprowski, and Kenneth Takemoto generously provided antisera and fluorescent conjugates that helped us establish that the virus was not SV40, mouse polyoma virus, or mouse K virus. At this point we had to bring SV40 and mouse polyoma viruses into our laboratory in order to compare what we now called JC virus (JCV) with those viruses. PHFG cell cultures that showed good evidence that JCV infection did not react with virus-specific antiserum against SV40, mouse polyoma virus, or K virus when tested by the indirect immunofluorescence method or with fluorescein-conjugated antiserum, nor did these antisera react with the virus in frozen sections of J.C. brain tissue. Antiserum that we had recently produced in rabbits by intravenous injection of J.C. brain extracts did react with oligodendrocytes in frozen sections of J.C. brain tissue and with enlarged spongioblasts in infected PHFG cell cultures. We produced antiserum in rabbits by injecting them intravenously with semipurified, concentrated virus from PHFG cell cultures.

This antiserum did react with both enlarged spongioblasts in infected PHFG cell cultures and enlarged oligodendrocytes in frozen sections of J.C. brain tissue, thus identifying the virus in cultures as the virus in J.C. brain tissue. The antiserum did not react with SV40 or mouse polyoma virus in cell cultures. JCV from PHFG cell cultures did not produce cytopathic effects in cultures of primary mouse embryo, BSC-1, Vero, or primary African green monkey kidney cells, and no cells in these cultures developed antigen reactive with antiserum against SV40 or mouse polyoma virus. JCV did not produce cytopathic effects in cell cultures of primary human embryonic kidney, primary human amnion, or an established human embryonic cell line. All evidence to this point indi-

cated that JCV was a newly recognized virus and not one of the previously known polyomaviruses. It was also becoming clear that JCV had a remarkably restricted host cell range.

5. SURPRISES

About a month after our first successful isolation of virus from J.C. brain tissue, Gabriele received a telephone call from Richard Johnson at Johns Hopkins Medical School. He invited her to attend a workshop on the epidemiology of multiple sclerosis at Easton, Maryland, and to visit his laboratory where she ''would be able to see the virus of PML.'' After Gabriele conveyed this news to me I talked by phone with Johnson and learned that Leslie Weiner and he had a papova-like agent growing in primary African green monkey kidney cells. They had made isolations from two cases of PML using cell fusion and co-cultivation techniques. Just the fact that their isolates grew well in green monkey kidney cells made it unlikely that their virus was the same as ours. I told him we had an isolate also, and he and I agreed that Gabriele and Billie both should attend the workshop so their isolates and ours could be compared.

At the workshop Weiner presented the data on the Baltimore isolates (Weiner et al., 1972), and Billie and Gabriele presented information on JC virus. One of the Baltimore isolates came from tissue sent to Johnson by Lucien Rubinstein from Stanford, while the other isolation was made from a brain biopsy of a case of PML in Baltimore. All data were preliminary at this time, but the Baltimore isolates were acting very much like SV40 while, at that time, we had found no antigenic relationship to SV40 for our isolate and in cultural characteristics it looked to be very different from SV40.

We were not through hearing about new papovavirus isolates. A month after learning about the Baltimore isolates we heard a rumor that investigators in London, England, had isolated a papovavirus from the urine of a patient with a kidney transplant and that the investigators thought it could be the virus of PML.

We learned no more about the London isolate at that time, but in the meantime we had assembled a manuscript describing our isolation and cultivation of what we now called JCV. We submitted the manuscript to *Lancet* for publication (Padgett et al., 1971). To our surprise, the editor told us that *Lancet* had received another manuscript describing the isolation of a papovavirus from a human patient and that the two papers were accepted and would be published together.

The two manuscripts going to the same publisher at approximately the same time was entirely a coincidence. The other paper was by Sylvia Gardner and Anne Field at the Virus Reference Laboratory in London and Dulcie Coleman and B. Hulme at St. Mary's Hospital, London (Gardner et al., 1971). We had not communicated and knew nothing of each other's work except what we in Madison had heard as a rumor.

The London isolate was initiated in the continuous line of African green monkey kidney cells called Vero cells and could be serially passaged in Vero cells. It had a minor antigenic relationship to SV40. Like mouse polyoma virus, it agglutinated human and guinea pig erythrocytes at 4°C, but was antigenically unrelated to mouse polyoma virus. Gardner and her associates had done as we did and used the patient's initials as a label in work with the virus, so they called it BK virus (BKV).

6. PROGRESS AT A SOMEWHAT FASTER PACE

We had not previously tested JCV for its hemagglutinating capacity, but if BKV caused hemagglutination, it seemed likely that JCV would. It was a great relief to find that, like BKV and mouse polyoma virus, JCV agglutinated human, guinea pig, and chicken erythrocytes at 4°C. This was very fortunate because finally we had a technique that offered some speed. Everything else about JCV was slow, and slower, but with hemagglutination and hemagglutination inhibition we could measure virus and antibody in hours rather than weeks or months.

After determining that antibody measured by hemagglutination inhibition correlated well with neutralizing antibody in human serum, we were able to perform a serum antibody survey to determine the prevalence of antibodies in human and animal populations. We were surprised to find that even though PML disease is uncommon, antibodies against the virus are very common in the human population. The presence of antibodies as evidence of past or present infection indicated that infection begins in early childhood and that by middle age 75% of persons have been infected. On the other hand, no antibodies were found in the sera of 12 animal species, including five primates. This suggested that JCV had a very narrow host range and that animals were not the source of infection for people.

Almost all of the persons working in our laboratories had good levels of serum antibody against JCV. To a considerable extent this relieved anxiety about working with the virus. Curiously, Billie and I were among the few without serum antibody, and we never did develop it during years of working with the virus. Billie and I have chosen to attribute our antibody-negative state to clean living and good laboratory technique. My reward for being JCV antibody-negative and having type O erythrocytes was to become the designated blood donor every Monday morning when the week's supply of red blood cells was collected for hemagglutination tests.

7. TUMOR PRODUCTION

Because mouse polyoma virus and SV40 were known to be highly oncogenic viruses, it was important to test the oncogenicity of JCV. Our previous injec-

tions of multiple animal species with New Jersey and J. C. brain extracts had not resulted in recognized tumors, but it seemed likely that the amount of virus in those inocula was quite small. Therefore experiments were planned as a direct test of oncogenicity.

Virus from the third passage in PHFG cells was concentrated and partially purified by differential centrifugation and tested to be certain that there was no contaminating SV40 or mouse polyoma virus in it. Newborn hamsters were inoculated both intracerebrally and subcutaneously with 10^6 infectious doses in each site. The control inoculum was a concentrated and partially purified extract of uninfected PHFG cells.

There was no acute disease caused by the inocula, but approximately 4 months after inoculation the virus-inoculated hamsters began showing overt signs of central nervous system disease. The first hamster to show clear signs and increasing illness did so while I was on vacation in San Francisco. An autopsy by Gabriele revealed a sizable brain tumor, so I received a telephone call from an excited group of people in Madison telling me about the tumor and the increasing number of neurologically sick hamsters. At first, one or two hamsters per week showed signs but soon the rate increased to five or six per week. Gabriele autopsied every hamster and examined each very closely for both gross and microscopic tumors. At 6 months after inoculation, the surviving animals were killed and autopsied.

The only significant pathogenic findings were tumors in the JCV-inoculated group. Eighty-three percent of 63 JCV-inoculated animals had developed brain tumors within the 6-month period, a remarkably high level of oncogenicity. None of 39 controls had tumors. The tumors ranged from 2 to 7 mm in diameter. On microscopy, Gabriele found them to be malignant and glial in origin. Most were glioblastomas, medulloblastomas, or unclassified primitive tumors (Walker et al., 1973).

JCV was isolated from some of these tumors by serially subculturing the tumor cells. Tumor cells did not contain JCV coat protein when tested by indirect immunofluorescence, nor did they contain coat protein of SV40 or mouse polyoma virus. They did, however, contain an intranuclear antigen that reacted with serum from certain of the tumor-bearing hamsters. This intranuclear antigen had the characteristics of the tumor antigens (T antigens) described for other polyomaviruses. When tumor cells were reacted with antiserum against SV40 and mouse polyoma virus it became clear that there was a strong cross reaction between the T antigens of JCV and SV40. Later it was found that the T antigen of BKV also cross reacted with JCV and SV40.

The demonstration of the high degree of oncogenicity of JCV in hamsters opened up a whole new area for additional research. Inoculation of newborn hamsters by various routes resulted in many varieties of tumors. Gabriele made an extensive and detailed study of the tumors over the next several years. Interest in JCV-induced tumors was further enhanced when, in a collaborative project with William London, John Sever, and associates at NIH it was found that intracerebral inoculation of JCV in adult owl monkeys resulted in malig-

nant brain tumors in about 25% of the animals after an incubation period of 16 to 25 months. Another experiment showed that JCV also induced brain tumors in adult squirrel monkeys.

Soon after our paper appeared in *Lancet* we began receiving requests for JCV from federally supported juggernaut laboratories. These requests increased after our reports on the oncogenicity of JCV. This was at a time when the National Cancer Institute was pouring many millions of dollars into a frontal assault on viruses as a major cause of cancer and had established multiple strongly funded satellite laboratories to study the role of viruses in cancer. It was clear from most of these requests that those asking for the virus were confident that they could get this obstinate virus to multiply in cells other than PHFG cells. Some mentioned human glial cell lines that they had developed. As we produced new virus we shipped starter samples to these laboratories. Almost invariably we never heard from them again. I inquired about the results of their efforts from a few of them and was usually told that they did not have time to spend on a virus like JCV so they had given up trying to cultivate it. They had greater interest in fast viruses than in slow viruses.

8. COMPARISON OF THE NEW VIRUSES

After our papers appeared together in *Lancet* we exchanged viruses and information with the London group so that the viruses could be compared in each laboratory. As these comparisons progressed it was clear that JCV and BKV were distinct virus species, but they had many similarities. Both viruses circulated widely in the human population. Infection begins to occur in early childhood and persists, probably for life, as a seemingly harmless latent infection of the kidneys unless host immunity is impaired. During periods of impaired immunity virus is frequently released in urine, and overt disease can develop. The overt disease produced by the two viruses is, however, very different. JCV causes the lethal brain infection PML, while disease caused by BKV is usually limited to the urinary tract. JCV is highly oncogenic in hamsters, owl monkeys, and squirrel monkeys, while BKV is much less oncogenic.

It was important to clarify the antigenic relationships of JCV, BKV, and SV40, and this was worked out in several laboratories including those of Sylvia Gardner and associates, Keerti Shah, Opendra Narayan and associates in Baltimore, Kenneth Takemoto and M.F. Mullarkey at NIH, as well as ours. It was gradually determined that there is at least one antigen on the surface of JCV, BKV, and SV40 virions that is specific for each virus and allows them to be distinguished from each other by serologic methods. Antiserum against the species-specific antigen can be produced in rabbits by one or two intravenous injections of purified, intact virions. Multiple injections of virus or use of adjuvants leads to the appearance of cross-reacting antibodies. JCV, BKV, and SV40 share a minor surface antigen that sets them apart as a subgroup from other polyomaviruses. This was the minor relatedness first recognized by Gard-

ner as a weak cross reaction between BKV and SV40. Use of intact virions in immunizing rabbits will produce antibodies against this antigen, but hyperimmunization is required. Keerti Shah and associates demonstrated that all members of the polyomavirus genus share a genus-specific cross-reacting antigen that is best demonstrated by the immunofluorescent technique in the nuclei of infected cells. Antibody can be produced in rabbits by immunizing with virions disrupted by detergent or alkali. This antigen is particularly useful because, unlike the other antigens, it is resistant to formalin and can be detected in the nuclei of infected cells in formalin-fixed, paraffin-embedded tissue using the peroxidase–antiperoxidase immunostaining technique.

Like SV40, JCV and BKV were shown to induce nonstructural T antigen in the nuclei of lytically infected cells, transformed cells, and tumor cells. The T antigens of JCV, BKV, and SV40 are antigenically very similar. Antiserum developed in tumor-bearing hamsters against the T antigen of one virus reacts strongly with the T antigens of all three viruses.

It gradually became clear that correct identification of one of the polyomaviruses by serologic methods requires recognition of these specific and cross-reacting antigens and requires reliable information about how an antiserum was produced plus appropriate tests and controls to certify the specificity or breadth of reactivity of an antiserum. Use of antisera of uncertain specificity has sometimes led to erroneous conclusions about the identity of viruses of this group.

After we had improved our isolation and cultivation techniques with JCV and had developed good virus-specific antisera for JCV, BKV, and SV40, we set about identifying the virus in more cases of PML. We went back to the New Jersey tissue and, using virus-specific antiserum and fluorescence microscopy, we identified the virus in the tissues as a JCV type of virus and were able to isolate and cultivate it. We also succeeded in cultivating virus from the small pieces of tissue we had received from Sam Chou and found it to be antigenically identical to JCV. Using frozen sections of the diseased tissue and virus-specific antisera, we found the virus in the brain tissue to be a JCV type of virus, and we were even able to do the same with the tissue that George Ellison had given us. This was the PML tissue that had been stored in 50% glycerin-saline at 4°C since 1965. This gave a good indication that JCV was a very hardy virus.

With virus-specific antisera, frozen sections, and immunofluorescence techniques it became possible for us to confirm a diagnosis of PML in biopsy and autopsy tissue in a few hours to a few days. When pathologists, neurologists, and other physicians learned this, we began receiving specimens of PML tissue at an increasing pace. We chose to cultivate viruses from about 15 cases so that we could compare isolates. We sometimes went through the work of isolating and cultivating the virus because the case was of special interest, such as the cases of two children who developed PML. All isolates were antigenically similar.

In the 30 years since JCV was isolated and cultivated, the identity of the virus in PML lesions has been determined many times. Most of these cases

have gone unreported because JCV in PML is no longer news, but the number must be approaching 200 because it was over 100 in 1988 when I retired and stopped receiving reports. Identification has been accomplished by a variety of methods in laboratories in many countries on several continents. In no case, thus far, has BKV been found in the brain of a patient with PML, and all available evidence indicates that BKV has no part in PML. With the exception of a handful of cases, JCV has always been the virus in the brain lesions of PML patients.

However, in six cases the virus has been reported to be SV40. The SV40 identifications have been from laboratories working with SV40 or with monkey cells that frequently harbor latent SV40 and where cross contamination is a risk. Furthermore, antiserum used in these identifications usually has not been shown with certainty to be virus specific. The cross-reacting antibodies common in antiserum developed against any one of SV40, BKV, or JCV can lead to mistaken identification. When Gerald Stoner and Caroline Ryschkewitsch (Stoner and Ryschkewitsch, 1998) re-examined diseased brain tissue from three of the six supposed SV40 cases, they found that these three cases were indeed mistakenly identified as due to SV40. Stoner and Ryschkewitsch used virus-specific monoclonal antibodies, virus-specific DNA probes for in situ hybridization, and virus-specific primers in the polymerase chain reaction. These are techniques that were not available when the cases were originally studied. In each of these cases, one of which was Rubinstein's case from Stanford reported in 1972, the virus in the brain lesions was unequivocally shown to be JCV rather than SV40. Tissues from the other three cases were not available for examination by Stoner and Ryschkewitsch, but it is quite likely that had they been re-examined they, too, would have been found to be mistaken identifications and that JCV is the sole etiologic agent of PML.

ACKNOWLEDGMENTS

I gratefully acknowledge the help of Dr. Billie Padgett, who read the manuscript and made helpful suggestions.

REFERENCES

Gardner SD, Field AM, Coleman DV, Hulme B (1971): New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1:1253–1257.

Padgett BL, Walker DL, Zu Rhein GM, Eckroade RJ, Dessel BH (1971): Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet 1:1257–1260.

Stoner GL, Ryschkewitsch CF (1998): Reappraisal of progressive multifocal leukoencephalopathy due to simian virus 40. Acta Neuropathol 96:271–278.

Walker DL, Padgett BL, Zu Rhein GM, Albert AE, Marsh RF (1973): Human papovavirus (JC): Induction of brain tumors in hamsters. Science 181:674–676.

Weiner LP, Herndon RM, Narayan O, Johnson RT, Shah K, Rubinstein LJ, Pregiosi TJ, Conley FK (1972): Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy. N Engl J Med 286:385–390.

Zu Rhein GM, Chou SM (1965): Particles resembling papova viruses in human cerebral demyelinating disease. Science 148:1477–1479.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

4

SERENDIPITY—THE FORTUITOUS DISCOVERY OF BK VIRUS

WENDY A. KNOWLES, PH.D.

1. INTRODUCTION

It is a remarkable fact that the first isolations of the two human polyomaviruses, BK virus (BKV) and JC virus (JCV), were reported simultaneously in *The Lancet* (Gardner et al., 1971; Padgett et al., 1971), the work of each group unbeknown to the other, and even more interesting to contrast the route by which each virus was discovered. Whereas the history of JCV can be followed from disease to virus over a protracted course (see Chapters 1 to 3), the discovery of BKV arose from observations made during work on an unrelated virus and was followed by a search for a disease. This is an account of the discovery of a virus taken from notes, specimen forms, and laboratory notebooks of the time, with the personal recollections of Dr. Sylvia Gardner. I was then working on cytomegalovirus for my Master of Philosophy degree with Sylvia as my supervisor, and was not directly involved in the initial polyomavirus studies, but switched to BK virus later when I began work on my doctorate.

2. THE INSTITUTE AND PEOPLE INVOLVED

The Public Health Laboratory Service (PHLS) began as a network of bacteriology laboratories in England and Wales, the Emergency Public Health Laboratory Service (EPHLS), brought together in 1939 to combat the threat of epidemics during the Second World War (Williams, 1985). In 1946 a permanent service was established and subsequently enlarged to include 63 laboratories by 1969 to monitor and control the spread of infectious disease in peacetime. In 1946 a collection of reference laboratories was assembled on the site of the Government Lymph Establishment at Colindale in North West London, where previously smallpox vaccine was produced. This formed the Central Public Health Laboratory (CPHL) and included the Virus Reference Laboratory (VRL), the initial function of which was to set up diagnostic facilities for smallpox. The work of CPHL expanded, and in 1951 the building of the ''tower block'' (Fig. 4.1), was begun. VRL was housed on the third floor of this building, and much of the early work on BKV was done in laboratory 316 (Fig. 4.2). The building was later demolished when CPHL relocated in 1985.

By the early 1970s the work of VRL was dominated by cell culture for virus isolation and virus neutralization tests, electron microscopy, and complement fixation tests. Hot rooms and incubators contained rack upon rack or rollers of cell culture tubes that had to be examined and medium laboriously changed once or twice a week, maybe for several months. There was a large tissue

Figure 4.1. The 1951 "tower block" at CPHL. BKV was first isolated in a laboratory on the third floor of this building. Courtesy of Mr. J. Gibson.

Figure 4.2. The laboratory (room 316) in the "tower block" where much of the early work on BKV was carried out. Courtesy of Dr. W.A. Knowles.

culture preparation laboratory and sections devoted to enteroviruses, respiratory viruses, smallpox, and rabies diagnosis.

Consultant Virologist Dr. Sylvia Gardner (Fig. 4.3) joined VRL in 1962, having studied medicine at Birmingham University. Her early interests were in respiratory viruses, and she ran a small virology unit in the hospital of a large childrens' home organized as a village. There was always a strong clinical aspect to her work, and she later became involved with investigations in VRL on transplant patients and pregnant women. This brought her into contact with Professor K.A. Porter, a pathologist at St. Mary's Hospital, Paddington, who, with colleagues in the United States was researching cystic duct obstruction in the first liver transplant recipients, and who was anxious for the role of viruses to be studied. Dr. Gardner retired from VRL in 1989.

Dr. Anne Field (Fig. 4.4) studied Microbiology at Bristol University and came to CPHL in 1959. She originally worked in the enterovirus laboratory, but in 1968 was appointed the first electron microscopist at CPHL and was responsible for setting up and running the electron microscope unit (EMU) until retirement in 1994. The first electron microscope in use at CPHL was an AEI 6B.

3. IDENTIFICATION OF A NEW VIRUS

In the late 1960s and early 1970s much work was being done to determine the epidemiology of cytomegalovirus (CMV), first isolated a decade earlier, and

Figure 4.3. Dr. Sylvia Gardner. Courtesy of Mr. J. Gibson.

its transmission in donated blood and organs. A collaboration was set up between Dr. Dulcie Coleman in the Department of Histopathology and Cytology at St. Mary's Hospital and Sylvia Gardner at Colindale to investigate, both cytologically and virologically, the presence of CMV infection in renal transplant patients.

On October 7, 1970, a midstream urine sample, collected a day earlier, arrived in the laboratory from a Sudanese patient who had received a renal transplant from his brother on June 24, 1970. A phone call the same day from St. Mary's Hospital informed us that this urine contained many inclusion-bearing cells, and so electron microscopy examination would be well worthwhile. Two days later, Anne Field saw very large numbers of papovavirus particles in the high-speed urine pellet and suggested that they were particles of common wart virus (the only human papovavirus known at the time). It was queried whether the patient had genital warts; a report earlier that year (Spencer and Andersen, 1970) had described a high incidence of warts after renal transplantation. Ultrathin sectioning of the cells in a subsequent urine sample collected on October 12 from the same patient showed many virus particles within enlarged cell nuclei.

On the day of receipt, the first urine was inoculated into tubes of both secondary rhesus monkey kidney (MK) cells and human embryo lung fibro-

Figure 4.4. Dr. Anne Field at a Philips 420 transmission electron microscope. Courtesy of Mr. J. Gibson.

blasts (HEL) and observed every 3 or 4 days for a cytopathic effect (CPE). On day 19 a CPE appeared in the inoculated MK cell cultures, and the culture fluid was found to contain papovavirus particles. The fluid from uninoculated cells of the same batch was also examined as it was known that SV40, a monkey polyomavirus, was sometimes a contaminant in these cells; however, the controls were negative. In contrast, no CPE was seen in the HEL cells up to 27 days after inoculation. Vero cells were also inoculated on October 12, and on day 37 a CPE consisting of rounded cells was clearly visible.

The isolate was subsequently passaged in MK and Vero cells but appeared to die out in the MK cultures, and by the third passage CPE was no longer detectable even after 47 days in culture. Papovavirus was subsequently isolated from many more urine samples from this patient and others, but was always slow growing in culture, requiring immense patience. Virus harvested from the second passage in Vero cells was used as an antigen in complement fixation tests, and a seroconversion to the agent was demonstrated in the patient at the time the virus was isolated. The agent was named BK, the patient's initials, and the original isolate is known as the Gardner strain.

Measurement of the virus particles (45 nm diameter) showed the isolate to be a polyomavirus, as distinct from a papillomavirus, and work was undertaken

to distinguish it from the only known polyomaviruses at the time: mouse polyoma, K virus of mice, monkey SV40, and rabbit kidney vacuolating agent. In early 1971 a range of biologic properties was investigated to characterize the new agent. It was found to agglutinate erythrocytes from different animal species, including humans, which immediately distinguished it from SV40, and enabled a hemagglutination inhibition (HI) test to be developed to add to the standard complement fixation test. Seroprevalence studies were later undertaken in various populations. The tumorigenic property of BKV in hamsters was also established early on.

After the virus was isolated and shown not to be human papillomavirus or SV40, it was thought that it might be the virus that had been described in association with the rare neurologic disease progressive multifocal leucoencephalopathy (PML) but that had thus far not been grown. Sylvia Gardner, therefore, wrote to laboratories in the United States that had published cases of PML and requested some PML brain tissue from them (hence the rumors heard by Duard Walker in Wisconsin!). No brain tissue was forthcoming, but we were later able to study patients in the United Kingdom with PML.

After our manuscript was submitted to *The Lancet*, the editors informed us that they had received another paper from the United States on the isolation of a human polyomavirus. Both papers had been accepted and would be published together.

Following the publication of the two original papers, it was obviously important to find out as soon as possible whether BK and JC viruses were related or even identical, and immediate collaboration was set up across ''the pond'' between Sylvia Gardner, Duard Walker, Billie Padgett, and Gabriele Zu Rhein. Viruses were exchanged, and it was established that, indeed, the isolates were two new human polyomaviruses, related to each other and also to SV40.

The original BKV paper was identified in 1989 as a ''Citation Classic'' by the Institute for Scientific Information.

4. SUBSEQUENT WORK ON THE HUMAN POLYOMAVIRUSES AT COLINDALE

Diagnostic and epidemiologic work on both BKV and JCV continued in VRL over the next 30 years, alongside developmental work that reflected the advances in new virologic techniques. BKV was always easier to grow than JCV, the latter requiring fetal brain cells. We became expert at growing these cells, which are no longer available. Large studies involving the original methods of cytology, electron microscopy, virus isolation, and serology were undertaken on renal transplant and PML patients, pregnant women, and children. BKV- and JCV-specific IgM detection methods were developed, and the molecular detection of BKV and JCV DNA was done first by hybridot and later by polymerase chain reaction. Antigenic subtypes of BKV were described, and subsequent work demonstrated the molecular basis for the observed variations.

Very recently collaborative studies have been undertaken on BKV and JCV VP1 expression in a yeast cell system.

ACKNOWLEDGMENTS

I am indebted to Dr. Sylvia Gardner for her recollections of events 30 years ago, which are unobtainable from any records or publications.

REFERENCES

Gardner SD, Field AM, Coleman DV, Hulme B (1971): New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet i:1253–1257.

Padgett BL, Walker DL, Zu Rhein GM, Eckroade RJ, Dessel BH (1971): Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet i:1257–1260.

Spencer ES, Andersen HK (1970): Clinically evident, non-terminal infections with herpesviruses and the wart virus in immunosuppressed renal allograft recipients. BMJ iii:251–254.

Williams REO (1985): Microbiology for the Public Health. Public Health Laboratory Service: London.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

5

THE HUMAN POLYOMAVIRUSES: AN OVERVIEW

MICHAEL J. IMPERIALE, PH.D.

1. INTRODUCTION

The subfamily of viruses named *Polyomavirinae* are small, naked viruses with icosahedral capsids and circular, double-stranded DNA genomes. These viruses have been isolated from a number of species, including human, monkey, rabbit, rodents, and birds. Their host range is rather restricted, and they generally do not infect other species productively. The first of these viruses to be identified was mouse polyoma virus (PyV), which, along with simian virus 40 (SV40), have been two of the most intensely studied viruses since their discovery 40 years ago (Sweet and Hilleman, 1960; Stewart et al., 1958). The study of these two viruses has led to major advances in our understanding of a variety of eukaryotic cell processes, including transcription, DNA replication, translation, signal transduction, cell growth, and oncogenic transformation. Indeed, the name *polyomavirus* is derived from the fact that the mouse virus causes a spectrum of tumor types when inoculated into newborn mice. Two polyomaviruses that solely infect humans have been identified, BKV and JCV. Both of these viruses were first isolated in 1971 (Padgett et al., 1971; Gardner et al., 1971), BKV from the urine of a renal transplant patient and JCV from brain tissue derived from a patient with progressive multifocal leukoencephalopathy

(PML), of which JCV is clearly identified as the causative agent. In this chapter, the biology of the polyomaviruses is reviewed, with an emphasis on these two human viruses and on SV40, which historically has not been thought to be a human pathogen but, according to accumulating recent evidence, may well be. For a detailed treatment of the other polyomaviruses, the reader is referred to the chapter by Cole (1996) in *Fields Virology*.

2. POLYOMAVIRUS STRUCTURE

The icosahedral capsids of polyomaviruses are composed of three structural proteins, VP1, VP2, and VP3. VP1 is the major component, with 360 molecules per capsid, and VP2 and VP3 contribute 30–60 molecules each to the capsid. The VP1 protein is the most highly homologous among the three viruses (Table 5.1). The significant degree of homology among these viruses has complicated somewhat the development of highly specific serologic assays, an issue that is relevant to ongoing epidemiologic studies relating to the involvement of these viruses in human cancer and the possible spread of SV40 in the human population. These viruses have a somewhat unique structure in that the icosahedron is composed of 72 pentamers, with no apparent hexamers (Rayment et al., 1982; Liddington et al., 1991). Each pentamer consists of five VP1 molecules and one molecule of VP2 or VP3. The capsid surrounds the viral DNA, which is a supercoiled, circular, double-stranded molecule of approximately 5200 base pairs in the case of the primate viruses. Histones H2A, H2B, H3, and H4 are associated with the DNA in the virion. In the cell, histone H1 is also bound to the viral DNA, and in fact the viral chromosome in the cell is structurally indistinguishable from host cell chromatin (Louie, 1975; Keller et al., 1978; Zentgraf et al., 1978; Muller et al., 1978). The DNA sequence identity among the primate viruses is approximately 70% (Table 5.1).

The genomic organization of these viruses is also conserved (Figure 5.1). The early and late promoters, along with the origin of DNA replication, are

Table 5.1. Sequence Homologies Among BKV, JCV, and SV40

	BKV–JCV	BKV–SV40	JCV–SV40
Proteins[a]			
VP1	87	88	85
VP2	88	86	83
VP3	84	81	78
T antigen	90	84	82
t antigen	86	78	76
DNA[b]	72	69	68

[a]Percent amino acid homology.
[b]Percent sequence identity.

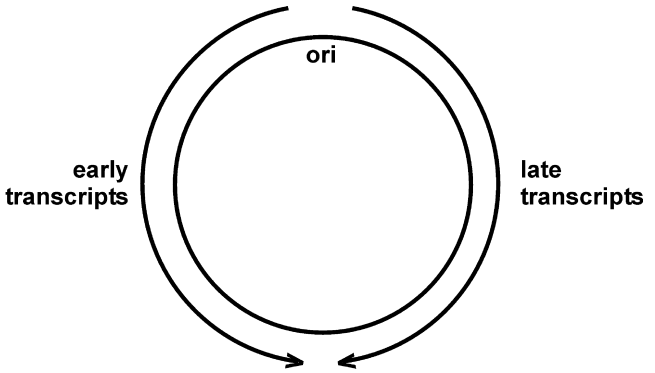


Figure 5.1. *Genomic organization of polyomaviruses.* The double-stranded, circular DNA genome is depicted with the origin of DNA replication (ori, which overlaps the transcriptional control region) at the top. The direction of transcription for the early and late transcription units is indicated by the arrows.

clustered together into a so-called viral regulatory or transcriptional control region. The early genes are transcribed from one strand of the genome, and the late genes are transcribed in the opposite direction from the complementary strand. In effect, then, the genetic map of the viruses has two halves, early and late, that are divided by the promoter/origin region on one side and the mRNA polyadenylation sites on the other.

3. THE POLYOMAVIRUS LIFE CYCLE

While the polyomaviruses can bind to and enter cells of multiple species, the outcome of the infection is species specific (Fig. 5.2). In permissive hosts, one generally obtains a lytic infection, while in nonpermissive hosts (e.g., rodents in the case of the primate viruses), there is a block to viral replication, leading to abortive infection or oncogenesis. For example, SV40 replication is restricted in mouse cells due to an inability to use the host DNA polymerase for viral DNA synthesis (Murakami et al., 1986). Infection of human cells by the human viruses is generally limited to epithelial cells, fibroblasts, lymphocytes, or cells derived from the nervous system. The outcome of that infection is also cell type specific. For example, while JCV persists in the urinary tract, infection of glial cells can be cytolytic. Most recently, it has been reported that cell type differences may dictate the outcome of SV40 infection. Specifically, it has been shown that while SV40 infection of human fibroblasts leads to a productive outcome, which has been known for some time, in mesothelial cells it established a persistent, nonlytic infection (Bocchetta et al., 2000). This result has important implications for the possible role of SV40 in human mesothelioma, as is discussed below. It should be noted that the concept of differences in the progression of the infection in different cell types within a given organism is

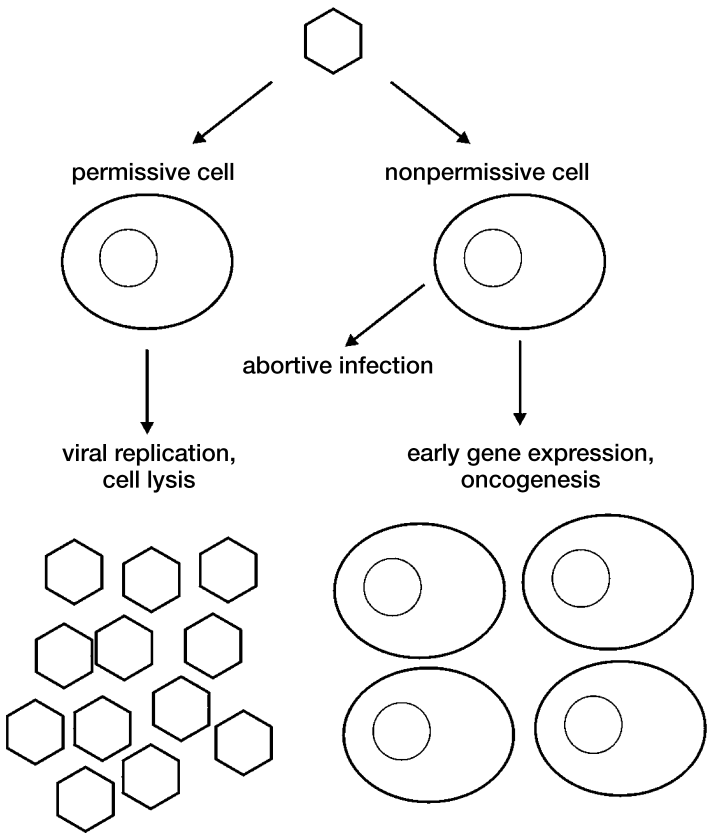


Figure 5.2. *Infection outcomes*. The two major outcomes of infections are shown. In a permissive cell (left side), the viral life cycle is completed and progeny virions are produced. In nonpermissive cells (right side), replication is blocked and abortive infection or transformation may ensue.

not limited to tissue specificity, as it has been known for some time that polyomaviruses require differentiated cells within a given tissue to provide a fertile environment for replication (Villarreal, 1991).

Infection of cells is initiated by binding of the virion to specific cell surface receptors. It is believed that SV40 binds MHC molecules (Norkin, 1999), although a differential ability to infect polarized epithelial cells via the apical or basolateral surfaces has been reported, even though MHC expression on the two membranes is the same (Basak et al., 1992). This may be a reflection of expression, or lack thereof, of additional molecules that are required for entry once the virus has bound. The receptors for BKV and JCV are not known, although it has been demonstrated that JCV binds specifically to glial cells, tonsilar stromal cells, and B lymphocytes, closely mirroring the observed cell tropism with respect to persistent and lytic infection (Wei et al., 2000). The

viral capsids are then internalized by endocytosis and are transported to the nucleus, where the DNA is uncoated. The mechanism by which the virions escape endocytic vesicles is not clear. Once the DNA reaches the nucleus, however, transcription of the early genes begins, driven by promoter and enhancer sequences that overlap the origin of DNA replication. These promoter elements bind a large variety of ubiquitous and cell type-specific transcription factors (see Chapter 6). Early gene transcription has been demonstrated to play a regulatory role in infection in the replication of JCV in glial cells, as discussed below.

The early genes of the human polyomaviruses encode two major proteins, the large T and small t antigens, which are expressed from two mRNAs that are derived by alternative splicing of a single primary transcript (Fig. 5.3A). These proteins are so named because of the early finding that they were the dominant tumor antigens recognized by the immune systems of animals harboring virally induced tumors (Black et al., 1963). Overall, the degree of amino acid homology among the T antigens is similar to that observed for the capsid

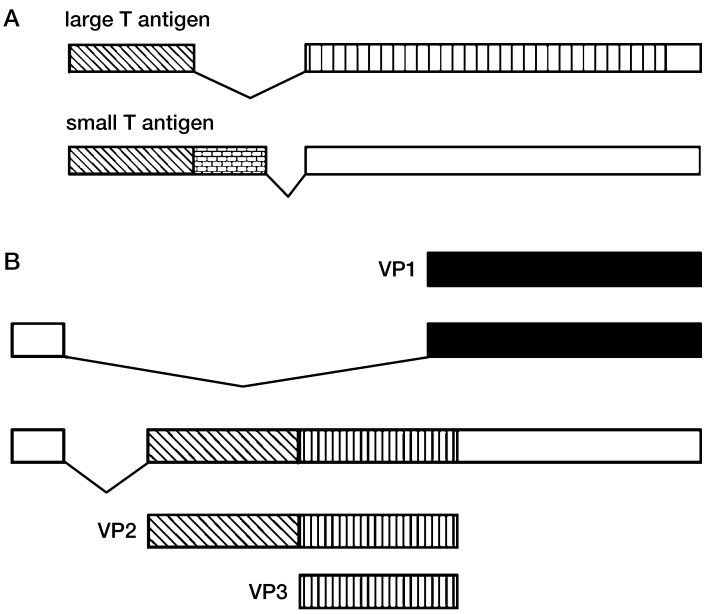


Figure 5.3. *Major viral genes.* **(A)** Early transcripts and proteins. The two major T antigens are expressed from two differentially spliced mRNAs. The angled lines indicate introns. The angled lines indicate introns that are removed by splicing. Hatched boxes, sequences shared in common by large T antigen and small t antigen; bricked box, small t-unique sequences; lined box, large T-unique sequences. **(B)** Late transcripts and proteins. The three late proteins VP1, VP2, and VP3 are expressed from two mRNAs, the shorter of which encodes VP1 and the longer of which encodes the other two proteins. The angled lines indicate introns. Black box, VP1; hatched box, VP2-unique sequence; lined box, sequences shared by VP2 and VP3.

proteins (Table 5.1), but if one examines the protein domains that have been shown to be functionally important for viral DNA replication and oncogenic transformation, the homology is much higher, closer to 100%. Mouse polyoma virus encodes a third T antigen molecule, middle T antigen, distinguishing it from the primate viruses. Most recently, an additional early mRNA has been identified in SV40 that would encode a T antigen with a predicted molecular weight of 17 kDa, sharing the first 131 amino acids of TAg and then terminating after four unique amino acids (Zerrahn et al., 1993). The role of this T antigen, sometimes referred to as "tiny t," has not yet been investigated in detail. For additional details on early mRNAs of JCV see Chapter 6.

Large T antigen (TAg), a nuclear phosphoprotein of approximately 700 amino acids in the primate viruses, is a critical player in the life cycle of the virus. One of its major roles during the early phase of the infection is to induce the cell to enter S phase. The virus must accomplish this because it does not encode any of the DNA synthetic machinery required for replication of its genome and therefore relies on the cell's machinery, which is expressed at high levels in S phase. Because the virus generally infects cells that are not actively cycling, it must provide them with a means to do so. The major mechanism by which the virus accomplishes this is by binding to and inactivating the retinoblastoma susceptibility protein, pRb, and its two related family members, p107 and p130 (DeCaprio et al., 1988; Ewen et al., 1989; Hannon et al., 1993; Dyson et al., 1989, 1990; Harris et al., 1996; Bollag et al., 1989; Trowbridge and Frisque, 1993). Inactivation results in the release of E2F transcription factors, which stimulate transcription of genes involved in entry into S phase and DNA synthesis (for review, see Cress and Nevins, 1996). The release of E2F is mediated by binding of TAg to pRb family members resulting in direct physical displacement of E2F, as well as TAg-mediated induction of the degradation of pRb family member proteins (Campbell et al., 1997; Stubdal et al., 1997; Zalvide et al., 1998; Sock et al., 1999; Harris et al., 1998). Both of these functions require a conserved LXCXE motif that is involved in pRb family protein binding, and degradation also requires the J domain, so named due to its homology to the DnaJ family of molecular chaperones (Rutila et al., 1986; Kalderon and Smith, 1984; Ewen et al., 1989; Christensen and Imperiale, 1995; Zalvide and DeCaprio, 1995; Brodsky and Pipas, 1998; Campbell et al., 1997; Stubdal et al., 1997; Sheng et al., 1997; Zalvide et al., 1998; Harris et al., 1998). The exact mechanism by which the J domain targets these tumor suppressors for degradation is currently unclear. TAg also plays a second, more direct, role in DNA replication, acting as an important initiation factor for DNA synthesis. First, TAg binds to specific sequences in the viral DNA at the origin of replication and recruits the DNA synthetic machinery to the origin of replication by directly binding to the DNA polymerase α/primase complex and replication protein A (RPA), a single-stranded DNA-binding protein that is required for efficient replication (Dornreiter et al., 1990; Melendy and Stillman, 1993). Second, TAg has helicase activity, unwinding the DNA at the origin and setting the stage for the initiation of DNA synthesis (Dean et al., 1987; Stahl

et al., 1986). Indeed, much of what we know about eukaryotic DNA replication comes from in vitro studies of SV40 replication, and the entire system has been reconstituted from purified factors in recent years (Waga et al., 1994).

Small t antigen is a cysteine-rich protein of 172 amino acids in BKV and JCV (174 in the case of SV40), the first 80 of which (82 in SV40) are shared with TAg. It appears to be dispensable for lytic infection in cultured cells. However, whether it plays a role in vivo is not known (Shenk et al., 1976; Staneloni et al., 1977). It has the ability to bind the cellular phosphatase PP2A, inhibiting its function. This results in a signal transduction cascade that stimulates cell growth, implying that, like TAg, small t antigen may be important in setting up the cellular environment for efficient replication under certain conditions (Sontag et al., 1993; Pallas et al., 1990). Middle T antigen of mouse PyV, as its name implies, is intermediate in size to the large and small T antigens. This cytoplasmic protein is localized to the plasma membrane and is identical to small t antigen in its amino terminus but has a unique carboxy terminus, and it is also required for viral replication in mice (Freund et al., 1992b).

Once DNA replication begins, the infection enters the late phase. At the same time, TAg acts to stimulate transcription of the late genes and to repress transcription of the early genes. While the mechanism of repression is poorly understood, activation of late gene expression is accomplished through the interaction of TAg and components of the cellular basal transcription machinery (TAFs; Gruda et al., 1993; Berger et al., 1996). The late genes encode the three structural proteins described above, VP1, VP2, and VP3, which are also expressed from alternatively spliced mRNAs whose transcription initiation and polyadenylation sites are identical (Fig. 5.3B). Based on studies in vitro and using baculovirus-expressed capsid proteins, it is believed that these proteins assemble into capsid structures and the DNA is then inserted into the capsids (Sandalon et al., 1997; Forstova et al., 1993; Salunke et al., 1986). It has been speculated that the infected cell is killed through an apoptotic mechanism. It has been argued, for example, that the reason the virus inhibits p53 function (see below) is to keep the cell alive longer to maximize the yield of progeny virions. There is direct evidence from analyses of adenovirus, whose E1A and E1B proteins share many functions with TAg, to support this idea (Rao et al., 1992). Studies have shown SV40 virions being released directly from the plasma membranes of intact epithelial cells, however (Clayson et al., 1989). Therefore, it is not clear that cell lysis is required for release of progeny virions.

4. CLASSIC INFECTIOUS DISEASE

The major clear-cut associations between the human polyomaviruses and classically defined infectious diseases are those of JCV with PML and BKV with hemorrhagic cystitis (Weber and Major, 1997; Arthur et al., 1988; Barbanti-Brodano et al., 1998). BKV and JCV are ubiquitous in most human populations,

infecting most individuals in early and late childhood, respectively (Gardner, 1973; Padgett and Walker, 1973; Taguchi et al., 1982). BKV is thought to cause mild respiratory illness, which would correlate with its appearance in the population at early ages (Goudsmit et al., 1981, 1982; Hashida et al., 1976; Mininberg et al., 1982; Padgett et al., 1983). Indeed, both viruses can be detected in tonsillar tissue, and JCV has been shown to infect tonsillar lymphocytes and stromal cells (Goudsmit et al., 1982; Monaco et al., 1998a,b). The viruses then disseminate and establish a subclinical, persistent infection in the urinary tract and lymphocytes (and, for JCV, the brain). To obtain overt disease, the individual usually must be immunocompromised. Most commonly affected are patients with AIDS or transplant recipients taking immunosuppressive drugs. The pathology of the disease is thought to be due to destruction of the infected tissue due to viral replication. The molecular mechanisms of disease have been described in a fair amount of detail for JCV (Chapter 12). For a detailed discussion of BKV and clinical disease see Chapter 14.

The pathology of PML is due to viral replication in oligodendrocytes, which are the cells in the brain that produce myelin. Numerous studies have demonstrated that the JCV promoter and enhancer contain binding sites for a variety of transcription factors that are expressed in glial cells, although only one of these appears to be truly glial cell specific (Frisque and White, 1992; Raj and Khalili, 1995). Disease is also often accompanied by rearrangements in the transcriptional control region that appear to enhance TAg expression and, subsequently, replication (Dörries, 1997, 1998; Weber and Major, 1997). The BKV promoter and enhancer have not been dissected in nearly as much detail, making the molecular pathogenesis of hemorrhagic cystitis less well understood.

5. ONCOGENIC TRANSFORMATION

Large T antigen is the major transforming protein of the primate viruses. Its expression is both necessary and sufficient for the oncogenic transformation of most cell types (Martin and Chou, 1975; Brockman, 1978; Tegtmeyer, 1975; Brugge and Butel, 1975). The TAg of PyV differs from its primate cousins in that it is not required for maintenance of the transformed state: that function falls upon middle T antigen (Di Mayorca et al., 1969; Eckhart, 1969; Fried, 1965; Basilico et al., 1980; Lania et al., 1980). Small t antigen is required for transformation under certain conditions, working through its interaction with PP2A. The ability of small t to bind PP2A correlates with its ability to induce cell growth (Mungre et al., 1994).

The capacity of TAg to transform cells maps to at least three domains of the molecule: the pRb binding and J domains discussed above and a more carboxy-terminal domain that allows TAg to bind to the tumor suppressor protein p53 (for review, see Pipas, 1992). It is worth noting here that p53 was originally identified through its ability to coprecipitate with TAg (Lane and Crawford, 1979; McCormick and Harlow, 1980; Linzer and Levine, 1979). Our

current understanding of the mechanism of transformation is as follows. TAg expression leads to the induction of S phase through the pRb-binding and J domain functions described above. The cell responds to this abnormal signal to divide by turning on a p53 response, which would normally lead to cell cycle arrest or apoptosis. TAg overcomes this roadblock, however, by binding to p53 and inhibiting its function. Thus, the cell is induced to proliferate, and p53-mediated check point control is abrogated. The purpose of the interaction with pRb and p53 pathways during lytic infection is therefore to push the cell into a maximal environment for the support of viral DNA replication and to keep it alive as long as possible to optimize viral yields before the cell is killed. Oncogenic transformation occurs when there is a block to productive infection, but continued expression of TAg. This leads to cell proliferation without killing of the cell, ultimately resulting in tumor formation in the nonpermissive host due to the restrictive nature of the virus–cell interaction relating to DNA replication. One can also obtain oncogenic transformation in a permissive host if viral replication is blocked by other means, such as mutations in the origin of replication (Gluzman, 1981). Finally, one must keep in mind that TAg itself can induce DNA damage, making its inactivation of p53 doubly dangerous to the cell (Theile and Grabowski, 1990; Lazutka et al., 1996; Tognon et al., 1996; Trabanelli et al., 1998; Ray et al., 1990).

In addition to the large and small T antigens, the third PyV T antigen, middle T antigen, is required for transformation by the mouse virus. While PyV large T antigen does bind to and deregulate pRb family members, working through the same functional domains as the primate TAg (Larose et al., 1991; Freund et al., 1992a; Sheng et al., 1997), it does not bind to p53. PyV small t antigen does affect PP2A function and appears to serve an identical function to SV40 small t antigen (Pallas et al., 1988). Middle T antigen acts through a distinctly different mechanism than the other two oncoproteins. Its membrane-bound location puts it in a position to bind to and activate Src family kinases, thereby signaling the cell to grow and divide via a kinase cascade pathway (Kaplan et al., 1989).

6. HUMAN CANCER

Until recently, evidence for the involvement of JCV, BKV, and SV40 in human cancer was virtually nonexistent. The advent of polymerase chain reaction (PCR) technology, however, has sparked renewed interest in the issue and provided provocative evidence that these viruses may indeed play a role in certain neoplasms. The fact that these viruses can indeed cause tumors is not disputed, based on the in vitro studies described above as well as experiments in animals. For example, SV40 induces osteosarcomas and mesotheliomas in infected hamsters and causes brain tumors in transgenic mice; JCV also causes brain tumors in transgenic mice and infected hamsters; and BKV transgenic mice get kidney tumors and hepatocellular carcinomas (Cicala et al., 1993; Diamandopoulos,

1972; Brinster et al., 1984; Walker et al., 1973; Zu Rhein, 1983; Franks et al., 1996; Krynska et al., 1999; Small et al., 1986; Dalrymple and Beemon, 1990). The crucial questions with respect to their potential role in human cancer are (1) Is the detection of viral sequences in tumor samples reliable? (2) Is the association of viral sequences with human tumor samples a reflection of a causal role, or is the virus an innocent bystander?

The use of PCR to detect sequences in biopsy samples is somewhat tricky in that often only small amounts of tissue are available, requiring large numbers of cycles in the reaction. Such large numbers increase the possibility of detecting contaminants that might be inadvertently introduced into the reactions. While this may have called into question some early reports, most laboratories now take great care in segregating the various sample manipulations so as to prevent contamination problems. In addition, there have now been a number of cases in which viral sequences isolated from tissues have been sequenced and shown to be distinct from laboratory strains of the viruses, thereby ruling out simple contamination with cloned DNAs.

BKV sequences have been detected in a variety of human tumors, including pancreas, neuroblastoma, and urinary tract, as well as samples isolated from tissues that the virus has not been previously thought to infect (for review, see Imperiale, 2000). Two of these studies are notable as the conclusions were not solely based on PCR analysis. First is an analysis of urinary tract tumors by Monini et al. (1995) at the University of Ferrara, in which they were able to detect BK viral sequences in some tumors using Southern blotting. Analysis of these sequences indicated that the viral DNA had integrated into the host chromosome and also that rearrangements in the origin of replication were present that would be predicted to interfere with productive infection. As discussed above, a block to viral replication concomitant with TAg expression could lead the infection down the path to oncogenic transformation. Flaegstad et al. (1999), in Norway, detected BKV DNA sequences in 17 of 18 neuroblastomas by in situ PCR, BKV TAg by immunohistochemistry, and TAg–p53 complexes by immunoprecipitation.

JCV sequences have been reported in colorectal tumors (Laghi et al., 1999; Ricciardiello, et al., 2001), although this location, like that of some of the sites in which BKV has been detected, does not represent a known site of infection by the virus. JCV has also been detected in various neuronal tumors, and T antigen expression can be demonstrated by immunohistochemistry in some of these samples (see Khalili et al., 1999, and references therein). With both viruses, the analysis of whether they are truly involved in cancer is complicated by the fact that they are also detected in a large percentage of normal tissue: For example, in the urinary tract study of BKV discussed above, approximately 60% of the normal tissues were positive for the viral early region by PCR. Thus, although viral sequences can be detected in tumors derived from tissues in which the virus is known to persist, expression can be detected in some cases, and the state of the viral chromosome appears to be conducive to trans-

formation in some cases, proving a role for these viruses in human cancer will require further study.

A possible role of SV40 in human cancer has also been postulated recently (Levine et al., 1998; Barbanti-Brodano et al., 1998; Lednicky and Butel, 2001). Viral sequences have been reported in three types of human cancer, brain tumors, osteosarcomas, and mesotheliomas, but have not been found at various other sites (Bergsagel et al., 1992; Lednicky et al., 1995; Carbone et al., 1994, 1996; Pass et al., 1996; Testa et al., 1998). Interestingly, brain tumors and mesotheliomas are the two most common tumors induced by SV40 in transgenic mice and infected hamsters, respectively (Cicala et al., 1993; Brinster et al., 1984). T antigen has been shown to be expressed in these human samples (Carbone et al., 1997, 1999; Butel and Lednicky, 1999). The argument for a role of SV40 in mesothelioma has received a strong boost recently from the finding that the virus can establish what appears to be a persistent infection in primary human mesothelial cell cultures and that these infected cells score positive in transformation assays in culture (Bocchetta et al., 2000). Again, this raises the possibility that nonproductive infection may predispose the cell to travel down an oncogenic path. In addition, viral DNA has been cloned from a number of these human tumors, and two findings have been described. First, the viruses isolated from humans do not have a characteristic duplication of the enhancer sequences that is found in laboratory strains after passage in tissue culture (Lednicky et al., 1995; Lednicky and Butel, 2001). Second, there is quite a bit of variability in the extreme carboxy-terminus of the T antigen gene (Stewart et al., 1996). These findings indicate that the samples have not been contaminated with laboratory stocks of the virus or plasmids containing viral sequences. It is clear that a large number of people may have been exposed to SV40 through administration of contaminated poliovirus vaccines in the late 1950s and early 1960s, but perhaps of more concern is the fact that the virus is being isolated from individuals who are too young to have received these contaminated lots of vaccine. Serologic studies also indicate the presence of SV40 infection in nonvaccinated populations (Butel et al., 1999). Thus, it is quite possible that SV40 is being transmitted horizontally throughout the population, and even vertical transmission cannot be ruled out. With the evidence for SV40 as a human pathogen growing, attempts to develop an SV40 vaccine may take on added importance (Xie et al., 1999; Imperiale et al., 2001).

7. CONCLUSIONS

The polyomaviruses have a long history of providing unique insights into how viruses interact with their host cells and organisms. Early investigations lent tremendous insight into basic molecular events that are critical to both viral replication and host cell biology. More recently, the mechanisms behind the role of JCV in PML have been teased apart. Finally, the evidence implicating BKV, JCV, and SV40 in human cancer has reached a point where the possibility

of their contributing to neoplastic disease cannot be ignored. Continued studies into the molecular pathogenesis of these cancers will no doubt allow a more definitive conclusion in the near future. In addition, an increased understanding of the biology of these viruses will assist in the development of antiviral drugs and vaccines, holding promise that the human diseases associated with these viruses can be treated or prevented.

REFERENCES

Arthur RR, Shah KV, Charache P, Saral R (1988): BK and JC virus infections in recipients of bone marrow transplants. J Infect Dis 158:563–569.

Barbanti-Brodano G, Martini F, De Mattei M, Lazzarin L, Corallini A, Tognon M (1998): BK and JC human polyomaviruses and simian virus 40: Natural history of infection in humans, experimental oncogenicity, and association with human tumors. Adv Virus Res 50:69–99.

Basak S, Turner H, Compans RW (1992): Expression of SV40 receptors on apical surfaces of polarized epithelial cells. Virology 190:393–402.

Basilico C, Zouzias D, Della-Valle G, Gattoni S, Colantuoni V, Fenton R, Dailey L (1980): Integration and excision of polyoma virus genomes. Cold Spring Harb Symp Quant Biol 44(Pt 1):611–620.

Berger LC, Smith DB, Davidson I, Hwang JJ, Fanning E, Wildeman AG (1996): Interaction between T antigen and TEA domain of the factor TEF-1 derepresses simian virus 40 late promoter in vitro: Identification of T-antigen domains important for transcription control. J Virol 70:1203–1212.

Bergsagel DJ, Finegold MJ, Butel JS, Kupsky WJ, Garcea RL (1992): DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N Engl J Med 326:988–993.

Black PH, Rowe WP, Turner HC, Huebner RJ (1963): A specific complement-fixing antigen present in SV40 tumor and transformed cells. Proc Natl Acad Sci USA 50: 1148–1156.

Bocchetta M, Di Resta I, Powers A, Fresco R, Tosolini A, Testa JR, Pass HI, Rizzo P, Carbone M (2000): Human mesothelial cells are unusually susceptible to simian virus 40–mediated transformation and asbestos cocarcinogenicity. Proc Natl Acad Sci USA 97:10214–10219.

Bollag B, Chuke WF, Frisque RJ (1989): Hybrid genomes of the polyomaviruses JC virus, BK virus, and simian virus 40: Identification of sequences important for efficient transformation. J Virol 63:863–872.

Brinster RL, Chen HY, Messing A, Van Dyke T, Levine AJ, Palmiter RD (1984): Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. Cell 37:367–379.

Brockman WW (1978): Transformation of BALB/c-3T3 cells by tsA mutants of simian virus 40: Temperature sensitivity of the transformed phenotype and retransformation by wild-type virus. J Virol 25:860–870.

Brodsky JL, Pipas JM (1998): Polyomavirus T antigens: Molecular chaperones for multiprotein complexes. J Virol 72:5329–5334.

Brugge JS, Butel JS (1975): Role of simian virus 40 gene A function in maintenance of transformation. J Virol 15:619–635.

Butel JS, Jafar S, Wong C, Arrington AS, Opekun AR, Finegold MJ, Adam E (1999): Evidence of SV40 infections in hospitalized children. Hum Pathol 30:1496–1502.

Butel JS, Lednicky JA (1999): Cell and molecular biology of simian virus 40: Implications for human infections and disease. J Natl Cancer Inst 91:119–134.

Campbell KS, Mullane KP, Aksoy IA, Stubdal H, Zalvide J, Pipas JM, Silver PA, Roberts TM, Schaffhausen BS, DeCaprio JA (1997): DnaJ/hsp40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication. Genes Dev 11: 1098–1110.

Carbone M, Fisher S, Powers A, Pass HI, Rizzo P (1999): New molecular and epidemiological issues in mesothelioma: Role of SV40. J Cell Physiol 180:167–172.

Carbone M, Pass HI, Rizzo P, Marinetti M, Di Muzio M, Mew DJ, Levine AS, Procopio A (1994): Simian virus 40–like DNA sequences in human pleural mesothelioma. Oncogene 9:1781–1790.

Carbone M, Rizzo P, Grimley PM, Procopio A, Mew DJ, Shridhar V, de Bartolomeis A, Esposito V, Giuliano MT, Steinberg SM, Levine AS, Giordano A, Pass HI (1997): Simian virus–40 large-T antigen binds p53 in human mesotheliomas. Nat Med 3: 908–912.

Carbone M, Rizzo P, Procopio A, Giuliano M, Pass HI, Gebhardt MC, Mangham C, Hansen M, Malkin DF, Bushart G, Pompetti F, Picci P, Levine AS, Bergsagel JD, Garcea RL (1996): SV40-like sequences in human bone tumors. Oncogene 13:527–535.

Christensen JB, Imperiale MJ (1995): Inactivation of the retinoblastoma susceptibility protein is not sufficient for the transforming function of the conserved region 2-like domain of simian virus 40 large T antigen. J Virol 69:3945–3948.

Cicala C, Pompetti F, Carbone M (1993): SV40 induces mesotheliomas in hamsters. Am J Pathol 142:1524–1533.

Clayson ET, Brando LV, Compans RW (1989): Release of simian virus 40 virions from epithelial cells is polarized and occurs without cell lysis. J Virol 63:2278–2288.

Cole CN (1996): Polyomavirinae: The viruses and their replication. In Fields Virology; Fields BN, Knipe DM, Howley PM, Eds. Lippincott-Raven: Philadelphia, pp. 1997–2026.

Cress WD, Nevins JR (1996): Use of the E2F transcription factor by DNA tumor virus regulatory proteins. Curr Top Microbiol Immunol 208:63–78.

Dalrymple SA, Beemon KL (1990): BK virus T antigens induce kidney carcinomas and thymoproliferative disorders in transgenic mice. J Virol 64:1182–1191.

Dean FB, Bullock P, Murakami Y, Wobbe CR, Weissbach L, Hurwitz J (1987): Simian virus 40 (SV40) DNA replication: SV40 large T antigen unwinds DNA containing the SV40 origin of replication. Proc Natl Acad Sci USA 84:16–20.

DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, Marsilio E, Paucha E, Livingston DM (1988): SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 54:275–283.

Diamandopoulos GT (1972): Leukemia, lymphoma, and osteosarcoma induced in the Syrian golden hamster by simian virus 40. Science 176:173–175.

Di Mayorca G, Callender J, Marin G, Giordano R (1969): Temperature-sensitive mutants of polyoma virus. Virology 38:126–133.

Dornreiter I, Hoss A, Arthur AK, Fanning E (1990): SV40 T antigen binds directly to the large subunit of purified DNA polymerase alpha. EMBO J 9:3329–3336.

Dörries K (1997): New aspects in the pathogenesis of polyomavirus-induced disease. Adv Virus Res 48:205–261.

Dörries K (1998): Molecular biology and pathogenesis of human polyomavirus infections. Dev Biol Stand 94:71–79.

Dyson N, Bernards R, Friend SH, Gooding LR, Hassell JA, Major EO, Pipas JM, Van Dyke T, Harlow E (1990): Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. J Virol 64:1353–1356.

Dyson N, Buchkovich K, Whyte P, Harlow E (1989): The cellular 107K protein that binds to adenovirus E1A also associates with the large T antigens of SV40 and JC virus. Cell 58:249–255.

Eckhart W (1969): Complementation and transformation by temperature-sensitive mutants of polyoma virus. Virology 38:120–125.

Ewen ME, Ludlow JW, Marsilio E, DeCaprio JA, Millikan RC, Cheng SH, Paucha E, Livingston DM (1989): An N-terminal transformation-governing sequence of SV40 large T antigen contributes to the binding of both p110Rb and a second cellular protein, p120. Cell 58:257–267.

Flaegstad T, Andresen PA, Johnsen JI, Asomani SK, Jorgensen GE, Vignarajan S, Kjuul A, Kogner P, Traavik T (1999): A possible contributory role of BK virus infection in neuroblastoma development. Cancer Res 59:1160–1163.

Forstova J, Krauzewicz N, Wallace S, Street AJ, Dilworth SM, Beard S, Griffin BE (1993): Cooperation of structural proteins during late events in the life cycle of polyomavirus. J Virol 67:1405–1413.

Franks RR, Rencic A, Gordon J, Zoltick PW, Curtis M, Knobler RL, Khalili K (1996): Formation of undifferentiated mesenteric tumors in transgenic mice expressing human neurotropic polyomavirus early protein. Oncogene 12:2573–2578.

Freund R, Bronson RT, Benjamin TL (1992a): Separation of immortalization from tumor induction with polyoma large T mutants that fail to bind the retinoblastoma gene product. Oncogene 7:1979–1987.

Freund R, Sotnikov A, Bronson RT, Benjamin TL (1992b): Polyoma virus middle T is essential for virus replication and persistence as well as for tumor induction in mice. Virology 191:716–723.

Fried M (1965): Cell-transforming ability of a temperature-sensitive mutant of polyoma virus. Proc Natl Acad Sci USA 53:486–491.

Frisque RJ, White FA, III (1992): The molecular biology of JC virus, causative agent of progressive multifocal leukoencephalopathy. In Molecular Neurovirology; Ross R, Ed. Humana Press: Clifton, NJ, pp 25–158.

Gardner SD (1973): Prevalence in England of antibody to human polyomavirus (B.K.) BMJ 1:77–78.

Gardner SD, Field AM, Coleman DV, Hulme B (1971): New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1:1253–1257.

Gluzman Y (1981): SV40-transformed simian cells support the replication of early SV40 mutants. Cell 23:175–182.

Goudsmit J, Baak ML, Sleterus KW, van der Noordaa J (1981): Human papovavirus isolated from urine of a child with acute tonsillitis. Br Med J (Clin Res Ed) 283: 1363–1364.

Goudsmit J, Wertheim-van Dillen P, van Strien A, van der Noordaa J (1982): The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. J Med Virol 10:91–99.

Gruda MC, Zabolotny JM, Xiao JH, Davidson I, Alwine JC (1993): Transcriptional activation by simian virus 40 large T antigen: Interactions with multiple components of the transcription complex. Mol Cell Biol 13:961–969.

Hannon GJ, Demetrick D, Beach D (1993): Isolation of the Rb-related p130 through its interaction with CDK2 and cyclins. Genes Dev 7:2378–2391.

Harris KF, Christensen JB, Imperiale MJ (1996): BK virus large T antigen: Interactions with the retinoblastoma family of tumor suppressor proteins and effects on cellular growth control. J Virol 70:2378–2386.

Harris KF, Christensen JB, Radany EH, Imperiale MJ (1998): Novel mechanisms of E2F induction by BK virus large T antigen: Requirement of both the pRb binding and J domains. Mol Cell Biol 18:1746–1756.

Hashida Y, Gaffney PC, Yunis EJ (1976): Acute hemorrhagic cystitis of childhood and papovavirus-like particles. J Pediatr 89:85–87.

Imperiale MJ (2000): The human polyomaviruses, BKV and JCV: Molecular pathogenesis of acute disease and potential role in cancer. Virology 267:1–7.

Imperiale MJ, Pass HI, Sanda MG (2001): Prospects for an SV40 vaccine. Semin Cancer Biol 11:81–85.

Kalderon D, Smith AE (1984): In vitro mutagenesis of a putative DNA binding domain of SV40 large-T. Virology 139:109–137.

Kaplan DR, Pallas DC, Morgan W, Schaffhausen B, Roberts TM (1989): Mechanisms of transformation by polyoma virus middle T antigen. Biochim Biophys Acta 948: 345–364.

Keller W, Muller U, Eicken I, Wendel I, Zentgraf H (1978): Biochemical and ultrastructural analysis of SV40 chromatin. Cold Spring Harb Symp Quant Biol 42(Pt 1):227–244.

Khalili K, Krynska B, Del Valle L, Katsetos CD, Croul S (1999): Medulloblastomas and the human neurotropic polyomavirus JC virus. Lancet 353:1152–1153.

Krynska B, Otte J, Franks R, Khalili K, Croul S (1999): Human ubiquitous JCV(CY) T-antigen gene induces brain tumors in experimental animals. Oncogene 18:39–46.

Laghi L, Randolph AE, Chauhan DP, Marra G, Major EO, Neel JV, Boland CR (1999): JC virus DNA is present in the mucosa of the human colon and in colorectral cancers. Proc Natl Acad Sci USA 96:7484–7489.

Lane DP, Crawford LV (1979): T antigen is bound to a host protein in SV40-transformed cells. Nature 278:261–263.

Lania L, Hayday A, Bjursell G, Gandini-Attardi D, Fried M (1980): Organization and expression of integrated polyoma virus DNA sequences in transformed rodent cells. Cold Spring Harbor Symp Quant Biol 44(Pt 1):597–603.

Larose A, Dyson N, Sullivan M, Harlow E, Bastin M (1991): Polyomavirus large T mutants affected in retinoblastoma protein binding are defective in immortalization. J Virol 65:2308–2313.

Lazutka JR, Neel JV, Major EO, Dedonyte V, Mierauskine J, Slapsyte G, Kesminiene A (1996): High titers of antibodies to two human polyomaviruses, JCV and BKV, correlate with increased frequency of chromosomal damage in human lymphocytes. Cancer Lett 109:177–183.

Lednicky JA, Butel JS (2001): Simian virus 40 regulatory region structural diversity and the association of viral archetypal regulatory regions with human brain tumors. Sem Cancer Biol 11:39–47.

Lednicky JA, Garcea RL, Bergsagel DJ, Butel JS (1995): Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. Virology 212:710–717.

Levine A, Butel J, Dörries K, Goedert J, Frisque R, Garcea R, Morris A, O'Neill F, Shah K (1998): SV40 as a putative human commensal. Dev Biol Stand 94:245–269.

Liddington RC, Yan Y, Moulai J, Sahli R, Benjamin TL, Harrison SC (1991): Structure of simian virus 40 at 3.8-Å resolution. Nature 354:278–284.

Linzer DI, Levine AJ (1979): Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 17:43–52.

Louie AJ (1975): The organization of proteins in polyoma and cellular chromatin. Cold Spring Harbor Symp Quant Biol 39(Pt 1):259–266.

Martin RG, Chou JY (1975): Simian virus 40 functions required for the establishment and maintenance of malignant transformation. J Virol 15:599–612.

McCormick F, Harlow E (1980): Association of a murine 53,000-dalton phosphoprotein with simian virus 40 large-T antigen in transformed cells. J Virol 34:213–224.

Melendy T, Stillman B (1993): An interaction between replication protein A and SV40 T antigen appears essential for primosome assembly during SV40 DNA replication. J Biol Chem 268:3389–3395.

Mininberg DT, Watson C, Desquitado M (1982): Viral cystitis with transient secondary vesicoureteral reflux. J Urol 127:983–985.

Monaco MC, Jensen PN, Hou J, Durham LC, Major EO (1998a): Detection of JC virus DNA in human tonsil tissue: Evidence for site of initial viral infection. J Virol 72: 9918–9923.

Monaco MC, Shin J, Major EO (1998b): JC virus infection in cells from lymphoid tissue. Dev Biol Stand 94:115–122.

Monini P, Rotola A, Di Luca D, De Lellis L, Chiari E, Corallini A, Cassai E (1995): DNA rearrangements impairing BK virus productive infection in urinary tract tumors. Virology 214:273–279.

Muller U, Zentgraf H, Eicken I, Keller W (1978): Higher order structure of simian virus 40 chromatin. Science 20:406–415.

Mungre S, Enderle K, Turk B, Porras A, Wu YQ, Mumby MC, Rundell K (1994): Mutations which affect the inhibition of protein phosphatase 2A by simian virus 40 small-t antigen in vitro decrease viral transformation. J Virol 68:1675–1681.

Murakami Y, Wobbe CR, Weissbach L, Dean FB, Hurwitz J (1986): Role of DNA polymerase alpha and DNA primase in simian virus 40 DNA replication in vitro. Proc Natl Acad Sci USA 83:2869–2873.

Norkin LC (1999): Simian virus 40 infection via MHC class I molecules and caveolae. Immunol Rev 168:13–22.

Padgett BL, Walker DL (1973): Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J Infect Dis 127:467–470.

Padgett BL, Walker DL, Desquitado MM, Kim DU (1983): BK virus and nonhaemorrhagic cystitis in a child. Lancet 1:770.

Padgett BL, Walker DL, Zu Rhein GM, Eckroade RJ, Dessel BH (1971): Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet 1:1257–1260.

Pallas DC, Cherington V, Morgan W, DeAnda J, Kaplan D, Schaffhausen B, Roberts TM (1988): Cellular proteins that associate with the middle and small T antigens of polyomavirus. J Viol 62:3934–3940.

Pallas DC, Shahrik LK, Martin BL, Jaspers S, Miller TB, Brautigan DL, Roberts TM (1990): Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. Cell 60:167–176.

Pass HI, Kennedy RC, Carbone M (1996): Evidence for and implications of SV40-like sequences in human mesotheliomas. Important Adv Oncol 89–108.

Pipas JM (1992): Common and unique features of T antigens encoded by the polyomavirus group. J Virol 66:3979–3985.

Raj GV, Khalili K (1995): Transcriptional regulation: Lessons from the human neurotropic polyomavirus, JCV. Virology 213:283–291.

Rao L, Debbas M, Sabbatini P, Hockenbery D, Korsmeyer S, White E (1992): The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. Proc Natl Acad Sci USA 89:7742–7746.

Ray FA, Peabody DS, Copper JL, Cram LS, Kraemer PM (1990): SV40 T antigen alone drives karyotype instability that precedes neoplastic transformation of human diploid fibroblasts. J Cell Biochem 42:13–31.

Rayment I, Baker TS, Caspar DL, Murakami WT (1982): Polyoma virus capsid structure at 22.5 Å resolution. Nature 295:110–115.

Ricciardiello L, Chang DK, Laghi L, Goel A, Chang CL, Boland CR (2001): Mad-1 is the exclusive JC virus strain present in the human colon, and its transcriptional control region has a deleted 98-base-pair sequence in colon cancer tissues. J Virol 75:1996–2001.

Rutila JE, Imperiale MJ, Brockman WW (1986): Replication and transformation functions of in vitro–generated simian virus 40 large T antigen mutants. J Virol 58:526–535.

Salunke DM, Caspar DL, Garcea RL (1986): Self-assembly of purified polyomavirus capsid protein VP1. Cell 46:895–904.

Sandalon Z, Dalyot-Herman N, Oppenheim AB, Oppenheim A (1997): In vitro assembly of SV40 virions and pseudovirions: Vector development for gene therapy. Hum Gene Ther 8:843–849.

Sheng Q, Denis D, Ratnofsky M, Roberts TM, DeCaprio JA, Schaffhausen B (1997): The DnaJ domain of polyomavirus large T antigen is required to regulate Rb family tumor suppressor function. J Virol 71:9410–9416.

Shenk TE, Carbon J, Berg P (1976): Construction and analysis of viable deletion mutants of simian virus 40. J Virol 18:664–671.

Small JA, Khoury G, Jay G, Howley PM, Scangos GA (1986): Early regions of JC virus and BK virus induce distinct and tissue-specific tumors in transgenic mice. Proc Natl Acad Sci USA 83:8288–8292.

Sock E, Enderich J, Wegner M (1999): The J domain of papovaviral large tumor antigen is required for synergistic interaction with the POU-domain protein Tst-1/Oct6/SCIP. Mol Cell Biol 19:2455–2464.

Sontag E, Fedorov S, Kamibayashi C, Robbins D, Cobb M, Mumby M (1993): The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. Cell 75:887–897.

Stahl H, Droge P, Knippers R (1986): DNA helicase activity of SV40 large tumor antigen. EMBO J 5:1939–1944.

Staneloni RJ, Fluck MM, Benjamin TL (1977): Host range selection of transformation-defective hr-t mutants of polyoma virus. Virology 77:598–609.

Stewart AR, Lednicky JA, Benzick US, Tevethia MJ, Butel JS (1996): Identification of a variable region at the carboxy terminus of SV40 large T-antigen. Virology 221: 355–361.

Stewart SE, Eddy BE, Borgese NG (1958): Neoplasms in mice inoculated with a tumor agent carried in tissue culture. J Natl Cancer Inst 20:1223–1243.

Stubdal H, Zalvide J, Campbell KS, Schweitzer C, Roberts TM, DeCaprio JA (1997): Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen. Mol Cell Biol 17:4979–4990.

Sweet BH, Hilleman MR (1960): The vacuolating virus, SV40. Proc Soc Exp Biol Med 105:420–427.

Taguchi F, Kajioka J, Miyamura T (1982): Prevalence rate and age of acquisition of antibodies against JC virus and BK virus in human sera. Microbiol Immunol 26: 1057–1064.

Tegtmeyer P (1975): Function of simian virus 40 gene A in transforming infection. J Virol 15:613–618.

Testa JR, Carbone M, Hirvonen A, Khalili K, Krynska B, Linnainmaa K, Pooley FD, Rizzo P, Rusch V, Xiao GH (1998): A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas. Cancer Res 58:4505–4509.

Theile M, Grabowski G (1990): Mutagenic activity of BKV and JCV in human and other mammalian cells. Arch Virol 113:221–233.

Tognon M, Casalone R, Martini F, De Mattei M, Granata P, Minelli E, Arcuri C, Collini P, Bocchini V (1996): Large T antigen coding sequences of two DNA tumor viruses, BK and SV40, and nonrandom chromosome changes in two glioblastoma cell lines. Cancer Genet Cytogenet 90:17–23.

Trabanelli C, Corallini A, Gruppioni R, Sensi A, Bonfatti A, Campioni D, Merlin M, Calza N, Possati L, Barbanti-Brodano G (1998): Chromosomal aberrations induced by BK virus T antigen in human fibroblasts. Virology 243:492–496.

Trowbridge PW, Frisque RJ (1993): Analysis of G418-selected Rat2 cells containing prototype, variant, mutant, and chimeric JC virus and SV40 genomes. Virology 196: 458–474.

Villarreal LP (1991): Relationship of eukaryotic DNA replication to committed gene expression: General theory for gene control. Microbiol Rev 55:512–542.

Waga S, Bauer G, Stillman B (1994): Reconstitution of complete SV40 DNA replication with purified replication factors. J Biol Chem 269:10923–10934.

Walker DL, Padgett BL, Zu Rhein GM, Albert AE, Marsh RF (1973): Human papovavirus (JC): Induction of brain tumors in hamsters. Science 181:674–676.

Weber T, Major EO (1997): Progressive multifocal leukoencephalopathy: Molecular biology, pathogenesis and clinical impact. Intervirology 40:98–111.

Wei G, Liu CK, Atwood WJ (2000): JC virus binds to primary human glial cells, tonsillar stromal cells, and B-lymphocytes, but not to T lymphocytes. J Neurovirol 6:127–136.

Xie YC, Hwang C, Overwijk W, Zeng Z, Eng MH, Mule JJ, Imperiale MJ, Restifo NP, Sanda MG (1999): Induction of tumor antigen-specific immunity in vivo by a novel vaccinia vector encoding safety-modified simian virus 40 T antigen. J Natl Cancer Inst 91:169–175.

Zalvide J, DeCaprio JA (1995): Role of pRb-related proteins in simian virus 40 large-T-antigen–mediated transformation. Mol Cell Biol 15:5800–5810.

Zalvide J, Stubdal H, DeCaprio JA (1998): The J domain of simian virus 40 large T antigen is required to functionally inactivate RB family proteins. Mol Cell Biol 18: 1408–1415.

Zentgraf H, Keller W, Muller U (1978): The structure of SV40 chromatin. Philos Trans R Soc Lond B Biol Sci 283:299–303.

Zerrahn J, Knippschild U, Winkler T, Deppert W (1993): Independent expression of the transforming amino-terminal domain of SV40 large T antigen from an alternatively spliced third SV40 early mRNA. EMBO J 12:4739–4746.

Zu Rhein GM (1983): Studies of JC virus-induced tumors in the Syrian hamster. In Polyomaviruses and Human Neurological Disease; Sever JL, Madden DM, Eds. Alan R. Liss: New York, pp 205–221.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

6

TRANSCRIPTION AND REPLICATION IN THE HUMAN POLYOMAVIRUSES

HEE-SUN KIM, PH.D., JOHN W. HENSON, M.D.,
and RICHARD J. FRISQUE, PH.D.

1. INTRODUCTION

JC virus (JCV) is a 5 kb circular double-stranded DNA virus that causes the fatal human disease progressive multifocal leukoencephalopathy (PML) by selective destruction of oligodendrocytes, leading to multiple areas of demyelination and attendant loss of brain function (Åström et al., 1958; Zu Rhein and Chou, 1965). The viral genome is divided into early and late gene coding regions, between which lies a regulatory region containing a bidirectional promoter and the viral origin of replication (Fig. 6.1). The JCV early promoter directs glial-specific expression of large T antigen (TAg). TAg is the major viral protein, containing several activities, through which the virus commandeers the cellular metabolic machinery for production of virions (Henson, 1996). Thus, glial-specific transcriptional regulation of TAg expression is believed to constitute a major mechanism of neural tropism in PML.

The promoters of numerous brain-specific genes have been partially characterized with respect to the ability of the 5′ untranslated region (UTR) to drive expression of a reporter gene in transgenic mice or in transient transfection assays. Studies of a number of viral and cellular genes have indicated that the

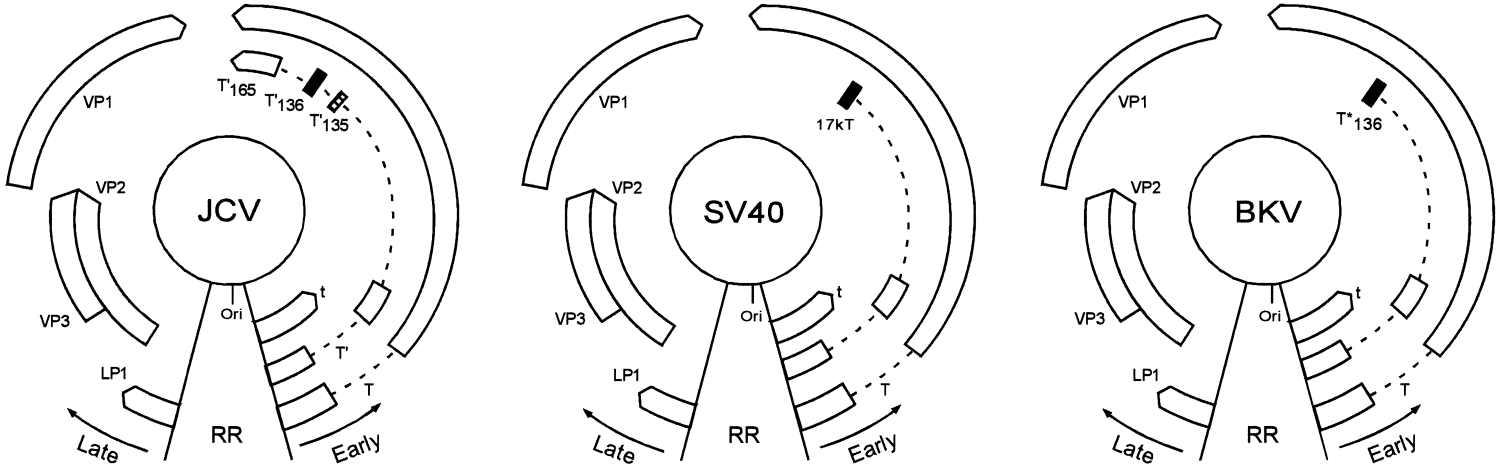


Figure 6.1. Comparisons of the JCV, BKV, and SV40 genomes. The organizations of these primate polyomavirus genomes are nearly identical. The inner circles represent the double-stranded DNA genomes (5130 bp, JCV[Mad-1]; 5098 bp, BKV[AS]; 5243 SV40[776]), and the outer arcs denote the encoded viral proteins. The genomes are divided into three regions. The early region specifies five (JCV) or three (BKV, SV40) regulatory proteins produced by translation of alternatively spliced early mRNAs. T′, T*, and 17kT on the JCV, BKV, and SV40 maps, respectively, represent proteins encoded by mRNAs composed of three exons that may or may not be shared with the TAg transcript. Each genome may encode an early leader peptide (JELP, BELP, or SELP) of unknown function. The late region specifies four proteins required for capsid assembly, VP1–3 and LP1. The regulatory region (RR) contains the *cis*-acting elements that control viral DNA replication (Ori) and transcription. The promoter/enhancer signals for transcription are the least conserved sequences in the three genomes.

temporal and cell-specific activation of eukaryotic genes by enhancer/promoter elements requires association of *cis*-acting DNA elements with *trans*-acting cellular proteins that recognize such DNA sequences. Specificity may arise from the expression of a specific transcriptional regulator in a restricted fashion or by virtue of a cell-specific combination of transcription factors and transcription factor binding sites within the promoter. For instance, a combination of NF-1 and AP1 transcription factor binding sites appears in numerous brain-specific promoters (Amemiya et al., 1992).

In the first half of this chapter, the transcriptional regulation of JCV is reviewed. First, the glial-specific nature of the JCV early promoter is described. Second, regulation by transcription factors, including TAg, are described. Third, cross-regulation by cytokines and HIV protein is reviewed. Finally, the possibility of regulation by epigenetic factors such as DNA methylation or chromatin structure is discussed.

In the second half of this chapter the regulation of primate polyomavirus DNA replication is reviewed. Our present understanding of JCV and BK virus (BKV) DNA replication relies in large part on studies involving the closely related monkey polyomavirus, simian virus 40 (SV40). SV40 has served as an important model with which to unravel the process by which small DNA tumor viruses duplicate their genomes; it has also served as a model to identify the mechanisms by which the DNA of eukaryotic cells is copied. A variety of molecular genetic and biochemical approaches have been employed to dissect the DNA replication process of this small, circular, double-stranded DNA genome. These approaches are now being redirected toward an investigation of human polyomavirus replication, and, while it is expected that the major events affecting replication of the human viruses will closely parallel those events already shown to influence SV40 replication, specific details unique to JCV and BKV have already been uncovered.

2. GLIAL-SPECIFIC NATURE OF JCV EARLY PROMOTER

A large number of observations have demonstrated the glial-specific nature of the JCV early promoter. Although oligodendrocytes are the only cells in the body known to be lytically infected by JCV, astrocytes also express viral early genes (i.e., large and small TAgs) in PML, whereas neurons do not (Itoyama et al., 1981). Successful JCV isolation followed the discovery that primary human fetal glial (PHFG) cells were permissive for infection in vivo, further demonstrating the restricted nature of the virus infection (Padgett et al., 1971). The expression of the TAg early gene was abolished following fusion of JCV-transformed hamster glial cells with mouse fibroblasts, and the degree of reduction of TAg expression correlated with the number of fibroblast nuclei in the heterokaryon fusion (Beggs et al., 1988). As discussed in more detail below, this was early experimental evidence in favor of JCV early promoter repression by a nonglial cell protein. In 1984 transcriptional analysis of the early promoter,

using a transient transfection assay, demonstrated that the JCV early promoter directed higher levels of expression of a reporter gene in glial cells than in nonglial cells (Kenny et al., 1984). This latter observation has been confirmed in a wide range of glial and nonglial cells types and has led to detailed analyses of the promoter in vitro. Mice carrying a JCV early promoter–TAg transgene selectively expressed TAg in oligodendrocytes, giving rise to a phenotype of demyelination (Trapp et al., 1988). Additional transgenic experiments, in which the early promoters and TAg genes of JCV and SV40 were exchanged, demonstrated that the JCV early promoter was responsible for glial-specific expression of TAg (Feigenbaum et al., 1992).

Thus, it is now established that the JCV early promoter directs glial-selective gene expression. A large number of transcription factors are known to regulate the early promoter, some of which activate expression in a cell-specific way.

3. STRUCTURE OF THE JCV PROMOTER

A promoter region of 293 bp was isolated from Mad-1 JCV obtained from PML brain tissue (Kenny et al., 1984; Tada et al., 1991). The Mad-1 promoter contains two characteristic tandem 98 bp repeats and functions in opposite orientations to regulate the early and late gene expression (Figs. 6.2 and 6.3). Distinct transcription initiation start sites have been detected from the viral promoter in the early and late phases of infection. Additional promoters cloned directly from brain (MH1, GS/B, Mad-11.3) contain an identical 222 bp 5′ UTR region from the first codon of TAg (Loeber and Dörries, 1988; Yogo et al., 1990). Thus, each isolate is identical to the end of the tandem repeat that is adjacent to the TATA box (Fig. 6.2), but several isolates diverge somewhat from the original Mad-1 promoter (Henson et al., 1992). Most isolates contain an Sp1 binding site upstream of the TATA box in a location identical to the first of six Sp1 binding sites in the SV40 promoter. By comparison, the original Mad-1 JCV promoter had sequence identity only through 13 bp upstream of the TATA box, and it then diverged from the direct isolates due to a 23 bp deletion (lower case letters in the Mad-1 sequence in Fig. 6.2). It is important to note that while the structure of the Mad-1 promoter does not alter its glial specificity, it does serve to complicate functional analysis of the Mad-1 promoter since the TATA region is duplicated in the upstream repeat. On the other hand, the other promoters can be easily divided into proximal and upstream regions in a manner similar to that employed in earlier studies of the SV40 promoter (Ondek et al., 1988).

4. CELL-SPECIFIC TRANSCRIPTION ANALYSIS

Introduction of Mad-1 and MH1 (Mad-11) JCV early promoter reporter gene plasmids into glial (U87MG glioma cells) and nonglial (HeLa) cells demon-

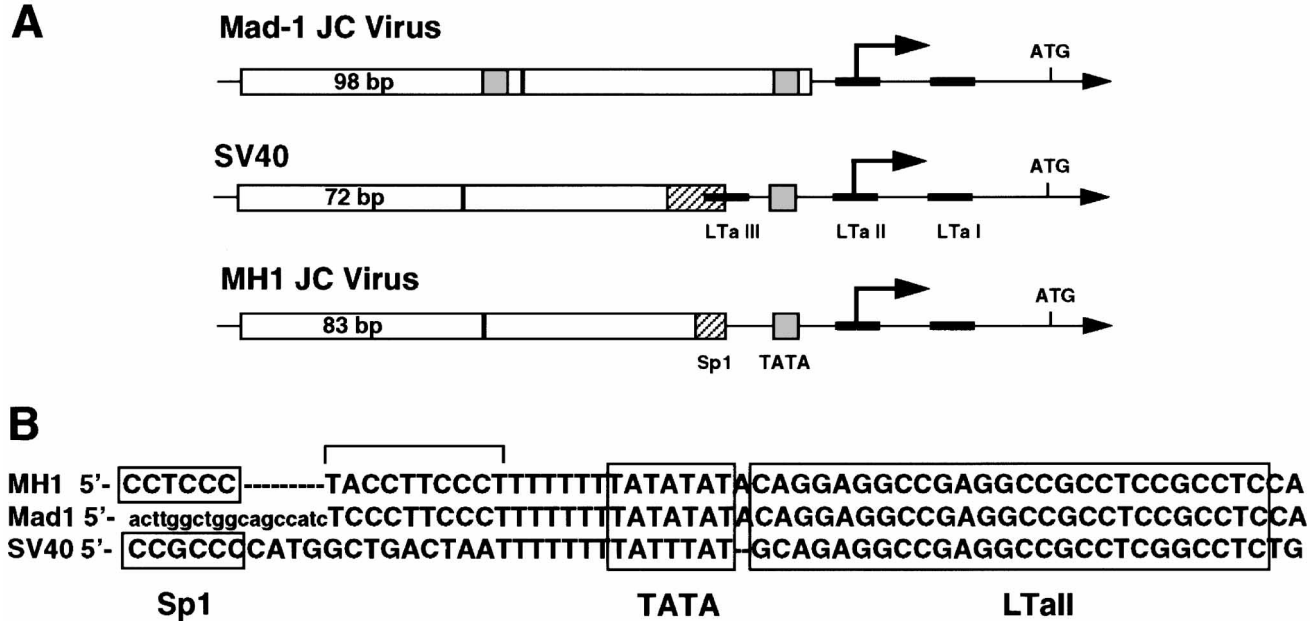


Figure 6.2. **(A)** Schematics of the Mad-1 JCV early promoter, the MH-1 JCV early promoter, and the SV40 promoter. Open boxes indicate direct tandem repeats of the indicated number of base pairs, dotted boxes represent TATA homologies, and the striped boxes represent Sp1 binding sites. The SV40 promoter contains three sequences that constitute binding sites for the viral protein large TAg (black boxes), whereas the JC virus early promoter contains only sites I and II. **(B)** Comparison of the basal promoter region DNA sequence reveals differences between JCV and SV40 in the region between the Sp1 binding sites and the second TAg binding site. MH1 and Mad-1 diverge upstream of a 5 bp TC(A)CCT repeat (horizontal bracket, B).

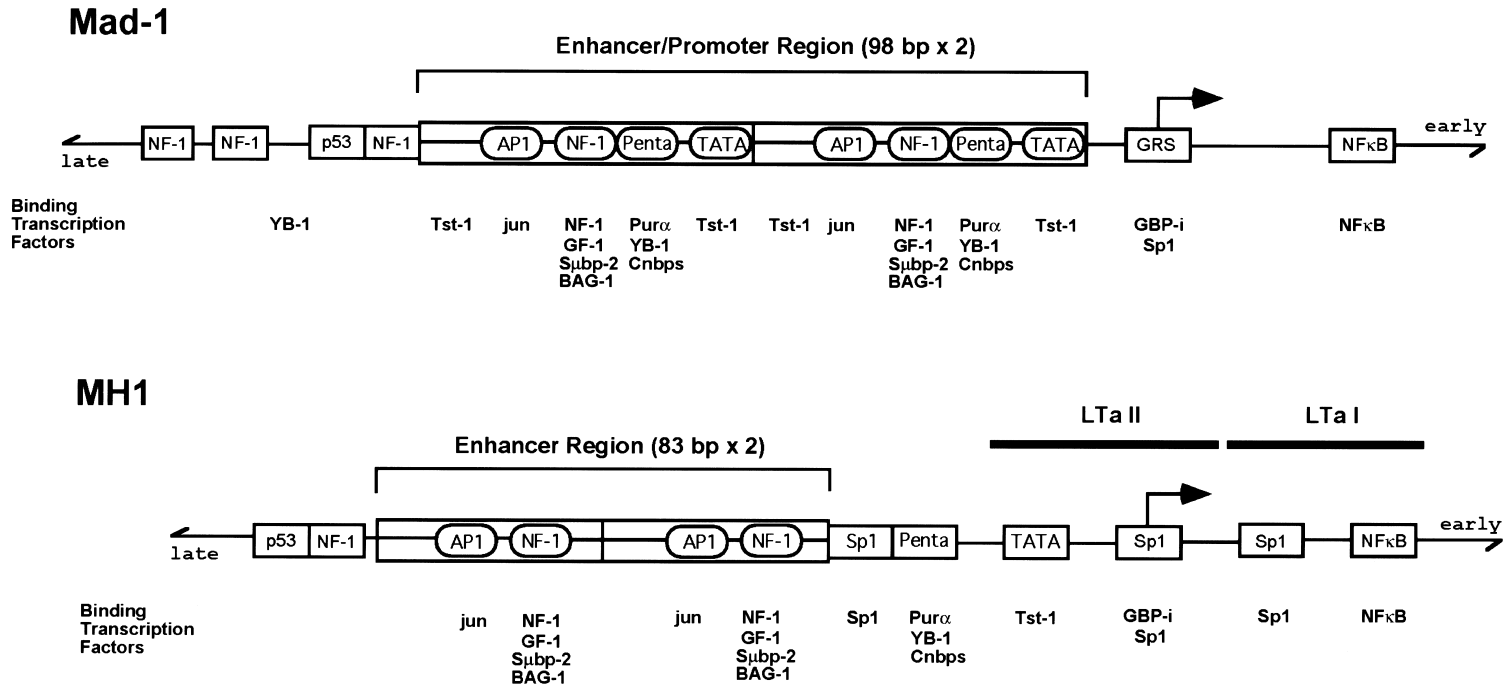


Figure 6.3. Multiple transcription factor binding sites in the enhancer/promoter regions of Mad-1 and MH1 JCV. Transcription factors that have been shown to bind the promoters are illustrated at the bottom of each schematic. Potential transcription factor binding sites derived from computer-aided analyses are depicted in the open boxes or circles in the schematics of two promoters. Two TAg binding sites (LTa I, LTa II) identified by DNase I footprinting analysis are exhibited on the MH1 promoter. Three Sp1 binding sites are also exhibited in the schematic of the MH1 promoter. The arrow indicates the transcription start site.

strated that the promoters directed 30- to 40-fold stronger expression in glial cells than in nonglial cells (Henson, 1994). More importantly, this analysis demonstrated that the MH-1 tandem repeats activated expression in both glial and nonglial cells, thus suggesting that the repeats are not the major determinant of cell specificity. Consistent with this, the proximal promoter region alone directed 19-fold more activity in glial cells than in nonglial cells (Henson et al., 1995; Krebs et al., 1995). Thus, the JCV basal promoter region is able to direct glial-specific gene expression, whereas the major role of the tandem repeats may be to increase promoter strength.

JCV early promoter function has been tested by in vitro transcription assays using whole cell extracts. A strong run-off transcript was identified across increasing concentrations of HJC (hamster glial cell) extract, whereas increasing HeLa whole cell extract concentration produced loss of the run-off transcript (Ahmed et al., 1990a,b). This not only suggested that the in vitro assay was glial specific but also provided additional evidence for a repressor in HeLa cells.

The expression of JCV is regulated by transcription factors, including activators and silencers. Many studies have been done to identify glial-specific transcription factors, as is discussed in the following section.

5. REGULATION OF JCV PROMOTER BY KNOWN TRANSCRIPTION FACTORS

Sp1, NF1, AP1, GF1, Sμbp-2

Sp1. Three Sp1 sites have been identified on the MH1 JCV early promoter region by gel-retardation and DNase I footprinting analysis, using recombinant protein and nuclear extracts (Sp1-I, Sp1-II, Sp1-III) (Henson et al., 1992; Henson, 1994; Kim et al., 2000). Sp1-I is located immediately upstream of the TATA box and appears to activate the JCV early promoter in both glial and nonglial cells (Henson, 1994) (Fig. 6.3). Sp1-II and Sp1-III sites are located downstream of the TATA box. Sp1-II was shown to be important for TAg-mediated transactivation of the JCV early promoter, but it is not involved in the regulation of basal expression (Kim et al., 2000).

Recently, Sp1 was shown to associate with Purα and regulate myelin basic protein (MBP) expression during brain development (Tretiakova et al., 1999a,b). TAg also appears to downregulate MBP transcription by interacting with Purα, leading to hypomyelination (Tretiakova et al., 1999a,b).

Based on its frequent occurrence in CpG islands, one possible mechanism underlying transcriptional control by Sp1 may be its role in maintaining methylation-free CpG islands in active genes (Graff et al., 1997; Macleod et al., 1994). In vitro methylation of the MH1 JCV early promoter leads to very strong repression of transcription after transfection into glial cells. Thus, cell-specific

methylation is another potential mechanism regulating JCV early gene expression (see below).

NF1, AP1. The Mad-1 promoter has been subjected to extensive molecular analysis. Several investigators have demonstrated the lack of a glial-specific DNase I footprint on the enhancer region of the Mad-1 JCV early promoter (Amemiya et al., 1989, 1992; Tada et al., 1989). A possible explanation for the lack of a glial-specific DNA binding activity would be that there is a glial-specific form of a well-known factor, such as NF1. The presence of closely apposed NF1 and AP1 binding sites in many brain-specific promoters has led to the search for such gene families (Amemiya et al., 1992; Sumner et al., 1996). Specifically, NF1/AT1(NF1-A1) was shown to be expressed at a higher level in human fetal brain cells compared with the ubiquitous NF1/CTF1(NF1-C1) in HeLa cells (Sumner et al., 1996). In addition, the cerebellum-enriched form of NF1-A1 transactivated two gliotropic JCV early promoters to a greater extent than NF1-C1 in U87MG and HeLa cells (Krebs et al., 1996).

Recently, BAG-1, a novel Bcl-2–interacting protein, was cloned from retinoic acid–differentiated P19 embryonic carcinoma (EC) cells using the JCV NF1 binding site as a probe sequence (Devireddy et al., 2000). This protein was shown to bind to the NF1 site and activate JCV early and late promoters. However, BAG-1 is ubiquitously expressed in neuronal and non-neuronal cells, arguing that it is not a cell-specific transactivator.

Next to the NF-1 binding site are cAMP response elements (overlapping with the AP1 site) that appear to regulate the JCV early promoter and enhancer in a tissue-specific manner (Kumar et al., 1996a,b). Addition of cAMP or forskolin enhanced the expression of the JCV early promoter by two- to threefold only in glial cells (Kumar et al., 1996a,b). However, the sequence of the cAMP response elements (TGAGCTCA) of the JCV promoter deviates by two bases from the consensus CRE sequence (TGACGTCA), which usually requires a very stringent match (Faisst and Meyer, 1992; Tinti et al., 1997).

GF1 and Sμbp-2. In gel-shift experiments, the central portion of the tandem repeats was found to bind a 45 kDa protein, glial-specific protein (GF1), that activated the late promoter and to a lesser extent the early promoter in glial cells (Ahmed et al., 1990a,b; Kerr and Khalili, 1991; Khalili et al., 1988). Following its cloning and expression, GF1 was found to activate the early promoter weakly in glioma cells. This latter result was interesting because if glial specificity relied solely on the presence or absence of an activating factor such as GF1, ectopic expression of that factor in nonglial cells would be expected to activate the promoter.

Because GF1 appeared to be a partial cDNA for human Sμbp-2, a transfection assay was employed to compare the effects of GF1 and its full-length form Sμbp-2 on JCV regulation (N.N. Chen et al., 1997). Sμbp-2 activated only the JCV late promoter in glial cells and was weaker compared with GF1. In addition, Northern blot analysis (N.N. Chen et al., 1997) showed that GF1 and

Sμbp-2 are expressed in both glial and nonglial cells, suggesting that, if they contributed to cell specificity, they might interact with cell-specific coactivators to induce glial cell-specific expression.

Tst-1/SCIP/Oct-6. Tst-1/SCIP/Oct-6 is a POU domain protein that activates the JCV early promoter in conjunction with MHG-I/Y through sequences adjacent to the TATA box (Leger et al., 1995; Renner et al., 1994; Wegner et al., 1993). Tst-1 also physically interacts with TAg, resulting in a synergistic activation of both early and late viral promoters (Renner et al., 1994). Interestingly, Tst-1 activates the basal promoter region more strongly in glial cells than in nonglial cells. Tst-1 is specifically expressed in myelinating glia, but it is not expressed in many glioma cell lines in which the JCV promoter is strongly and selectively active, nor is it expressed in adult glia. Thus, the role of Tst-1 in glial specificity remains unclear.

TAg. Analysis of the action of TAg on the JCV early promoter has provided some potentially important insights into cell-specific basal promoter regulation. Closely related SV40 TAg represses its own expression by cooperative binding to three sites in the basal region of the SV40 early promoter (Fig. 6.2, LTa I, II, and III) (Rio and Tjian, 1983). TAg repression of the SV40 early promoter is thought to result from blocking the transcription initiation complex through direct DNA binding to the basal promoter (steric hindrance model); however, there is little direct evidence for this mechanism. On the other hand, TAg produces DNA binding-independent activation of other promoters such as the cellular hsp70 promoter, the Rous sarcoma virus (RSV) promoter, and SV40 and JCV late promoters (Rice and Cole, 1993; Taylor et al., 1989). TAg can activate a basal hsp70 promoter consisting of a TATA sequence plus a single upstream element (e.g., Sp1 binding site) in a TATA sequence-dependent manner, and because TAg physically binds to TATA binding protein (TBP), it is likely that it acts during initiation complex formation (Gruda et al., 1993; Taylor et al., 1989).

TAg shares with adenovirus E1A protein the ability to stimulate transcription in the absence of sequence-specific DNA binding. E1A activation also requires a specific TATA sequence (Simon et al., 1988), and there is evidence that E1A activates through derepression (Horikoshi et al., 1995; Kraus et al., 1994). Each of these observations suggests that TAg promoter regulation depends on TATA-specific initiation complexes.

Co-transfection of a JCV TAg expression construct and the MH1luc reporter into U87MG glioma cells revealed that increasing levels of TAg produced fivefold repression of the JCV and SV40 early promoters (Henson et al., 1995; Kim et al., 2000) in a manner similar to that previously shown for the SV40 virus (Hansen et al., 1981). In HeLa cells, by comparison, 100–200-fold transcriptional activation was observed. The effect was specific to the JCV early promoter, as the SV40 promoter was still repressed fivefold in HeLa cells, and TAgs of both JCV and SV40 activated the JCV early promoter to a similar

degree. Deletion mutants in a co-transfection assay showed that TAg activation in nonglial cells required only the basal promoter region. Thus, TAg produced glial-specific, divergent regulation of the JCV basal promoter (Henson et al., 1995; Kim et al., 2000).

DNase I footprinting on the MH1 JCV basal promoter region using SV40 or JCV TAg revealed two protected domains (LTa I and LTa II) (Fig. 6.3) (Kim et al., 2000). Site-directed mutagenesis in the area of LTa II indicates that alteration of two specific bases in the second pentanucleotide repeat abolished TAg-induced transactivation while the mutation of the first repeat did not affect either basal or TAg-induced transactivation in HeLa cells (Kim et al., 2000). The change of the TATA to an irrelevant sequence also abolished TAg-induced transactivation. In U87MG cells, the mutations did not alter TAg repression. These results suggest an important role for the second pentanucleotide element and TATA sequence for TAg-induced transactivation in nonglial cells. Functional analysis of three new binding domains on the MH1 JCV promoter, which were identified by footprinting analysis with nuclear extracts, revealed that the Sp1-II and novel sequences are also involved in TAg-induced transactivation (Kim et al., 2000).

The binding of TAg to LTa I and LTa II was not significantly changed by the mutations. These results suggest that TAg regulates the JCV promoter largely by protein–protein interactions surrounding the TATA site rather than by direct DNA binding.

The activation of the JCV early promoter by TAg in nonglial cells could represent either transactivation or derepression. TAg activates a large number of cellular and viral promoters in vitro and in vivo (Rice and Cole, 1993; Taylor et al., 1989). Simple basal promoter regions are sufficient for transactivation, and a wide variety of transcription factor binding sites can cooperate with TAg in activation (Damania and Alwine, 1996; Gruda and Alwine, 1991). TAg lacks a strong activation domain, and DNA binding is not required. TAg can interact with TBP, can discriminate between TATA sequences for transactivation, and can substitute for $TAF_{II}250$ (Gruda and Alwine, 1991), strongly suggesting a role in transcription initiation. Thus, the divergent regulation of the JCV early promoter could reflect cell-specific or TATA-specific TFIID complexes. Indeed, the ability of TATA and pentanucleotide mutations to abolish TAg induction suggests that regions surrounding the TATA box are crucial for this effect. Also, the differences in the footprint analysis over the TATA region between the glial and HeLa nuclear extracts support this hypothesis (Kim et al., 2000).

Recently it was reported that TAg appears to transactivate the Mad-1 JCV late promoter by increasing expression from a basal transcriptional initiation site and through a novel TAg-dependent initiation site (TADI), which is homologous to initiator (Inr) sequences (Raj et al., 1998). The ability of TAg to activate the JCV late promoter might be attributed to the formation of specific protein complexes and increased transcriptional initiation from the TADI site on the late promoter.

Purα, YB-1, and TAg

Purα is a 322 amino acid sequence-specific single-stranded DNA binding protein that has been implicated in the control of both DNA replication and transcription. Purα has been implicated in control of gene transcription involving both viral and cellular promoters, including the JCV early promoter (Chen and Khalili, 1995), the human immunodeficiency virus type 1 promoter (Chepenik et al., 1998), the MBP promoter (Hass et al., 1995), and the neuron-specific FE65 gene promoter (Zambrano et al., 1997).

Purα stimulates transcription of the JCV early promoter up to sixfold. Moreover, TAg attenuates the Purα-induced level of early gene transcription. Although Purα alone has little effect on the late promoter, it is able to decrease TAg-mediated transactivation of the JCV late promoter (Chen and Khalili, 1995). Purα and TAg physically interact and antagonistically modulate each other's function on the JCV promoter (Gallia et al., 1998). According to recent reports, the association of TAg and Purα in vivo appears to downregulate the MBP gene and thus induce hypomyelination in brains of mice transgenically expressing TAg (Tretiakova et al., 1999a,b).

YB-1, a Y-box binding protein, is among the most evolutionarily conserved nucleic acid binding proteins in prokaryotes and eukaryotes (Wolffe, 1994; Wolffe et al., 1992). Y-box binding proteins appear to be involved in a wide variety of biologic functions, including regulation of gene expression at the transcriptional level (Kashanchi et al., 1994; Kerr et al., 1994; Li et al., 1997; Mertens et al., 1998), the translational level (Tafuri and Wolffe, 1993), DNA repair and DNA and RNA condensation (Grant and Deeley, 1993; Wolffe, 1994; Wolffe et al., 1992). The members of the Y-box family proteins are responsive to a wide spectrum of stress-related stimuli, including ultraviolet light radiation (Koike et al., 1997), drug treatment (Bargou et al., 1997), DNA damage-inducing antineoplastic agents (Ise et al., 1999), and interleukin-2 treatment in T cells (Sabath et al., 1990). Because viral infection induces cellular stress, YB-1 may be a candidate for an inducible protein in this setting. Recent data demonstrate that YB-1 is involved in transcriptional regulation of the JCV promoter (Chen and Khalili, 1995; Kerr et al., 1994; Safak et al., 1999a,c). YB-1 activates the JCV late promoter. In Mad-1 JCV, binding of YB-1 to its DNA target within the lytic control element (LCE) is increased by Purα. In contrast, interaction of Purα with the LCE motif is diminished once YB-1 is included in the reaction mixture.

Purα and YB-1 bind to the late (A/G-rich) and early (T/C-rich) strands of the LCE of Mad-1 JCV, respectively, and modulate JCV transcription in glial cells. The LCE is positioned within the enhancer repeat of the JCV promoter in close proximity to the origin of DNA replication and exhibits a remarkable effect on viral gene transcription and DNA replication. This region contains a pentanucleotide repeat sequence, AGGGAAGGGA, in juxtaposition to a poly(dT-dA) tract, which displays a single-stranded configuration. The interplay between Purα, YB-1, and TAg appears to dictate the level of association of

these proteins with their target DNA sequences and hence their regulatory action on viral early and late gene transcription (Chen and Khalili, 1995; Gallia et al., 1998; Safak et al., 1999a,c). Based on in vitro binding assays and transfection studies, a model has been proposed for the involvement of Purα, YB-1, and TAg in the transition of early to late gene transcription (Fig. 6.4). According to this model, efficient binding of Purα to the LCE late strand stimulates early gene transcription and facilitates the interaction of YB-1 with its target positioned on the LCE early strand. The association of YB-1 with the DNA, which is concurrent with TAg production and its binding to the origin of DNA replication, may result in dissociation of Purα from the LCE. This

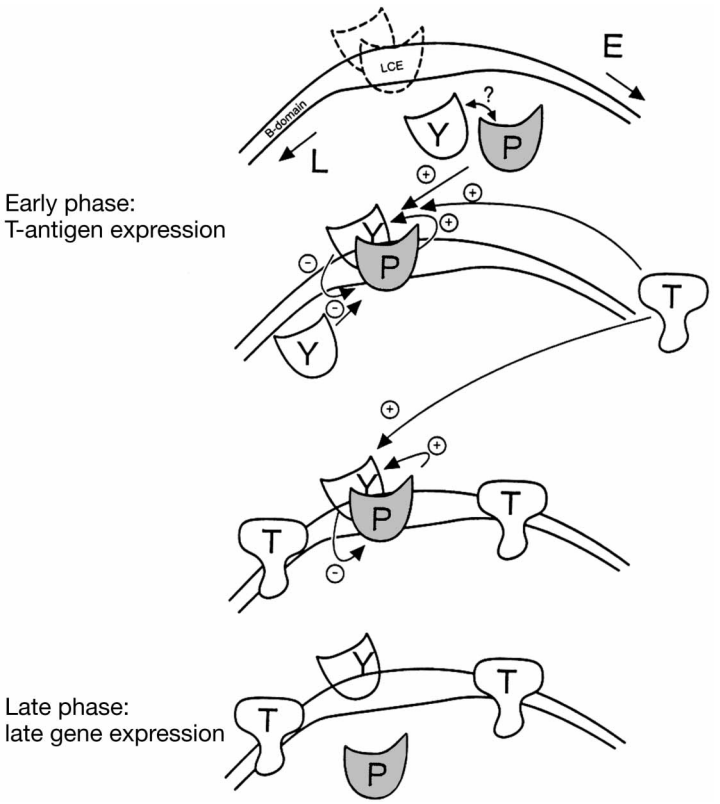


Figure 6.4. Proposed model of the involvement of Purα (P), YB-1 (Y), and TAg (T) in the transition of early to late gene transcription. Efficient binding of Purα to the LCE late strand (L) stimulates early gene transcription and facilitates binding of YB-1 to its target position of the LCE early strand (E). Binding of YB-1 to the DNA, which is concurrent with T antigen production, and its binding to the origin of DNA replication and the B region results in dissociation of Purα from the LCE late strand. This alteration in the pattern of DNA–protein complexes results in a decrease in the level of early gene transcription and an increase in late promoter activity in the late phase of infection.

alteration in the pattern of DNA–protein interaction can lead to a decrease in the level of early gene transcription and an increase in late promoter activity. Interestingly, none of these proteins affects the expression of interacting counterpart proteins (Chen and Khalili, 1995). This result suggests that Purα, YB-1, and TAg modulate JCV transcription through protein–protein interaction. With the concept that YB-1 is ubiquitously expressed in every cell and tissue, one might anticipate the involvement of a negative regulatory mechanism in nonglial cells that interferes with the positive activity of YB-1 on the basal and TAg-induced levels of virus late gene expression.

6. CELL-SPECIFIC REGULATION OF JCV PROMOTER BY SILENCERS

Cell-specific transcriptional regulation can result from selective activation or derepression. A region immediately upstream of the TATA sequence weakly repressed a heterologous promoter (Tada et al., 1991). Within the sequence adjacent to the TATA homology, there is a five base pair repeat (5′ TCCCTTCCCT), and because this region held sequence differences compared with the SV40 basal promoter (see Fig. 6.2), it was considered to be a potential binding site for a transcriptional repressor. Although several proteins have been identified that bind to this sequence, their identity and relevance to glial specificity remains unknown (Sharma and Kumar, 1991; Tada et al., 1991). It was demonstrated that point mutations within the pentanucleotide sequence reduced transcription in glial cells (Kumar et al., 1994). However, the mutations did not increase transcriptional strength in nonglial cells (HeLa cells), as would be expected from a mutation that abolished glial specificity. Recently, one cellular nucleic acid binding protein (Cnbps) was cloned from glial P19 mouse embryonal cells using a pentanucleotide oligonucleotide as a probe (Liu et al., 1998). It negatively regulated only the JCV early promoter and only in glial cells.

Transcriptional activation of the MH1 JCV early promoter by TAg in nonglial cells implies the presence of silencers (Henson et al., 1995; Kim et al., 2000). TAg has been shown to form complexes with pRb or p53 protein (Fanning and Knippers, 1992; Henson et al., 1995; Kim et al., 2000; Tavis et al., 1994). Transient transfection of a p53 expression plasmid with MH1 JCV promoter repressed promoter activity, and the activity was derepressed by TAg in nonglial cells (unpublished data). This result suggests that p53 might be a candidate silencer protein that represses JCV expression in nonglial cells.

7. REGULATION OF JCV TRANSCRIPTION BY CYTOKINES AND HIV

Regulation by Cytokine-Induced Transcription Factors

Immunosuppressive states with accompanying alterations in cytokine profiles have been postulated to play a vital role in the reactivation of viruses from

latency. Cytokines regulate gene expression by activating transcription factors via well-characterized signal transduction pathways. One of the factors involved in JCV transcription is GBP-i, a novel GGA/C binding protein (Raj and Khalili, 1994). The expression of GBP-i is induced by phorbol myristate acetate (PMA) and a variety of cytokines and immunomodulators, including interleukin-1β, tumor necrosis factor-α, interferon-γ, and transforming growth factor-β. GBP-i, unlike NFκB, acts as a transcriptional repressor of the JCV late promoter and is ubiquitously expressed in glial and nonglial cells. Interestingly, the GBP-i binding site, GRS, overlaps with the binding site of the JCV TAg and HIV-1 Tat-responsive region. It has been suggested that Tat upregulates the expression of several cytokines and might exert its effect indirectly on the JCV promoter, specifically through the GRS.

In other studies, the importance of the κB motif and the 23–bp sequence that interrupts the LCE motif was suggested in terms of cytokine-mediated regulation of JCV transcription (Safak et al., 1999b). The NFκB motif is located near the early gene translation start site, and the mutation of this motif completely abrogated the basal and PMA-induced levels of JCV late promoter transcription. The 23–bp sequence was critical for the observed inhibitory action. In the early phase of JCV reactivation inducible transcription factors such as NFκB may increase viral early gene transcription indirectly via the 23-bp sequence and to a much lesser degree through the NFκB motif. A 40 kDa protein has been identified that communicates between the κB and LCE regions in controlling viral gene transcription (Mayreddy et al., 1996; Safak et al., 1999b). Results from site-directed mutagenesis indicated that formation of a 40 kDa DNA–protein complex with the 23–bp sequence is critical for the transcriptional activation of the JCV promoter by PMA (Safak et al., 1999b).

Regulation by HIV-Encoded Regulatory Protein Tat and HTLV-1–Encoded Tax

The higher incidence of PML among individuals with AIDS compared with other immunocompromised patients implies that the presence of HIV type 1 (HIV-1) in the brains of infected individuals may directly contribute to the pathogenesis of this disease. In support of this model, earlier in vitro studies have indicated direct intercommunication between HIV-1 and JCV through the HIV-encoded regulatory protein Tat (Chowdhury et al., 1990, 1993). An upstream Tat-responsive DNA element (upTAR) of JCV has been shown to be important for HIV-1 Tat stimulation of the JCV late promoter. Specifically, Tat enhances the ability of Purα to bind the upTAR element and synergistically activates transcription (Krachmarov et al., 1996). Recent data indicate that Tat–Purα are exclusively nuclear and are co-localized in the extranucleolar chromatin structural elements (Wortman et al., 2000). These results also demonstrated that Tat–Purα interaction is direct rather than through an intermediary RNA or DNA molecule, and RNA binding configures Purα for optimal inter-

action with Tat. It has been postulated that RNA associates with Purα, stabilizing its structure and allowing it to interact with its protein partners (Gallia et al., 1999a,b). Specifically, the two acidic leucine-rich repeats of Purα are involved in the interaction (Krachmarov et al., 1996), and a polypeptide based on one such sequence inhibits binding (Wortman et al., 2000). Because Purα is ubiquitously expressed in human cells and because PUR elements are located near many promoters and origins of replication, the Tat–Purα interaction may be implicated in effects of HIV-1 throughout the full range of HIV-1–infected cells.

Recently it was demonstrated that human T-lymphotropic virus type 1 (HTLV-1)–encoded regulatory protein Tax activates JCV transcription in human neuronal cells but not in non-neuronal cells (Okada et al., 2000). Tax activated the transcription of both early and late promoters of Mad-1 and archetype JCV, and this activation was through the NFκB binding motif. A JCV promoter that lacks the NFκB binding motif could not be activated by Tax, and a Tax mutant lacking the potential for activation via the NFκB pathway did not activate the JCV promoter. From gel-shift assays, it was demonstrated that a Tax-bound protein(s) was present specifically in non-neuronal cells, suggesting the possibility of a repressor or silencer.

8. CHROMATIN AND DNA METHYLATION IN THE REGULATION OF TRANSCRIPTION

In addition to the direct regulation of transcription by nuclear proteins, gene expression is also controlled by molecular and structural modifications of promoter DNA. Chromatin packing and DNA methylation are two such mechanisms. Because these modifications are just beginning to be explored in depth, their significance for neural-specific gene expression remains largely speculative.

Chromatin

Chromatin occurs in two forms: as heterochromatin, which is densely packed and can be stained and visualized by light microscopy in the interphase nucleus; and euchromatin, which cannot be visualized (except in a highly condensed stage during mitosis). A major function of chromatin condensation is the efficient packing of long DNA molecules into the nucleus. However, cells also utilize chromatin as a mechanism to regulate gene expression (Felsenfeld, 1992; Lu et al., 1992; Surridge, 1996; Wolffe and Pruss, 1996). Genes residing within regions of heterochromatin are transcriptionally inactive, whereas actively expressed regions of the genome reside within euchromatin. It is well documented that histones repress gene expression when bound directly over promoters (Croston et al., 1991). There is competition between histones and transcription

factors for binding to promoter sequences because preincubation of promoter DNA with either excludes the alternate protein from binding to the DNA. Promoters residing within heterochromatin are presumably inactive because of the stereochemical restraint on transcription factor binding. The state of DNA binding to histones is controlled in part by the degree of histone acetylation (Wolffe and Pruss, 1996). Acetylases and deacetylases modify amino-terminal lysine residues on the outer surface of histone molecules. Acetylation destabilizes heterochromatin, thus providing increased access of transcription factors to promoters and allowing increased gene expression, whereas deacetylation of histones represses gene expression.

Polyomavirus DNA is assembled into a set of approximately 21 nucleosomes, in both the virion and the infected cell, with each nucleosome consisting of an octamer containing two copies of H2A, H2B, H2, and H4. In the infected nucleus, it appears that the histone H1 is associated with at least some of the ''minichromosomes'' (Bellard et al., 1976). Sequence-specific transcription factors can counteract histone-mediated transcriptional repression by displacing H1 (Croston et al., 1991; Felsenfeld, 1992). Therefore, because JCV basal promoter function is regulated in a cell-specific manner, it is possible that chromatin has a role in promoter specificity.

Methylation

Methylation of cytosine bases at CpG dinucleotides within promoters participates in the regulation of gene expression. Genomic methylation occurs immediately after DNA replication, producing a pattern of methylation that is stably inherited from mother cell to daughter cell (Bestor and Tycko, 1996). Semiconservative replication produces hemimethylated DNA, which is a strong substrate for DNA methyltransferase, thus leading to a fully methylated site.

The majority of genomic DNA contains CpG dinucleotides at a frequency of about 1 pair per 100 bp, which is a fivefold lower frequency than would be expected from the random occurrence of the sequence CpG (Antequera and Bird, 1993; Bird, 1992). These CpG pairs are methylated on both cytosines, and 5-methylcytosine can undergo spontaneous hydrolytic deamination to thymidines, perhaps explaining a loss of genomic CpG dinucleotides over time through mutation, as well as accounting for over one-third of all point mutations in human cancers. Methyl groups can interfere directly with the binding of transcription factors, presumably through steric hindrance or through competition from cellular methyl-CpG binding proteins (e.g., MeCP1 and MeCP2) (Boyes and Bird, 1992; Meehan et al., 1989). Finally, methylated CpG dinucleotides can repress transcription regardless of their location within the promoter, raising the possibility that MeCP1 could act directly as a transcriptional repressor or act indirectly by inhibiting the formation of the stereospecific complex of transcription factors required to activate a tissue-specific promoter.

A much smaller fraction of the genome, estimated at about 2%, contains stretches of approximately 1 kb in which CpG dinucleotides occur at a fre-

quency of 1 pair per 10 bases. These so-called CpG islands are located within the promoter and the first exon at the 5′ end of genes. There are instances in which de novo methylation of CpG islands occurs. Mutation of transcription factor binding sites within one part of a CpG island leads to methylation of the entire island (Macleod et al., 1994).

Three CpG dinucleotides in the JCV promoter occur in the region of the transcription initiation site, suggesting the possibility that JCV promoter cell specificity might be regulated through methylation. In vitro methylation of the MH1 JCV early promoter with SssI methylase leads to very strong repression of transcription in glial cells (unpublished data). Thus, cell-specific methylation is another potential mechanism for cell specificity.

9. JCV AND BKV DNA REPLICATION

It should be readily apparent that a discussion of JCV and BKV DNA replication must start with findings made previously with SV40. At the outset, it should be emphasized that polyomavirus DNA replication requires three distinct components: (1) a single, *trans*-acting viral protein called *large T antigen* (TAg); (2) a *cis*-acting viral DNA element termed the *core origin of replication*, which is necessary and sufficient for initiation of replication in vivo and in vitro; and (3) a collection of factors expressed in a eukaryotic cell known to be permissive (capable of supporting the entire viral life cycle) for the virus in question. Because of the limited coding capacity of the polyomavirus genomes, it is important to note that these viruses must commandeer the replication machinery of the host cell.

The importance of the two viral replication components first became apparent to investigators who infected or transfected cells in culture with mutant virus or viral DNA. Mutations in the TAg gene or the core origin often resulted in complete elimination of viral replication. Later, the development and biochemical characterization of cell-free replication systems allowed researchers to identify proteins in permissive cell extracts that were required for viral DNA replication in vitro. By employing techniques that permitted the detection of protein–protein interactions, it became clear that TAg physically interacted with some of these cellular factors to assemble a replication complex on the viral DNA template.

10. SV40 DNA REPLICATION MODEL

The multifunctional SV40 TAg regulates the initiation and elongation steps of viral DNA replication through a number of its intrinsic biochemical activities (reviewed by Virshup et al., 1992; Stillman, 1994; L. Chen et al., 1997; Smelkova and Borowiec, 1997; Brodsky and Pipas, 1998; Herbig et al., 1999; Kim et al., 1999; Weisshart et al., 1999; Simmons, 2000). To initiate replication, the

708 amino acid (a.a.) protein interacts with three sequence elements contained within the 64 bp viral core origin: (1) a 27–bp central dyad symmetry comprising TAg binding site II (BSII), which includes four copies of the pentameric recognition sequence, GAGGC; (2) an early-side 15–bp imperfect palindrome (IP); and (3) a late-side 17–bp adenine-thymine (AT)-rich sequence (Fig. 6.5) (Deb et al., 1986). Flanking this minimal origin are auxiliary sequences, called *aux*-1 and *aux*-2, that stimulate in vivo DNA replication activity. These elements contain binding sites for viral TAg (BSI) and the cellular transcription factor Sp1. Through its specific DNA binding domain (a.a. 147–246), TAg recognizes BSII and, in the presence of ATP, assembles cooperatively as a double hexamer structure (Fig. 6.6). Each hexamer unit is positioned over one half of the core origin and together they effect the distortion of both the IP and AT-rich regions by melting the former and untwisting the latter sequence. Mutational analyses indicate that these two activities are effected by different parts of TAg and that a.a. 121–135 influence AT untwisting but not IP melting (L. Chen, et al., 1997).

Following local destabilization of the helix, the TAg double hexamer acts as a helicase to promote unwinding of the origin DNA. Each hexamer appears to assemble as a propeller-shaped particle around a channel through which the DNA is reeled (San Martin et al., 1997; Valle et al., 2000). The unwinding reaction requires additional TAg functions, including ATPase activity and the ability to bind/recruit the cellular replication factors. One of these factors, topoisomerase I, may relieve torsional strain during unwinding of the circular genome and increase unwinding specificity (Simmons et al., 1998). A second cellular protein, RPA, binds single-stranded DNA and, together with TAg, recruits the DNA polymerase α–primase complex to the exposed single-stranded DNA of the replication bubble to generate short RNA–DNA primers.

TAg exhibits a 10-fold greater affinity for primate versus bovine DNA polymerase α, suggesting that this interaction contributes to primate-specific replication of SV40 DNA (Dornreiter et al., 1990). Elongation proceeds bidirectionally, with TAg continuing to act as a helicase at the two replication forks. Coupling of these replication forks through the TAg double hexamer structure stimulates the unwinding activity (Smelkova and Borowiec, 1997; Weisshart et al., 1999). Continuous synthesis of the leading strand is accomplished by cellular replication factor C (RFC), proliferating cell nuclear antigen (PCNA), and DNA polymerase δ; discontinuous synthesis of the lagging strand, involving the production of Okazaki fragments, requires the coordinated action of both DNA polymerases α and δ and their associated co-factors (Stillman, 1994). FEN-1, a cellular RNase, removes the short RNA primers, and DNA ligase 1 links the Okazaki fragments together. Termination of replication is not well understood, although it does require topoisomerase activity (Ishimi et al., 1992). This step is rate limiting in the replication process, and it is possible that steric constraints, leading to changes in TAg helicase structure, result in reduced replication fork movement (Smelkova and Borowiec, 1997). Termination oc-

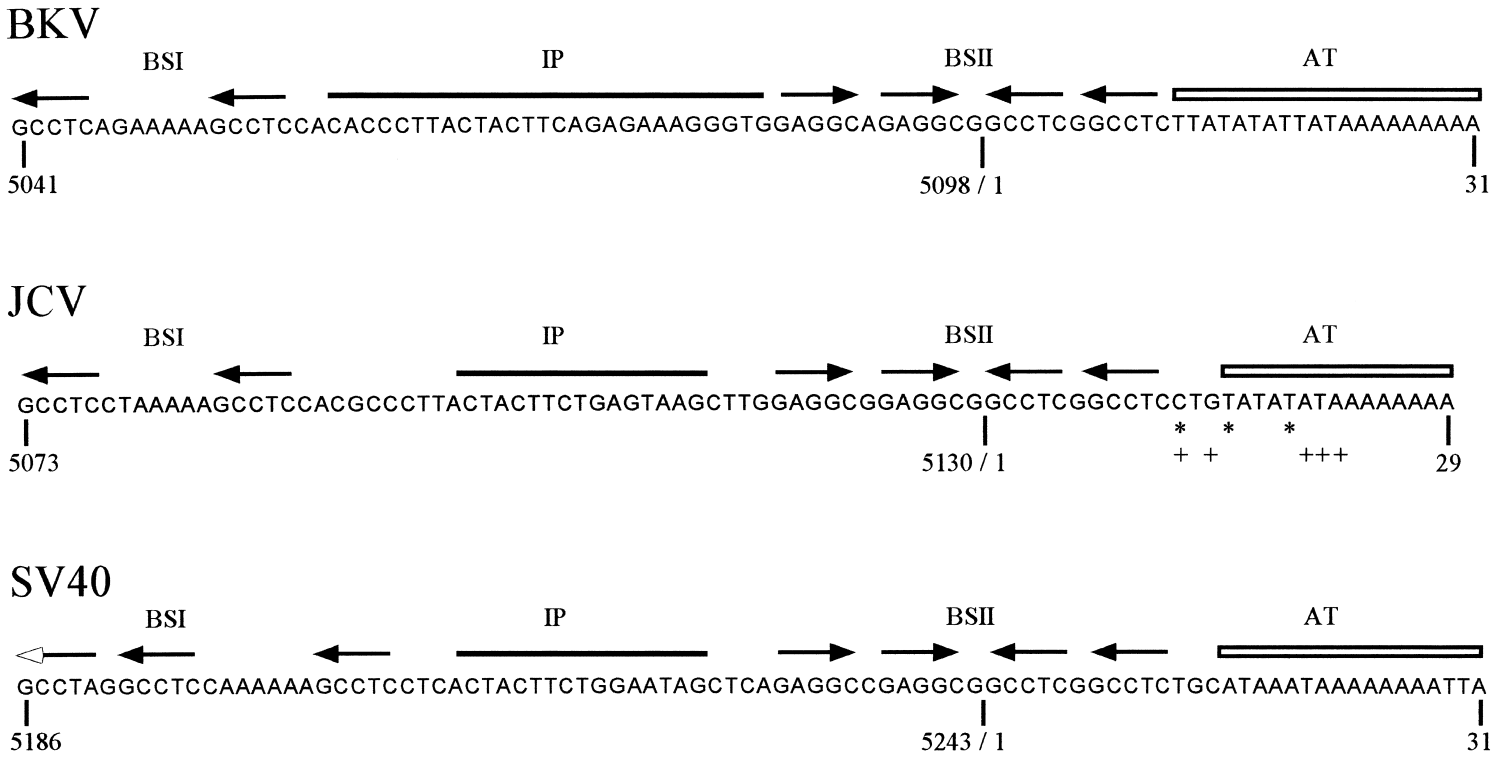


Figure 6.5. Comparisons of the JCV(Mad-1), BKV(AS), and SV40(776) origins of replication. The nucleotide sequences of each viral core origin and a portion of each auxiliary region 1 are shown. BSI and BSII contain multiple copies of the pentanucleotide sequence (denoted by arrows) recognized by TAg; the open arrowhead in SV40 BSI represents a variant consensus sequence. A closed or open bar denotes the imperfect palindrome (IP) or AT-rich region (AT), respectively, of each core origin. Nucleotide positions are indicated beneath each sequence; numbering begins at nucleotide 1 at the center of BSII and continues around the circular genome and ends at nucleotide 5098 (BKV), 5130 (JCV), or 5243 (SV40). Three asterisks (*) and five plus signs (+) mark the nucleotides on the late side of the JCV core origin that differ from the SV40 and BKV sequences, respectively. These differences are hypothesized to affect the ability of JCV TAg to productively interact with each origin.

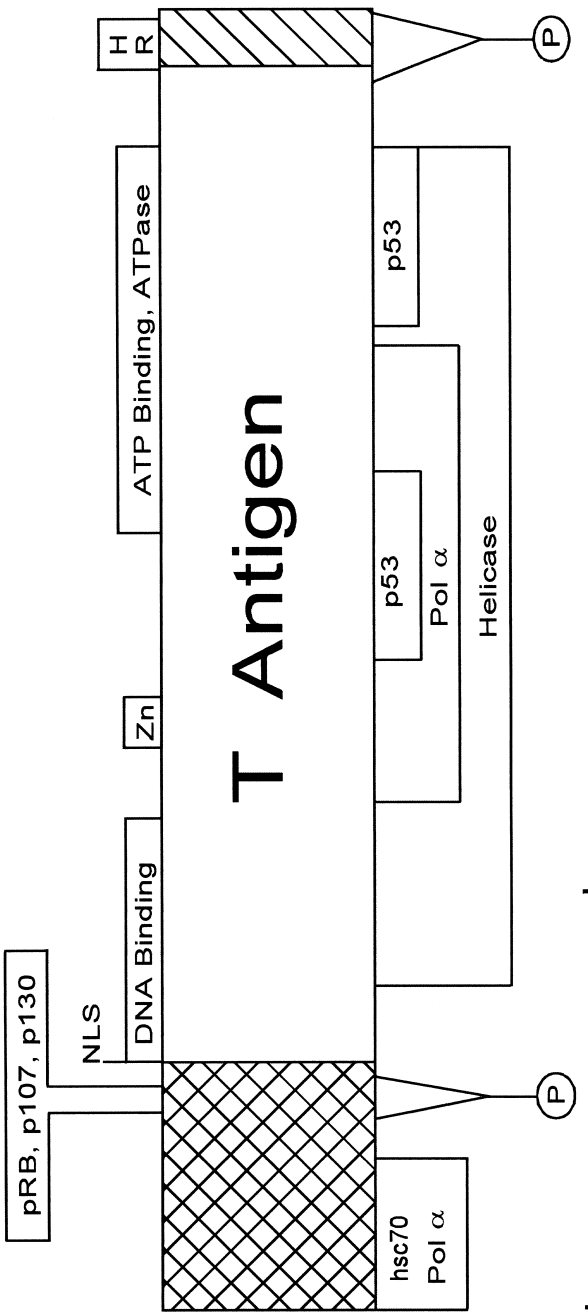


Figure 6.6. Functional domains of the primate polyomavirus TAgs. The JCV(Mad-1), BKV(AS), and SV40(776) TAgs are 688, 691, and 708 amino acids in size, respectively. The amino-terminal sequences of the TAgs (cross-hatched area) are shared with proteins T'_{135}, T'_{136}, T'_{165} (JCV; Trowbridge and Frisque, 1995), T^*_{136} (BKV; Prins and Frisque, unpublished data), and 17kT (SV40; Zerrahn et al., 1993) that are encoded by alternatively spliced early viral transcripts. This region of TAg interacts with cellular proteins involved in DNA replication and cellular transformation, including polymerase α (Pol α), hsc70 (via the J domain), and the pRB family of proteins (via the LXCXE domain). Sequences here also localize the proteins to the nucleus (NLS), overlap a transactivation domain, and include serine and threonine residues that are modified by phosphorylation (P). The central region of TAg binds the DNA sequence GAGGC, zinc (Zn), ATP, and the tumor suppressor p53 and exhibits ATPase and helicase activities. The carboxy-terminal 33 amino acids of the JCV TAg (diagonal lines) represent sequences shared with T'_{165}; this region includes a host range domain (HR) as well as a second set of phosphorylation sites.

curs approximately 180° around the circle from the core origin; specific DNA sequences do not appear to signal this event.

The DNA replication functions of TAg depend on the phosphorylation status of serine (Ser) and threonine (Thr) residues in the amino-terminal portion of the protein. Phosphorylation of Thr 124 causes a modest increase in binding of TAg to BSII and is required for origin unwinding and replication of the SV40 genome. In contrast, phosphorylation of TAg residues Ser 120 and Ser 123 interferes with double hexamer formation at the core origin, which correlates with inhibition of DNA replication. Dephosphorylation of these sites by the cellular phosphatase PP2A restores efficient replication function (Scheidtmann et al., 1991c; Virshup et al., 1992).

Several cellular factors involved in transcriptional activation are known to impact SV40 DNA replication. TBP binds the TATA box within the AT-rich region of the core origin; it also binds TAg. Herbig and co-workers (1999) have reported that the interaction of the viral and cellular proteins interferes with viral DNA replication, perhaps because TBP complexes associate with a TAg surface involved in unwinding origin sequences. Similarly, the transcription factor Oct1 has also been shown to bind the SV40 AT-rich region and to negatively regulate origin unwinding by TAg (Kilwinski et al., 1995). On the other hand, a number of transcription factors, including NF1, AP1, and Sp1, bind the SV40 *aux*-2 and enhancer elements and stimulate DNA replication (Cheng and Kelly, 1989; Guo and Depamphilis, 1992). The means by which this stimulation is achieved is not completely understood, although several mechanisms have been proposed (reviewed by Herbig et al., 1999). Recent evidence favors the hypothesis that direct interaction of certain transcription factors (e.g. NF1) with histone H3 activates SV40 DNA replication by altering the chromatin structure at the origin (Muller and Mermod, 2000).

TAg interacts with two additional classes of cellular factors, tumor suppressor proteins and molecular chaperones, which impact the DNA replication process. The former includes the pRB family of proteins that regulates a cell's entry into S phase of the cell cycle (reviewed by Kaelin, 1999). TAg overrides the normal function of these proteins through two domains at its amino terminus. Via its LXCXE domain (Fig. 6.6), TAg binds to pRB and the related proteins p107 and p130, and, in conjunction with its J domain, TAg effects S phase progression by causing the release of a heterodimeric complex composed of members of the E2F and DP families of transcription factors (Nevins, 1992; Zalvide et al., 1998; Kaelin, 1999). The J domain interacts with, and activates the ATPase activity of, the molecular chaperone hsc70. Mutation of a highly conserved HPD sequence in this domain alters TAg's ability to bind hsc70; the mutation also negatively influences SV40 DNA replication (Campbell et al., 1997). It is possible that efficient viral replication depends on the ability of hsc70 to alter the conformation of a replication initiation or elongation complex. In addition, the failure of the mutant TAg to effect release of E2F-DP from the pRB family of proteins might compound the replication defect by interfering with the transition of the cell into S phase (Ohtani, 1999).

11. HUMAN POLYOMAVIRUS DNA REPLICATION

Unique Features of JCV and BKV DNA Replication

It is expected that the mechanisms by which the primate polyomaviruses replicate their genomes will be highly conserved. However, specific differences have been observed between JCV, BKV, and SV40 DNA replication, and attempts have been made to explain the basis for this variability. For example, the host and tissue specificity of JCV replication is highly restricted relative to that of BKV and SV40. In addition, the TAg produced by each virus varies in its ability to support replication from the homologous and heterologous viral origins. Furthermore, considerable variation occurs in the ability of auxiliary sequences flanking the core origins of these three polyomaviruses to influence DNA replication efficiency. These differences suggest that modifications to the SV40 DNA replication model will be necessary to accurately describe the replication process for the human polyomaviruses.

Replication of the JCV genome has been examined more extensively than that of the BKV genome, and the following discussion centers on the former virus. Where appropriate, findings obtained with the BKV system are presented. Furthermore, it should be noted that most JCV studies cited here were conducted with the Mad-1 variant of JCV.

JCV Origin of Replication

The 67–bp core origin of JCV encompasses nucleotides 5094 to 30 (Lynch and Frisque, 1990; Sock et al., 1991, 1993) and includes the IP, BSII, and AT-rich elements described above for SV40 (Fig. 6.5). Although the sequences comprising the core origin of replication are highly conserved among the primate polyomaviruses (Deb et al., 1986; Deyerle et al., 1989; Lynch and Frisque, 1990), JCV's AT-rich element differs from that of SV40 with regard to its degree of DNA bending (Amirhaeri et al., 1988) and its interaction with TAg (Lynch and Frisque, 1990). The *aux*-1 and especially the *aux*-2 elements of the JCV replication origin differ significantly in sequence and in function relative to those of BKV and SV40. Dependence on these auxiliary elements for efficient DNA replication is most apparent for JCV and least apparent for BKV. A pentanucleotide repeat called the lytic control element (LCE; 5′-AGGGA-AGGGA-3′) is located immediately adjacent to the late side of the JCV core origin in *aux*-2 and has been shown to influence viral transcriptional activity (e.g., the transcription factors YB-1 and Purα bind this sequence) and to enhance replication behavior mediated by JCV, but not SV40 or BKV, TAg (Lynch and Frisque, 1990; Tada et al., 1991; Sock et al., 1993; Chang et al., 1994). A related sequence, *rep*, occurs at the same position in the BKV genome, and it too influences BKV replication and transcription (Del Vecchio et al., 1989).

Several approaches have been taken to examine how the JCV origin sequences contribute to replication differences observed between JCV and the

other two primate polyomaviruses. These approaches have relied on the characterization of mutant, chimeric, and naturally occurring variant replication origins in in vivo and in vitro assay systems. Early development of an in vitro DNA replication system indicated that a plasmid containing the JCV replication origin could be propagated in a reaction utilizing primate cell extracts and SV40 TAg, thus demonstrating that a productive interaction between the SV40 regulatory protein and the JCV *cis*-acting regulatory sequences occurred (Li and Kelly, 1985). A similar conclusion was reached in a number of studies conducted in vivo. Some of these experiments involved the transfection of a JCV origin-containing vector into monkey or human cells expressing SV40 TAg either constitutively (e.g., in COS and cPOS cells) or transiently (from a cotransfected vector) (Lynch and Frisque, 1990, 1991; Sock et al., 1991, 1993). Other experiments relied on the transfection of primate cells (primary human fetal glial [PHFG] and CV-1) with complete chimeric viruses composed of SV40 coding regions and the JCV regulatory region (Chuke et al., 1986; Lynch and Frisque, 1991; Lynch et al., 1994). These in vivo studies utilized a DpnI replication assay (Peden et al., 1980) that discriminates between input and replicated viral DNA on the basis of susceptibility to the restriction enzyme *Dpn*I. Because the JCV and SV40 TAgs and core origins exhibit a high degree of sequence similarity (Frisque et al., 1984), it was not surprising to find that SV40 TAg promoted replication of the JCV origin, although the ability of SV40 TAg to support this replication more efficiently than did the JCV TAg was unexpected (Chuke et al., 1986; Lynch and Frisque, 1991; Lynch et al., 1994). More surprising was the result obtained when attempts were made to mediate SV40 origin replication with the JCV TAg. In the initial study, a chimera composed of the JCV coding regions and the SV40 regulatory region failed to replicate in PHFG or CV-1 cells (Chuke et al., 1986). Subsequent experiments revealed that this chimera could replicate in cells that constitutively expressed SV40 TAg (COS cells), but not in cells that expressed JCV TAg (POJ cells) (Lynch and Frisque, 1991). Similarly, the BKV TAg was found to activate replication of the JCV origin, but the JCV TAg again failed to drive replication of the heterologous (BKV) origin (Lynch and Frisque, 1990; Sock et al., 1993). These observations have served as the impetus for a number of experiments designed to identify the basis for JCV TAg's ability to discriminate between the polyomavirus origins. This work suggests that sequence differences in the TAgs and replication origins of these three polyomaviruses contribute to this observation.

Replication of JCV Origins from Naturally Occurring Variants. Two types of sequence variation have been detected in the genome of JCV isolates. Single nucleotide polymorphisms in the coding sequences have been identified in JCV isolates obtained from different human populations, and these variations are used to classify JCV into specific genotypes (reviewed by Agostini et al., 1999 and Chapter 18). A second type of variation involves the JCV transcriptional control region (TCR) and reveals two forms of the virus in the human

host. Archetype JCV is detected in the kidneys and urine of its host, and it is thought to be the form of the virus that circulates in the population (see Chapter 7). Upon entry into a new susceptible host, it has been hypothesized that the archetype TCR undergoes a rearrangement process involving the deletion and duplication of promoter/enhancer sequences that yields a rearranged variant. The precise location of the deletion and duplication boundaries varies so that a large number of rearranged TCRs may be generated. These variants, compared with archetype, are detected at a wider variety of locations in the body (Newman and Frisque, 1997, 1999; Jensen and Major, 1999). JCV(Mad-1) is an example of a rearranged variant in which a 23−bp and a 66−bp sequence found in the archetype TCR have been deleted and a duplication of 98−bp has occurred. Differences between the archetype and rearranged TCRs primarily affect the *aux*-2 element; the core origin and *aux*-1 sequences remain largely unaffected by the rearrangement process.

One element that influences SV40 DNA replication, the Sp1 recognition sequence or GC box, is present in the *aux*-2 element of archetype, but in only a subset of rearranged variants. Constructs containing replication origins from archetype or rearranged forms of JCV exhibit equivalent replication efficiencies upon transfection into TAg-expressing cells, indicating that differences in the *aux*-2 elements have little influence on this activity (Lynch and Frisque, 1990; Daniel et al., 1996; Sock et al., 1996; Ault, 1997). However, transfection of intact archetype and rearranged JCV genomes into PHFG cells does reveal significant differences in replication behavior (Daniel et al., 1996). These data highlight the difficulty in separating transcription from replication effects when comparing overall accumulation of viral genomes in infected cells. Variations in the *aux*-2 element alter JCV transcriptional efficiency, thereby affecting the levels of early and late protein production. If this efficiency is reduced, lower levels of TAg and the capsid proteins are produced. This in turn leads to reduced replication because of the direct role that TAg plays in this process and because of the indirect role the capsid proteins play in facilitating secondary viral infections to yield biological amplification and spread of viral genomes during an infection.

Replication of JCV−SV40 Chimeric Origins. In an effort to delineate the JCV and SV40 origin sequences recognized differentially by the JCV TAg, JCV−SV40 chimeras were created. The first step in the construction scheme was to alter the only nucleotide that differed in the TAg BSII of JCV and SV40. SV40 nucleotide 5237 (a C) is included within the recognition site for the restriction enzyme *Bgl*I. The corresponding nucleotide in the JCV sequence (5124, a G) was converted to a C to create a *Bgl*I site at the center of the JCV origin (Lynch and Frisque, 1991). This mutation did not alter JCV replication potential (Lynch and Frisque, 1990). Both origin DNAs were cleaved with *Bgl*I, and the resulting restriction fragments were swapped to create two chimeras in which the early half of one origin was joined to the late half of the other origin (Lynch and Frisque, 1990). Analysis of the replication potential of the two

chimeras in POJ and COS cells indicated that SV40 sequences in the late half of the origin (including the AT-rich and *aux*-2 elements) were responsible for the inability of the JCV TAg to interact with the SV40 origin. In addition, sequences representing the early half of each origin (including the IP and *aux*-1 elements) only enhanced replication about twofold in the presence of the homologous TAg.

Vacante et al. (1989) also employed a chimera approach to investigate JCV host- and tissue-specific replication behavior. These investigators joined SV40 promoter/enhancer signals (nucleotides 37–270) to the JCV genome at nucleotide 268 within the regulatory region. Propagation of this chimera in PHFG cells resulted in the deletion of JCV nucleotides 90–268 and SV40 nucleotides 37–145 to yield M1-SVE(Δ). Compared with JCV(Mad-1), this stable chimeric virus exhibited accelerated growth kinetics and increased virus titers when propagated in PHFG cells. The host range of M1-SVE(Δ) was also expanded to include monkey cells. Presumably, acquisition of these new properties was a result of enhanced transcriptional activity leading to increased levels of TAg rather than direct alterations to the replication machinery.

Replication of Mutant JCV Origins. Mutational analyses of the JCV regulatory region have identified elements critical to understanding (1) TAg's ability to discriminate between homologous versus heterologous origins, (2) the relative contributions of core and auxiliary origin sequences to DNA replication, and (3) the influence of host/tissue specificity on JCV replication (Table 6.1). The first mutations introduced into the JCV core origin were small deletions that abolished DNA replication (Mandl et al., 1987). These mutants, like similar SV40 mutants used to create COS cells (Gluzman, 1981), were transfected into PHFG cells to generate TAg-expressing POJ cells (Mandl et al., 1987).

To examine the influence of the JCV *aux*-1, *aux*-2, and enhancer elements on the efficiency of DNA replication, two groups created three series of mutants by progressively deleting sequences from the outer boundaries of the JCV replication origin toward the core origin using exonuclease III (Lynch and Frisque, 1990) or Bal31 (Sock et al., 1991, 1993) digestion. Analysis of the *aux*-1 element indicated that TAg BSI stimulated JCV TAg-mediated replication severalfold relative to the minimal core origin, a finding paralleling that made with the SV40 system (Sock et al., 1993). In their study of the *aux*-2 and enhancer elements, Lynch and Frisque (1990) assessed the replication potentials of deletion mutants in POJ, COS, and cPOS cells. The results were compared with those obtained with the JCV(Mad-1) and M1(Δ98) origins. The latter construct is missing one of the two copies of the JCV(Mad-1) enhancer.

The replication patterns of the mutants were similar in the monkey and human cells (COS and cPOS) expressing SV40 TAg; replication activity decreased as the size of the deletion increased. Once the deletion reached the AT-rich region of the core origin, replication was abolished in these cells. In contrast, transfection of the mutant origins into human cells (POJ) expressing the

Table 6.1. JCV Regulatory Region Mutants

Name[a]	Sequence(s) Altered[b]	Type[c]	Location[d]	Replication[e]
1. M1(ΔNco)	RR, T/t/T′	Del	nt 4980–275	—
2. S-3	Core	Del	nt 5072–5130	NT
3. S-8	Core	Del	nt 5072–5	NT
4. S-15	Core	Del	nt 5093–5127	—
5. S-19	Core	Del	nt 5108–5118	—
6. S-27	Core	Del	nt 5107–5116	—
7. M1(*Bgl*I)	Core	Sub	nt 5124, G/C	+
8. M1(Δ98)	*aux*-2	Del	nt 57–154	↓
9. 3A	*aux*-2	Sub	nt30, G/T	↓
			nt 35, G/C	
10. 4A	*aux*-2	Sub	nt 31, G/T	↑
			nt 36, G/C	
11. 5A	*aux*-2	Sub	nt 32, G/T	↓
			nt 37, G/C	
12. pM1(Δ98)o	*aux*-2	Del	nt 57–154	↑
13. d56	*aux*-2	Del	nt 57–275	↑
14. d45	*aux*-2	Del	nt 46–275	↑
15. d38	*aux*-2	Del	nt 39–275	↑
16. d30	*aux*-2	Del	nt 31–275	↓
17. d17	Core, *aux*-2	Del	nt 18–275	—
18. B38C	Core, *aux*-2	Del	nt 39–275	↑
		Sub	nt 5124, G/C	
19. B33C	Core, *aux*-2	Del	nt 34–275	↑
		Sub	nt 5124, G/C	
20. B31C	Core, *aux*-2	Del	nt 32–275	+
		Sub	nt 5124, G/C	
21. B31C(TT)	Core, *aux*-2	Del	nt 32–275	+
		Sub	nt 5124, G/C	
			nt 30, G/T	
			nt 31, G/T	
22. B31C(dl)	Core, *aux*-2	Del	nt 12, 32–275	↓
		Sub	nt 5124, G/C	
23. B31C(SV)	Core, *aux*-2	Del	nt 12, 32–275	↓
		Sub	nt 5124, G/C	
			nt 15, T/C	
			nt 19, T/A	
			nt 30, G/T	
			nt 31, G/T	
24. Δ46	EL	Del	nt 4981–5026	+
25. Δ48	EL	Del	nt 4981–5028	+
26. Δ80	EL	Del	nt 4981–5060	+
27. Δ91	EL	Del	nt 4981–5071	+
28. Δ112	*aux*-1	Del	nt 4981–5092	↓
29. Δ131	*aux*-1, core	Del	nt 4981–5111	—
30. Δ136	*aux*-1, core	Del	nt 4981–5116	—
31. Δ146	*aux*-1, core	Del	nt 4981–5126	—

Table 6.1. (*Continued*)

Name[a]	Sequence(s) Altered[b]	Type[c]	Location[d]	Replication[e]
32. Δ179	*aux*-1, core	Del	nt 4981–30	—
33. Δ48*	EL, *aux*-2	Del	nt 4981–5028 nt 31–279	↓
34. Δ91*	EL, *aux*-2	Del	nt 4981–5071 nt 31–279	↓
35. Δ112*	*aux*-1, 2	Del	nt 4981–5092 nt 31–279	↓
36. Δ131*	Core, *aux*-1, -2	Del	nt 4981–5111 nt 31–279	—
37. pJC264	*aux*-2	Del	nt 111–279	↓
38. pJC264Δpl	*aux*-2	Del Sub	nt 111–279 nt 42, C/A nt 43, T/A nt 44, G/A	↓
39. pJC264Δnf	*aux*-2	Del Sub	nt 111–279 nt 49, C/A nt 50, C/A	↓
40. pJC264Δnp	*aux*-2	Del Sub	nt 111–279 nt 39, T/G nt 40, G/T nt 41, G/T	↓
41–76. 36 mutants[f]	Core, *aux*-1, -2	Del	nt 5090–279 to nt 241–279	—/↓/+

[a] Replication mutants are described by Chuke et al. (1986; mutant 1), Mandl et al. (1987; mutants 2–6), Lynch and Frisque (1991; mutant 7), Daniel et al. (1996; mutant 8), Chang et al. (1994; mutants 9–11), Lynch and Frisque (1990; mutants 12–23), Sock et al. (1993; mutants 24–36), and Sock et al. (1991; mutants 37–76).

[b] Mutations altered one or more of the following JCV coding or regulatory sequences: TAg (T), tAg (t), all three T′ proteins (T′), entire regulatory region (RR), early leader (EL), auxiliary replication sequences 1 and 2 (*aux*-1, -2), and core origin (core).

[c] Types of mutations include deletions (Del) and substitutions (Sub). Substitution mutants 7, 21, 22, and 23 were created to alter the JCV sequence to an SV40 sequence at the specified mutated position(s).

[d] The sites of the mutations are identified by nucleotide (nt) numbers (Frisque et al., 1984). Substitutions are denoted with the wild-type nucleotide shown first, followed by a slash and then the replacement nucleotide. Some mutants contain multiple alterations (e.g., mutant 21 has three substitutions and one deletion).

[e] Replication activity was measured in vivo using the *Dpn*I assay. Mutants either failed to replicate (—) or exhibited reduced (↓), elevated (↑), or similar (+) activity relative to the wild-type JCV genome or to isolated origin sequences. Mutations were introduced into the intact JCV genome to create mutants 1–8; however, an intact or partial JCV origin that had been cloned into a plasmid vector was altered to generate the majority of the mutants (9–76). Replication of the mutant origin–containing plasmids was tested in cells expressing JCV TAg.

[f] Mutants 41–76, which are not listed individually, contain deletions that begin on the late side of the JCV origin (nt 241–279; smallest deletion) and extend through the *aux*-2 sequences first, then the core origin, and finally the *aux*-1 sequences (nt 5090–279; largest deletion). Replication activity of this series of mutants varied from equal to wild type to undetectably low levels.

JCV TAg led to about a twofold increase in replication behavior until the deletion reached the pentanucleotide repeat (LCE; AGGGAAGGGA) immediately adjacent to the core origin, and then replication fell five- to sixfold. As seen in COS and cPOS cells, replication was abolished when the deletion extended into the AT-rich sequence of the core origin. These studies indicate that the LCE sequence stimulates JCV, but not SV40, TAg-mediated replication. Furthermore, effects on replication by other sequences within *aux*-2 and the enhancer also depended on the source of the TAg that was tested. Point mutations introduced into the LCE signal reduce replication potential in the presence of JCV, but not SV40, TAg (Chang et al., 1994). In addition, these mutations altered the structure of the adjacent AT-rich region. It is possible that the AGGGA repeat contributes to the unusual DNA structure detected previously in the JCV AT-rich region and that these alterations are important to JCV TAg's ability to function as a helicase (Amirhaeri et al., 1988; Chang et al., 1994).

Sock et al. (1991) also employed a deletion mutagenesis approach to examine the contributions of the *aux*-2 and enhancer elements to JCV replication. A recognition site for NF-1 partially overlaps the LCE sequence described above, and deletion of this site reduces replication three- to fourfold in COS cells (Lynch and Frisque, 1990; Sock et al., 1991). When these mutants were tested in a human glioblastoma cell line co-transfected with a JCV TAg-expressing plasmid, a reduction in replication was again observed. However, using the complementing POJ cell system, Lynch and Frisque (1990) had not observed this reduction in activity using similar mutants (see above). Because the experimental protocols followed by these two laboratories differed in a number of ways, it is not possible to explain the discrepancies at this time. Sock et al. (1991) did go on to perform site-specific mutagenesis of the NF-1 site that prevented detectable NF-1 binding. These mutants again exhibited reduced SV40 TAg-mediated DNA replication of the JCV sequences (Sock et al., 1991). In contrast to these in vivo results, NF-1 did not have an effect on in vitro DNA replication, leading to the suggestion that this transcription factor exerts its influence via effects on chromatin accessibility (Sock et al., 1991). Finally, several forms of NF-1 have been identified in human tissues. NF-1/AT1, produced in human glial cells, binds JCV DNA and may influence tissue-specific replication behavior (Sumner et al., 1996).

To pursue the observation that the failure of the JCV TAg to support SV40 replication involves sequences within the late half of the origin, cassette mutagenesis was employed. The late half of the JCV and SV40 core origins differ at three positions; nucleotides 12, 15, and 19 (JCV numbering; Fig. 6.5). The JCV origin was converted to an SV40-like origin (Lynch and Frisque, 1990), and this mutant sequence replicated nearly 10-fold less efficiently than the corresponding JCV origin in the presence of JCV TAg; in the presence of SV40 TAg both origins displayed similar levels of amplification. These results identify three specific nucleotide differences in the late halves of the two viral core origins that are in large part responsible for JCV TAg's ability to discriminate

between the JCV and SV40 *cis*-acting replication signals. It is interesting to speculate that the basis for this discrimination involves an Oct-1 binding site found in the late half of the SV40 core origin. The Oct-1 recognition sequence is altered in the corresponding region of the JCV origin (nucleotides 10–21), perhaps precluding the transcription factor from recognizing the JCV sequences. A second octamer-binding transcription factor, Tst-1 (also called Oct-6 or SCIP), does bind the JCV origin (nucleotides 10–25) and JCV TAg (Wegner et al., 1993; Renner et al., 1994; Sock et al., 1999). Because Oct-1 interferes with SV40 TAg's ability to unwind the SV40 origin, the JCV TAg may be unable to compete with Oct-1 bound to the SV40 sequences and thus fails to unwind the origin and initiate replication. The JCV protein may not face a similar barrier with its own origin if, in fact, it is true that the Oct-1 recognition site is missing.

JCV Replication Protein(s)

The multifunctional TAg is necessary and sufficient to mediate JCV DNA replication (Tavis and Frisque, 1991; Nesper et al., 1997). However, three recently discovered JCV early proteins, T'_{135}, T'_{136}, and T'_{165}, produced by alternative splicing of the early precursor mRNA (Fig. 6.1), modulate TAg-mediated replication (Trowbridge and Frisque, 1995; Prins and Frisque, 2001). The ability of JCV tAg, a fifth early protein, to influence DNA replication has not been investigated, although studies of the corresponding SV40 protein suggest that it may affect this process indirectly via effects on cellular gene expression and interaction with PP2A (Loeken et al., 1988; Scheidtmann et al., 1991b; Yang et al., 1991). Most of the studies that have examined the role of the JCV early proteins in DNA replication have relied on the extensive SV40 literature to predict functional domains and to target specific sequences for mutagenesis and chimeric approaches.

Replication Induced by TAgs from Naturally Occurring Variants. JCV variants are classified into several genotypes and subtypes based on nucleotide polymorphisms detected in the coding sequences. These point changes are associated with variants isolated from specific human populations and are thought to have arisen as the virus co-evolved with its host. While considerable effort has been invested in determining the influence of TCR variations on JCV replication and transcription behaviors, few studies have investigated whether variation in the coding sequences influence these processes. It is known that archetype and rearranged JCV variants exhibit different replication potentials. Chimeras in which the regulatory and coding regions have been exchanged indicate that changes in the former region are chiefly responsible for these differences, although the data do suggest that alterations in the latter region may have some influence as well. For example, the Mad-1 variant replicates efficiently, whereas the archetype CY strain and the rearranged Mad-8 strain exhibit barely detectable activities. Chimeras containing a CY or Mad-8 reg-

ulatory region linked to the Mad-1 coding region exhibit behavior similar to the parental CY and Mad-8 genomes, highlighting the importance of the TCR sequences. On the other hand, replication of chimeric genomes in which the Mad-1 regulatory region is linked to the CY or Mad-8 coding region is readily detectable, although it is not restored to Mad-1 levels. This result suggests that the CY and Mad-8 proteins function less efficiently than those of Mad-1 (Daniel et al., 1996) and that coding region variations are responsible, in part, for altered replication potential of JCV variants. At this time the specific CY and Mad-8 protein(s) and the specific sequence alterations that effect reduced replication activity have not been identified.

A zinc finger motif in the SV40 TAg (Fig. 5.6) contributes to viral replication and transformation activity (Loeber et al., 1989). Agostini and colleagues (1997, 1999) have suggested that sequence variations within the JCV TAg zinc finger domain may influence the replication behavior of the Type 2B form of the virus. Unlike the Asian Type 2A and 2C forms of JCV that have a hydrophobic leucine residue at a.a. position 301 in TAg's zinc finger motif, Type 2B has a hydrophilic glutamine residue. Although this genotypic variant has not yet been shown to exhibit altered replication activity relative to the other Type 2 genotypes, it does appear to be associated with PML more often than would be expected based on its overall prevalence in the population (Agostini et al., 1999).

Major and coworkers (1984, 1987) isolated, cloned, and partially sequenced episomal JCV DNA that was present in cultured cells derived from an owl monkey tumor induced by JCV (Mad-1). The regulatory region of this cloned genome, called JCV-586, contained a 19–bp deletion characteristic of the JCV(Mad-4) variant. In addition, changes in the carboxy terminus of TAg apparently had occurred because the TAg exhibited altered reactivity with several monoclonal antibodies and with cellular p53. JCV-586 is the only JCV isolate reported to replicate in monkey brain cells, and it is possible that changes in the viral TAg contribute to this extended host range property.

Replication Induced by JCV-SV40 Chimeric TAgs. The regulatory regions of primate polyomavirus genomes were exchanged to produce the first JCV–SV40 and JCV–BKV chimeras (Chuke et al., 1986; Bollag et al., 1989; Haggerty et al., 1989). This work suggested that the JCV early coding region, not just the regulatory region, contributed significantly to the unique behavior of JCV; therefore a series of early region chimeras were constructed (Haggerty et al., 1989). To create these JCV–SV40 chimeras, two restriction endonuclease recognition sites (for *Bst*XI and *Nsi*I) found at the corresponding position in each genome were utilized. Each chimeric early region was linked to either a JCV or SV40 regulatory region. As a consequence of the construction scheme, sequences in the VP1 coding region (at an *Eco*RI site) were also swapped, an alteration that precluded virion production but not DNA replication capability. The DNA replication potential of these eight chimeric genomes were compared with the activities of the two parental genomes and the two regulatory region

chimeras (Lynch et al., 1994). To ensure that the comparisons were equivalent, sequences were inserted into the VP1 gene of the latter four genomes to prevent virion production. Transfection of the 12 DNAs into PHFG and CV-1 cells yielded the following observations relevant to TAg replication function: (1) the JCV(Mad-1) TCR promoted expression of the SV40 TAg at levels sufficient to support viral DNA replication in the monkey cells; (2) the failure of the JCV TAg to interact with the SV40 origin mapped to its central region (a.a. 82–411), which includes DNA binding, zinc finger, and helicase domains (Fig. 6.6); (3) the ability to replicate in monkey cells was limited to constructs expressing TAgs that contained the carboxy-terminal host range domain of SV40 (Fig. 6.6); and (4) SV40 DNA replication was 10-fold higher than that of JCV in PHFG cells. This replication difference was much less than that observed using intact, parental viral genomes, presumably because replication measurements for the latter DNAs are influenced by the rapid secondary infection of cells by viable SV40.

Replication Induced by Mutant JCV TAgs

Mutation of the Specific DNA Binding Domain of JCV TAg. Mutagenesis of the JCV early coding region has been guided by using the SV40 TAg as a template and by the earlier studies utilizing variant and chimeric viruses (Table 6.2). Initial experiments targeted the specific DNA binding domain of JCV TAg because its function is critical to replication initiation and because its sequence in the JCV and SV40 TAgs differs. Furthermore, JCV TAg had already been shown to bind less efficiently than SV40 TAg to each viral origin (Lynch and Frisque, 1991). Interestingly, JCV TAg interacts with both DNAs with similar efficiencies, ruling out the possibility that differential binding activity is the basis for JCV TAg's inability to stimulate replication of the SV40 origin. Tavis and Frisque (1991) created 10 JCV TAg mutants by introducing single or multiple mutations into the DNA binding domain (a.a. 145, 149, 157, 159, 162, and 168) or the nuclear localization signal (NLS; a.a. 131). Nine of these alterations converted the JCV sequence to an SV40 sequence. The tenth mutation, a lysine (Lys) to arginine (Arg) change at a.a. 168, was created in an initial step of the mutagenesis scheme and did not result in a JCV TAg that was more SV40-like (the corresponding residue in the SV40 TAg is also a Lys). The 10 mutant TAgs bound JCV and SV40 BSI with efficiencies ranging from 44% to 301% of the wild-type JCV protein. Nine of the mutants replicated in PHFG cells (22–220% of wild-type activity); the Arg 168 mutant, which exhibited the most efficient DNA binding activity, was replicative negative. In contrast to previous reports, wild-type JCV, as well as the nine viable TAg mutants, was shown to replicate in CV-1 cells. Although highly inefficient, this activity could be enhanced by propagating the cells in medium containing calf serum rather than fetal bovine serum. Finally, the mutant TAgs were tested to see if any had acquired the ability to drive replication of the SV40 origin. Again a new observation was made—wild type JCV TAg did mediate repli-

Table 6.2. JCV Early Region Mutants

Name[a]	Protein(s) Altered[b]	Type[c]	Location[d]	SV40-like[e]	Replication[f]
1. K-2	T/t/T′	Dup	nt 4915–4918	N	NT
2. S-1	T/t/T′	Del	nt 4871–4937	N	—
3. S-18	T/t/T′	Del	nt 4908–4922	N	NT
4. S-36	T/t/T′	Del	nt 4912–4924	N	NT
5. K-1	t	Dup	nt 4499–4502	N	NT
6. S-6	t	Del	nt 4467–4538	N	NT
7. S-21	t	Del	nt 4486–4511	N	NT
8. S-44	t	Del	nt 4498–4505	N	NT
9. M1(B-B)	T/T′	Del	nt 4244–4309	N	—
10. JCVΔT′	T′	Sub	nt 4274, G/A	N	↓
11. JCV-H42Q	T/t/T′	Sub	a.a. 42, H/Q	N	NT
12. JCTAg-RbS	T/T′	Sub	a.a. 104, D/N	Y	↓
			a.a. 108, H/S		
			a.a. 112, F/P		
			a.a. 113, A/S		
			a.a. 118, N/A		
			a.a. 120, G/D		
		Ins	a.a. 119/120, A		
13. JCTAg-RbN	T/T′	Sub	a.a. 104, D/V	N	↓
			a.a. 105, L/N		
			a.a. 107, C/A		
			a.a. 109, E/Q		
		Ins	a.a. 119/120, A		
14. M1(R131-T)	T/T′	Sub	a.a. 131, K/R	Y	+
15. M1(S145-T)	T	Sub	a.a. 145, A/S	Y	+
16. M1(H149-T)	T	Sub	a.a. 149, Q/H	Y	↓
17. M1(L157-T)	T	Sub	a.a. 157, V/L	Y	↑
18. M1(C159-T)	T	Sub	a.a. 159, S/C	Y	↓
19. M1(I162-T)	T	Sub	a.a. 162, V/I	Y	↓
20. M1(R168-T)	T	Sub	a.a. 168, K/R	Y	—
21. M1(HL-T)	T	Sub	a.a. 149, Q/H	Y	↓
			a.a. 157, V/L		
22. M1(HLC-T)	T	Sub	a.a. 149, Q/H	Y	↑
			a.a. 157, V/L		
			a.a. 159, S/C		
23. M1(SHLCI-T)	T	Sub	a.a. 145, A/S	Y	+
			a.a. 149, Q/H		
			a.a. 157, V/L		
			a.a. 159, S/C		
			a.a. 162, V/I		
24. JCV-N316K	T	Sub	a.a. 316, N/K	Y	+
25. JCV-H317Y	T	Sub	a.a. 317, H/Y	Y	+
26. JCV-NHKY	T	Sub	a.a. 316, N/K	Y	+
			a.a. 317, H/Y		
27. JCV-T125A	T/T′	Sub	a.a. 125, T/A	N	—
28. JCV-T664A	T/T'_{165}	Sub	a.a. 664, T/A	N	+

Table 6.2. (*Continued*)

Name[a]	Protein(s) Altered[b]	Type[c]	Location[d]	SV40-like[e]	Replication[f]
29. JCV-T664S	T/T'_{165}	Sub	a.a. 664, T/S	Y	+
30. JCV-E666A	T/T'_{165}	Sub	a.a. 666, E/A	N	+
31. JCV-E666S	T/T'_{165}	Sub	a.a. 666, E/S	Y	+

[a] Mutants are described by Mandl et al. (1987; mutants 1–8), Lynch and Frisque (1991; mutant 9), Trowbridge and Frisque (1995; mutant 10), Kelley and Georgopoulus (1997; mutant 11), Tavis et al. (1994; mutants 12 and 13), Tavis and Frisque (1991; mutants 14–23), and Swenson et al. (1996; mutants 24–31).

[b] Mutations alter one or more of the following JCV early proteins: TAg (T), tAg (t), all three T′ proteins (T′), or a single T′ protein (T'_{165}). Introducing insertions or deletions at convenient restriction enzyme cleavage sites yielded mutants 1–9. Specific functional domains or sequences were targeted in mutants 10–31 and included the shared donor splice site for T'_{135}, T'_{136}, and T'_{165} (mutant 10), J domain (mutant 11), pRB binding and amino-terminal phosphorylation domains (mutants 12 and 13), NLS (mutant 14), specific DNA binding domain (mutants 15–23), zinc finger domain (mutants 24–26), amino-terminal phosphorylation site (mutant 27), and potential carboxy-terminal phosphorylation sites (mutants 28–31).

[c] Types of mutations include duplications (Dup), deletions (Del), substitutions (Sub), and insertions (Ins).

[d] The sites of the mutations are identified either by nucleotide (nt) or amino acid (a.a.) numbers (Frisque et al., 1984). Nucleotide and amino acid substitutions are denoted using single letter codes, with the wild-type residue shown first, followed by a slash and then the replacement residue. Some mutants contain multiple mutations (e.g., mutant 12 has six substitutions and one insertion).

[e] Mutants created to alter the JCV sequence to an SV40 sequence at a specific position are identified as SV40-like (Y).

[f] Replication activity was measured in vivo using the *Dpn*I assay. Mutants either failed to replicate (—) or exhibited reduced (↓), elevated (↑), or similar (+) activity relative to wild-type JCV. NT = not tested. The mutation in mutant 11 was not introduced into an intact JCV genome, but was created in a clone encoding an *E. coli* DnaJ chaperone protein, thus precluding an analysis of replication potential.

cation of the SV40 origin, but at a very low level that was not altered significantly by changing its specific DNA binding domain to be more similar to that of SV40 TAg. This finding does not alter the earlier conclusion that the interaction between the JCV TAg and the SV40 origin is defective.

Mutation of the pRB Binding Domain of JCV TAg. In a second mutagenesis study, Tavis et al. (1994) investigated the observations that JCV TAg binds the pRB tumor suppressor protein and transforms cells in culture inefficiently. Again the approach was to convert the JCV TAg to an SV40-like protein, this time within the pRB binding domain. JCV and SV40 TAg differ at seven positions between residues 103 and 120, although the critical LXCXE sequence is conserved in both proteins. A JCV TAg mutant altered at each of these seven positions was tested in several assays relevant to either transforming activity or DNA replication behavior. Relative to wild type, the mutant TAg bound JCV

and SV40 BSI more efficiently, but surprisingly bound pRB less efficiently. Furthermore, while transforming activity of the mutant was unaffected, DNA replication was decreased 25- to 50-fold and virus production was not detected. In addition to altering the pRB binding domain, the mutations also affected the predicted amino-terminal phosphorylation domain of JCV TAg. Serine residues 106 and 111 in this region of the SV40 TAg are phosphorylated, whereas the corresponding amino acids in the JCV TAg, a histidine (His) and an alanine (Ala), are not expected to be post-translationally modified. Because the pRB mutant JCV TAg was constructed by converting the JCV sequences to SV40 sequences, the mutant TAg may be phosphorylated at these serines. It is known that changes in the phosphorylation pattern of SV40 TAg alter its replication activity and its interaction with BSII; therefore, changes in JCV TAg phosphorylation may have been responsible for enhanced binding of the mutant TAg to BSI. Unfortunately, it was not possible to determine whether the mutant TAg, obtained from cell extracts, was altered in its ability to bind BSII DNA, an interaction that would be more relevant to understanding the basis for reduced DNA replication potential of the mutant. Another possible explanation for the reduced replication potential of the mutant is that precise regulation of pRB activity in the slowly growing PHFG cells may be critical to replication in this system, and the mutant TAg might be defective in this regard.

Mutation of Residues Within the Two Clusters of Phosphorylation Sites in the JCV TAg. Phosphorylation sites have been mapped to Ser and Thr residues within the amino- and carboxy-terminal portions of the JCV TAg (Fig. 6.6; Swenson and Frisque, 1995). Relative to SV40 TAg, JCV TAg is modified at fewer residues, suggesting that the absence of phosphorylation at critical sites might contribute to the less robust activity of the JCV protein. To test this possibility, three residues in the JCV TAg corresponding to three phosphorylation sites in the SV40 TAg were mutated (Swenson and Frisque, 1995; Swenson et al., 1996). Phosphorylation of Ser 677 in the SV40 TAg influences the modification of several amino-terminal regulatory phosphorylation sites, which in turn affects the DNA binding and replication activities of TAg (Schneider and Fanning, 1988; Scheidtmann et al., 1991a). Mutation of its counterpart in the JCV TAg, Thr 664, to a Ser or Ala indicated that this site is not phosphorylated and does not significantly influence DNA replication activity. A second carboxy-terminal phosphorylation site in the SV40 TAg, serine 679, is a glutamic acid (Glu) (a.a. 666) in JCV TAg. Converting the latter residue to a Ser or Ala again did not alter replication behavior, and the Ser 666 mutant did not become phosphorylated. These results suggest that important differences exist in the way the two TAgs are regulated by this post-translational modification. A critical phosphorylation site in the amino-terminal region of SV40 TAg, Thr 124, contributes to stable origin binding and is essential for unwinding and DNA replication (Scheidtmann et al., 1984, 1991a; McVey et al., 1993; Moarefi et al., 1993). Mutation of the conserved Thr 125 residue in the JCV TAg to

Ala abolishes DNA replication and viability, suggesting that phosphorylation of this site plays a similar key role in JCV TAg function.

Mutation of the Zinc Finger Domain of JCV TAg. The zinc finger motif is highly conserved in polyomavirus TAgs (Pipas, 1992), and mutational analysis of this domain in the SV40 TAg (a.a. 302-320) demonstrates its importance in the formation of stable hexamers and modulation of specific DNA binding and origin unwinding (Arthur et al., 1988; Hoss et al., 1990; Loeber et al., 1991). Alterations to any of the predicted zinc coordinating residues or the nearby His residue in the major loop (a.a. 302, 305, 313, 317, and 320) diminish transforming activity and abolish viral infectivity (Loeber et al., 1989). Mutations within the carboxy-terminal region of the major loop (a.a. 312–316) reduce the replication and transforming activities of SV40 TAg. These zinc finger mutants exhibit phenotypes similar to those displayed by wild-type JCV TAg. JCV TAg differs from SV40 TAg at four of the five positions in the major loop (JCV a.a. 313–317). Based on previous SV40 work, three JCV TAg mutants were made by converting residues 316, 317, or 316 + 317 to the corresponding SV40 sequence. Contrary to expectation, these mutations did not lead to enhanced JCV DNA replication activity or to the ability of JCV TAg to interact with the SV40 origin (Swenson et al., 1996). The latter possibility had been examined because the zinc finger domain lies within the central region of the JCV TAg known to negatively regulate its interaction with the SV40 origin.

Biochemical Analyses Relevant to JCV TAg Replication Functions. Biochemical approaches have been employed to examine JCV TAg's stability, phosphorylation status, and ability to bind cellular proteins and viral DNA. This work has utilized TAg obtained from extracts of infected and transformed cells or purified from bacterial, insect, and mammalian sources. Information derived from these experiments is relevant to our understanding of JCV DNA replication.

JCV TAg Stability. Early studies (Cikes et al., 1977; Bollag et al., 1989; Haggerty et al., 1989) suggested that JCV TAg was more labile than its SV40 counterpart. Pulse-chase experiments indicated that the half-life of the SV40 protein was twice as long as that of the JCV protein isolated from transformed human cells (Lynch and Frisque, 1991). Greater instability, coupled with reduced expression from JCV transcription signals, is responsible for the lower levels of JCV TAg relative to SV40 TAg in infected and transformed cells and likely contributes to the less robust activity of the JCV replication protein. In addition, the appearance of a 17 kD T′ protein in JCV-transformed cells (Bollag et al., 1989; Haggerty et al., 1989) was attributed initially to the proteolytic degradation of the large T protein. However, additional pulse-chase experiments comparing the degradation profiles of TAg and the T′ protein suggested that the latter protein was not a breakdown product of the former protein (Trowbridge and Frisque, 1995).

Oligomerization of JCV TAg. Interactions of the SV40 TAg with viral DNA and cellular proteins involve higher order structures of the viral protein, and sequences within the zinc finger and phosphorylation domains influence the formation of these oligomers (Arthur et al., 1988; Schneider and Fanning, 1988; Mastrangelo et al., 1989; Hoss et al., 1990; Loeber et al., 1991; Parsons et al., 1991; Scheidtmann et al., 1991c). Tavis and co-workers (1994) employed sucrose density gradient centrifugation to investigate the quaternary structure of JCV and SV40 TAgs present in extracts of transformed cells. They demonstrated that the unpurified JCV protein oligomerized less efficiently than the SV40 protein under nonreplication conditions; JCV TAg sedimented predominantly as monomers/dimers, whereas SV40 TAg yielded a mixture of monomers/dimers and higher oligomers. Because the TAgs differed significantly in this important property, additional experiments were conducted using purified JCV and SV40 TAgs under replication conditions (Bollag et al., 1996). In the presence of ATP and at 37°C, JCV TAg did form hexamers and double hexamers, albeit less efficiently than did SV40 TAg. In a second experiment, the oligomerization and DNA binding functions of TAg were analyzed in a DNA mobility shift assay using purified proteins and origin-containing DNA fragments. The baculovirus-expressed JCV and SV40 TAgs both behaved in a highly cooperative manner to form double hexamers on the origin DNA.

Binding of JCV TAg to BSI and BSII. The binding of the polyomavirus TAgs to BSII in the core origin is necessary but not sufficient to initiate DNA replication. It should be noted that the arrangement of TAg binding sites differs in the JCV and SV40 origins; JCV TAg BSI contains two, instead of three, pentanucleotide recognition sequences (Fig. 6.5), and a third binding site (BSIII), present in the SV40 origin, appears to be missing altogether. These differences were predicted to affect interactions between JCV TAg and DNA, and this possibility was first investigated using the McKay assay (McKay, 1981) with unpurified protein from transformed cell extracts (Lynch and Frisque, 1991). The JCV TAg exhibited lower binding activity than did the SV40 TAg, especially to BSII, suggesting one possibility for reduced DNA replicating potential of JCV. Both viral proteins bound to BSI with higher affinity than to BSII in this assay, and both bound the SV40 BSI with twofold higher affinity than the JCV site. The latter observation likely reflects differences in the sequences of the two sites. An expectation in these studies was that reduced binding activity was responsible for the nonproductive interaction between JCV Ag and the SV40 origin. However, the JCV protein recognized both origins with similar efficiencies, leading to the hypothesis that JCV TAg fails to promote replication of an SV40 origin because of a step subsequent to specific binding. Similar findings were made in a second study in which the predicted DNA binding domain of JCV TAg was mutated (Tavis and Frisque, 1991). Because binding of purified SV40 TAg to BSII is stimulated by ATP (Dean et al., 1987; Deb and Tegtmeyer, 1987; Borowiec and Hurwitz, 1988;

Mastrangelo et al., 1989), a more thorough investigation of JCV TAg binding to BSII awaited the availability of purified protein.

Windl and Dörries (1995) examined the binding activity of JCV TAg purified from human 293 cells infected with a recombinant adenovirus Ad5-JCVTAg. With a modified McKay assay and JCV TAg immunoprecipitated from the infected cell extracts, the viral protein was shown to interact with DNA fragments containing the JCV BSI and BSII or BSI alone. Reduced binding to the latter fragment was taken as evidence that BSII contributed to the overall binding pattern, but binding to a fragment containing only BSII could not be demonstrated. Thus the results were similar to those obtained previously with unpurified viral protein. In a second approach using purified TAg, Bollag and co-workers (1996) infected Sf9 insect cells with the recombinant baculovirus B-JCT. JCV TAg was purified by immunoaffinity chromatography and tested for specific DNA binding in the presence of ATP and at 37°C (McVey et al., 1993). Under these conditions, JCV TAg was shown capable of interacting with BSII and in fact exhibited a greater affinity for this site than for BSI of either viral origin. In agreement with earlier studies, SV40 TAg preferentially bound BSI (Tjian, 1978; Shalloway et al., 1980; Tegtmeyer et al., 1981), and both TAgs exhibited a slight preference for the SV40 origin. JCV TAg also bound nonorigin DNA (lacking the GAGGC sequence) more efficiently than did the SV40 protein. One could speculate that JCV TAg's altered behavior relative to that of SV40 TAg reflects an adaptation to lower levels of TAg produced during an infection (Bollag et al., 1996). To enhance initiation, JCV TAg may need to display a higher affinity to the core origin sequences, and to ensure efficient elongation the viral protein may require a tighter association with sequences beyond the origin region.

More recently, Nesper et al. (1997) re-examined the interaction between purified JCV TAg and JCV BSI and BSII. Under nonreplication conditions, the TAg was found to bind predominantly to BSI, in agreement with the work of Windl and Dörries (1995). Under replication conditions, however, TAg was found to bind tightly to BSII, confirming the work of Bollag et al. (1996).

Interactions Between JCV TAg and Cellular Proteins. Although not as extensive as the analyses conducted with SV40 TAg, the JCV protein has been shown to bind several cellular factors, including members of the pRB family of tumor suppressor proteins (pRB, p107, p130; Dyson et al., 1989, 1990; Howard et al., 1998); a second tumor suppressor, p53 (Bollag et al., 1989); the molecular chaperone hsc70 (Bollag and Frisque, unpublished data); and Tst-1, a member of the POU family of transcription factors (Renner et al., 1994; Sock et al., 1999). The latter interaction does not appear to alter DNA replication directly; rather, by stimulating early viral transcription, TAg levels increase, thereby elevating replication activity (Wegner et al., 1993). Based on SV40 studies (Campbell et al., 1997), the interaction between the J domain of the JCV TAg and the DnaK protein hsc70 might also be expected to enhance viral DNA replication, possibly by altering the conformation of the replication com-

plex at the core origin. A great deal of effort has been expended examining SV40 TAg's binding to the cell cycle regulatory proteins p53 and the pRB family members. SV40 TAg, like the oncoproteins encoded by a number of other DNA tumor viruses, promotes S phase progression and inhibits apoptosis of cells by binding and inactivating these cellular proteins (reviewed by Brodsky and Pipas, 1998). Although these events have not been demonstrated to enhance SV40 DNA replication directly, most of the experiments have been conducted in exponentially growing cells. Replication of JCV occurs in cells that may be quiescent or slowly growing, and one might speculate that the interaction of JCV TAg with cellular tumor suppressor proteins is critical to ensuring the proper environment to support viral DNA replication.

Contribution of Other JCV Early Proteins to DNA Replication. Five proteins are encoded by JCV mRNAs generated by alternative splicing of the early precursor mRNA (Trowbridge and Frisque, 1995). In addition to large and small T antigens (TAg, tAg), three T′ proteins are expressed in infected human cells (Fig. 6.1). These latter proteins are translated from mRNAs in which two introns have been removed. The intron proximal to the 5′ end of the message is the same one removed from the TAg mRNA. The distal intron excised from each T′ mRNA utilizes a shared donor site but a unique acceptor site. This arrangement results in the production of three T′ proteins that share their amino-terminal 132 a.a. with TAg and have unique carboxy termini (either 3, 4, or 33 a.a. in T'_{135}, T'_{136}, or T'_{165}, respectively). The overlapping sequences include a number of functional domains important to TAg's transforming and replication activities, including sequences predicted or shown to (1) bind Tst-1, DNA polymerase α, pRB, p107, p130, and hsc70; (2) localize the protein to the nucleus (NLS); and (3) transactivate viral and cellular genes (Fig. 6.6). It would be reasonable to expect that the three T′ proteins would exhibit some of these TAg characteristics.

To investigate the possibility that T'_{135}, T'_{136}, and/or T'_{165} influences DNA replication, the shared distal donor splice site of the T′ mRNAs was mutated while preserving the authentic TAg a.a. sequence. This mutant, JCV(ΔT′), failed to express the three T′ proteins and was reduced 10-fold in DNA replicating activity compared with JCV(Mad-1) (Trowbridge and Frisque, 1995). Seven additional T′ mutants were constructed in which the three unique acceptor sites were targeted individually or in combination (Prins and Frisque, 2001). The triple mutant JCV($\Delta T'_{135/136/165}$) had a replication phenotype similar to the donor site mutant, whereas the three single mutants and one double acceptor site mutant ($\Delta T'_{135/165}$) replicated with nearly normal efficiency. The other two double acceptor site mutants ($\Delta T'_{135/136}$ and $\Delta T'_{136/165}$) exhibited partial replication defects, suggesting that T'_{136} might influence replication behavior to a greater extent than does T'_{135} and T'_{165}. These results support the hypothesis that JCV T′ proteins encode TAg replication functions. Similar "truncated" TAgs are also translated from alternatively spliced SV40, BKV, and mouse polyoma virus (PyV) early transcripts (Fig. 6.1; Zerrahn et al., 1993; Riley et al., 1997; Prins

and Frisque, unpublished data), but the role of these T′-like proteins in viral DNA replication has not been established. It should be emphasized that there are important differences between TAg and the T′ proteins, including altered structures, phosphorylation status, and temporal and cell-specific expression patterns that are likely to result in functional differences (Swenson and Frisque, 1995; Trowbridge and Frisque, 1995; Prins, Jones and Frisque, unpublished data). This expectation was confirmed recently when these four early proteins where found to differ in the affinity with which they bound members of the pRB family (Bollag et al., 2000).

Cells Permissive for JCV DNA Replication

The initial success in recovering JCV from PML brain tissue was due to the identification of PHFG cells as a culture system that would support the complete lytic cycle of the virus. This heterogeneous population of glial cells remains the most permissive system in which to propagate the virus, although transformed derivatives of these cells (e.g., POJ, SVG, and POS cells) and human tonsillar stromal cells support moderate to efficient production of infectious virions. Additional human cell types have been found that support low levels of virus replication. Jensen and Major (1999) provide an extensive list of permissive and semipermissive cells in their recent review of JCV biology.

A large number of studies have been conducted to uncover the basis for the restricted growth of JCV. Feigenbaum et al. (1987) proposed that the restricted host range behavior of the virus is the result of regulation at the levels of transcription and replication. Their model suggests that because signals within the JCV TCR are glial cell specific (Kenney et al., 1984), early gene expression is limited to these cells, and, because JCV TAg interactions with host cell replication machinery is species (primate) specific, DNA replication is restricted to primate cells. Based on this model, one would predict that JCV replication would be confined to primate glial cells. Given the available data, some modifications to this model are necessary. For example, JCV replicates in cells outside the human CNS in vivo (Jensen and Major, 1999), and in vitro a chimera with a JCV regulatory region linked to the SV40 coding regions replicates efficiently in monkey kidney (CV-1) cells (Chuke et al., 1986; Lynch and Frisque, 1991; Lynch et al., 1994). Both examples indicate that TAg is expressed via the JCV TCR in amounts adequate to support multiplication of the virus in a nonglial cell. Furthermore, in the in vitro experiment, replication of intact JCV was highly inefficient in CV-1 cells, suggesting that JCV TAg, unlike SV40 TAg, interacted only weakly with the replication machinery of the monkey cells. Therefore, while most investigators agree that JCV's restricted host range is a function of regulation at both the transcription and replication levels, the former is not limited to (but is most efficient in) glial cells and the latter is most effective with (although not limited to) the replication machinery of human cells.

In Vitro DNA Replication

The development of cell-free replication systems has permitted investigators to identify cellular factors required for replication of polyomavirus genomes. Because naked viral DNA has been used routinely in this work, the influence of chromatin structure can not be fully assessed in in vitro systems, and therefore some differences in replication requirements in vivo versus in vitro have been recognized (Cheng and Kelly, 1989; Bullock et al., 1997; Halmer and Gruss, 1997; Nesper et al., 1997; Herbig et al., 1999; Muller and Mermod, 2000).

Using a soluble cell-free system derived from primate cells, Li and Kelly (1985) tested the replication of the SV40, JCV, and BKV origins in the presence of SV40 TAg. In extracts of human HeLa cells, constructs containing the core origins and auxiliary sequences of the two human polyomavirus genomes replicated with an efficiency 10–20% that of a plasmid containing the corresponding SV40 sequences. When monkey cell extracts (COS cells) were used, replication of the JCV and BKV origin plasmids was reduced another 5–10-fold relative to that of the SV40 origin-containing vector. In contrast, the SV40 construct replicated with similar efficiency in both the human and monkey cell extracts.

More recently Nesper and colleagues (1997) described two in vitro DNA replication systems containing purified JCV TAg and either purified cellular replication factors or crude extracts of human cells. Replication initiation was examined using the system containing DNA polymerase α-primase, RPA, and topoisomerase I, while replication elongation was measured in the system employing HeLa cell extracts. Sequence requirements for JCV DNA replication were examined with these assays. Deletion of TAg BSI resulted in approximately a twofold reduction in replication, whereas constructs lacking the IP or BSII elements of the core failed to exhibit any significant replication activity. Similar findings were reported when the BSI and BSII mutants were tested in an in vivo replication assay. In addition, a construct that lacked one copy of the JCV(Mad-1) 98 bp enhancer element replicated in vitro slightly better than the intact construct, in agreement with an earlier in vivo study (Lynch and Frisque, 1990). Replacement of the JCV TAg with either the SV40 or PyV TAgs in the in vitro system confirmed that the SV40 protein was a functional substitute, but yielded the new information that the PyV protein did not interact productively with the JCV sequences.

Other Factors Influencing JCV DNA Replication

A limited number of studies have identified factors that inhibit JCV DNA replication. Kerr and colleagues (1993) confirmed their prediction that the antitumor drug camptothecin would block replication of JCV DNA in vivo. The primary target of the drug appears to be topoisomerase I of the cell (Li and Liu, 2001). Pulse treatment of glial cells with nontoxic doses of camptothecin

specifically inhibited viral replication; repeated pulse treatment of the cells with the drug was required to maintain the inhibitory effect. The use of camptothecin in the treatment of PML was suggested. In a second study, Staib et al. (1996) determined that exogenous addition of the human and murine tumor suppressor protein p53 strongly inhibited JCV DNA replication in vivo. Their results suggest that the basis for the inhibition was the binding of p53, via its highly conserved central region, to JCV TAg. It was postulated that this interaction might interfere with the ability of TAg to unwind the JCV origin during replication initiation. In work relevant to JCV reactivation in immunocompromised individuals, Chang and co-workers (1996) detected soluble factor(s) secreted by activated T cells that suppress replication of JCV DNA in glial cells. The factor(s) were found to be heat labile and to range in size from 30 to 100 kDa. Finally, N.N. Chen et al. (1997) identified a cellular factor, Sμbp-2, that stimulated JCV late transcription but decreased JCV DNA replication activity in glial cells. A truncated version of Sμbp-2, GF-1, induced JCV promoter function more efficiently than the intact human protein and also had a slight stimulatory effect on JCV DNA replication in vivo.

Evidence has been presented that virus–virus interactions may influence replication of JCV in its human host. The relatively high incidence of PML in HIV-infected individuals was recognized early in the AIDS pandemic, and several studies have indicated that the Tat protein of HIV-1 stimulates JCV transcriptional activity in vitro (Chowdhury et al., 1990, 1993; Tada et al., 1990). Recently, Mock and co-workers (1999) reported that JCV and human herpesvirus 6 (HHV6) co-infect oligodendrocytes within and around PML lesions. These investigators suggest that HHV6 might contribute to central nervous system demyelination, and one could speculate that such an effect might be the result of a direct action of HHV6 on the glial cells or an indirect action via stimulation of JCV replication. A clear demonstration of activation of JCV DNA replication by a second human virus is provided in the study of Heilbronn et al. (1993). These investigators hypothesized that human cytomegalovirus (HCMV) might serve as a helper virus for JCV replication in human fibroblasts, a cell type considered nonpermissive for JCV growth. Indeed, JCV replication was activated in HCMV-infected fibroblasts in culture. Furthermore, ganciclovir-induced inhibition of HCMV replication led to the concomitant inhibition of JCV DNA replication. These results might be relevant to mechanisms of JCV reactivation in immunocompromised, HCMV-infected individuals.

12. CONCLUSIONS

JCV transcription is regulated by complicated mechanisms. Many cell-specific and general transcription factors appear to regulate the JCV promoter via protein–protein interaction or by binding to promoter DNA elements. We have summarized in Figure 6.3 the transcription factor binding sites on the JCV promoter that have been reported. Multiple transcription factors interact with

each other and regulate the transcription of JCV. In addition, chromatin structure and DNA methylation might be other factors controlling cell-specific expression. In the future, identification of novel aspects of JCV neurotropism should shed light not only on our understanding of the biochemical mechanism of virus infection, but also on the therapy of glial-specific diseases such as PML, multiple sclerosis, and other demyelinating diseases.

SV40 serves as a model for studying DNA replication in the eukaryotic cell. Its study has also provided a foundation on which to investigate this process in the human polyomaviruses. JCV and BKV are closely related to this monkey virus, and the sequences of the *cis* (origin) and *trans* (TAg) acting replication components of each virus are highly conserved. In addition, all three viruses replicate in primate cells, so it is not surprising that key features of the replication process are shared. However, differences in replication functions and potential do exist, thereby contributing to the unique biology of each virus. Significant variation has been observed in the ability of each TAg to interact with the homologous and heterologous core and auxiliary origin sequences. These variations may reflect differences in the communication between TAg and the viral DNA or TAg and permissive cell factors; they might also signal differences in the ability of core and auxiliary origin sequences to bind one or more cellular factors. Identifying the outcomes of these interactions will be critical to our understanding of how these viruses establish an asymptomatic infection or a pathogenic state. The recent discovery of multiple ''truncated'' versions of the polyomavirus TAgs that exhibit alterations in sequence and expression levels adds yet another dimension to the regulation of viral DNA replication that investigators have only recently begun to assess.

Acquiring a better understanding of viral DNA replication will be central to our ability to control the pathogenic potential of JCV and BKV in their human host. Preliminary studies based on such information have already been successful in identifying inhibitors that limit DNA replication of these viruses in cell culture systems. Furthermore, as we begin to understand the mechanisms by which other viruses activate JCV and BKV replication, we should be better able to devise the means to prevent the pathogenic consequences of these coinfections.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health grant NS35735 to J.W.H. and grant CA44970 to R.J.F.

REFERENCES

Agostini HT, Jobes DV, Chima SC, Ryschkewitsch CF, Stoner GL (1999): Natural and pathogenic variation in the JC virus genome. Recent Res Dev Virol 1:683–701.

Agostini HT, Ryschkewitsch CF, Mory R, Singer EJ, Stoner GL (1997): JC virus (JCV) genotypes in brain tissue from patients with progressive multifocal leukoencephalopathy (PML) and in urine from controls without PML: Increased frequency of JCV Type 2 in PML. J Infect Dis 176:1–8.

Ahmed S, Chowdhury M, Khalili K (1990a): Regulation of a human neurotropic virus promoter, JCV(E): Identification of a novel activator domain located upstream from the 98 bp enhancer promoter region. Nucleic Acids Res 18:7417–7423.

Ahmed S, Rappaport J, Tada H, Kerr D, Khalili K (1990b): A nuclear protein derived from brain cells stimulates transcription of the human neurotropic virus promoter, JCVE, in vitro. J Biol Chem 265:13899–13905.

Amemiya K, Traub R, Durham L, Major EO (1989): Interaction of a nuclear factor-1–like protein with the regulatory region of the human polyomavirus JC virus. J Biol Chem 264:7025–7032.

Amemiya K, Traub R, Durham L, Major EO (1992): Adjacent nuclear factor-1 and activator protein binding sites in the enhancer of the neurotropic JC virus. J Biol Chem 267:14204–14211.

Amirhaeri S, Wohlrab F, Major EO, Wells R (1988): Unusual DNA structure in the regulatory region of the human papovavirus JC virus. J Virol 62:922–931.

Antequera F, Bird A (1993): Number of CpG islands and genes in human and mouse. Proc Natl Acad Sci USA 90:11995–11999.

Arthur AK, Hoss A, Fanning E (1988): Expression of simian virus 40 T antigen in *Escherichia coli*: Localization of T-antigen origin DNA-binding domain to within 129 amino acids. J Virol 62:1999–2006.

Åström K-E, Mancall EL, Richardson EP (1958): Progressive multifocal leukoencephalopathy. Brain 81:93–111.

Ault GS (1997): Activity of JC virus archetype and PML-type regulatory regions in glial cells. J Gen Virol 78:163–169.

Bargou RC, Jurchott K, Wagener C, Bergmann S, Metzner S, Bommert K, Mapara MY, Winzer KJ, Dietel M, Dorken B, Royer HD (1997): Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic MDR1 gene expression. Nat Med 3:447–450.

Beggs AH, Frisque RJ, Scangos GA (1988): Extinction of JC virus tumor-antigen expression in glial cell-fibroblast hybrids. Proc Natl Acad Sci USA 85:7632–7636.

Bellard M, Oudet P, Germond JE, Chambon P (1976): Subunit structure of simian-virus-40 minichromosome. Eur J Biochem 70:543–553.

Bestor TH, Tycko B (1996): Creation of genomic methylation patterns. Nat Genet 12: 363–367.

Bird A (1992): The essentials of DNA methylation. Cell 70:5–8.

Bollag B, Chuke WF, Frisque RJ (1989): Hybrid genomes of the polyomaviruses JC virus, BK virus, and simian virus 40: Identification of sequences important for efficient transformation. J Virol 63:863–872.

Bollag B, MacKeen PC, Frisque RJ (1996): Purified JC virus T antigen derived from insect cells preferentially interacts with binding site II of the viral core origin under replication conditions. Virology 218:81–93.

Bollag B, Prins C, Snyder EL, Frisque RJ (2000): Purified JC virus T and T′ proteins differentially interact with the retinoblastoma family of tumor suppressor proteins. Virology 274:165–178.

Borowiec JA, Hurwitz J (1988): ATP stimulates the binding of simian virus 40 (SV40) large tumor antigen to the SV40 origin of replication. Proc Natl Acad Sci USA 85: 64–68.

Boyes J, Bird A (1992): Repression of genes by DNA methylation depends on CpG density and promoter strength: Evidence for involvement of a methyl-CpG binding protein. EMBO J 11:327–333.

Brodsky JL, Pipas JM (1998): Polyomavirus T antigens: Molecular chaperones for multiprotein complexes. J Virol 72:5329–5334.

Bullock PA, Joo WS, Sreekumar KR, Mello C (1997): Initiation of SV40 DNA replication in vitro: Analysis of the role played by sequences flanking the core origin on initial synthesis events. Virology 227:460–473.

Campbell KS, Mullane KP, Aksoy IA, Stubdal H, Zalvide J, Pipas JM, Silver PA, Roberts TM, Schaffhausen BS, DeCaprio JA (1997): DnaJ/hsp40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication. Genes Dev 11: 1098–1110.

Chang CF, Tada H, Khalili K (1994): The role of a pentanucleotide repeat sequence, AGGGAAGGGA, in the regulation of JC virus DNA replication. Gene 148:309–314.

Chang CF, Otte J, Kerr DA, Valkkila M, Calkins CE, Khalili K (1996): Evidence that the soluble factors secreted by activated immune cells suppress replication of human neurotropic JC virus DNA in glial cells. Virology 221:226–231.

Chen L, Joo WS, Bullock PA, Simmons DT (1997): The N-terminal side of the origin-binding domain of simian virus 40 large T antigen is involved in A/T untwisting. J Virol 71:8743–8749.

Chen NN, Kerr D, Chang CF, Honjo T, Khalili K (1997): Evidence for regulation of transcription and replication of the human neurotropic virus JCV genome by the human S(mu)bp-2 protein in glial cells. Gene 185:55–62.

Chen NN, Khalili K (1995): Transcriptional regulation of human JC polyomavirus promoters by cellular proteins YB-1 and Purα in glial cells. J Virol 69:5843–5848.

Cheng L, Kelly TJ (1989): Transcriptional activator nuclear factor I stimulates the replication of SV40 minichromosomes in vivo and in vitro. Cell 59:541–551.

Chepenik LG, Tretiakova AP, Krachmarov CP, Johnson EM, Khalili K (1998): The single-stranded DNA binding protein, Purα, binds HIV-1 TAR RNA and activates HIV-1 transcription. Gene 210:37–44.

Chowdhury M, Kundu M, Khalili K (1993): GA/GC-rich sequence confers Tat responsiveness to human neurotropic virus promoter, JCVL, in cells derived from central nervous system. Oncogene 8:887–892.

Chowdhury M, Taylor JP, Tada H, Rappaport J, Wong-Staal F, Amini S, Khalili K (1990): Regulation of the human neurotropic virus promoter by JCV-T antigen and HIV-1 Tat protein. Oncogene 15:1737–1742.

Chuke WF, Walker DL, Peitzman LB, Frisque RJ (1986): Construction and characterization of hybrid polyomavirus genomes. J Virol 60:960–971.

Cikes M, Beth E, Guignard N, Walker DL, Padgett BL, Giraldo G (1977): Purification of simian virus 40 and JC T-antigens from transformed cells. J Natl Cancer Inst 59: 889–894.

Croston GE, Kerrigan LA, Lira LM, Marshak DR, Kadonaga JT (1991): Sequence-specific antirepression of histone H1-mediated inhibition of basal RNA polymerase II transcription. Science 251:643–649.

Damania B, Alwine JC (1996): TAF-like function of SV40 large T antigen. Genes Dev 10:1369–1381.

Daniel AM, Swenson JJ, Mayreddy RPR, Khalili K, Frisque RJ (1996): Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. Virology 216:90–101.

Dean FB, Dodson M, Echols H, Hurwitz J (1987): ATP-dependent formation of a specialized nucleoprotein structure by simian virus 40 (SV40) large tumor antigen at the SV40 replication origin. Proc Natl Acad Sci USA 84:8981–8985.

Deb S, DeLucia AL, Baur CP, Koff A, Tegtmeyer P (1986): Domain structure of the simian virus 40 core origin of replication. Mol Cell Biol 6:1663–1670.

Deb SP, Tegtmeyer P (1987): ATP enhances the binding of simian virus 40 large T antigen to the origin of replication. J Virol 61:3649–3654.

Del Vecchio AM, Steinman RA, Ricciardi RP (1989): An element of the BK virus enhancer required for DNA replication. J Virol 63:1514–1524.

Devireddy LR, Kumar KU, Pater MM, Pater A (2000): BAG-1, a novel Bcl-2–interacting protein, activates expression of human JC virus. J Gen Virol 81:351–357.

Deyerle KL, Sajjadi FG, Subramani S (1989): Analysis of origin of DNA replication of human papovavirus BK. J Virol 63:356–365.

Dornreiter I, Hoss A, Arthur AK, Fanning E. (1990): SV40 T antigen binds directly to the large subunit of purified DNA polymerase alpha. EMBO J 9:3329–3336.

Dyson N, Bernards R, Friend SH, Gooding LR, Hassell JA, Major EO, Pipas JM, Vandyke T, Harlow E (1990): Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. J Virol 64:1353–1356.

Dyson N, Buchkovich K, Whyte P, Harlow E (1989): The cellular 107K protein that binds to adenovirus E1A also associates with the large T antigens of SV40 and JC virus. Cell 58:249–255.

Faisst S, Meyer S (1992): Compilation of vertebrate-encoded transcription factors. Nucleic Acids Res 20:3–26.

Fanning E, Knippers R (1992): Structure and function of simian virus 40 large tumor antigen. Annu Rev Biochem 61:55–85.

Feigenbaum L, Hinrichs SH, Jay G (1992): JC virus and simian virus 40 enhancers and transforming proteins: Role in determining tissue specificity and pathogenicity in transgenic mice. J Virol 66:1176–1182.

Feigenbaum L, Khalili K, Major E, Khoury G (1987): Regulation of the host range of human papovavirus JCV. Proc Natl Acad Sci USA 84:3695–3698.

Felsenfeld G (1992): Chromatin as an essential part of the transcriptional mechanism. Nature 355:219–224.

Frisque RJ, Bream GL, Cannella MT (1984): Human polyomavirus JC virus genome. J Virol 51:458–469.

Gallia GL, Darbinian N, Johnson EM, Khalili K (1999a): Self-association of Purα is mediated by RNA. J Cell Biochem 74:334–348.

Gallia GL, Darbinian N, Tretiakova A, Ansari SA, Rappaport J, Brady J, Wortman MJ, Johnson EM, Khalili K (1999b): Association of HIV-1 tat with the cellular protein, Purα, is mediated by RNA. Proc Natl Acad Sci USA 96:11572–11577.

Gallia GL, Safak M, Khalili K (1998): Interaction of the single-stranded DNA-binding protein Purα with the human polyomavirus JC virus early protein T-antigen. J Biol Chem 273:32662–32669.

Gluzman Y (1981): SV40 transformed cells support the replication of early SV40 mutants. Cell 23:175–182.

Graff JR, Herman JG, Myohanen S, Baylin SB, Vertino PM (1997): Mapping patterns of CpG island methylation in normal and neoplastic cells implicates both upstream and downstream regions in de novo methylation. J Biol Chem 272:22322–22329.

Grant CE, Deeley RG (1993): Cloning and characterization of chicken YB-1: Regulation of expression in the liver. Mol Cell Biol 13:4186–4196.

Gruda MC, Alwine JC (1991): Simian virus 40 (SV40) T-antigen transcriptional activation mediated through the Oct/SPH region of the SV40 late promoter. J Virol 65: 3553–3558.

Gruda MC, Zabolotny JM, Xiao JH, Davidson I, Alwine JC (1993): Transcriptional activation by simian virus large T antigen: Interactions with multiple components of the transcription complex. Mol Cell Biol 13:961–969.

Guo ZS, Depamphilis ML (1992): Specific transcription factors stimulate simian virus 40 and polyomavirus origins of DNA replication. Mol Cell Biol 12:2514–2524.

Haggerty S, Walker DL, Frisque RJ (1989): JC virus-simian virus 40 genomes containing heterologous regulatory signals and chimeric early regions: Identification of regions restricting transformation by JC virus. J Virol 63:2180–2190.

Halmer L, Gruss C (1997): Accessibility to topoisomerases I and II regulates the replication efficiency of simian virus 40 minichromosomes. Mol Cell Biol 17:2624–2630.

Hansen U, Tenen DG, Livingston DM, Sharp PA (1981): T antigen repression of the SV40 early transcription from two promoters. Cell 27:603–612.

Hass S, Thatikunta P, Steplewski A, Johnson EM, Khalili K, Amini S (1995): A 39-kD DNA-binding protein from mouse brain stimulates transcription of myelin basic protein gene in oligodendrocytes. J Cell Biol 130:1171–1179.

Heilbronn R, Albrecht I, Stephan S, Burkle A, Zurhausen H (1993): Human cytomegalovirus induces JC virus DNA replication in human fibroblasts. Proc Natl Acad Sci USA 90:11406–11410.

Henson JW (1994): Regulation of the glial-specific JC virus early promoter by the transcription factor Sp1. J Biol Chem 269:1046–1050.

Henson JW (1996): Coupling JC virus transcription and replication. J Neurovirol 2:57–59.

Henson J, Saffer J, Furneaux H (1992): The transcriptional factor Sp1 binds to the JC virus promoter and is selectively expressed in glial cells in human brain. Ann Neurol 32:72–77.

Henson JW, Schnitker BL, Lee T-S, McAllister J (1995): Cell-specific activation of the glial-specific JC virus early promoter by large T antigen. J Biol Chem 270:13240–13245.

Herbig U, Weisshart K, Taneja W, Fanning E (1999): Interaction of the transcription factor TFIID with simian virus 40 (SV40) large T antigen interferes with replication of SV40 DNA in vitro. J Virol 73:1099–1107.

Horikoshi N, Usheva A, Chen J, Levine AJ, Weinmann R, Shenk T (1995): Two domains of p53 interact with the TATA-binding protein, and the adenovirus 13S E1A protein disrupts the association, relieving p53-mediated transcriptional repression. Mol Cell Biol 15:227–234.

Hoss A, Moarefi IF, Fanning E, Arthur AK (1990): The finger domain of simian virus 40 large T antigen controls DNA-binding specificity. J Virol 64:6291–6296.

Howard CM, Claudio PP, Gallia GL, Gordon J, Giordano GG, Hauck WW, Khalili K, Giordano, A. (1998). Retinoblastoma-related protein pRb2/p130 and suppression of tumor growth in vivo. J Natl Cancer Inst 90:1451–1460.

Ise T, Nagatani G, Imamura T, Kato K, Takano H, Nomoto M, Izumi H, Ohmori H, Okamota T, Ohga T, Uchimi T, Kuwano M, Kohno K (1999): Transcription factor Y-box binding protein 1 binds preferentially to cisplatin-modified DNA and interacts with proliferating cell nuclear antigen. Can Res 59:342–346.

Ishimi Y, Sugasawa K, Hanaoka F, Eki T, Hurwitz J (1992): Topoisomerase-II plays an essential role as a swivelase in the late stage of SV40 chromosome replication in vitro. J Biol Chem 267:462–466.

Itoyama Y, Webster HdeF, Sternberger NH, Richardson EP, Walker DL, Quarles RH, Padgett BL (1981): Distribution of papovavirus, myelin-associated glycoprotein, and myelin basic protein in progressive multifocal leukoencephalopathy lesions. Ann Neurol 11:396–407.

Jensen PN, Major EO (1999): Viral variant nucleotide sequences help expose leukocytic positioning in the JC virus pathway to the CNS. J Leukocyte Biol 65:428–438.

Kaelin WG (1999): Functions of the retinoblastoma protein. Bioessays 21:950–958.

Kashanchi F, Duvall JF, Dittmer J, Mireskandari A, Reid RL, Gitlin SD, Brady JN (1994): Involvement of transcription factor YB-1 in human T-cell lymphotropic virus type I basal gene expression. J Virol 68:7637–7643.

Kelley WL, Georgopoulos C (1997): The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the *Escherichia coli* DnaJ molecular chaperone. Proc Natl Acad Sci. USA 94:3679–3684.

Kenny S, Natarajan V, Strike D, Khoury G, Salzman NP (1984): JC virus enhancer–promoter active in human brain cells. Science 226:1337–1339.

Kerr D, Chang CF, Chen N, Gallia G, Raj G, Schwartz B, Khalili K (1994): Transcription of a human neurotropic virus promoter in glial cells: Effect of YB-1 on expression of the JC virus late gene. J Virol 68:7637–7643.

Kerr DA, Chang CF, Gordon J, Bjornsti MA, Khalili K (1993): Inhibition of human neurotropic virus (JCV) DNA replication in glial cells by camptothecin. Virology 196:612–618.

Kerr D, Khalili K (1991): A recombinant cDNA derived from human brain encodes a DNA binding protein that stimulates transcription of the human neurotropic virus JCV. J Biol Chem 266:15876–15881.

Khalili K, Rappaport J, Khoury G (1988): Nuclear factors in human brain cells bind specifically to the JCV regulatory region. EMBO J 7:1205–1210.

Kilwinski J, Baack M, Heiland S, Knippers R (1995): Transcription factor Oct1 binds to the AT-rich segment of the simian virus 40 replication origin. J Virol 69:575–578.

Kim HS, Goncalves NM, Henson JW (2000): Glial cell-specific regulation of the JC virus early promoter by large T antigen. J Virol 74:755–763.

Kim HY, Barbaro BA, Joo WS, Prack AE, Sreekumar KR, Bullock PA (1999): Sequence requirements for the assembly of simian virus 40 T antigen and the T-antigen origin binding domain on the viral core origin of replication. J Virol 73:7543–7555.

Koike K, Uchiumi T, Ohga T, Toh S, Wada M, Kohno K, Kuwano M (1997): Nuclear translocation of the Y-box binding protein by ultraviolet irradiation. FEBS Letter 417:390–394.

Krachmarov CP, Chepenik LG, Barr-Vagell S, Khalili K, Johnson EM (1996): Activation of the JC virus Tat-responsive transcriptional control element by association of Tat protein of human immunodeficiency virus 1 with cellular protein Purα. Proc Natl Acad Sci USA 93:14112–14117.

Kraus VB, Inostroza JA, Yeung K, Reinberg D, Nevins JR (1994): Interaction of the Dr1 inhibitory factor with the TATA binding protein is disrupted by adenovirus E1A. Proc Natl Acad Sci USA 91:6279–6282.

Krebs CJ, Dey B, Kumar G (1996): The cerebellum-enriched form of nuclear factor I is functionally different from ubiquitous nuclear factor I in glial-specific promoter regulation. J Neurochem 66:1354–1361.

Krebs CJ, McAvoy MT, Kumar G (1995): The JC virus minimal core promoter is glial cell specific in vivo. J Virol 69:2434–2442.

Kumar G, Srivastava DK, Tefera W (1994): A 70- to 80-kDa glial cell protein interacts with the AGGGAAGGGA domain of the JC virus early promoter only in the presence of the neighboring cis elements. Virology 203:116–124.

Kumar KU, Reddy DLN, Pater MM, Pater A (1996a): Human JC virus cAMP response elements functional for enhanced glial cell expression in differentiating embryonal carcinoma cells. Virology 215:178–185.

Kumar KU, Tang SC, Pater MM, Pater A (1996b). Glial and muscle embryonal carcinoma cell-specific independent regulation of expression of human JC virus early promoter by cyclic AMP response elements and adjacent nuclear factor 1 binding sites. J Med Virol 49:199–204.

Leger H, Sock E, Renner K, Grummt F, Wegner M (1995): Functional interaction between the POU domain protein Tst-1/Oct-6 and the high-mobility-group protein HMG-I/Y. Mol Cell Biol 15:3738–3747.

Li JJ, Kelly TJ (1985): Simian virus 40 DNA replication in vitro: Specificity of initiation and evidence for bidirectional replication. Mol Cell Biol 5:1238–1246.

Li WW, Hsiung Y, Wong V, Galvin K, Zhou Y, Shi Y, Lee AS (1997): Suppression of grp78 core promoter element-mediated stress induction by the dbpA and dbpB (YB-1) cold shock domain proteins. Mol Cell Biol 17:61–68.

Li TK, Liu LF (2001): Tumor cell death induced by topoisomerase-targeting drugs. Annu Rev Pharmacol 41:53–77.

Liu M, Kumar KU, Pater MM, Pater A (1998): Identification and characterization of a JC virus pentanucleotide repeat element binding protein: Cellular nucleic acid binding protein. Virus Res 58:73–82.

Loeber G, Dörries K (1988): DNA rearrangement in organ-specific variants of polyomavirus JC strain GS. J Virol 62:1730–1735.

Loeber G, Parsons R, Tegtmeyer P (1989): The zinc finger region of simian virus 40 large T antigen. J Virol 63:94–100.

Loeber G, Stenger JE, Ray S, Parsons RE, Anderson ME, Tegtmeyer P (1991): The zinc finger region of simian virus-40 large T-antigen is needed for hexamer assembly and origin melting. J Virol 65:3167–3174.

Loeken M, Bikel I, Livingston DM, Brady J (1988): Trans-activation of RNA polymerase II and III promoters by SV40 small t antigen. Cell 55:1171–1177.

Lu H, Zawel L, Fisher L, Egly J-M, Reinberg D (1992): Human general transcription factor IIH phosphorylates the C-terminal domain of RNA polymerase II. Nature 358: 641–645.

Lynch KJ, Frisque RJ (1990): Identification of critical elements within the JC virus DNA replication origin. J Virol 64:5812–5822.

Lynch KJ, Frisque RJ (1991): Factors contributing to the restricted DNA replicating activity of JC virus. Virology 180:306–317.

Lynch KJ, Haggerty S, Frisque RJ (1994): DNA replication of chimeric JC virus–simian virus 40 genomes. Virology 204:819–822.

Macleod D, Charlton J, Mullins J, Bird AP (1994): Sp1 sites in the mouse aprt gene promoter are required to prevent methylation of the CpG island. Genes Dev 8:2282–2292.

Major EO, Mourrain P, Cummins C (1984): JC virus–induced owl monkey glioblastoma cells in culture: Biological properties associated with the viral early gene product. Virology 136:359–367.

Major EO, Vacante DA, Traub RG, London WT, Sever JL (1987): Owl monkey astrocytoma cells in culture spontaneously produce infectious JC virus which demonstrates altered biological properties. J Virol 61:1435–1441.

Mandl C, Walker DL, Frisque RJ (1987): Derivation and characterization of POJ cells, transformed human fetal glial cells that retain their permissivity for JC virus. J Virol 61:755–763.

Mastrangelo IA, Hough PVC, Wall JS, Dodson M, Dean FB, Hurwitz J (1989): ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication. Nature 338:658–662.

Mayreddy RPR, Safak M, Razmara M, Zoltick P, Khalili K (1996): Transcription of the JC virus archetype late genome: Importance of the kB and the 23-base-pair motifs in late promoter activity in glial cells. J Virol 70:2387–2393.

McKay RDG (1981): Binding of simian virus 40 T antigen-related protein to DNA. J Mol Biol 145:471–488.

McVey D, Ray S, Gluzman Y, Berger L, Wildeman AG, Marshak DR, Tegtmeyer P (1993): cdc2 phosphorylation of threonine 124 activates the origin-unwinding functions of simian virus 40 T antigen. J Virol 67:5206–5215.

Meehan RR, Lewis JD, McKay S, Kleiner EL, Bird AP (1989): Identification of a mammalian protein that binds specifically to DNA containing methylated CpGs. Cell 58:499–507.

Mertens PR, Alfonso-Jaume MA, Steinmann K, Lovett DH (1998): A synergistic interaction of transcription factors AP2 and YB-1 regulates gelatinase A enhancer–dependent transcription. J Biol Chem 273:32957–32965.

Moarefi IF, Small D, Gilbert I, Hopfner M, Randall SK, Schneider C, Russo AAR, Ramsperger U, Arthur AK, Stahl H, Kelly TJ, Fanning E (1993): Mutation of the cyclin-dependent kinase phosphorylation site in simian virus 40 (SV40) large T-antigen specifically blocks SV40 origin DNA unwinding. J Virol 67:4992–5002.

Mock DJ, Powers JM, Goodman AD, Blumenthal SR, Ergin N, Baker JV, Mattson DH, Assouline JG, Bergey EJ, Chen BJ, Epstein LG, Blumberg BM (1999): Association of human herpesvirus 6 with the demyelinative lesions of progressive multifocal leukoencephalopathy. J Neurovirol 5:363–373.

Muller K, Mermod N (2000): The histone-interacting domain of nuclear factor I activates simian virus 40 DNA replication in vivo. J Biol Chem 275:1645–1650.

Nesper J, Smith RWP, Kautz AR, Sock E, Wegner M, Grummt F, Nasheuer HP (1997): A cell-free replication system for human polyomavirus JC DNA. J Virol 71:7421–7428.

Nevins JR (1992): E2F—A link between the Rb tumor suppressor protein and viral oncoproteins. Science 258:424–429.

Newman JT, Frisque RJ (1997): Detection of archetype and rearranged variants of JC virus in multiple tissues from a pediatric PML patient. J Med Virol 52:243–252.

Newman JT, Frisque RJ (1999): Identification of JC virus variants in multiple tissues of pediatric and adult PML patients. J Med Virol 58:79–86.

Ohtani K (1999): Implication of transcription factor E2F in regulation of DNA replication. Frontiers Biosci 4:793–804.

Okada Y, Sawa H, Tanaka S, Takada A, Suzuki S, Hasegawa H, Umemura T, Fujisawa J, Tanaka Y, Hall WW, Nagashima K (2000): Transcriptional activation of JC virus by human T-lymphotropic virus type 1 Tax protein in human neuronal cell lines. J Biol Chem 275:17016–17023.

Ondek B, Gloss L, Herr W (1988): The SV40 enhancer contains two distinct levels of organization. Nature 333:40–45.

Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH (1971): Cultivation of a papova-like virus from human brain with progressive multifocal leukoenchephalopathy. Lancet 1:1257–1260.

Parsons RE, Stenger JE, Ray S, Welker R, Anderson ME, Tegtmeyer P (1991): Cooperative assembly of simian virus-40 T-antigen hexamers on functional halves of the replication origin. J Virol 65:2798–2806.

Peden KWC, Pipas JM, Pearson-White S, Nathans D (1980): Isolation of mutants of an animal virus in bacteria. Science 209:1392–1396.

Pipas JM (1992): Common and unique features of T-antigens encoded by the polyomavirus group. J Virol 66:3979–3985.

Prins C, Frisque RJ (2001): JC virus T′ proteins encoded by alternatively spliced early mRNAs enhance T antigen-mediated viral DNA replication in human cells. J Neurovirol 7:250–264.

Raj GV, Khalili K (1994): Identification and characterization of a novel GGA/C-binding protein, GBP-i, that is rapidly inducible by cytokines. Mol Cell Biol 14:7770–7781.

Raj GV, Gallia GL, Chang CF, Khalili K (1998): T-antigen–dependent transcriptional initiation and its role in the regulation of human neurotropic JC virus late gene expression. J Gen Virol 79:2147–2155.

Renner K, Leger H, Wegner M (1994): The POU domain protein Tst-1 and papovaviral large tumor antigen function synergistically to stimulate glia-specific gene expression of JC virus. Proc Natl Acad Sci USA 91:6433–6437.

Rice PW, Cole CN (1993): Efficient transcriptional activation of many simple modular promoters by simian virus 40 large T antigen. J Virol 76:6689–6697.

Riley MI, Yoo W, Mda NY, Folk WR (1997): Tiny T antigen: An autonomous polyomavirus T antigen amino-terminal domain. J Virol 71:6068–6074.

Rio DC, Tjian R (1983): SV40 T antigen binding site mutations that affect autoregulation. Cell 32:1227–1240.

Sabath DE, Podolin PL, Comber PG, Prystowsky MB (1990): cDNA cloning and characterization of interleukin 2-induced genes in a cloned T helper lymphocyte. J Biol Chem 265:12671–12678.

Safak M, Gallia GL, Ansari SA, Khalili K (1999a): Physical and functional interaction between the Y-box binding protein YB-1 and human polyomavirus JC virus large T antigen. J Virol 73:10146–10157.

Safak M, Gallia GL, Khalili K (1999b): A 23-bp sequence element from human neurotropic JC virus is responsive to NF-kB subunits. Virology 262:178–189.

Safak M, Gallia GL, Khalili K (1999c): Reciprocal interaction between two cellular proteins, Purα and YB-1, modulates transcriptional activity of JCV$_{CY}$ in glial cells. Mol Cell Biol 19:2712–2723.

San Martin MC, Gruss C, Carazo JM (1997): Six molecules of SV40 large T antigen assemble in a propeller-shaped particle around a channel. J Mol Biol 268:15–20.

Scheidtmann KH, Buck M, Schneider J, Kalderon D, Fanning E, Smith AE (1991a): Biochemical characterization of phosphorylation site mutants of simian virus 40 large T-antigen: Evidence for interaction between amino- and carboxy-terminal domains. J Virol 65:1479–1490.

Scheidtmann KH, Hardung M, Echle B, Walter G (1984): DNA binding activity of simian virus 40 large T antigen correlates with a distinct phosphorylation state. J Virol 50:1–12.

Scheidtmann KH, Mumby MC, Rundell K, Walter G (1991b): Dephosphorylation of simian virus-40 large-T antigen and p53 protein by protein phosphatase-2A: Inhibition by small-T antigen. Mol Cell Biol 11:1996–2003.

Scheidtmann KH, Virshup DM, Kelly TJ (1991c): Protein phosphatase-2A dephosphorylates simian virus-40 large T-antigen specifically at residues involved in regulation of DNA-binding activity. J Virol 65:2098–2101.

Schneider J, Fanning E (1988): Mutations in the phosphorylation sites of simian virus 40 (SV40) T antigen alter its origin DNA-binding specificity for site I or II and affect SV40 DNA replication activity. J Virol 62:1598–1605.

Shalloway D, Kleinberger T, Livingston DM (1980): Mapping of SV40 DNA replication origin region binding sites for the SV40 T antigen by protection against exonuclease III digestion. Cell 20:411–422.

Sharma AK, Kumar G (1991): A 53 kDa protein binds to the negative regulatory region of the JC virus early promoter. FEBS 281:272–274.

Simmons DT (2000): SV40 large T antigen functions in DNA replication and transformation. Adv Virus Res 55:75–134.

Simmons DT, Trowbridge PW, Roy R (1998): Topoisomerase I stimulates SV40 T antigen-mediated DNA replication and inhibits T antigen's ability to unwind DNA at nonorigin sites. Virology 242:435–443.

Simon MC, Fisch TM, Benecke BJ, Nevins JR, Heintz N (1988): Definition of multiple, functionally distinct TATA elements, one of which is a target in the hsp70 promoter for E1A regulation. Cell 52:723–729.

Smelkova NV, Borowiec JA (1997): Dimerization of simian virus 40 T-antigen hexamers activates T-antigen DNA helicase activity. J Virol 71:8766–8773.

Sock E, Enderich J, Wegner M (1999): The J domain of papovaviral large tumor antigen is required for synergistic interaction with the POU-domain protein Tst-1/Oct6/SCIP. Mol Cell Biol 19:2455–2464.

Sock E, Renner K, Feist D, Leger H, Wegner M (1996): Functional comparison of PML-type and archetype strains of JC virus. J Virol 70:1512–1520.

Sock E, Wegner M, Fortunato EA, Grummt F (1993): Large T-antigen and sequences within the regulatory region of JC virus both contribute to the features of JC virus DNA replication. Virology 197:537–548.

Sock E, Wegner M, Grummt F (1991): DNA replication of human polyomavirus-JC is stimulated by NF-I in vivo. Virology 182:298–308.

Staib C, Pesch J, Gerwig R, Gerber JK, Brehm U, Stangl A, Grummt F (1996): p53 inhibits JC virus DNA replication in vivo and interacts with JC virus large T-antigen. Virology 219:237–246.

Stillman B (1994): Smart machines at the replication fork. Cell 78:725–728.

Sumner C, Shinohara T, Durham L, Traub R, Major EO, Amemiya K (1996): Expression of multiple classes of the nuclear factor-1 family in the developing brain: Differential expression of two classes of NF-1 genes. J Neurovirol 2:87–100.

Surridge C (1996): Transcription. The core curriculum. Nature 380:287–288.

Swenson JJ, Frisque RJ (1995): Biochemical characterization and localization of JC virus large T antigen phosphorylation domains. Virology 212:295–308.

Swenson JJ, Trowbridge PW, Frisque RJ (1996): Replication activity of JC virus large T antigen phosphorylation and zinc finger domain mutants. J Neurovirol 2:78–86.

Tada H, Lashgari MS, Khalili K (1991): Regulation of JCVL promoter function: Evidence that a pentanucleotide ''silencer'' repeat sequence AGGGAAGGGA downregulates transcription of the JC virus late promoter. Virology 180:327–338.

Tada H, Lashgari M, Rappaport J, Khalili K (1989): Cell type-specific expression of JC virus early promoter is determined by positive and negative regulation. J Virol 63:463–466.

Tada H, Rappaport J, Lashgari M, Amini S, Wong-Staal F, Khalili K (1990): Transactivation of the JC virus late promoter by the Tat protein of type 1 human immunodeficiency virus in glial cells. Proc Natl Acad Sci USA 87:3479–3483.

Tafuri SR, Wolffe AP (1993): Selective recruitment of masked maternal mRNA from messanger ribonuclearprotein particles containing FRGY2(mRNP4). J Biol Chem 268:24255–24261.

Tavis JE, Frisque RJ (1991): Altered DNA binding and replication activities of JC virus T-antigen mutants. Virology 183:239–250.

Tavis JE, Trowbridge PW, Frisque RJ (1994): Converting the JCV T antigen Rb binding domain to that of SV40 does not alter JCV's limited transforming activity but does eliminate viral viability. Virology 199:384–392.

Taylor IAC, Solomon W, Weiner BM, Paucha P, Bradley M, Kingston RE (1989): Stimulation of the human heat shock 70 promoter in vitro by simian virus 40 large T antigen. J Biol Chem 264:16160–16164.

Tegtmeyer P, Anderson B, Shaw SB, Wilson VG (1981): Alternative interactions of the SV40 A protein with DNA. Virology 115:75–87.

Tinti C, Yang C, Seo H, Conti B, Kim C, Joh TH, Kim KS (1997): Structure/function relationship of the cAMP response element in tyrosine hydroxylase gene transcription. J Biol Chem 272:19158–19164.

Tjian R (1978): Protein–DNA interactions at the origin of simian virus 40 DNA replication. Cold Spring Harbor Symp Quant Biol 43:655–662.

Trapp BD, Small JA, Pulley M, Khoury G, Scangos GA (1988): Dysmyelination in transgenic mice containing JC virus early region. Ann Neurol 23:38–48.

Tretiakova A, Otte J, Croul SE, Kim JH, Johnson EM, Amini S, Khalili K (1999a): Association of JC virus large T antigen with myelin basic protein transcription factor(MEF-1/Purα) in hypomyelinated brains of mice transgenically expressing T antigen. J Virol 73:6076–6084.

Tretiakova A, Steplewski A, Johnson EM, Khalili K, Amini S (1999b): Regulation of myelin basic protein gene transcription by Sp1 and Purα: Evidence for association of Sp1 and Purα in brain. J Cell Physiol 181:160–168.

Trowbridge PW, Frisque RJ (1995): Identification of three new JC virus proteins generated by alternative splicing of the early viral mRNA. J Neurovirol 1:195–206.

Vacante DA, Traub R, Major EO (1989): Extension of JC virus host range to monkey cells by insertion of a simian virus 40 enhancer into the JC virus regulatory region. Virology 170:353–361.

Valle M, Gruss C, Halmer L, Carazo JM, Donate LE (2000): Large T-antigen double hexamers imaged at the simian virus 40 origin of replication. Mol Cell Biol 20:34–41.

Virshup DM, Russo AAR, Kelly TJ (1992): Mechanism of activation of simian virus 40 DNA replication by protein phosphatase-2A. Mol Cell Biol 12:4883–4895.

Wegner M, Drolet DW, Rosenfeld MG (1993): Regulation of JC virus by the POU-domain transcription factor Tst-1: Implications for progressive multifocal leukoencephalopathy. Proc Natl Acad Sci USA 90:4743–4747.

Weisshart K, Taneja P, Jenne A, Herbig U, Simmons DT, Fanning E (1999): Two regions of simian virus 40 T antigen determine cooperativity of double-hexamer assembly on the viral origin of DNA replication and promote hexamer interactions during bidirectional origin DNA unwinding. J Virol 73:2201–2211.

Windl O, Dörries K (1995): Expression of human polyomavirus JC T antigen by an adenovirus hybrid vector and its binding to DNA sequences encompassing the JC virus origin of DNA replication. J Gen Virol 76:83–92.

Wolffe AP (1994): Structural and functional properties of the evolutionarily ancient Y-box family of nucleic acid binding proteins. Bioessays 16:245–251.

Wolffe AP, Pruss D (1996): Targeting chromatin disruption: Transcription regulators that acetylate histones. Cell 84:817–819.

Wolffe AP, Tafuri S, Ranjan M, Familari M (1992): The Y-box factors: A family of nucleic acid binding proteins conserved from *Escherichia coli* to man. New Biol 4: 290–298.

Wortman MJ, Krachmarov CP, Kim JH, Gordon RG, Chepenik LG, Brady JN, Gallia GL, Khalili K, Johnson EM (2000): Interaction of HIV-tat with Purα in nuclei of human glial cells: Characterization of RNA-mediated protein–protein binding. J Cell Biochem 77:65–74.

Yang SI, Lickteig RL, Estes R, Rundell K, Walter G, Mumby MC (1991): Control of protein phosphatase-2A by simian virus-40 small-t antigen. Mol Cell Biol 11:1988–1995.

Yogo Y, Kitamura T, Sugimoto C, Ueki T, Aso Y, Hara K, Taguchi F (1990): Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J Virol 64:3139–3143.

Zalvide J, Stubdal H, Decaprio JA (1998): The J domain of simian virus 40 large T antigen is required to functionally inactivate RB family proteins. Mol Cell Biol 18: 1408–1415.

Zambrano N, DeRenzis S, Minopoli G, Faraonio R, Donini V, Scaloni A, Cimino F, Russo T (1997): DNA-binding protein Purα and transcription factor YY1 function as transcription activators of neuron-specific FE65 gene promoter. Biochem J 328: 293–300.

Zerrahn J, Knippschild U, Winkler T, Deppert W (1993): Independent expression of the transforming amino-terminal domain of SV40 large T-antigen from an alternatively spliced third SV40 early messenger RNA. EMBO J 12:4739–4746.

Zu Rhein GM, Chou SM (1965): Particles resembling papova viruses in human cerebral demyelinating disease. Science 148:1477–1479.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

7

THE ARCHETYPE CONCEPT AND REGULATORY REGION REARRANGEMENT

YOSHIAKI YOGO, PH.D., and CHIE SUGIMOTO, PH.D.

1. HYPERVARIABLE PML-TYPE REGULATORY SEQUENCES

JC virus (JCV) was first isolated in 1971 from the brain of a patient with a fatal demyelinating disease in the brain, progressive multifocal leukoencephalopathy (PML) (Padgett et al., 1971). Since then, JCV has repeatedly been isolated from the brains of PML patients, and it is now established as the etiologic agent of PML (Berger and Major, 1999). Here we designate JCV isolates from the brains of PML patients as PML-type isolates. The complete sequence of the genome of a PML-type isolate (Mad-1) was reported in 1984 by Frisque et al. To date, the complete sequences of six PML-type isolates have been reported, and analyses of these sequences have revealed that the overall genome organization of JCV is consistent among PML-type JCV isolates (Agostini et al., 1998a,d; Frisque et al., 1984; Loeber and Dörries, 1988; Kato et al., 2000).

However, the promoter/enhancer region (designated here as the regulatory region) located between the DNA replication origin and the agnoprotein gene (see Chapter 5) is hypervariable among PML-type isolates. Variation in the regulatory region of PML-type JCVs was first demonstrated in 1981 by Rentier-Delrue et al. To exclude the possibility that the changes were introduced into

JCV DNAs during propagation of JCV in culture, they extracted viral DNA from the brain tissues of two PML patients. Each of the viral genomes was cloned intact in *Escherichia coli*, using a plasmid vector. The two JCV genomes were approximately 50 bp larger or approximately 50 bp smaller than Mad-1 DNA. Analysis of the restriction endonuclease cleavage fragments of these two DNAs and Mad-1 DNA revealed that the slight differences were mapped to within the regulatory region. Two years later, Grinnell et al. (1983) directly cloned JCV DNAs from the brains of 10 PML patients and compared the restriction enzyme cleavage profiles among them. They concluded that a genome area corresponding to the regulatory region is hypervariable. Furthermore, Martin et al. (1985) determined the regulatory sequences of several PML-type JCVs and confirmed the hypervariation in the regulatory sequence among PML-type JCVs.

2. ARCHETYPE REGULATORY SEQUENCE

Sero-epidemiologic studies have shown that JCV circulates in humans without any obvious symptoms (Padgett and Walker, 1973, 1976). Indeed, JCV is frequently excreted in the urine of healthy individuals as well as patients without PML (Kitamura et al., 1990, 1994a; Agostini et al., 1996). Yogo et al. (1990) cloned JCV DNA directly from two healthy volunteers and eight nonimmunocompromised patients. Restriction enzyme analysis confirmed that the overall genome structure of the urine-derived isolates was identical with that of PML-type isolates. The regulatory region sequences of the 10 urine-derived isolates were then analyzed. The basic structure of the regulatory region was identical among clones derived from all individuals, with a few nucleotide mismatches (the regulatory sequence of a clone from a healthy individual, CY, is shown in Fig. 7.1). Where multiple clones from the same individual were examined, the structure of the regulatory region was identical among clones examined, with a single exception. Thus, it was concluded that the regulatory regions of JCVs derived from the urine of nonimmunocompromised individuals are highly homogeneous, in marked contrast with the hypervariable regulatory regions of PML-type JCVs.

The regulatory region of the JCV DNA cloned by Yogo et al. (1990) lacked any repetition of a sequence of significant length (Fig. 7.1). It contained 23 and 66 bp sequences, which were inserted into the 98 bp sequence present in a tandem repeat in Mad-1. As a result, the 98 bp sequence was split into three portions of 25, 55, and 18 bp (Fig. 7.1). The 23 bp sequence had been identified in a majority of PML-derived variants, although it was absent in a few, including Mad-1 (Frisque et al., 1984; Martin et al., 1985). As described below, it was later found that a significant number of isolates from the brain and cerebrospinal fluid (CSF) of PML patients retained a region encompassing the 66 bp sequence (Ault and Stoner, 1993; Agostini et al., 1997a; Sugimoto et al., 1998). The regulatory sequence depicted in Figure 7.1 was designated as

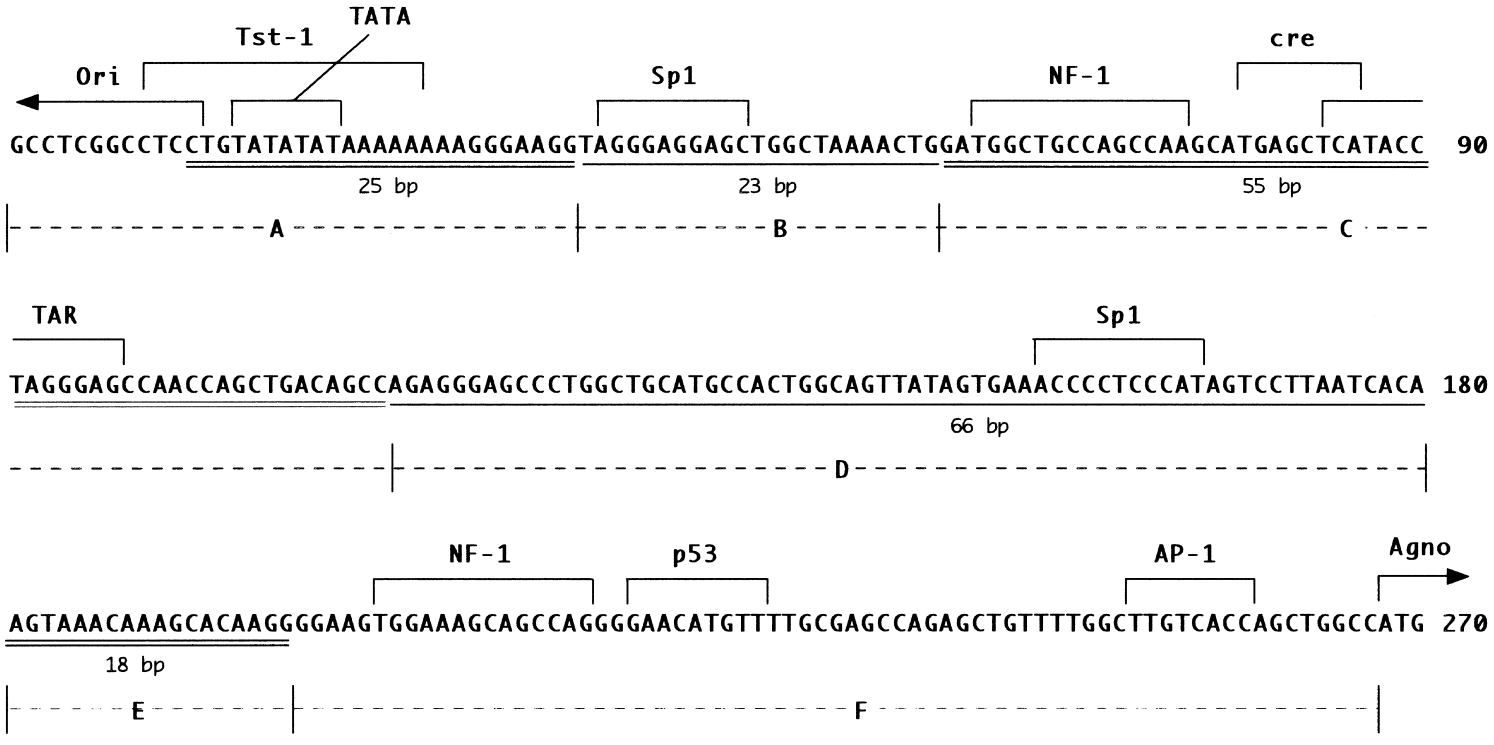


Figure 7.1. Structure of the regulatory region of JCV DNA cloned from the urine of a healthy individual (CY). The nucleotide sequence shown is from the origin of DNA replication (Ori) to the start site of the late leader protein, agnoprotein (Agno). The 25, 55, and 18 bp sequences present in the 98 bp repeated element of Mad-1 (Frisque et al., 1984) are shown by double straight lines, and the 23 and 66 bp sequences not present in Mad-1 are shown by single straight lines. Regions A–F are shown below the sequence (Ault and Stoner, 1993). TATA sequence and several transcriptional factor binding sites or homologies are indicated above the sequence.

the archetype regulatory sequence because, as described below, this sequence contained all sequences required to generate various PML-type sequences (Yogo et al., 1990).

Many complete JCV DNA clones have been established from urine collected in various areas of the Old World, and their regulatory region structures were examined (Yogo et al., 1991a,b; Guo et al., 1986). Furthermore, JCV regulatory regions were amplified by polymerase chain reaction (PCR) from urine collected in the United States (Markowitz et al., 1991; Agostini et al., 1996), Europe (Flægstad et al., 1991), and Taiwan (Chang et al., 1996a,b, 1999; Tsai et al., 1997), and their sequences were examined. These studies indicated that, without exception, JCV DNAs derived from the urine collected throughout the world carry the archetype regulatory sequence or archetype-like regulatory sequences that deviate only slightly from the archetype.

Tominaga et al. (1992) examined whether JCV persisting in normal human kidney tissue contains the archetype regulatory region. Renal medulla, cortex, and tumor tissue from 32 patients bearing renal tumors were screened for JCV DNA by Southern blot hybridization. Viral DNA was detected in the medulla in 13 cases (41%), in the cortex in 2 cases (6%), but in none of the tumor tissue specimens. Representative JCV DNA-positive specimens were used for PCR amplification and sequence analysis of the JCV regulatory regions. Structures of the regulatory regions from all specimens were, with a few nucleotide variations, that of the archetype. These observations indicated that JCVs persisting in the kidney have the archetype regulatory region and that in adults these JCVs actively replicate and excrete progeny in the urine.

In summary, (1) the archetype regulatory sequence is highly conserved, in marked contrast to PML-type regulatory sequences; (2) it contains all sequences present in various PML-type regulatory sequences, with a single exception (see below); (3) JCV DNAs derived from the urine collected throughout the world carry the archetype regulatory sequence or archetype-like regulatory sequences that deviate only slightly from the archetype; and (4) JCVs persisting in the kidney have the archetype regulatory sequence. Thus, it is very likely that JCV with the archetype regulatory sequence (or archetype-like sequences deviating slightly from it) represents the JCV circulating in the human population.

3. ALIGNMENT OF VARIOUS PML-TYPE REGULATORY SEQUENCES WITH THE ARCHETYPE

Several complete DNA clones of PML-type JCVs have been obtained in the United States, Japan, and Germany, and the regulatory sequences of these clones have been clarified (Kato et al., 1994; Loeber and Dörries, 1988; Martin et al., 1985; Matsuda et al., 1987; Takahashi et al., 1992; Yogo et al., 1994). Furthermore, owing to the advent of the PCR technology, many regulatory region structures of PML-type JCVs have also been determined (Agostini et al., 1997a, 1998a; Ault and Stoner, 1993; Chima et al., 1999; Newman and

Frisque, 1997, 1999; Stoner et al. 1998; Wakutani et al., 1998). In addition, some regulatory sequences have been detected from CSF of PML patients for diagnostic purposes (Sugimoto et al., 1998). All of these regulatory sequences (77 in total) were aligned with the archetype sequence by placing duplicated segments on separate lines and leaving gaps where sequences were deleted relative to the archetype (Fig. 7.2).

Each of these rearranged regulatory sequences was unique, that is, no identical regulatory sequences have occurred in the brain or CSF of different PML patients. Nevertheless, they could be categorized into a few groups according to their structural features (Fig. 7.2). To classify the 77 rearranged forms we use here the system introduced by Ault and Stoner (1993). They divided the archetypal regulatory region into five blocks of sequences, A to F (Fig. 7.1), based on sequences in the archetype that are lacking from, or duplicated in, the Mad-1 regulatory region.

The first pattern, designated as ''long duplicate,'' shows duplication of region C and deletion of region D (Fig. 7.2A). The regulatory sequences with this pattern account for about half of the rearranged regulatory sequences shown in Figure 7.2. The second pattern, the ''short triplicate'' pattern, is characterized by having the first half of region C duplicated or triplicated, the second half of C and all of D deleted, and region E present in three or four copies (Fig. 7.2B). The main feature of the third pattern, ''D retaining,'' was retention of region D with a duplication of sequences before or after it (Fig. 7.2C). We also included regulatory sequences, retaining region D but not having any duplication, in the ''D-retaining'' group. Regulatory sequences with miscellaneous sequences not included in any of the three groups are shown in Figure 7.2D.

Some regulatory regions (designated as pseudo-archetypes) belonging to the ''D-retaining'' group had only deletions (see Fig. 7.2C). These deletions destroyed some transcriptional factor binding elements (Table 7.1). In contrast, these elements are conserved in archetype-like regulatory regions (see above) found in the JCV isolates from the urine of non-PML individuals throughout the world (Agostini et al., 1996; Chang et al., 1996a,b; Guo et al., 1996; Yogo et al., 1990, 1991a,b). Thus, pseudo-archetype regulatory regions can be distinguished from the archetype-like regulatory region.

4. ARCHETYPE SEQUENCE CAN GENERATE VARIOUS REARRANGED FORMS

The line drawings in Figure 7.2 indicate that each of the rearranged regulatory sequences detected in the brain or CSF of PML patients can be produced from the archetype by deletions and duplications (or, in one case, by recombination with a coding region of the JCV genome). For example, ''long duplicate'' structures usually contain one or two identical deletions, with exactly the same 5′ and 3′ end points. This can simply be explained by hypothesizing that, in the archetype sequence, deletion occurred first and the segment carrying the

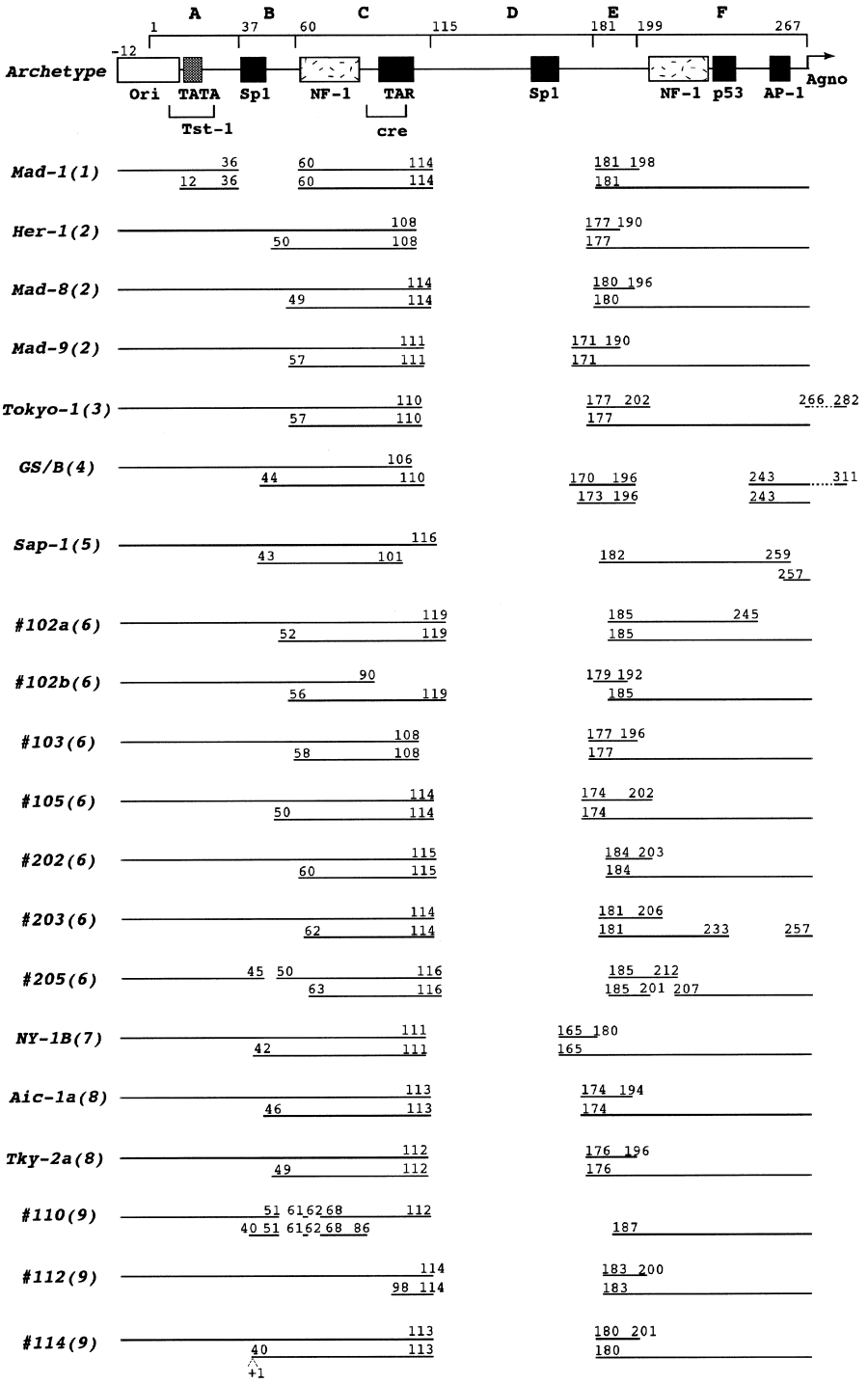

A
B
C
D
E
F
1
37
60
115
181
199
267
-12
Archetype
Ori
TATA
Sp1
NF-1
TAR
Sp1
NF-1
p53
AP-1
Agno
Tst-1
cre
Mad-1(1)
36
12 36
60 114
60 114
181 198
181
Her-1(2)
108
50 108
177 190
177
Mad-8(2)
114
49 114
180 196
180
Mad-9(2)
111
57 111
171 190
171
Tokyo-1(3)
110
57 110
177 202
266 282
177
GS/B(4)
106
44 110
170 196
173 196
243 311
243
Sap-1(5)
116
43 101
182 259
257
#102a(6)
119
52 119
185 245
185
#102b(6)
90
56 119
179 192
185
#103(6)
108
58 108
177 196
177
#105(6)
114
50 114
174 202
174
#202(6)
115
60 115
184 203
184
#203(6)
114
62 114
181 206
181 233 257
#205(6)
45 50 116
63 116
185 212
185 201 207
NY-1B(7)
111
42 111
165 180
165
Aic-1a(8)
113
46 113
174 194
174
Tky-2a(8)
112
49 112
176 196
176
#110(9)
51 61 62 68 112
40 51 61 62 68 86
187
#112(9)
114
98 114
183 200
183
#114(9)
113
40 113
+1
180 201
180

Figure 7.2A

deletion was subsequently duplicated (the example of Mad-1 is shown in Fig. 7.3A). Many ''long duplicate'' structures appear to have been complicated by further deletions or duplications. In general, ''short triplicate'' structures may have been produced by the duplication of segments containing previously generated deletions and duplications (the example of Matsue-1 is shown in Fig. 7.3B). Likewise, most of the rearranged regulatory sequences of ''D-retaining'' and miscellaneous groups (Fig. 7.3C,D), except for the NY-1A regulatory sequence, could have been generated from the archetype by deletions and duplications (or only by deletions). As shown in Figure 7.3C, it is very likely that the NY-1A regulatory sequence was generated by recombination between the archetype regulatory region and the T-antigen gene.

Martin et al. (1985) argued that the hypervariation of PML-type regulatory regions arose by complex alterations of the 98 bp repeat of Mad-1 DNA (Fig. 7.2). More recently, Monaco et al. (1998), who reported the detection of JCV DNA in human tonsil tissue, concluded that the Mad-1 regulatory sequence can rearrange to generate multiple genotypes observed in other infected host cells. Nevertheless, there is no simple way in which the various structures shown in Figure 7.2 can be generated from any of the arrangements other than the archetype.

5. SITE SELECTION FOR DNA BREAKAGE AND REJOINING

The presence of the major patterns of deletions and duplications (Fig. 7.2) suggests that there are preferred areas for DNA breakage and rejoining (Ault

Figure 7.2. Alignment of various PML-type regulatory sequences with the archetype. The structure of the archetypal regulatory region is schematically shown at the top. Regions A–F and some transcriptional factor binding sites are indicated. The origin of replication (Ori) and the start site of the late leader protein, agnoprotein (Agno), are indicated. The structures of various rearranged regulatory regions derived from the brain and CSF are shown below, with deletions relative to the archetype shown as gaps. On reading from left to right, when a repeat is encountered, the linear representation is displaced to the line below and to positions corresponding to the sequence of the archetype. Numbers indicate end points of segments present in each regulatory region. Arrowheads represent the insertion of sequences, the sizes of which are indicated below in base pairs. The regulatory regions classified as "long duplicate," "short triplicate," and "D retaining" are shown in **A**, **B**, and **C**, respectively, and those with miscellaneous sequences not included in any of the three groups are shown in **D**. The last three structures in **D** were tentatively designated as AC/Br-1, AC/Br-2, and AC/Br-3, and the last structure in **A** was tentatively designated as AH/Br because they were not named in the original paper (Newman and Frisque, 1999). References for regulatory regions are shown in parentheses by numbers 1, Frisque et al. (1984); 2, Martin et al. (1985); 3, Matsuda et al. (1987); 4, Loeber and Dörries (1988); 5, Takahashi et al. (1992); 6, Ault and Stoner (1993); 7, Yogo et al. (1994); 8, Kato et al. (1994); 9, Agostini et al. (1997a); 10, Newman and Frisque (1997); 11, Agostini et al. (1998a); 12, Wakutani et al. (1998); 13, Stoner et al. (1998); 14, Sugimoto et al. (1998); 15, Chima et al. (1999); 16, Newman and Frisque (1999).

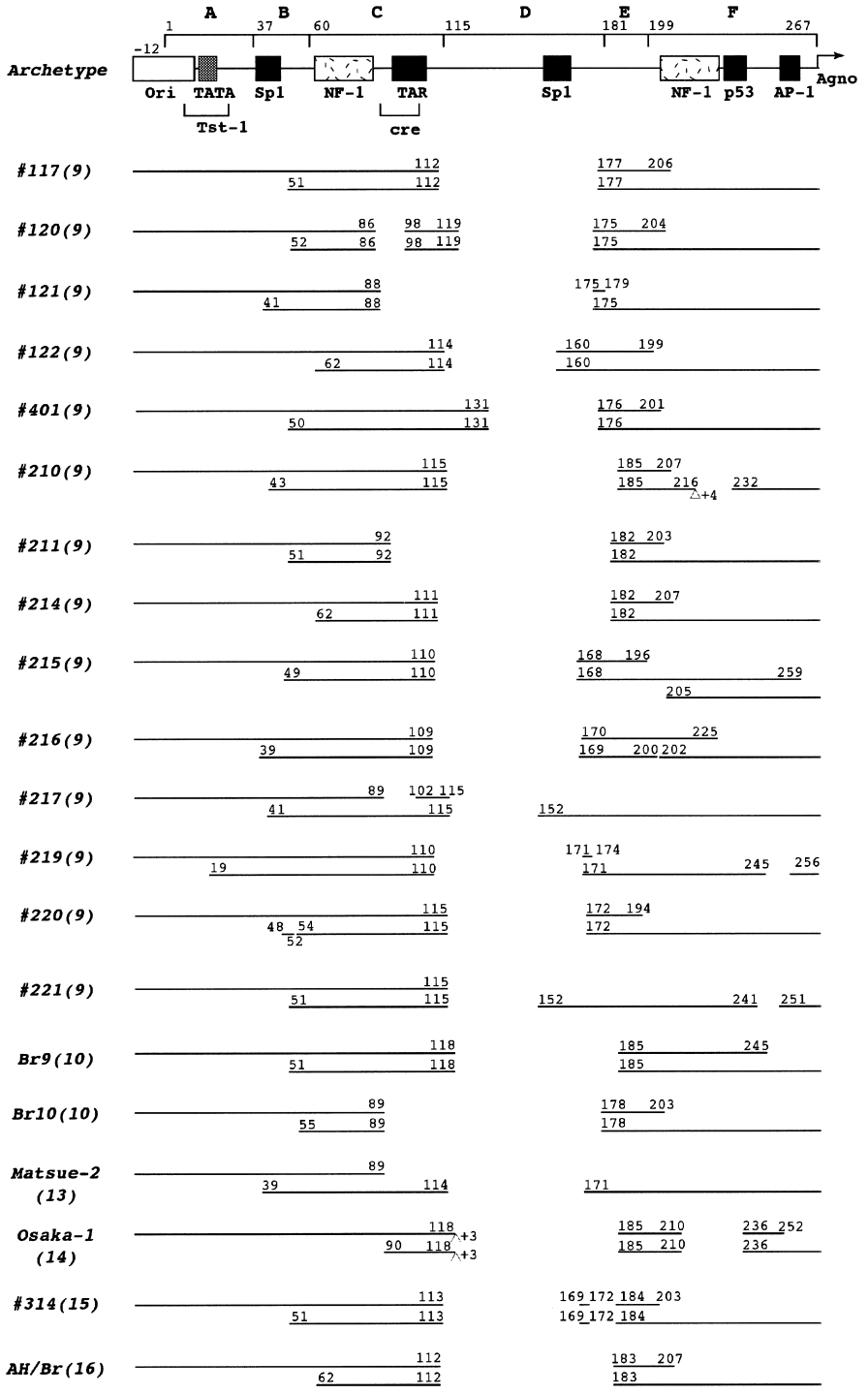


Figure 7.2A. (*Continued*)

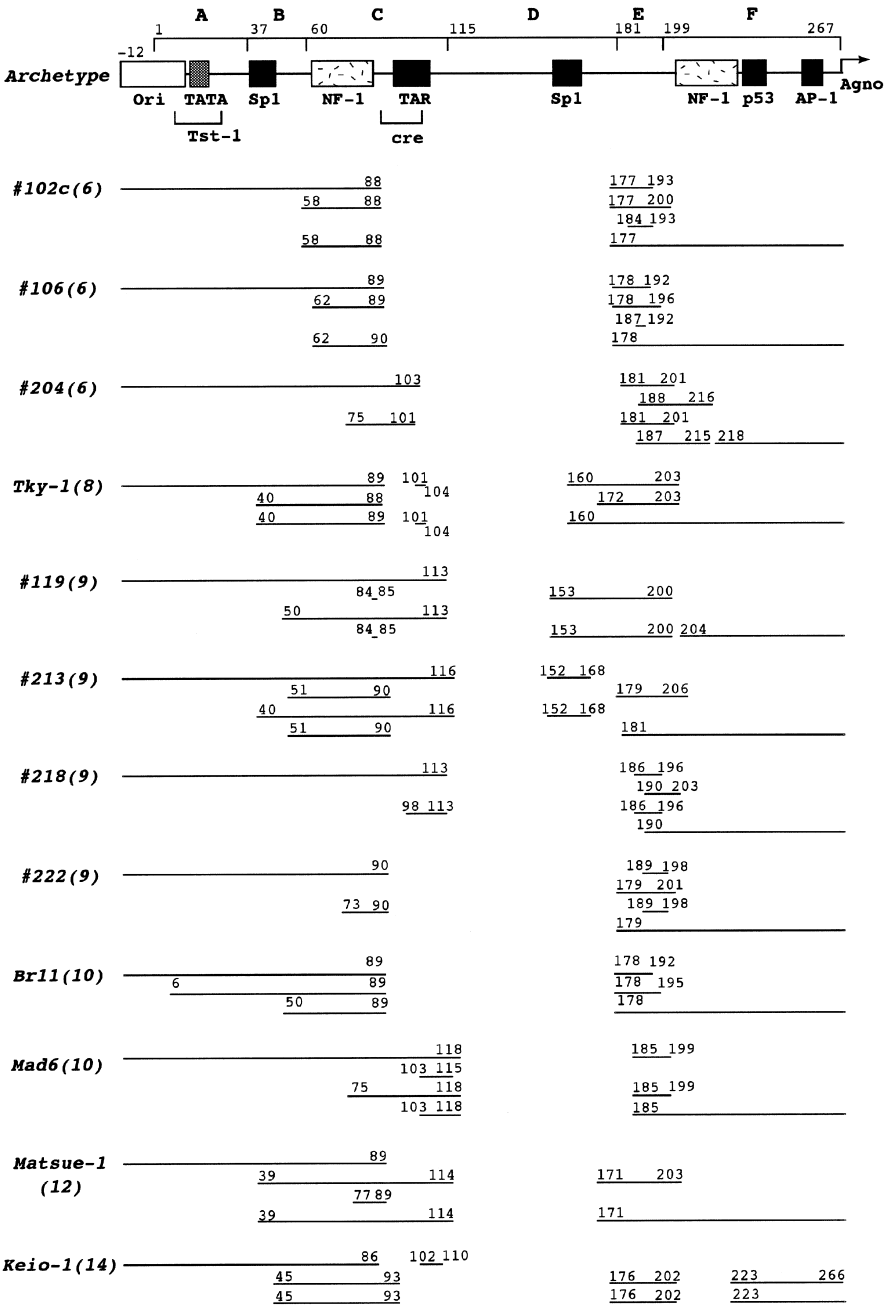

A
B
C
D
E
F
1
37
60
115
181
199
267
-12
Archetype
Ori
TATA
Spl
NF-1
TAR
Spl
NF-1
p53
AP-1
Agno
Tst-1
cre
#102c(6)
#106(6)
#204(6)
Tky-1(8)
#119(9)
#213(9)
#218(9)
#222(9)
Br11(10)
Mad6(10)
Matsue-1
(12)
Keio-1(14)

Figure 7.2B

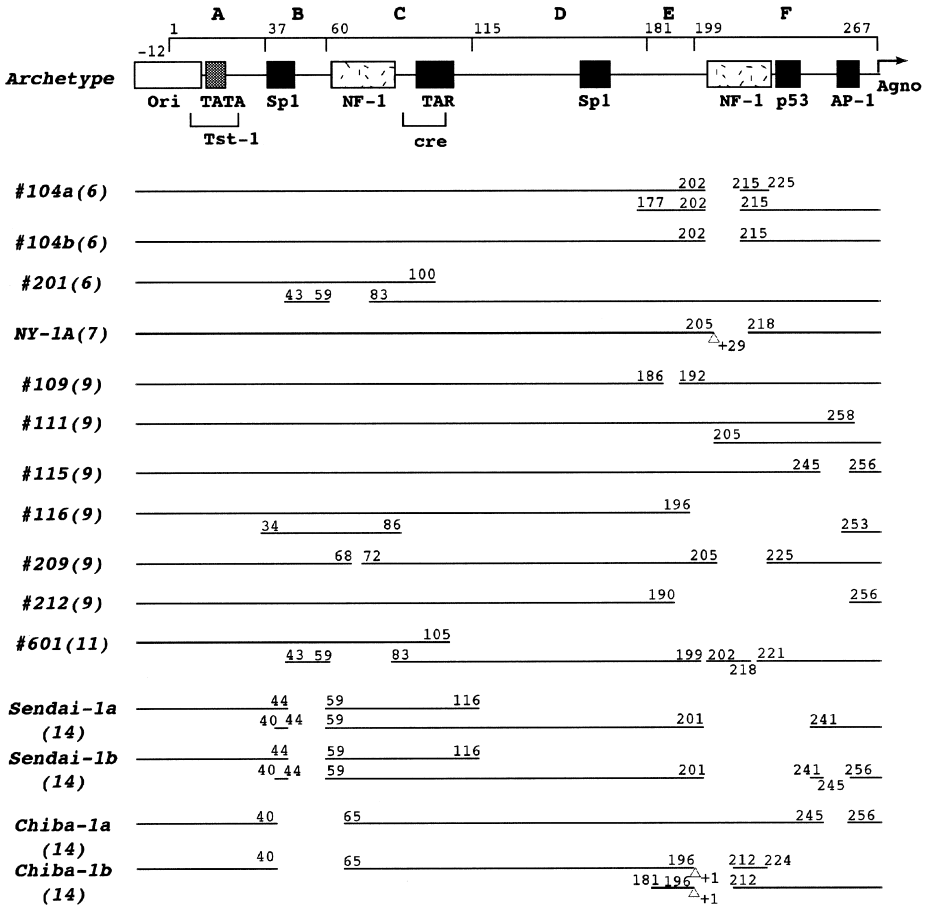


Figure 7.2C

and Stoner, 1993). Thus, most frequently targeted are the junctions of regions B/C, C/D, D/E, E/F, and the middle of region C. One reason for the selection of these particular areas is the pressure to retain sequences necessary for promoter function. The TATA box and the origin-proximal half of region C appear to be indispensable (Fig. 7.2). The latter contains an NF-1 binding site and a CRE-like element (Fig. 7.1). Other transcription factor recognition elements exist in other parts of the archetype regulatory region as well, but are not consistently retained (see Chapter 6).

The existence of preferred breakage and rejoining may be conferred by one of the cellular recombination activities, as discussed by Ault and Stoner (1993). Nevertheless, it is possible that junctions between B/C and C/D are not preferentially targeted, as suggested by Kitamura et al. (1994b). Thus, variants in

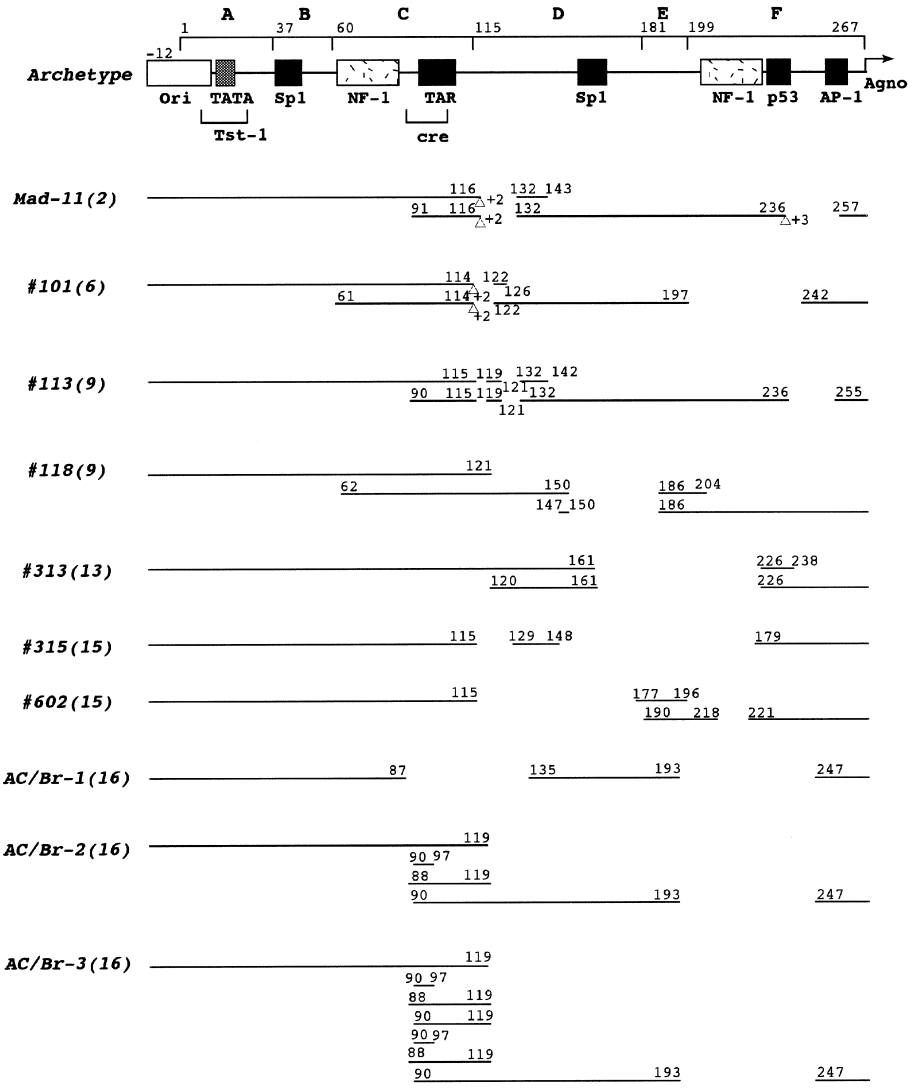


Figure 7.2D

which region C is duplicated and region D is deleted may be generated as a rare event. Once generated, however, these variants may become a major population, if they have selective replication advantage over the others in the target tissue (brain).

Ault and Stoner (1993) suggested that total length constraints may limit the lengths of segments to be duplicated or deleted. This factor may not be very

Table 7.1. Affected Elements in PML-Type Regulatory Regions Carrying Only Deletions

Isolate	Deletions[a]	Affected Elements[b]	Reference for Isolate
104b	nt 203–214	Second NF-1	Ault and Stoner (1993)
109	nt 187–191	Unknown	Agsotini et al. (1997a)
115	nt 246–255	AP-1	Agsotini et al. (1997a)
209	nt 69–71	First NF-1	Agsotini et al. (1997a)
	nt 206–224	Second NF-1, p53	
212	nt 191–255	Second NF-1, p53, AP-1	Agsotini et al. (1997a)
Chiba-1a	nt 41–64	First Sp-1, first NF-1	Sugimoto et al. (1998)
	nt 246–255	AP-1	

[a]Deletion end points are shown. See Figure 7.1 for nucleotide numbers.
[b]The position of each element is shown in Figure 7.1.

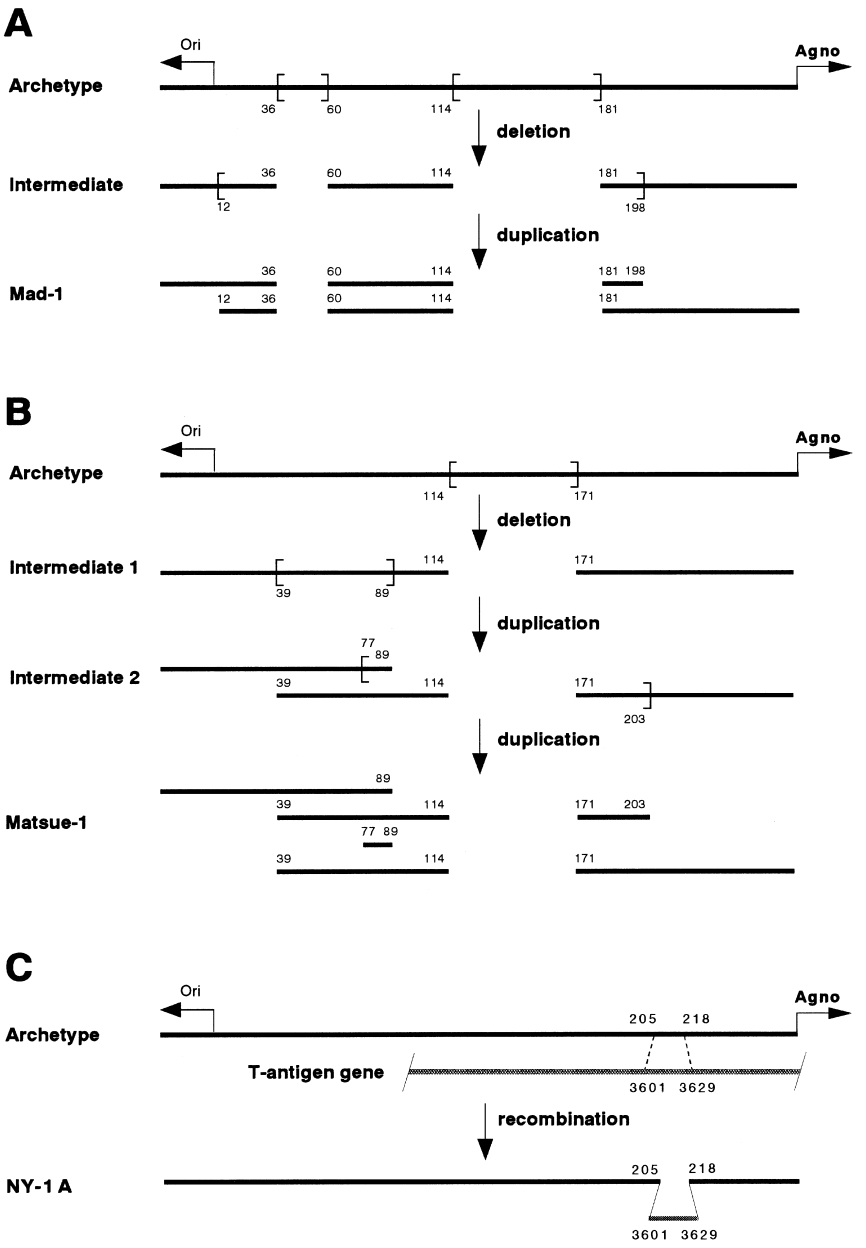
rigid, however, because the length of a rearranged regulatory region (213) was increased by 172 bp and that of another (212) was decreased by 65 bp (Fig. 7.3).

6. MOLECULAR MECHANISM OF REGULATORY REGION REARRANGEMENT

It remains unclear what molecular mechanism operates to generate rearranged regulatory regions. As described above, however, it is very likely that the NY-1A regulatory region was produced by nonhomologous recombination between two JCV DNA molecules. Whether JCV usually uses a host recombination system to cause deletions and duplications is not clear.

Yoshiike and Takemoto (1986) proposed an interesting mechanism of BK virus (BKV) regulatory region rearrangement. JCV and BKV are related polyomaviruses infecting only humans, and their overall genome structures are very similar (see Chapters 6 and 14). The archetype regulatory region was found in BKV earlier than in JCV (Rubinstein and Harley 1989; Rubinstein et al., 1987;

Figure 7.3. (**A**) A possible pathway that might have generated Mad-1 regulatory region. The two bracketed regions of the archetype were first deleted to generate the intermediate structure. The bracketed region of the intermediate was then duplicated to generate the Mad-1 regulatory region. (**B**) A possible pathway that might have generated the Matsue-1 regulatory region. The bracketed region of the archetype was first deleted to generate intermediate 1. The bracketed region of intermediate 1 was then duplicated to generate intermediate 2. Finally, the bracketed region of intermediate 1 was duplicated to generate Matsue-1 regulatory region. (**C**) A possible pathway that might have generated NY-1A regulatory region. Nonhomologous recombination occurred between the archetype regulatory region and the large T antigen gene at the indicated sites.


A
Ori
Agno
Archetype
36
60
114
181
deletion
Intermediate
36
60
114
181
12
198
duplication
Mad-1
36
60
114
181 198
12 36
60
114
181
B
Ori
Agno
Archetype
114
171
deletion
Intermediate 1
114
171
39
89
duplication
Intermediate 2
77 89
39
114
171
203
duplication
Matsue-1
89
39
114
171 203
77 89
39
114
171
C
Ori
Agno
Archetype
205 218
T-antigen gene
3601 3629
recombination
NY-1 A
205 218
3601 3629

Sugimoto et al., 1989, 1990; Yoshiike and Takemoto, 1986). The BKV archetype undergoes deletions and duplications, particularly during passage of viruses in cell culture. The mechanism suggested by Yoshiike and Takmoto (1986) is as follows. Recombination may occur between two newly synthesized daughter segments of a replicating DNA molecule at nonhomologous points. The resulting molecule will be a dimer composed of one molecule with deletion and the other with duplication. Dimers may be converted to monomers by homologous recombination that may subsequently occur at any two points within a dimer. It is possible that this mechanism also functions in JCV regulatory region rearrangement.

Another possible mechanism is an error in viral DNA replication. If a replicating point is shifted downstream along the template and replication is restarted, a deletion will be generated. Alternatively, if a replicating point goes back along the template and replication is restarted, a duplication will be generated. However, it is unlikely that this mechanism operates in JCV DNA replication that uses the strictly controlled host DNA replication system.

7. PML-TYPE JCV EVOLVE WITHIN PATIENTS

In the argument given above, we assumed that the genesis of JCV with rearranged regulatory sequences occurred within the patients. This assumption was examined by Iida et al. (1993). They constructed a phylogenetic tree for seven archetype and seven PML-type strains using DNA sequence data on the VP1 (major capsid protein) gene. However, it was recently shown that the whole-genome approach to phylogeny reconstruction offers significant improvement over earlier studies that were limited to partial JCV sequences (Hatwell and Sharp, 2000; Jobes et al., 1998). We therefore performed phylogenetic analysis of 28 JCV isolates, including 6 PML types, complete DNA sequences of which were reported previously (Agostini et al., 1998a,d; Frisque et al. 1994; Kato et al., 2000; Loeber and Dörries, 1988). A phylogenetic tree (Fig. 7.4) constructed by the neighbor-joining method (Saitou and Nei, 1987) revealed that the 28 isolates diverged into eight previously described genotypes (EU, Af1, Af2, SC, CY, MY, B1-b, and B1-c) (Guo et al., 1998; Jobes et al., 1998; Sugimoto et al. 1997). Five (EU, Af1, CY, MY, and B1-c) contained both archetype and PML-type isolates. Thus, we concluded that PML-type isolates are polyphyletic in origin and do not constitute a unique lineage. This conclusion supports the view that PML-type JCVs are generated from archetype JCVs during persistence in the host.

Of the three genotypes (Af2, SC, and B1-b) that contained only archetypes in the phylogenetic tree (Fig. 7.4), a PML-type JCV was detected from the genotype Af2 (Type 3) by analyzing its partial genome sequences (Stoner et al., 1998). However, there have been no reports of the isolation of PML-type JCVs belonging to genotypes Af3, SC, B1-a, B1-b or B1-d. It remains to be clarified whether archetype JCVs belonging to these genotypes are resistant to

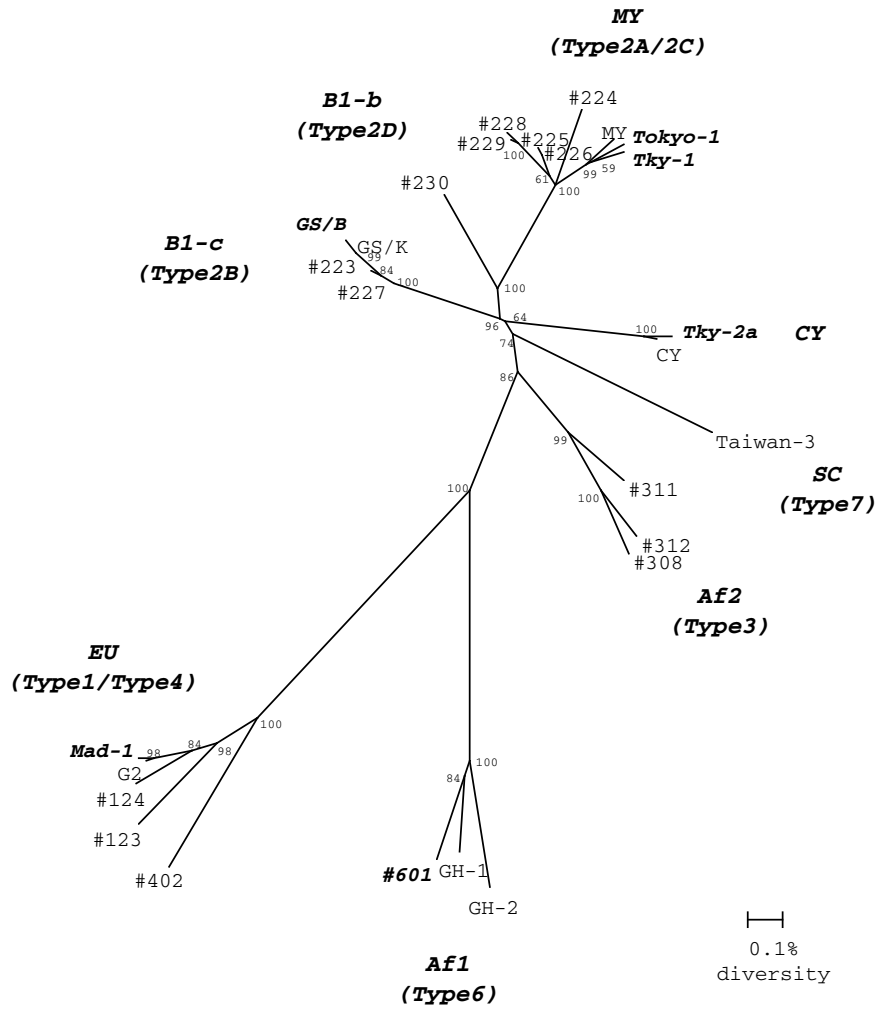


Figure 7.4. Evolutionary tree relating archetype and PML-type JCV isolates. From reported complete JCV DNA sequences, an unrooted evolutionary tree was constructed using the neighbor-joining method (Saitou and Nei, 1987). PML-type isolates are shown in boldface and italics. A genotype is indicated for each cluster according to the classification system of Sugimoto et al. (1997) and Guo et al. (1998) and that of Jobes et al. (1998) (the latter is indicated in parentheses). Numbers at the nodes show bootstrap confidence levels obtained by 100 replicates. The analyzed sequences are from Agostini et al. (1997b, 1998a,b,d), Kato et al. (2000), Loeber and Dörries (1988), and Ou et al. (1997).

conversion to PML-type counterparts. In this connection, it is relevant to mention the study by Agostini et al. (1998c), who reported that the proportion of the JCV genotype B1-c (or Type 2B) in the PML brain (36%) was significantly increased relative to its occurrence in control urine samples (5.9%). This finding suggested that the change of the JCV regulatory region may be influenced by the genotypes of JCV themselves or by the human populations carrying JCV.

8. LACK OF DISEASE-SPECIFIC AMINO ACID CHANGES IN THE VIRAL PROTEINS OF PML-TYPE JCVS

Although the structural differences in the regulatory region between PML-type and archetype isolates are striking, it is possible that PML-type JCVs undergo some functionally significant mutations in their protein-coding regions. Loeber and Dörries (1988) molecularly isolated two JCV variants in the same PML patient, one from the kidney (variant GS/K) and the other from the brain (variant GS/B). GS/K carried an archetype regulatory region, while GS/B carried a unique rearrangement that may have been produced from the GS/K regulatory region. There were two nucleotide differences in their coding regions, one in the VP1 gene and the other in a portion shared by the small t and large T antigen genes. Although the latter nucleotide difference caused no amino acid difference, the former caused a change from Leu to GS/K to Phe in GS/B. This amino acid change does not appear to be associated with PML because another PML-type isolate (Mad-1) did not carry this change. Kato et al. (2000) further examined whether PML-associated amino acid changes occurred in the viral proteins of PML-type JCV. Amino acid sequences of individual viral proteins were deduced from complete DNA sequences and were compared among 16 isolates (6 PML types and 10 archetypes). From the data obtained, it was concluded that PML-associated amino acid changes did not occur in the viral proteins of PML-type JCV.

9. BIOLOGICAL SIGNIFICANCE OF REGULATORY REGION REARRANGEMENT

It has been postulated that the nonpathogenic archetype form is converted to the pathogenic PML-type, which acquires a higher growth capacity in glial cells (i.e., oligodendrocytes) in the brain (Iida et al., 1993; Loeber and Dörries, 1988; Shah, 1996; Yogo et al., 1990, 1991b). A few studies were carried out to examine this hypothesis.

Sock et al. (1996) compared, in glial and nonglial cells, the expression of a reporter gene driven by early- and late-gene promoters present in three typical archetype regulatory regions and two PML-type regulatory regions (Mad-1 and GS/B) with ''long duplicate'' structures (Fig. 7.2A). Surprisingly, early- and late-gene promoters from not only PML-type but also archetype strains exhib-

ited significantly higher activity in glial than in nonglial cells. Sock et al. (1996) concluded that the archetype is more or less competent to infect the brain without the need for modification of the viral regulatory region. This conclusion argues against any hypothesis postulating that regulatory region rearrangements would be required for infection in oligodendrocytes in the brain. This conclusion, however, does not exclude the possibility that JCV is endowed with more active promoters by regulatory region rearrangement that may occur during viral replication in vivo in glial cells.

Ault (1997) compared the replicating and promoter activities in human glial cells between one archetype and four PML-type regulatory regions (Mad-1, Nos. 104a, 203, and 102). According to the classification system of Ault and Stoner (1993), the regulatory regions of Mad-1/203, 102, and 104a belong to ''long duplicate,'' ''short triplicate,'' and ''D retaining'' (see Fig. 7.2). All five regulatory regions demonstrated similar levels of DNA replicating activity (this was also reported by Sock et al. [1996] and Daniel et al. [1996]). Surprisingly, the reporter gene expression differed within a fivefold range, and the archetype was intermediate in strength to the PML-type regulatory regions. This finding indicated that some PML types are more active, but some are less active, than the archetype promoter.

10. DUAL REARRANGEMENT MODEL

Major et al. (1992) proposed a hypothesis that some peripheral blood lymphocytes (PBL) harbor JCV in a latent form and that upon activation they carry JCV to the brain. Extending this hypothesis, we propose a dual rearrangement model that can explain both the presence of a variety of PML-type regulatory regions (Fig. 7.2) and the findings reported by Sock et al. (1996) and Ault (1997).

After primary infection, JCV multiplies without severe restriction in infected hosts. This viral growth generates JCV variants with rearranged regulatory regions. Of these variants, those with decreased growth capacity persist in PBL without being detected by the immune surveillance established by stimulation of initial viral growth. Rearranged regulatory regions produced by the initial event are probably those carrying only deletions.

Upon a decrease in host immune activity, the persisting JCV variants begin to replicate actively, resulting in expansion of JCV infection in the peripheral blood. Some of the infected lymphocytes enter the central nervous system (CNS), and transmission of JCV from lymphocytes to more favorable cells (i.e., oligodendrocytes) occurs. In the course of viral replication in the CNS, secondary regulatory region rearrangement frequently, but not always, occurs to generate variants with more complicated regulatory regions that now acquire more active promoters for early and late transcription. This highly pathogenic variant causes rapid expansion of brain lesions. Rearranged regulatory regions

produced by the second event in the CNS include those belonging to ''long duplicate'' and ''short triplicate'' groups (Fig. 7.3A,B).

11. CONCLUSIONS

The findings described in this chapter can be summarized as the archetype concept as formulated below:

1. JCV with the archetype regulatory sequence is circulating in the human population.
2. The archetype regulatory sequence is highly conserved, in marked contrast to the hypervariable regulatory sequences of PML-derived isolates.
3. Each of the PML-type regulatory sequences is produced from the archetype by deletion and duplication or by deletion alone.
4. The shift of the regulatory region from archetype to PML type occurs during persistence in the host.
5. PML-type JCVs never return to the human population.

With respect to the biologic significance of regulatory region rearrangement, we have presented a dual rearrangement model postulating two independent rearrangements, one occurring in an immunocompetent state and the other in an immunosuppressed state. This model remains to be tested in future studies.

REFERENCES

Agostini HT, Ryschkewitsch CF, Stoner GL (1996): Genotype profile of human polyomavirus JC excreted in urine of immunocompetent individuals. J Clin Microbiol 34:159–164.

Agostini HT, Ryschkewitsch CF, Singer EJ, Stoner GL (1997a): JC virus regulatory region rearrangements and genotypes in progressive multifocal leukoencephalopathy: Two independent aspects of virus variation. J Gen Virol 78:659–664.

Agostini HT, Ryschkewitsch CF, Brubaker GR, Shao J, Stoner GL (1997b): Five complete genomes of JC virus Type 3 from Africans and African Americans. Arch Virol 142:637–655.

Agostini HT, Ryschkewitsch CF, Stoner GL (1998a): Complete genome of a JC virus genotype Type 6 from the brain of an African American with progressive multifocal leukoencephalopathy. J Hum Virol 1:267–272.

Agostini HT, Ryschkewitsch CF, Stoner GL (1998b): JC virus Type 1 has multiple subtypes: Three new complete genomes. J Gen Virol 79:801–805.

Agostini HT, Ryschkewitsch CF, Singer EJ, Baumhefner RW, Stoner GL (1998c): JC virus Type 2B is found more frequently in brain tissue of progressive multifocal leukoencephalopathy patients than in urine from controls. J Hum Virol 1:200–206.

Agostini HT, Shishido-Hara Y, Baumhefner RW, Singer EJ, Ryschkewitsch CF, Stoner GL (1998d): JC virus Type 2: Definition of subtypes based on DNA sequence analysis of ten complete genomes. J Gen Virol 79:1143–1151.

Ault GS (1997): Activity of JC virus archetype and PML-type regulatory regions in glial cells. J Gen Virol 78:163–169.

Ault GS, Stoner GL (1993): Human polyomavirus JC promoter/enhancer rearrangement patterns from progressive multifocal leukoencephalopathy brain are unique derivatives of a single archetypal structure. J Gen Virol 74:1499–1507.

Berger JR, Major EO (1999): Progressive multifocal leukoencephalopathy. Semin Neurol 19:193–200.

Chang D, Sugimoto C, Wang M, Tsai RT, Yogo Y (1999): JC virus genotypes in a Taiwan aboriginal tribe (Bunun): Implications for its population history. Arch Virol 144:1081–1090.

Chang D, Tsai RT, Wang M, Ou WC (1996a): Different genotypes of human polyomaviruses found in patients with autoimmune diseases in Taiwan. J Med Virol 48:204–209.

Chang D, Wang M, Ou WC, Lee MS, Ho HN, Tsai RT (1996b): Genotypes of human polyomaviruses in urine samples of pregnant women in Taiwan. J Med Virol 48: 95–101.

Chima SC, Agostini HT, Ryschkewitsch CF, Lucas SB, Stoner GL (1999): Progressive multifocal leukoencephalopathy and JC virus genotypes in West African patients with acquired immunodeficiency syndrome: A pathologic and DNA sequence analysis of 4 cases. Arch Pathol Lab Med 123:395–403.

Daniel AM, Swenson JJ, Mayreddy RPR, Khalili K, Frisque RJ (1996): Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. Virology 216:90–101.

Flaegstad T, Sundsfjord A, Arthur RR, Pedersen M, Traavik T, Subramani S (1991): Amplification and sequencing of the control regions of BK and JC virus from human urine by polymerase chain reaction. Virology 180:553–560.

Frisque RJ, Bream GL, Cannella MT (1984): Human polyomavirus JC virus genome. J Virol 51:458–469.

Gallia GL, Houff SA, Major EO, Khalili K (1997): Review: JC virus infection of lymphocytes—Revisited. J Infect Dis 176:1603–1609.

Grinnell BW, Padgett BL, Walker DL (1983): Comparison of infectious JC virus DNAs cloned from human brain. J Virol 45:299–308.

Guo J, Sugimoto C, Kitamura T, Ebihara H, Kato A, Guo Z, Liu J, Zheng SP, Wang YL, Na YQ, Suzuki M, Taguchi F, Yogo Y. (1998): Four geographically distinct genotypes of JC virus are prevalent in China and Mongolia: Implications for the racial composition of modern China. J Gen Virol 79:2499–2505.

Guo J, Kitamura T, Ebihara H, Sugimoto C, Kunitake T, Takehisa J, Na YQ, Al-Ahdal MN, Hallin A, Kawabe K, Taguchi F, Yogo Y (1996): Geographical distribution of the human polyomavirus JC virus types A and B and isolation of a new type from Ghana. J Gen Virol 77:919–927.

Hatwell JN, Sharp PM (2000): Evolution of human polyomavirus JC. J Gen Virol 81: 1191–1200.

Iida T, Kitamura T, Guo J, Taguchi F, Aso Y, Nagashima K, Yogo Y (1993): Origin of JC polyomavirus variants associated with progressive multifocal leukoencephalopathy. Proc Natl Acad Sci USA 90:5062–5065.

Jobes DV, Chima SC, Ryschkewitsch CF, Stoner GL (1998): Phylogenetic analysis of 22 complete genomes of the human polyomavirus JC virus. J Gen Virol 79:2491–2498.

Kato K, Guo J, Taguchi F, Daimaru O, Tajima M, Haibara H, Matsuda J, Sumiya M, Yogo Y (1994): Phylogenetic comparison between archetypal and disease-associated JC virus isolates in Japan. Jpn J Med Sci Biol 47:167–178.

Kato A, Sugimoto C, Zheng H-Y, Kitamura T, Yogo Y (2000): Lack of disease-specific amino acid changes in the viral proteins of JC virus isolates from the brain with progressive multifocal leukoencephalopathy. Arch Virol 145:2173–2182.

Kitamura T, Aso Y, Kuniyoshi N, Hara K, Yogo Y (1990): High incidence of urinary JC virus excretion in nonimmunosuppressed older patients. J Infect Dis 161:1128–1133.

Kitamura T, Kunitake T, Guo J, Tominaga T, Kawabe K, Yogo Y (1994a): Transmission of the human polyomavirus JC virus occurs both within the family and outside the family. J Clin Microbiol 32:2359–2363.

Kitamura T, Satoh K, Tominaga T, Taguchi F, Tajima A, Suzuki K, Aso Y, Yogo Y (1994b): Alteration in the JC polyomavirus genome is enhanced in immunosuppressed renal transplant patients. Virology 198:341–345.

Loeber G, Dörries K (1988). DNA rearrangements in organ-specific variants of polyomavirus JC strain GS. J Virol 62:1730–1735.

Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR (1992): Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev 5:49–73.

Markowitz RB, Eaton BA, Kubik MF, Latorra D, McGregor JA, Dynan WS (1991): BK virus and JC virus shed during pregnancy have predominantly archetypal regulatory regions. J Virol 65:4515–4519.

Martin JD, King DM, Slauch JM, Frisque RJ (1985): Differences in regulatory sequences of naturally occurring JC virus variants. J Virol 53:306–311.

Matsuda M, Jona M, Yasui K, Nagashima K (1987): Genetic characterization of JC virus Tokyo-1 strain, a variant oncogenic in rodents. Virus Res 7:159–168.

Monaco MCG, Jensen PN, Hou J, Durham LC, Major EO (1998): Detection of JC virus DNA in human tonsil tissue: Evidence for site of initial viral infection. J Virol 72:9918–9923.

Newman JT, Frisque RJ (1997): Detection of archetype and rearranged variants of JC virus in multiple tissues from a pediatric PML patient. J Med Virol 52:243–252.

Newman JT, Frisque RJ (1999): Identification of JC virus variants in multiple tissues of pediatric and adult PML patients. J Med Virol 58:79–86.

Ou WC, Tsai RT, Wang M, Fung CY, Hseu TH, Chang D (1997): Genomic cloning and sequence analysis of Taiwan-3 human polyomavirus JC virus. J Formos Med Assoc 96:511–516.

Padgett BL, Walker DL (1973): Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J Infect Dis 127:467–470.

Padgett BL, Walker DL (1976): New human papovaviruses. Prog Med Virol 22:1–35.

Padgett BL, Walker DL, Zu Rhein GM, Eckroade RJ, Dessel BH (1971): Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet 1:1257–1260.

Rentier-Delrue F, Lubiniecki A, Howley PM (1981): Analysis of JC virus DNA purified directly from human progressive multifocal leukoencephalopathy brains. J Virol 38: 761–769.

Rubinstein R, Harley EH (1989): BK virus DNA cloned directly from human urine confirms an archetypal structure for the transcriptional control region. Virus Genes 2:157–165.

Rubinstein R, Pare N, Harley EH (1987). Structure and function of the transcriptional control region of nonpassaged BK virus. J Virol 61:1747–1750.

Saitou N, Nei M (1987): The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Shah KV (1996): In Fields BN, Knipe DM, Howley PM, Eds. Fields Virology. Lippincott-Raven: New York, pp 2027–2043.

Sock E, Renner K, Feist D, Leger H, Wegner M (1996): Functional comparison of PML-type and archetype strains of JC virus. J Virol 70:1512–1520.

Stoner GL, Agostini HT, Ryschkewitsch CF, Mazlo M, Gullotta F, Wamukota W, Lucas S (1998): Detection of JC virus in two African cases of progressive multifocal leukoencephalopathy including identification of JCV Type 3 in a Gambian AIDS patient. J Med Microbiol 47:733–742.

Sugimoto C, Hara K, Taguchi F, Yogo Y (1989): Growth efficiency of naturally occurring BK virus variants in vivo and in vitro. J Virol 63:3195–3199.

Sugimoto C, Hara K, Taguchi F, Yogo Y (1990): Regulatory DNA sequence conserved in the course of BK virus evolution. J Mol Evol 31:485–492.

Sugimoto C, Ito D, Tanaka K, Matsuda H, Saito H, Sakai H, Sakai H, Fujihara K, Itoyama Y, Yamada T, Kira J, Matsumoto R, Mori M, Nagashima K, Yogo Y (1998): Amplification of JC virus regulatory DNA sequences from cerebrospinal fluid: Diagnostic value for progressive multifocal leukoencephalopathy. Arch Virol 143: 249–262.

Sugimoto C, Kitamura T, Guo J, Al-Ahdal MN, Shchelkunov SN, Otova B, Ondrejka P, Chollet J-Y, El-Safi S, Ettayebi M, Grésenguet G, Kocagöz T, Chaiyarasamee S, Thant KZ, Thein S, Moe K, Kobayashi N, Taguchi F, Yogo Y (1997): Typing of urinary JC virus DNA offers a novel means of tracing human migrations. Proc Natl Acad Sci USA 94:9191–9196.

Takahashi H, Yogo Y, Furuta Y, Takada A, Irie T, Kasai M, Sano K, Fujioka Y, Nagashima K (1992): Molecular characterization of a JC virus (Sap-1) clone derived from a cerebellar form of progressive multifocal leukoencephalopathy. Acta Neuropathol 83:105–112.

Tominaga T, Yogo Y, Kitamura T, Aso Y (1992): Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. Virology 186:736–741.

Tsai RT, Wang M, Ou WC, Lee YL, Li SY, Fung CY, Huang YL, Tzeng TY, Chen Y, Chang D (1997): Incidence of JC viruria is higher than that of BK viruria in Taiwan. J Med Virol 52:253–257.

Wakutani Y, Shimizu Y, Miura H, Nakashima K, Nakano T, Ohama E, Sugimoto C, Yogo Y, Kobayashi Y, Nagashima K (1998): A case of brain-biopsy proven progressive multifocal leukoencephalopathy: Pathological findings and analysis of JC virus regulatory region. Neuropathology 18:347–351.

Yogo Y, Guo J, Iida T, Satoh K, Taguchi F, Takahashi H, Hall WW, Nagashima K (1994): Occurrence of multiple JC virus variants with distinctive regulatory sequences in the brain of a single patient with progressive multifocal leukoencephalopathy. Virus Genes 8:99–105.

Yogo Y, Iida T, Taguchi F, Kitamura T, Aso Y (1991a): Typing of human polyomavirus JC virus on the basis of restriction fragment length polymorphisms. J Clin Microbiol 29:2130–2138.

Yogo Y, Kitamura T, Sugimoto C, Ueki T, Aso Y, Hara K, Taguchi F (1990): Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J Virol 64:3139–3143.

Yogo Y, Kitamura T, Sugimoto C, Hara K, Iida T, Taguchi F, Tajima A, Kawabe K, Aso Y (1991b): Sequence rearrangement in JC virus DNAs molecularly cloned from immunosuppressed renal transplant patients. J Virol 65:2422–2428.

Yoshiike K, Takemoto KK (1986): Studies with BK virus and monkey lymphtropic papovavirus. The Papovaviridae, Volume 1, The Polyomaviruses; Salzman NP, Ed.; Plenum Press: New York, pp 295–326.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

8

SYNTHESIS AND ASSEMBLY OF POLYOMAVIRUS VIRIONS

YUKIKO SHISHIDO-HARA, M.D., PH.D., and
KAZUO NAGASHIMA, M.D., PH.D.

1. INTRODUCTION

The polyomaviruses are nonenveloped viruses whose capsid is composed of three capsid proteins: a major capsid protein, VP1, and minor capsid proteins, VP2 and VP3. The human polyomaviruses, JC (JCV) and BK (BKV) are closely related to simian virus 40 (SV40) and distantly related to murine polyoma virus. By X-ray crystallography, it has been shown that SV40 and murine polyoma virus have a T = 7d icosahedral structure, which is composed of 72 pentamers of VP1, with VP2 and VP3 inside in a specific stoichiometry (Liddington et al., 1991; Stehle et al., 1994, 1996; Stehle and Harrison, 1997).

JCV infects oligodendrocytes of human brain tissues and causes progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disorder in the central nervous system. By electron microscopy, JCV has been identified as round particles and filamentous forms in the nuclei of infected oligodendrocytes (Fig. 8.1) (Silverman and Rubinstein, 1965; Zu Rhein and Chou, 1965). Distribution and arrangement of the viral particles are variable in cells. The round JCV particles, which are relatively uniform in size, are occasionally arranged in crystalloid array, but in other cases they are distributed randomly. Filamentous forms, which are about one-half to two-thirds the diameter of the round

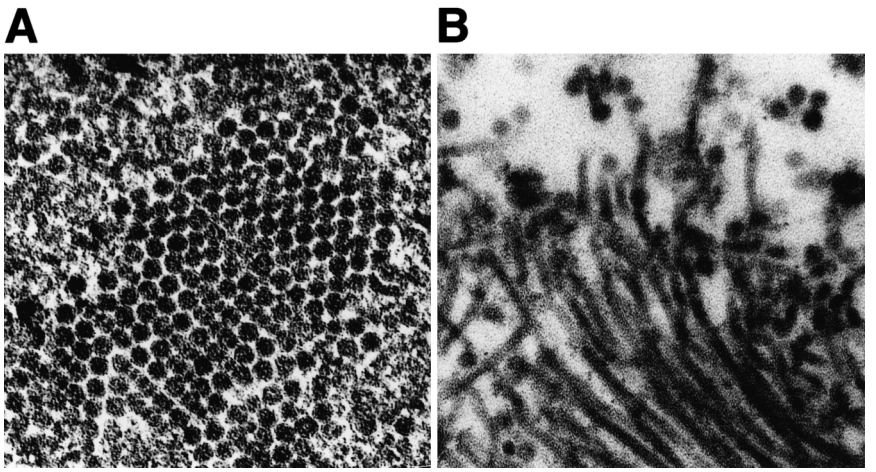


Figure 8.1. Ultrastructural features of JCV virions identified in PML patients' brains. (**A**) JCV round particles arranged in crystalloid array. JCV forms clusters in which the round virions are regularly arranged in a crystalloid array. Within the nucleus, clusters of regularly arranged round virions are isolated or fused with neighboring clusters. The round virions are quite homogeneous in size. (Courtesy of Drs. S. Takeda and H. Takahashi.) (**B**) JCV in filamentous forms. The filamentous forms are more variable in size than the round particles and arranged in strands, in spirals, or scattered irregularly with round particles. ×62,000.

particles, are arranged in strands, in spirals, or scattered irregularly with round particles. In some cells, both round and filamentous forms are distributed near the nuclear membrane. However, structures of the JCV virions and the mechanisms for their assembly are mostly unknown. The precise structures of the round and filamentous profiles have not been analyzed in a higher resolution. During the late stage of the virus replication cycle, the capsid proteins can be synthesized from alternatively spliced RNAs, transported from the cytoplasm to the nucleus, and assembled into progeny virions. Although the round particles are likely composed of the three capsid proteins, VP1, VP2, and VP3, in a specific stoichiometry, it is not known how expression of these component capsid proteins is regulated for efficient assembly into virions.

Recently, we reported unique features of JCV during the late stage of the virus replication cycle (Shishido-Hara et al., 2000). In cell culture systems, JCV replicates more slowly and less efficiently than BKV, SV40, and murine polyoma virus. This slow and inefficient replication of JCV has been a major barrier in studying the late stage of the virus replication cycle. To overcome this difficulty, we have developed a highly efficient eukaryotic expression system for the JCV capsid proteins by using pcDL-SRα296 (Takebe et al., 1988) and COS-7 cells (Gluzman, 1981). With this system, we have elucidated unique features of JCV in production of progeny virions. In this chapter, we review the regulated expression of the JCV capsid proteins, their nuclear transport,

and assembly of virions. In comparison with SV40 and murine polyoma virus, the unique features of JCV are described, and possible future studies in elucidating the pathogenesis of PML are discussed.

2. THE JCV LATE PROTEINS: AGNOPROTEIN, VP1, VP2, AND VP3

JCV has a circular DNA genome, which includes two coding regions: the early region and the late region. In the late region, JCV encodes the four late proteins: agnoprotein, VP1, VP2, and VP3 (Fig. 8.2). Agnoprotein is a short peptide, which is encoded upstream of the capsid proteins. Agnoprotein is encoded in JCV, BKV (Rinaldo et al., 1998), and SV40 (Jay et al., 1981). However, agnoprotein has not been reported in murine polyoma virus. The major capsid protein VP1 and the minor capsid proteins VP2 and VP3 are encoded in an overlapping manner. Downstream of the coding sequence for the agnoprotein, the coding sequence for VP2 is encoded, with that for VP3 completely overlapped in the 3′ terminus in the same reading frames. Therefore, the coding sequence for VP3 is identical to two-thirds of the 3′ terminal sequence for VP2. The 3′ termini of the coding sequences for VP2 and VP3 partly overlap the 5′ terminus of that for VP1 in different reading frames.

JCV Tokyo-1 strain has been isolated from the brain tissue of a Japanese patient with PML (Nagashima et al., 1981; Matsuda et al., 1987). JCV Tokyo-1 has a genomic DNA that is 5128 bp in length (Shishido-Hara et al., 2000). The amino acid sequences of the four late proteins have been deduced based on the sequence homology to BKV and SV40: agnoprotein, 71 amino acids, nt 275–490; VP1, 354 amino acids, nt 1467–2531; VP2, 344 amino acids, nt 524–1558; and VP3, 225 amino acids, nt 881–1558. Antibodies against these four late proteins were prepared by immunizing with synthetic peptides whose sequences are identical to a part of the amino acid sequences deduced from the nucleotide sequence data. With these antibodies, expression of the late proteins was analyzed in PML brain tissues by immunohistochemistry. JCV agnoprotein is detected predominantly in the cytoplasm of infected oligodendrocytes (Fig. 8.3A). Subcellular distribution of JCV agnoprotein is similar to

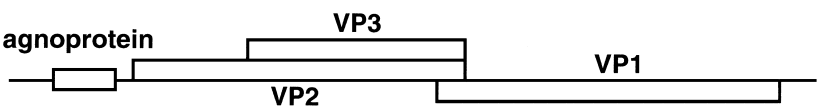


Figure 8.2. JCV genomic organization in the late region encoding the four late proteins: agnoprotein, VP1, VP2, and VP3. The coding sequence for agnoprotein is present in the 5′ end of the late region, followed by those for the three capsid proteins VP1, VP2, and VP3. The coding sequences for the capsid proteins are encoded in an overlapping manner. The coding sequence for VP2 completely includes that for VP3 in the 3′ terminus in the same reading frames. The 3′ termini of the coding sequences for VP2 and VP3 partly overlap the 5′ terminus of VP1 in different reading frames. Agnoprotein is present in JCV, BKV, and SV40, but not in murine polyoma virus.

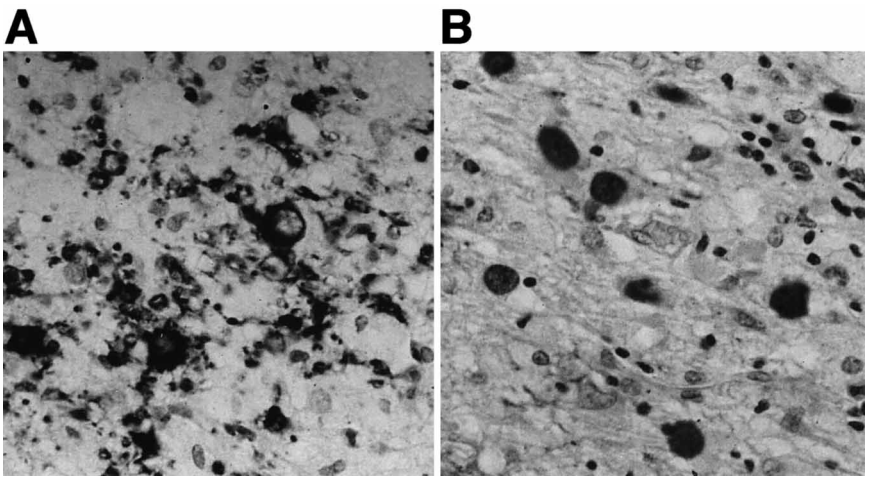


Figure 8.3. Immunostaining of PML brain tissues by using peptide antibodies against agnoprotein and VP1. (**A**) Expression of agnoprotein. Agnoprotein is widely expressed in the glial cytoplasmic processes in the demyelinating PML lesions. (**B**) Expression of VP1. VP1 was detected mostly in the nuclei of the infected glial cells by using the peptide antibodies against the residues in the potential loop structures (see text). Similarly, expression of VP2 or VP3 was also detected mostly in the nuclei of the infected cells.

that of BKV agnoprotein (Rinaldo et al., 1998) and SV40 agnoprotein (Nomura et al., 1983). All three capsid proteins, VP1, VP2, and VP3, are detected predominantly in the nucleus (Fig. 8.3B). Expression of VP2 and that of VP3 are not yet distinguished, because the antibody against VP3 also detects VP2, due to the identical amino acid sequence for VP3 to the carboxy-terminal sequence for VP2. However, these data consistently indicate that JCV encodes the four proteins, agnoprotein, VP1, VP2, and VP3, in the late region and that their amino acid sequences are consistent with those deduced from the nucleotide sequence.

3. AMINO ACID SEQUENCE COMPARISON OF THE LATE PROTEINS FOR JCV TO THOSE FOR THE OTHER POLYOMAVIRUSES: POTENTIAL STRUCTURES AND BIOLOGIC FUNCTIONS OF THE JCV LATE PROTEINS

Although the precise structure is not yet known for JCV virions, it is likely that JCV has some common features to SV40 and murine polyoma virus. The crystal structures of SV40 and murine polyoma virus have been reported (Rayment et al., 1982; Liddington et al., 1991; Stehle et al., 1994, 1996; Yan et al., 1996). In both viruses, the outer shell of the capsid is composed of 360 molecules of VP1 arranged in 12 pentameric and 60 hexameric pentamers on an

icosahedral lattice. Inside the capsid, each VP1 pentamer is associated with a single molecule of either VP2 or VP3 (Chen et al., 1998). From the inner surface of the capsid, VP2 or VP3 is extended inwardly and likely anchored to the viral minichromosome (Liddington et al., 1991; Griffith et al., 1992; Stehle et al., 1996). In JCV Tokyo-1 strain, potential functions of the late proteins have been analyzed based on the amino acid sequence comparison with SV40 and murine polyoma virus (Fig. 8.4).

VP1 comprises the capsid surface structures. In SV40, the crystal structure has shown that each VP1 molecule displays elaborate loop structures that emanate outward from the β-sheet framework (Liddington et al., 1991). The external loops were suggested to provide principal antigenic structures, receptor binding sites, or domains responsible for hemagglutination. Based on the amino acid sequence comparison, the VP1 sequence for JCV is divergent from those for other polyomaviruses primarily in the potential loops and is relatively conserved in the potential framework. We selected the peptide sequences from the potential BC, DE, and HI loops of JCV VP1 and prepared antibodies. These antibodies efficiently recognized the enlarged nuclei of the JCV-infected oligodendrocytes of PML brain tissues (Fig. 8.3B). Similarly, Aoki et al. (1996) prepared antibodies against the two peptide sequences from the potential BC loop of JCV VP1, KSISISDTFE (10 residues) and KSISISDTFESDSPNRDM (18 residues; the common residues in JCV and BKV are underlined). The antibody against the 10 residue peptide detected JCV, but not BKV. In contrast, the antibody against the 18 residue peptide cross reacted with both JCV and BKV due to the larger number of common residues. Therefore, in both JCV and BKV, the potential loop structures can provide antigenic domains, and specific sequences in this region can define distinctive antigenic structures.

VP2 and VP3 are internal proteins. These proteins are not yet well analyzed in JCV. In the carboxy-terminal regions for VP2 and VP3, JCV has the corresponding sequences to VP1 interactive domain, a DNA binding domain, and a nuclear localization signal (NLS), which are determined in SV40 or murine polyoma virus (Gharakhanian and Kasamatsu, 1990; Clever and Kasamatsu, 1991; Chang et al., 1992a; Clever et al., 1993; Barouch and Harrison, 1994; Dean et al., 1995). These observations indicate that VP2 and VP3 for JCV has some common functions with those for SV40 and murine polyoma virus in the structure and assembly of virions.

Agnoprotein is not a component of the capsid. Because the SV40 mutant lacking agnoprotein can replicate (Resnick and Shenk, 1986), this protein is not essential for viral replication, but rather regulates efficient production of progeny virions. In JCV, BKV, and SV40, the sequences of agnoprotein are homologous in the first two-thirds of the amino terminus, but are divergent in the carboxy terminal one-third. In SV40, two types of antibodies against agnoprotein were prepared by immunizing the whole agnoprotein and by immunizing a synthetic peptide, VLRRLSRQASVKVR, identical to the 14 residues in the amino-terminal region. In a comparison of these two antibodies, it was noted that SV40 agnoprotein has multiple subpopulations, the majority of

A: VP1


JCV MAPTKRKG.. .....ERKD ...PVQVPKL LIRGGVEVLE VKTGVDSITE VECFLTPEMG DP..... DEHLRGFS.K .SISISDTFE SDSPNKDMLP
BKV MAPTKRKGEC PGAAPKKPKE ...PVQVPKL LIKGGVEVLE VKTGVDAITE VECFLNPEMG DP..... DENLRGFSLK ..LSAENDFS SDSPERKMLP
SV40 MAPTKRKGSC PGAAPKKPKE ...PVQVPKL VIKGGIEVLG VKTGVDSFTE VECFLNPQMG NP..... DEHQKGLS.K .SLAAEKQFT DDSPDKEQLP
PyV MAP.KRKSGV SKCETKCTKA CPRPAPVPKL LIKGGMEVLD LVTGPDSVTE IEAFLNPRMG QPPTPESLTE GGQYYGWSRG INLATSDTWI ...PRNNTLP
disordered
N-arm
β-A
α-A
AB
β-B
BC loop
JCV CYSVARIPLP NLNEDLTCGN ILMWEAVTLK TEVLGVTTLM NVHS..NG.Q ATHDNGAGKP VQGTSFHFFS VGGEALELQG VVFNYRTKYP DG......TI
BKV CYSTARIPLP NLNEDLTCGN LLMWEAVTVQ TEVIGITSML NLHA...GSQ KVHEHGGGKP IQGSNFHFFA VGGEPLEMQG VLMNYRSKYP DG......TI
SV40 CYSVARIPLP NLNEDLTCGN ILMWEAVTVK TEVLGVTAML NLHS...GTQ KTHENGAGKP IQGSNFHFFA VGGEPLELQG VLANYRTKYP AQ......TV
PyV TWSMAKSSFP CLNEDLTCDT LQMWEAVSVK TEVVGSGSLL DVHGFNKTHR FSKHKGNSTP VEGSQYHVFA GGGEPLDLQG LVTDARTKYK EEGVVTIKTI
β-C
CD
β-D
α-B
DE loop
β-E
EF loop
JCV FPKNATVQSQ VMNTEHKAYL DKNKAYPVEC WVPDPTRNEN TRYFGTLTGG ENVPPVLHIT NTATTVLLDE FGVGPLCKGD NLYLSAVDVC GMFTNRSGS.
BKV TPKNPTAQSQ VMNTDHKAYL DKNNAYPVEC WVPDPSRNEN ARYFGTFTGG ENVPPVLHVT NTATTVLLDE QGVGPLCKAD SLYVSAADIC GLFTNSSGT.
SV40 TPKNATVDSQ QMNTDHKAVL DKDNAYPVEC WVPDPSKNEN TRYFGTYTGG ENVPPVLHIT NTATTVLLDE QGVGPLCKAD SLYVSAVDIC GLFTNTSGT.
PyV TKKDMVNKDQ VLNPISKAKL DKDGMYPVEI WHPDPAKNEN TRYFGNYTGG TTAPPVLQFT NTLTTVLLDE NGVGPLCKGE GLYLSCVDIM GWRVTRNYVS
EF loop
β-F
β-G1
β-G2
GH loop
β-H
HI loop
JCV QQWRGLSRYF KVQLRKRRVK NPYPISFLLT DLINRRTPRV DGQPMYGMDA QVEEVRVFEG TEELPGDPDM MRYVDRYGQL QTKML*
BKV QQWRGLARYF KIRLRKRSVK NPYPISFLLS DLINRRTQRV DGQPMYGMES QVEEVRVFDG TERLPGDPDM IRYIDKQGQL QTKML*
SV40 QQWKGLPRYF KITLRKRSVK NPYPISFLLS DLINRRTQRV DGQPMIGMSS QVEEVRVYED TEELPGDPDM IRYIDEFGQT TTRMQ*
PyV SLEKGFPRYF KITLRKRWVK NPYPMASLIS SLFNNMLPQV QGQPMEGENT QVEEVRVYDG TEPVPGDPDM TRYVDRFGKT KTVFPGN*
β-I
...
α-C
...C arm...
β-J
...C arm...
C-loop

B: VP2/VP3

```
     VP2
JCV  MGAALALLGD LVATVSEAAA ATGFSVAE.I AAGEAAATIE VEIASLATVE GITSTSEAIA AIGLTPETYA VITGAPGAV. ..AGFAALVQ TVTGGSAIAQ
BKV  MGAALALLGD LVASVSEAAA ATGFSVAE.I AAGEAAAAIE VQIASLATVE GITSTSEAIA AIGLTPQTYA VIAGAPGAI. ..AGFAALIQ TVSGISSLAQ
SV40 MGAALTLLGD LIATVSEAAA ATGFSVAE.I AAGEAAAAIE VQLASVATVE GLT.TSEAIA AIGLTPQAYA VISGAPAAI. ..AGFAALLQ TVTGVSAVAQ
PyV  MGAALTILVD LIEGLAEVST LTGLS.AEAI LSGEALAALD GEITAL.TLE GVMSSETALA TMGISEEVYG FVSTVPVFVN RTAGAIWLMQ TVQGASTIS.

                                VP3
JCV  LGYRFFADWD HKVSTVGLFQ QPAMALQLFN PEDYYDILFP GVNAFVNNIH YLDPRHWGPS LFSTISQAFW .NLVRDDLPS LTSQ..EI.Q RRTQKLFVET
BKV  VGYKFFDDWD HKVSTVGLYQ QSGMALELFN PDEYYDILFP GVNTFVNNIQ YLDPRHWGPS LFATISQALW .HVIRDDIPS ITSQ..EL.Q RRTERFFRDS
SV40 VGYRFFSDWD HKVSTVGLYQ QPGMAVDLYR PDDYYDILFP GVQTFVHSVQ YLDPRHWGPT LFNAISQAFW .RVIQNDIPR LTSQ..EL.E RRTQRYLRDS
PyV  LGIQRYLHNE E.VPTVNRN. ...MALIPWR DPALLDIYFP GVNQFAH..A LNVVHDWGHG LLHSVGRYVW QMVVQETQHR LEGAVRELTV RQT.HTFLDG

JCV  LARFLEETTW AIVNSP.... .......... VNLYNYISDY YSRLSPVRPS MVRQVAQR.E GTYISFGHSY TQSIDNADSI QEVTQRLDLK N..PNVQ...
BKV  LARFLEETTW TIVNAP.... .......... INFYNYIQQY YSDLSPIRPS MVRQVAER.E GTRVHFGHTY ..SIDDADSI EEVTQRMDLR ....NQQSVH
SV40 LARFLEETTW TVINAP.... .......... VNWYNSLQDY YSTLSPIRPT MVRQVANR.E GLQISFGHTY .DNIDEADSI QQVTERWEAQ SQSPNVQ...
PyV  LARLLENTRW VVSNAPQSAI DAINRGASSV SSGYSSLSDY YRQLG.LNPP QRRALFNRIE GSMGNGGPTP AAHIQDE... .......... ..........

JCV  SGEFIEKSFA PGGANQRSAP QWMLPLLLGL YG..TVTPAL EAYEDGPNKK KRRKE..... ...GPRASSK TSYKRRSRSS RS*
BKV  SGEFIEKTIA PGGANQRTAP QWMLPLLLGL YG..TVTPAL EAYEDGPNQK KRRVSRGSSQ KAKGTRASAK TTNKRRSRSS RS*
SV40 SGEFIEKFEA PGGANQRTAP QWMLPLLLGL YG..SVTSAL KAYEDGPNKK KRKLSRGSSQ KTKGTSASAK ARHKRRNRSS RS*
PyV  SGEVIKFYQA PGGAHQRVTP DWMLPLILGL YGDITPTWAT VIEEDGPQKK KRRL*
```

C: agnoprotein

```
JCV  MVLRQLSRKA SVKVSKTWSG TKKRAQRILI FLLEFLLDFC TGEDSVDGKK RQKHSGLTQQ TYSALPEPKA T*
BKV  MVLRQLSRQA SVKVGKTWTG TKKRAQRIFI FILELLLEFC RGEDSVDGKN K......... STTALPAVKD SVKDS*
SV40 MVLRRLSRQA SVKVRRSWTE SKKTAQRLFV FVLELLLQFC EGEDTVDGKR K.KPERLTEK PES*
```

Figure 8.4. Amino acid sequence comparison of the late proteins of JCV, BKV, SV40, and murine polyoma virus (PyV). (**A**) Comparison of the amino acid sequences for VP1. Potential structure of JCV VP1 is analyzed based on the crystal structure determined in SV40 VP1 (Liddington et al., 1991). JCV VP1 potentially has loop structures, that project from the protein framework. The potential loop structures can provide principal antigenic structures, receptor binding sites, or domains responsible for hemagglutination. (**B**) Comparison of the amino acid sequences for VP2/VP3. The amino acid sequence of VP3 is identical to two thirds of the carboxy terminus of VP2 and therefore is designated as VP2/VP3. The sequences are divergent in the carboxy-terminal regions of VP2/VP3. (**C**) Comparison of the amino acid sequences for agnoprotein. Agnoprotein is encoded in JCV, BKV, and SV40, but not reported in murine polyoma virus. The amino acid sequences of the agnoproteins are highly divergent in the carboxy-terminal one third.

which is not readily detected by the peptide antibody against the amino-terminal 14 residues (Nomura et al., 1983). BKV agnoprotein has been shown to be phosphorylated (Rinaldo et al., 1998). Similarly, JCV agnoprotein may also have multiple isoelectric forms that potentially play different roles in the regulation of the viral replication.

4. STRUCTURES OF THE LATE RNAs ARE DIVERGENT IN JCV, SV40, AND MURINE POLYOMA VIRUS

During the late stage of the virus replication cycle, the capsid proteins are synthesized from the late RNAs generated by alternative splicing. In JCV, SV40, and murine polyoma virus, the structures of the late RNAs are divergent to each other due to different patterns in transcription, extension of the pre-RNAs, and splicing. In particular, the divergence in structures of the 5′ leader sequence affects the presence or absence of the open reading frame (ORF) of agnoprotein. Therefore, the JCV late RNAs and some species of the SV40 late RNAs are in polycistronic structures; in contrast, the murine polyoma virus late RNAs are in monocistronic structures. For convenience in this chapter the 5′ untranslated region (5′-UTR) indicates the sequence upstream of the ORF of agnoprotein, and the term *leader sequence* indicates the sequence upstream of the ORFs of the capsid proteins.

JCV has at least four species of the late RNAs, M1–M4, that encode agnoprotein in the leader sequence (Fig. 8.5) (Shishido-Hara et al., 2000). Transcription of the late RNAs is initiated from the heterogeneous RNA start sites,

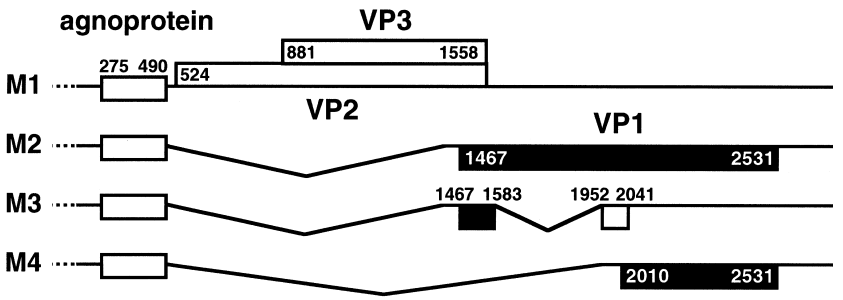
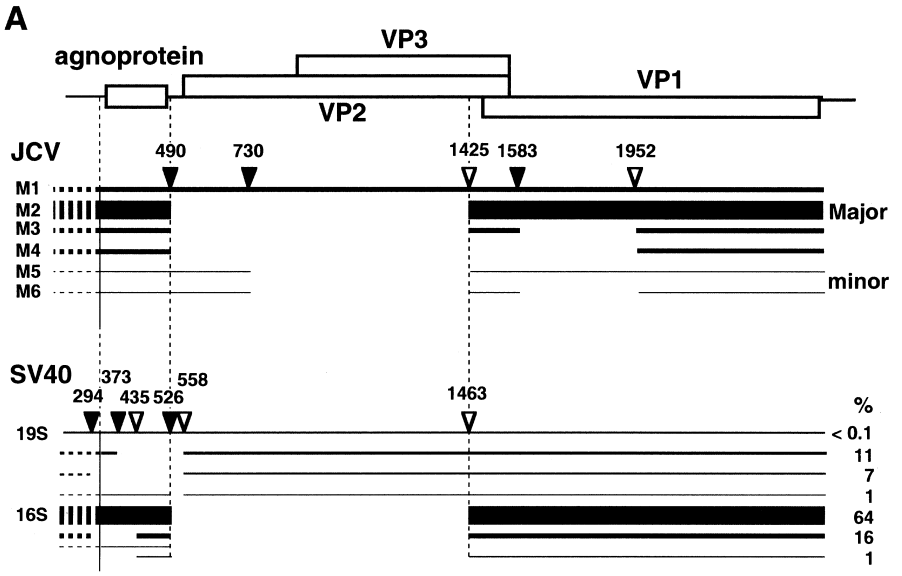


Figure 8.5. Structures of the JCV late RNAs. JCV has at least four species of the late RNAs, M1–M4, in polycistronic structures. JCV has distinctive RNA structures from SV40 and murine polyoma virus. Because most of the late RNAs are not spliced in the leader sequence, the ORF of agnoprotein is encoded. M1 is an unspliced RNA, and M2–M4 are generated by alternative splicing. M1 can produce agnoprotein, VP2, and VP3. M2 can produce agnoprotein and VP1. M3 and M4 can encode potentially the new ORFs in addition to the ORF of agnoprotein. The 5′ terminus of each of the late RNAs has not been analyzed as indicated by dotted lines. The numbering system refers to the JCV Tokyo-1 strain. (Taken with permission from Shishido-Hara et al., 2000.)

as determined in JCV Mad 1 strain by two groups of investigators. Daniel and Frisque (1993) reported the RNA start sites at nt 124–129, 191–192, 200–203, and 5118–5121. Kenney et al. (1986) reported the RNA start sites at nt 5114–5117, 90–98, 198–203, 224, and 236–242. (The sequence numbering follows that of JCV Mad 1 [Frisque et al., 1984].) The JCV late RNAs are generated by alternative splicing, and positions of the splice sites have been mapped in JCV Tokyo-1 strain. M1 is an unspliced RNA. M2 is spliced at nt 490/1425 (934 nt intron); M3, at nt 490/1425 (934 nt intron) and nt 1583/1952 (368 nt introns); and M4 at nt 490/1952 (1461 nt intron). (The sequence numbering system follows JCV Tokyo-1, and paired numbers separated by a slash indicate 5′ and 3′ splice sites.) Most of the JCV late RNAs are not spliced in the leader sequence, and therefore the ORF of agnoprotein can be encoded. M1 can produce agnoprotein, VP2, and VP3. M2 can produce agnoprotein and VP1. M3 and M4 are generated by using splice sites at nt 1583 or nt 1952, and cannot produce intact VP1. The splice sites corresponding to these positions have not been reported in SV40. Thus, M3 and M4 are unique RNA species to JCV and may potentially encode new ORFs in addition to the ORF of agnoprotein. It is not known if each of the M1–M4 RNAs has the same sequence in the 5′-UTR or contains heterogeneous species of different 5′-UTRs. The length of the 5′-UTR can affect translation efficiency of the capsid proteins, as described in detail later.

SV40 has two classes of the late RNAs, 16S and 19S. The majority of the late RNAs encode the ORF of agnoprotein, but some species lack its ORF due to splicing in the leader sequence (Fig. 8.6). Transcription of the SV40 late RNAs is initiated from multiple RNA start sites between nt 28 and 325 (Ghosh et al., 1978; Haegeman and Fiers, 1978; Canaani et al., 1979). However, unlike JCV, transcription of 70–90% of the SV40 late RNAs is initiated from the major RNA start site at nt 325, which is located 25–30 nucleotides downstream of the surrogate TATA signal, TACCTA (Brady et al., 1982; Nandi et al., 1985). The two classes of late RNAs, 16S and 19S, are generated by alternative splicing from common pre-RNAs (Lai et al., 1978; Good et al., 1988b). The 16S RNAs are relatively homogeneous compared with the 19S RNAs. On the 16S RNAs transcription is initiated mostly from the major RNA start site at nt 325. In contrast, on the 19S RNAs it is initiated from multiple RNA start sites between nt 28 and nt 325. The ORF of agnoprotein is encoded on 80% of the 16S RNAs (64% of the total late RNAs) and on 5% of the 19S RNAs (1% of the total late RNAs; Somasekhar and Mertz, 1985b; Good et al., 1988b). Therefore, VP1 is produced from the 16S RNAs, and VP2 and VP3 are produced from the 19S RNAs, in both the presence and absence of the upstream ORF of agnoprotein.

Murine polyoma virus has distinctive RNAs for VP1, VP2, and VP3 (Siddell and Smith, 1978; Hunter and Gibson, 1978). Unlike JCV and SV40, the late RNAs of murine polyoma virus are monocistronic, and the presence of agnoprotein is not reported in the leader sequence. Transcription initiates from the heterogeneous RNA start sites from nt 5075 to 5168 (Flavell et al., 1980; Cowie



B

5' splice sites

	nt	$^{A}_{C}$AG/GU$^{A}_{G}$AGU
JCV		
▼SV40	294	**AAG/GUA**ccU
JCV	313	**AA**a **GU**u**AGU**
▼SV40	373	**AAG/GU**uc**GU**
▼ JCV	490	u**AG/GUAAGU**
▼SV40	526	**C**u**G/GUAAGU**
▼ JCV	730	gcu**/GUAA**u**U**
SV40	765	gcu **GUGA**ua
▼ JCV	1583	g**AG/GUA**gaa
SV40	1645	g**AG GUG**gag

3' splice sites

	nt	UUUUUUUUUUUUUUU$_{N}$C$^{}_{U}$AG/G CCCCCCCCCCCCCCC
JCV	375	**UUUU**aa**UUUUUUU**g**UUAG** a
∇SV40	435	**UUUUU**g**U**g**UUU**g**UUUUAG/**a
JCV	520	**UUUUUUUU**g**U**g**UUUUCAG G**
∇SV40	558	**UUUU**g**UCUUUU**a**UUUCAG/G**
∇ JCV	1425	**U**g**UU**g**CCUUU**a**CUUUUAG/G**
∇SV40	1463	**U**g**UU**g**CCUUU**a**CUUCUAG/G**
∇ JCV	1952	gg**U**gg**UUUUU**aa**UU**a**CAG/**a
SV40	2014	**U**g**U**g**UU**ag**C**aaa**CU**a**CAG G**

Figure 8.6. Structures and splice sites of the late RNAs of JCV and SV40. (**A**) JCV and SV40 have distinctive structures in the late RNAs despite similar genomic organizations in the late region. The divergence in the RNA structures is due to different patterns in transcription initiation and alternative splicing. In the 5′ terminal region, the heterogeneous sequences resulting from the multiple RNA start sites in SV40 and the sequences that have not been determined for each of the RNA species in JCV are indicated with the dotted lines. The 5′ and 3′ splice sites are indicated by closed and open triangles, respectively. In JCV, M5 and M6 are potential RNAs that were detected in cells transfected with a eukaryotic vector but not in PML brain tissues (Shishido-Hara et al., 2000). The levels of the different species of the SV40 late RNAs are from Good et al. (1988b) and Somasekhar and Mertz (1985b). (**B**) Alignment of nucleotide sequences of the splice sites and the corresponding sequences between JCV and SV40. Nucleotides identical to the consensus sequences defined for splice sites are indicated by bold upper case letters. Exon–intron boundaries are indicated by a slash. The 5′ and 3′ splice sites are marked with closed and open triangles, respectively. (Taken with permission from Shishido-Hara et al., 2000.)

et al., 1981). Pre-RNAs of murine polyoma virus are extended around the circular viral genome multiple times (Acheson, 1978), and are extremely large in size. These giant pre-RNAs are spliced from the leader sequence to the leader sequence. Therefore, the late RNAs processed by leader-to-leader splicing encode multiple copies of the leader sequence and a single ORF of either VP1, VP2, or VP3 (Legon et al., 1979; Kamen et al., 1980). The distinct late RNAs, 16S, 19S, and 18S, respectively encode VP1, VP2, and VP3 (Siddell and Smith, 1978) and represent approximately 80%, 5%, and 15% of the total late RNAs (Batt et al., 1994). The ratios of individual RNAs reflect the ratios of the capsid proteins composed in the virions.

Therefore, in JCV, SV40, and murine polyoma virus, the structures of the late RNAs are highly divergent from each other. The heterogeneity is generated by different patterns in transcription, pre-RNA extension, and splicing. Most importantly, different splicing patterns in the leader sequence determine the presence or absence of the ORF of agnoprotein.

5. THE LEADER SEQUENCE INCLUDING THE ORF OF AGNOPROTEIN *IN CIS* AND AGNOPROTEIN *IN TRANS*, CAN REGULATE VIRUS REPRODUCTION IN MULTIPLE WAYS

JCV has the late RNAs in polycistronic structures, as in some species of the SV40 late RNAs. Polycistronic RNAs are unusual in eukaryotes as most of the eukaryotic RNAs are monocistronic. The polyomaviruses have distinct structures in the late RNAs, especially in the leader sequences. This indicates that expression of the capsid proteins is regulated in a different manner. Although few studies have focused on this aspect of JCV, in SV40 the way in which *cis*-acting DNA or RNA leader sequence or *trans*-acting agnoprotein encoded in this region regulates expression of the capsid proteins has been studied extensively.

In SV40, transcription initiation of the late RNAs was affected by *cis*-acting elements of the DNA sequence. Many investigators reported that the relative frequency in utilization of the heterogeneous RNA start sites was altered by mutating the leader sequence (Piatak et al., 1981, 1983; Ghosh et al., 1982; Haegeman et al., 1979a,b; Somasekhar and Mertz, 1985b). It was also suggested that transcription of the late SV40 RNAs can be attenuated within agnoprotein coding sequence at the U residues downstream of the potential stem-and-loop structure (Hay et al., 1982). The late viral transcription may be also affected by *trans*-acting diffusible factors (Alwine, 1982).

Processing and stability of the SV40 late RNAs was affected by the RNA leader sequence. The relative frequency in utilizing the splice sites was dramatically altered by mutating the leader sequence (Ghosh et al., 1982; Somasekhar and Mertz, 1985a; Good et al., 1988a). It was also suggested that the leader sequence affects the stability of the SV40 late RNAs by splicing (Ryu and Mertz, 1989) and polyadenylation (Chiou et al., 1991).

The translation efficiency from the downstream AUG is generally inefficient in the presence of the upstream AUG on polycistronic RNAs. The translation efficiency of SV40 VP1 was reduced due to the presence of the AUG start codon for agnoprotein on the major 16S RNA (Grass and Manley, 1987; Sedman et al., 1989). Similarly, translation of VP2 and VP3 was reduced in the presence of the AUG for agnoprotein on the 19S RNA (Dabrowski and Alwine, 1988; Good et al., 1988a; Sedman et al., 1989). The minor 16S RNA (16% of the total late RNAs), in which the AUG start codon for agnoprotein is removed by splicing, was more frequently used for VP1 translation than the major 16S RNA encoding the ORF of agnoprotein (64% of the total late RNAs; Barkan and Mertz, 1984). Despite the lower level of RNA expression, the minor 16S RNA produced almost half the amount of VP1 (Sedman et al., 1989).

To analyze functions of SV40 agnoprotein *in trans*, the AUG start codon for agnoprotein was mutated to UUG (Resnick and Shenk, 1986). Detectable differences could not be seen in transcription initiation sites, expression levels of 16S and 19S late RNAs, and expression levels of VP1 protein. Agnoprotein facilitated nuclear transport of VP1 and cell-to-cell spread of virions rather than regulating expression levels of the capsid proteins.

The roles of the SV40 leader sequence *in cis* and agnoprotein encoded in this region *in trans* were controversial in some points. It is likely because expression of the capsid proteins and production of viral progeny are regulated in multiple processes at different levels. Thus, the conclusions of some investigators contradicted each other by using different experimental systems for analysis. However, it has been consistently indicated that the leader sequence greatly affects expression levels of the capsid proteins and it is in this region that agnoprotein, which can regulate virus reproduction *in trans*, is encoded. JCV, SV40, and murine polyoma virus have divergent structures in the late RNAs. The divergence in the leader sequence may be one of the important factors that explain distinct regulatory mechanisms in the production of progeny virions.

6. HOW ARE VP1, VP2, AND VP3 SYNTHESIZED FROM THE POLYCISTRONIC RNAS?

Expression of the three capsid proteins roughly corresponds to the ratio of the capsid proteins incorporated into the virions. Although the mechanism for proportional expression of VP1, VP2, and VP3 is not completely understood, translation can be one of the important processes. Translation on polycistronic RNAs has been well studied with respect to the leaky scanning model (Kozak, 1986, 1996, 1999). In this model, it is proposed that 40S ribosomal subunits bind near the 5′ end of the RNA and scan downstream until they encounter the AUG start codon for translation. The most favorable sequence for translation initiation is GCC $\mathbf{A}^{-3}$CC AUG $\mathbf{G}^{+4}$. Counting A of AUG as +1, a purine, preferably

A at the −3 position ($\mathbf{A}^{-3}$ or $\mathbf{G}^{-3}$) and G at the +4 position ($\mathbf{G}^{+4}$) are especially important (as indicated with bold letters). In general, translation is preferentially initiated from the upstream AUG and rarely initiated from the downstream AUG. However, in some cases ribosomes bypass the upstream AUG and efficiently initiate translation from the downstream AUG (bypassing). Ribosomes also can reinitiate translation from the downstream AUG after completing translation of the upstream ORF (reinitiation). However, translation by reinitiation is much less efficient than by bypassing (Fig. 8.7).

On the SV40 bicistronic 16S RNA, it has been shown that ribosomes frequently bypass the AUG start codon for agnoprotein and efficiently synthesize VP1 from the downstream AUG (Grass and Manley, 1987). At least one-third of 40S ribosomal subunits bypass the AUG start codon for agnoprotein despite its strong context for translation, $\mathbf{G}^{-3}$CC AUG $\mathbf{G}^{+4}$ (Sedman et al., 1989). It has been suggested that this is due to the extremely short 5′-UTR of only 10 nucleotides on this 16S RNA. Because the major RNA start site, nt 325, is

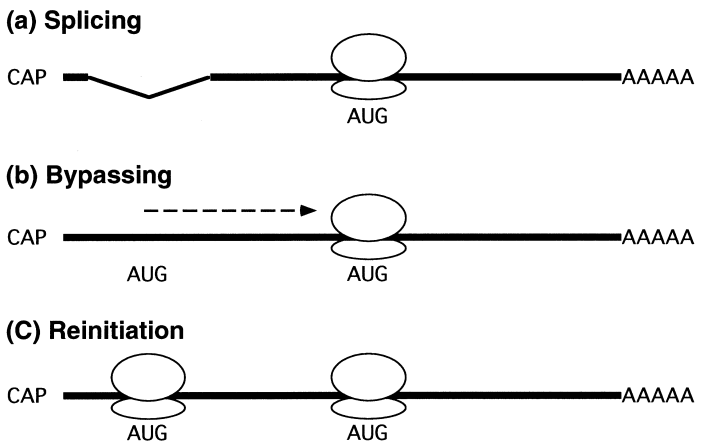


Figure 8.7. Leaky scanning models for translation. According to the model, ribosomes bind to the 5′ terminal region of the RNAs and scan downstream until they encounter the AUG start codon for translation. The presence of the upstream AUG, in general, reduces translation efficiency from the downstream AUG. However, in SV40, the capsid proteins are produced relatively efficiently from the downstream AUG due to the structure of its late RNAs. (**a**) On some species of SV40 late RNAs, the AUG start codon for agnoprotein is removed by splicing in the leader sequence. (**b**) Even if the AUG start codon for SV40 agnoprotein is present in the leader sequence, ribosomes can bypass its AUG, likely due to an extremely short 5′-UTR of only 10 nucleotides. Ribosomes that bypassed the AUG for agnoprotein initiate translation for the capsid proteins from the downstream AUG. (**c**) Ribosomes also re-initiate translation after completing translation of the upstream ORF for agnoprotein. However, it is not as efficient to re-initiate translation from the downstream AUG for the capsid proteins.

predominantly used on the 16S RNA, there are only 10 nucleotides between the 5′ cap site and the AUG start codon for the agnoprotein (Sedman et al., 1990). This extremely short 5′-UTR is quite unique because eukaryotic RNAs generally have 5′-UTR of 20–100 nucleotides in length (Kozak, 1987). When the adenovirus type 2 late promoter was inserted into the SV40 late promoter region, synthesis of SV40 VP1 was dramatically reduced in the presence of the 5′-UTR, which is 33 nucleotides in length (Grass and Manley, 1986). A similar observation has been reported for translation of VP2 and VP3 under the RSV promoter, which generates a 5′-UTR of 40 nucleotides (Dabrowski and Alwine, 1988). Therefore, although SV40 encodes agnoprotein on its genome, the structures of the SV40 late RNAs provide the mechanisms to produce the capsid proteins efficiently. On some species of the late RNAs, the AUG for agnoprotein is removed by splicing. On other species of the late RNAs, even in the presence of the AUG for agnoprotein, ribosomes can easily bypass its AUG under the extremely short 5′-UTR. These mechanisms are more efficient in expression of the capsid proteins than reinitiation of translation after the upstream ORF (Grass and Manley, 1987).

Both SV40 VP2 and VP3 are synthesized from the 19S RNAs by leaky scanning (Sedman and Mertz, 1988). Unlike the 16S RNAs, the 5′-UTRs of the 19S RNAs are heterogeneous in length, which influences utilization of the AUG start codon for agnoprotein (Dabrowski and Alwine, 1988) and those for VP2 and VP3 (Good et al., 1988a). The AUG start codon for VP2 is in a weak context for translation, UCC AUG $\mathbf{G}^{+4}$ compared with that for VP3, $\mathbf{G}^{-3}$ GA AUG $\mathbf{G}^{+4}$. Approximately 70% of the scanning ribosomes bypass the AUG start codon for VP2 and initiate translation from the downstream AUG for VP3. The AUG codon for VP3 was 3.1 times more frequently used for translation than that for VP2 (Dabrowski and Alwine, 1988), corresponding to their ratio in the cytoplasm of infected cells, which ranges from 2.7 to 3.1 (Lin et al., 1984). Therefore, translation is an important process to determine the proportional expression levels of VP2 and VP3.

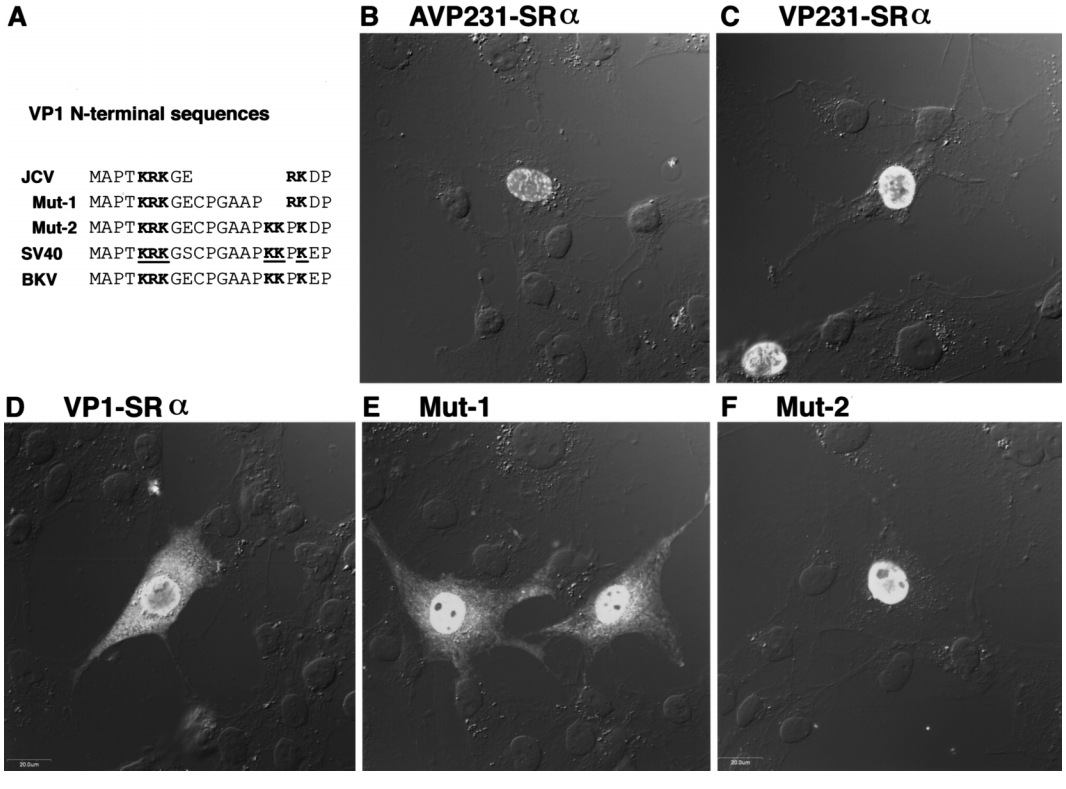
How is expression of the JCV capsid proteins regulated in the process of translation? It has been suggested that the JCV leader sequence (nt 275–409) or the presence of the ORF for agnoprotein may decrease the expression level of VP1 in the process of translation (Shishido-Hara et al., 2000). Unlike SV40, most of the JCV late RNAs are not spliced in the leader sequence, and the ORF of agnoprotein is encoded upstream of the ORFs of the capsid proteins. In addition, unlike SV40, the 5′-UTRs of the JCV late RNAs are, at minimum, 74 or 35 nucleotides in length, depending on the RNA start sites reported by two groups of investigators (Daniel and Frisque, 1993; Kenney et al., 1986). Therefore, on the JCV polycistronic RNAs, ribosomes can be more frequently intercepted at the AUG for agnoprotein than on the SV40 RNAs before arriving at the downstream AUG for the capsid proteins. Thus, translation efficiency of the capsid proteins may be lower in JCV than in SV40.

7. THE NUCLEAR LOCALIZATION SIGNALS OF VP1, VP2, AND VP3

The polyomaviruses reproduce progeny virions in the nucleus. Thus, after the process of translation, the capsid proteins synthesized in the cytoplasm are transported to the nucleus for subsequent assembly. The proteins targeted to the nucleus, in general, contain a nuclear localization signal (NLS), which is usually a stretch of basic amino acids such as **K** (lysine) or **R** (arginine). These basic amino acids are arranged in either one or two clusters and called a *monopartite* or a *bipartite signal*, respectively. By crystallography, it has been shown that karyopherin α, which recognizes the NLS, has two binding sites for basic amino acids (Conti et al., 1998), suggesting a difference in the binding patterns to the NLS in a monopartite and a bipartite structure. JCV VP1 encodes the basic amino acids in a monopartite structure in the amino-terminal sequence, while SV40 VP1 encodes the basic amino acids in a bipartite structure. A difference in alignment of the basic amino acids caused different efficiencies in nuclear transport between JCV VP1 and SV40 VP1.

JCV VP1 has been shown to be inefficient in nuclear transport (Shishido-Hara et al., 2000). This is due to the unique structure in the amino-terminal sequence. The amino-terminal sequence of JCV VP1, MAPT**KRK**GE**RK**D, encodes the basic amino acids, $\mathbf{K^5R^6K^7}$ and $\mathbf{R^{10}K^{11}}$ interrupted by G^8E^9 (superscripts indicate positions of residues in the amino acid sequence). In comparison to SV40 VP1 and BKV VP1, the eight residues CPGAAPKK are missing between the two clusters of the basic amino acids. JCV VP1 was distributed both in the cytoplasm and in the nucleus when it was expressed in the absence of VP2 and VP3 in COS-7 cells (Fig. 8.8A,D). To analyze the effects of the amino-terminal sequence in nuclear transport, JCV VP1 was mutated by inserting the missing six amino acids CPGAAP between **KRK**GE and **RK**. The VP1 mutant, designated as Mut-1, has the sequence MAPT**KRK**GECP GAAP**RK**D in which **KRK** and **RK** are encoded in a bipartite structure spanning eight residues. When Mut-1 was expressed in COS-7 cells, Mut-1 was transported to the nucleus more efficiently and detected more prominently in the nucleus than wild-type VP1. However, Mut-1 was still distributed in both the cytoplasm and the nucleus (Fig. 8.8A,E). Therefore, the basic amino acids **RK** in Mut-1 were replaced with **KKPK** and designated as Mut-2. Mut-2 has the sequence MAPT**KRK**GECPGAAP**KKPK**D in which **KRK** and **KKPK** are encoded in a bipartite structure spanning eight residues, as in BKV VP1 and SV40 VP1. When Mut-2 was expressed, Mut-2 was localized to the nucleus (Fig. 8.8A,F). Thus, inefficiency in nuclear transport of JCV VP1 is due to the unique amino-terminal sequence **KRK**GE**RK**, a monopartite structure of basic amino acids of **KRK** and **RK**, and low potency of the **RK** residues in nuclear transport compared with **KKPK** (Shishido-Hara et al., 2000).

In contrast, SV40 VP1 is localized to the nucleus in the absence of VP2 and VP3 when it is expressed alone in TC7 cells, a derivative of African green monkey kidney cells like COS-7 cells (Ishii et al., 1996). SV40 VP1 has the


A
VP1 N-terminal sequences
JCV MAPTKRKGE RKDP
Mut-1 MAPTKRKGECPGAAP RKDP
Mut-2 MAPTKRKGECPGAAPKKPKDP
SV40 MAPTKRKGSCPGAAPKKPKEP
BKV MAPTKRKGECPGAAPKKPKEP
B AVP231-SRα
C VP231-SRα
D VP1-SRα
E Mut-1
F Mut-2

sequence MAPT**KRK**GSCPGAAP**KK**P**K**E in which the clusters of the basic amino acids $\mathbf{K^5R^6K^7}$ and $\mathbf{K^{16}K^{17}}$, $\mathbf{K^{19}}$ are encoded in a bipartite structure spanning the eight residues GSCPGAAP. These two clusters of basic amino acids have been identified as the NLS of SV40 VP1 (Ishii et al., 1996) (Fig. 8.8A). In BKV VP1, a nearly identical sequence MAPT**KRK**GECPGAAP**KK**P**K**E is present in the amino-terminal region, suggesting that BKV VP1 can be transported to the nucleus in the absence of VP2 and VP3 as in SV40 VP1.

For VP2 and VP3, JCV has the putative NLS that corresponds to the NLS determined in SV40 and murine polyoma virus in TC7 cells and COS-7 cells, respectively (Clever and Kasamatsu, 1991; Chang et al., 1992a). In SV40, the NLS of VP2 and VP3 has been determined in the carboxy-terminal region (Gharakhanian et al., 1987; Wychowski et al., 1987), and it has been shown that the sequence of GPNKKKRKL is especially important for nuclear targeting (Clever and Kasamatsu, 1991). In JCV, a nearly identical sequence GPNKKKRRK is present in the corresponding region, suggesting that JCV VP2 and VP3 can be transported to the nucleus autonomously.

Therefore, JCV VP1 is not efficient in nuclear transport, although VP2 and VP3 are likely transported to the nucleus efficiently. However, in oligodendrocytes of PML brain tissues, the JCV capsid proteins, including VP1, are expressed predominantly in the nucleus. Because JCV VP1 was localized predominantly to the nucleus when all the three capsid proteins were expressed together (Fig. 8.8B,C), the presence of VP2 and/or VP3 is likely important for predominant nuclear localization of JCV VP1.

Figure 8.8. Nuclear transport of the JCV capsid proteins. (**A**) JCV VP1 has a distinctive sequence from SV40 VP1 and BKV VP1 in the amino-terminal sequence. In JCV VP1, the basic amino acids **KRK** and **RK** are encoded spanning the two residues GE. In contrast, in SV40 VP1, the two clusters of basic amino acids **KRK** and **KK**, **K** are encoded in a bipartite structure spanning eight residues and identified as the NLS (Ishii et al., 1996). A nearly identical sequence is present in BKV VP1. To investigate the effects of this amino-terminal sequence on nuclear transport, two mutants were constructed for JCV VP1. Mut-1 encodes the basic amino acids **KRK** and **RK**, and Mut-2 encodes **KRK** and **KK**P**K** in bipartite structures. VP1 and VP1 mutants were expressed in COS-7 cells, and their distribution was analyzed by immunocytochemistry with a confocal microscope. (**B**) When cells were transfected with an expression vector, AVP231-SRα, which encodes agnoprotein, VP1, VP2, and VP3, VP1 was efficiently transported to the nucleus and identified as speckles in the nucleus. (**C**) When cells were transfected with an expression vector, VP231-SRα, which encodes VP1, VP2, and VP3, VP1 was also efficiently transported to the nucleus and identified as speckles in the absence of agnoprotein. (**D**) When cells were transfected with VP1-SRα, which encodes VP1 alone, VP1 was distributed in both the cytoplasm and the nucleus (**E**) Mut-1 was transported to the nucleus more efficiently and detected more prominently in the nucleus than wild-type VP1. However, Mut-1 was still distributed in both the cytoplasm and the nucleus. (**F**) Mut-2 was efficiently transported to the nucleus and distributed diffusely except for nucleoli. Mut-2 was distributed in the nucleus diffusely. (Taken with permission from Shishido-Hara et al., 2000.)

8. THE VP1 PENTAMERS CAN BE TRANSPORTED TO THE NUCLEUS WITH VP2 OR VP3

How are the capsid proteins transported to the nucleus? It has been thought that VP1 is associated with VP2 or VP3 in the cytoplasm and transported to the nucleus cooperatively. JCV VP1 was distributed in both the cytoplasm and the nucleus in the absence of VP2 and VP3, but predominantly localized to the nucleus in their presence (Fig. 8.8B–D). The NLS-defective SV40 VP1 and murine polyoma virus VP1 were distributed in the cytoplasm, but localized to the nucleus in the presence of wild-type VP2 or VP3 (Ishii et al., 1994; Cai et al., 1994). Similarly, the NLS-defective SV40 VP2 and VP3 was distributed in the cytoplasm, but localized to the nucleus in the presence of wild-type VP1 (Ishii et al., 1994). In insect cells, murine polyoma virus VP2 and VP3 were distributed predominantly in the cytoplasm, but localized to the nucleus in the presence of VP1 (Delos et al., 1993; Forstova et al., 1993). Therefore, the capsid proteins are more efficiently localized to the nucleus when both major and minor capsid proteins are expressed together. Even if the NLS of one of the capsid proteins is defective or functionally weak, the capsid proteins are transported to the nucleus complimentarily. Based on these data, it has been inferred that the major and minor capsid proteins are associated in the cytoplasm and transported to the nucleus as a complex. However, it is not yet demonstrated, in a dynamic state, that the capsid proteins are migrating to the nucleus cooperatively in living cells.

Why are the capsid proteins transported to the nucleus together? In JCV, interaction of VP1 with VP2 or VP3 in the cytoplasm can be a critical step for efficient nuclear transport because VP1 is not efficiently localized to the nucleus in the absence of VP2 and VP3. However, in SV40 and murine polyoma virus, each capsid protein has its own NLS and has a potential to be transported to the nucleus individually (Gharakhanian et al., 1987; Clever and Kasamatsu, 1991; Moreland and Garcea, 1991; Chang et al., 1992a,b; Ishii et al., 1996). In SV40, the ratios of the three capsid proteins were analyzed through the replication cycle. The ratios of the capsid proteins in the nuclear fraction were constantly similar to their ratios incorporated into the virions, suggesting that the amounts of the capsid proteins within the nucleus can be regulated in the process of nuclear transport (Lin et al., 1984). In both SV40 and mouse polyoma virus, VP1 easily interacted with VP2 or VP3 and formed a complex, as demonstrated by co-immunoprecipitation (Gharakhanian et al., 1988, 1990; Barouch and Harrison, 1994; Cai et al., 1994; Forstova et al., 1993; Delos et al., 1995). In murine polyoma virus, interaction of VP1 and VP2 or VP3 within a complex was shown in a specific stoichiometry (Barouch and Harrison, 1994). The crystal structure of this complex indicated that each VP1 pentamer is associated with a single molecule of the carboxy-terminal region of VP2 or VP3 (Chen et al., 1998). Therefore, in the cytoplasm, the capsid proteins are associated and transported to the nucleus likely as a complex in a specific stoichiometry, which might be the VP1 pentamer associated with VP2 or VP3. The

complex may be a part of the capsid, which is further assembled into a complete capsid structure, and nuclear transport is likely the initial interaction of the capsid proteins prior to further assembly.

9. PRODUCTION OF PROGENY VIRIONS IN THE NUCLEUS

Where are the viral capsids or virions assembled? According to the crystal structure of the SV40 virions, the NLSs of both VP1 and VP2/VP3 are completely hidden inside the capsid (Liddington et al., 1991). Therefore, it is likely that the VP1 molecules associated with VP2 or VP3 are transported to the nucleus as a complex such as the pentamer that exposes the NLS, but not as the assembled capsid. Assembly may be retarded in the cytoplasm due to a deficiency of calcium ions. When murine polyoma virus VP1 was expressed in insect cells, VP1 was assembled into the virus-like particles in the nucleus, but not in the cytoplasm. However, by adding calcium, the virus-like particles were assembled in both the cytoplasm and the nucleus (Montross et al., 1991). Therefore, the nucleus can provide a more suitable environment for assembly with the proper concentration of calcium.

Agnoprotein is not essential for nuclear transport of the capsid protein, but it likely facilitates their entry. In the absence of agnoprotein, SV40 VP1 was delayed in nuclear transport and localized to the periphery of the nucleus (Resnick and Shenk, 1986; Carswell and Alwine, 1986). SV40 agnoprotein was suggested to interact with VP1 to prevent aberrant cytoplasmic aggregation of the VP1 pentamers and facilitate their nuclear transport (Margolskee and Nathans, 1983; Barkan et al., 1987). The distributions of JCV agnoprotein and SV40 agnoprotein were mostly cytoplasmic, especially in the perinuclear region (Fig. 8.3) (Nomura et al., 1983). Because some intermediate filaments in the perinuclear region are contiguous with the nuclear matrix (Lazarides, 1980; Capco et al., 1982), agnoprotein may facilitate transport of the VP1 pentamers associated with VP2 or VP3 from the perinucleus to the nucleus.

In which regions in the nucleus are the progeny virions generated? In both SV40 and murine polyoma virus, the capsid proteins or virions were associated with the nuclear framework (Ben-Ze'ev et al., 1982; Stamatos et al., 1987). In JCV also, when VP1 was expressed in the presence of VP2 and VP3 in COS-7 cells, VP1 was localized to discrete regions in the nucleus and identified as speckles by confocal microscopy. JCV VP1, identified as speckles, was spread from the nuclear membrane into the center of the nucleus, potentially along subnuclear structures. In contrast, when VP1 was expressed in the absence of VP2 and VP3, VP1 was distributed more diffusely in the nucleus (Fig. 8.8B–D). Therefore, the VP1 pentamers, likely with VP2 and VP3, may accumulate in distinct subnuclear regions and spread along the skeletal frameworks in the nucleus. For encapsidating the viral genome, it was indicated that the VP1 pentamers are added on the viral minichromosome that includes cellular histones possibly guided by VP2 and VP3, and are further assembled into the

mature virions (Coca-Prados and Hsu, 1979; Fanning and Baumgartner, 1980; Garber et al., 1980; Garcea and Benjamin, 1983; Blasquez et al., 1983; Ng and Bina, 1984). Because the viral genomic DNA was also associated with the nuclear framework (Buckler-White et al., 1980), the nucleus might be divided into functional domains, some of which may actively support maturation of virions.

Using electron microscopy of PML brain tissues, the JCV round virions were frequently identified as discrete clusters in the nucleus, in which the virions are regularly arranged in crystalloid array (Fig. 8.1A). This unique morphologic feature was observed in the initial phase of virus infection, when the virus was cultured in primary human fetal glial (PHFG) cells (Nagashima et al., 1981). This electron microscopic observation and the speckles identified by confocal microscopy consistently indicate that the three capsid proteins, VP1, VP2, and VP3, accumulate in discrete regions in the nucleus. In these regions, the capsid proteins may be actively assembled into the virions, or the virions assembled elsewhere may gather together and organize clusters.

10. ASSEMBLY OF RECOMBINANT VIRUS-LIKE PARTICLES IN VITRO AND IN VIVO

For the polyomaviruses there are many reports that virus-like particles are generated by molecular manipulation of the viral genome both in vitro and in vivo. Assembly of virus-like particles in defined environmental conditions can give us step-by-step understanding of the highly ordered assembly of virions in the natural viral replication system.

Murine polyoma virus VP1 expressed in *Escherichia coli* was isolated as the VP1 pentamers. In vitro, at high ionic strength, the VP1 pentamers were assembled into virus-like structures, but also into heterogeneous aggregates (Salunke et al., 1986). At low ionic strength and in the presence of calcium, the pentamers were assembled into more homogeneous virus-like structures similar to native virions. At various conditions, the VP1 pentamers were formed into several structures of capsid-like aggregates, such as a 12-pentamer icosahedron and a 24-pentamer octahedron (Salunke et al., 1989). Thus, VP1, even when it is not post-translationally modified in *E. coli*, is assembled into virus-like structures, in the absence of VP2 and VP3 and in appropriate environmental conditions. Conditions of proper ionic strength and the presence of calcium ions are especially important.

In cells VP1 alone expressed in the absence of VP2 and VP3 was also assembled into the virus-like particles. JCV VP1 was isolated as the virus-like particles from *E. coli* (Ou et al., 1999). In the nucleus of insect cells, VP1 expressed by baculovirus vectors was also assembled into virus-like particles in JCV (Chang et al., 1997; Goldmann et al., 1999), in SV40 (Kosukegawa et al., 1996), and in murine polyoma virus (Montross et al., 1991; Gillock et al., 1997). The virus-like particles assembled in cells were much more homoge-

neous than those assembled in vitro, suggesting additional factors in cells that facilitate assembly (Montross et al., 1991). The virus-like particles isolated from *E. coli* (Ou et al., 1999) and insect cells (Gillock et al., 1997; Goldmann et al., 1999) contained nonviral DNA in the absence of VP2 and VP3. It is not well understood how the viruses encapsidate their own genomic DNA specifically during a natural virus replication.

The three capsid proteins VP1, VP2, and VP3 were expressed together in insect cells by co-infection of the three recombinant baculoviruses. In insect cells, the recombinant capsid proteins were assembled into virus-like particles in SV40 (Colomar et al., 1993; Sandalon and Oppenheim, 1997; Sandalon et al., 1997) and in murine polyoma virus (Delos et al., 1993; Forstova et al., 1993). The proportion of the three capsid proteins in these virus-like particles was comparable to those in natural virions (Forstova et al., 1993). Therefore, even if expression of the capsid proteins is not regulated as in the natural regulatory system, the three capsid proteins are assembled into virus-like particles in an appropriate ratio. In JCV, a viral genomic fragment encoding the overlapping capsid proteins VP1, VP2, and VP3 was located downstream of the SRα promoter, and the plasmid vector was transfected into COS-7 cells (Shishido et al., 1997). The vector-derived RNA was alternatively spliced at the authentic splice sites, and the virus-like structures of both round particles and filamentous forms were assembled in the nucleus (Shishido-Hara et al., 2000). Therefore, the capsid proteins are assembled into virus-like structures, even if their proportional expression is not regulated by the native regulatory system.

Because VP1 alone is spontaneously assembled into the virus-like particles in vitro, the capsid assembly itself is able to occur in a simple environment. However, in a cellular environment, production of progeny virions is regulated in multiple ways, and levels of regulation can be distinctive in each regulatory step. The efficiency of the production of progeny virions influences the viral biologic activities and subsequent occurrence of the virus-induced disease. Therefore, studies on the underlying regulatory mechanism in the production of virions can give us a better understanding of the nature of virus assembly, and clues to the molecular pathogenesis of virus-induced disease.

11. QUESTIONS FOR FUTURE STUDIES

How differently is production of progeny virions regulated in PML patients and in healthy individuals? JCV persists in most healthy individuals in an asymptomatic state and causes PML in some immunodeficient patients. It has been shown that the virus isolated from healthy individuals and the virus from PML patients' brains have divergent sequences in the regulatory region. The virus from healthy individuals has a typical regulatory sequence, which is called the *archetype* (Yogo et al., 1990). In contrast, the viruses from PML patients' brains have divergent regulatory sequences, possibly derived from the arche-

type by rearrangement involving deletion and duplication. When the two types of viruses were cultured in cells, the virus from a PML patient's brain replicated more efficiently than the urinary virus from a healthy individual (Daniel et al., 1996). The viral transcription activities can be affected by rearrangement in the regulatory region. However, different groups of investigators reported contradictory results when using different experimental systems (Sock et al., 1996; Daniel et al., 1996; Ault, 1997). As we have reviewed in this chapter, production of the progeny virions is regulated in multiple steps during the late stage. It has not yet been investigated how the rearrangement in the regulatory region influences initiation of late RNA transcripts, utilization of splice sites, and translation efficiencies of the capsid proteins in the altered 5′-UTR. The rearrangement might also affect proportional expression levels of the capsid proteins. These subjects remain for future studies.

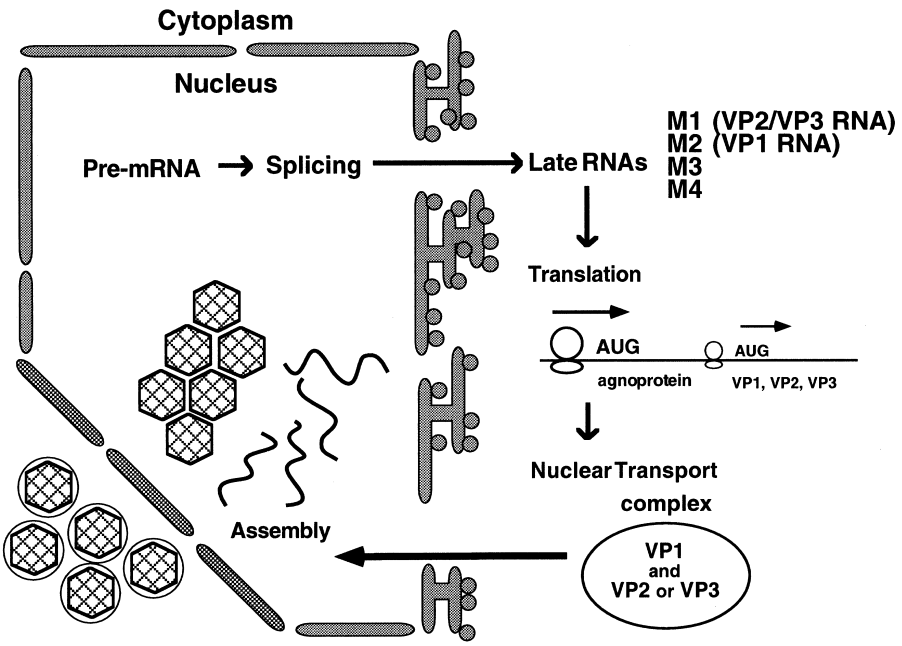


Figure 8.9. Schematic illustration of the late stage in the JCV replication cycle. Transcription occurs in the nucleus, and the pre-mRNAs are generated from heterogeneous RNA start sites. The JCV late RNAs are generated by alternative splicing. M1 can produce agnoprotein, VP2, and VP3. M2 can produce agnoprotein and VP1. Translation of the capsid proteins may be reduced due to the presence of the AUG start codon for agnoprotein in the leader sequence. JCV VP1 is not efficient in nuclear transport by itself, but can be efficiently transported to the nucleus in the presence of VP2 or VP3. In the nucleus, the capsid proteins are likely accumulated to discrete subnuclear regions, which may actively support production of progeny virions. JCV is identified as the round particles and the filamentous forms in the nucleus, and their distribution patterns are variable depending on cells.

In the nucleus of infected oligodendrocytes, JCV is identified as round and filamentous structures by electron microscopy. The proportions of round and filamentous forms are variable depending on the host cells. It has been suggested that filamentous forms result from aberrant assembly (Kiselev and Klug, 1969; Baker et al., 1983). The filamentous structures may be formed due to aberrant interaction of the first two VP1 pentamers (Stehle et al., 1996) or to different phosphorylation patterns of VP1 (Li et al., 1995). It is not known how the viruses in round and filamentous structures are different in nature. However, they likely have different biologic features, and the proportions of the round and filamentous forms may reflect the viral biologic activities as human pathogen. Few studies have focused on JCV virions in different structures.

In conclusion, during the late stage, production of progeny virions is regulated in multiple steps, including transcription, splicing, translation, nuclear transport, and maturation of virions (Fig. 8.9). We have shown in this chapter that JCV has distinctive regulatory mechanism from SV40 and murine polyoma virus. Studies on the underlying molecular mechanisms in the production of progeny virions and their structures can contribute to understanding the nature of JCV and the pathogenesis of PML. Inhibition of critical regulatory steps in the production of progeny virions may provide an efficient therapy for progressive demyelination in the JCV-infected human brain.

ACKNOWLEDGMENTS

We thank Dr. Y. Hara for helpful suggestions, Drs. S. Takeda and H. Takahashi for an electron micrograph, and Dr. S. Ichinose for cooperation in electron microscopy. This work is supported by grants from the Special Coordination Funds (SPSBS), the Science and Technology Agency of the Japanese Government, and the Japanese Ministry of Health, Labour and Welfare.

REFERENCES

Acheson NH (1978): Polyoma virus giant RNAs contain tandem repeats of the nucleotide sequence of the entire viral genome. Proc Natl Acad Sci USA 75:4754–4758.

Alwine JC (1982): Evidence for simian virus 40 late transcriptional control: Mixed infections of wild-type simian virus 40 and a late leader deletion mutant exhibit trans effects on late viral RNA synthesis. J Virol 42:798–803.

Aoki N, Mori M, Kato K, Sakamoto Y, Noda K, Tajima M, Shimada H (1996): Antibody against synthetic multiple antigen peptides (MAP) of JC virus capsid protein (VP1) without cross reaction to BK virus: A diagnostic tool for progressive multifocal leukoencephalopathy. Neurosci Lett 205:111–114.

Ault GS (1997): Activity of JC virus archetype and PML-type regulatory regions in glial cells. J Gen Virol 78:163–169.

Baker TS, Caspar DL, Murakami WT (1983): Polyoma virus ''hexamer'' tubes consist of paired pentamers. Nature 303:446–448.

Barkan A, Mertz JE (1984): The number of ribosomes on simian virus 40 late 16S mRNA is determined in part by the nucleotide sequence of its leader. Mol Cell Biol 4:813–816.

Barkan A, Welch RC, Mertz JE (1987): Missense mutations in the VP1 gene of simian virus 40 that compensate for defects caused by deletions in the viral agnogene. J Virol 61:3190–3198.

Barouch DH, Harrison SC (1994): Interactions among the major and minor coat proteins of polyomavirus. J Virol 68:3982–3989.

Batt DB, Rapp LM, Carmichael GG (1994): Splice site selection in polyomavirus late pre-mRNA processing. J Virol 68:1797–1804.

Ben-Ze'ev A, Abulafia R, Aloni Y (1982): SV40 virions and viral RNA metabolism are associated with cellular substructures. EMBO J 1:1225–1231.

Blasquez V, Beecher S, Bina M (1983): Simian virus 40 morphogenetic pathway. An analysis of assembly-defective tsB201 DNA protein complexes. J Biol Chem 258: 8477–8484.

Brady J, Radonovich M, Vodkin M, Natarajan V, Thoren M, Das G, Janik J, Salzman NP (1982): Site-specific base substitution and deletion mutations that enhance or suppress transcription of the SV40 major late RNA. Cell 31:625–633.

Buckler-White AJ, Humphrey GW, Pigiet V (1980): Association of polyoma T antigen and DNA with the nuclear matrix from lytically infected 3T6 cells. Cell 22:37–46.

Cai X, Chang D, Rottinghaus S, Consigli RA (1994): Expression and purification of recombinant polyomavirus VP2 protein and its interactions with polyomavirus proteins. J Virol 68:7609–7613.

Canaani D, Kahana C, Mukamel A, Groner Y (1979): Sequence heterogeneity at the 5′ termini of late simian virus 40 19S and 16S mRNAs. Proc Natl Acad Sci USA 76: 3078–3082.

Capco DG, Wan KM, Penman S (1982): The nuclear matrix: Three-dimensional architecture and protein composition. Cell 29:847–858.

Carswell S, Alwine JC (1986): Simian virus 40 agnoprotein facilitates perinuclear–nuclear localization of VP1, the major capsid protein. J Virol 60:1055–1061.

Chang D, Fung CY, Ou WC, Chao PC, Li SY, Wang M, Huang YL, Tzeng TY, Tsai RT (1997): Self-assembly of the JC virus major capsid protein, VP1, expressed in insect cells. J Gen Virol 78:1435–1439.

Chang D, Haynes JID, Brady JN, Consigli RA (1992a): Identification of a nuclear localization sequence in the polyomavirus capsid protein VP2. Virology 191:978–983.

Chang D, Haynes JID, Brady JN, Consigli RA (1992b): The use of additive and subtractive approaches to examine the nuclear localization sequence of the polyomavirus major capsid protein VP1. Virology 189:821–827.

Chen XS, Stehle T, Harrison SC (1998): Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry. EMBO J 17:3233–3240.

Chiou HC, Dabrowski C, Alwine JC (1991): Simian virus 40 late mRNA leader sequences involved in augmenting mRNA accumulation via multiple mechanisms, including increased polyadenylation efficiency. J Virol 65:6677–6685.

Clever J, Dean DA, Kasamatsu H (1993): Identification of a DNA binding domain in simian virus 40 capsid proteins Vp2 and Vp3. J Biol Chem 268:20877–20883.

Clever J, Kasamatsu H (1991): Simian virus 40 Vp2/3 small structural proteins harbor their own nuclear transport signal. Virology 181:78–90.

Coca-Prados M, Hsu MT (1979): Intracellular forms of simian virus 40 nucleoprotein complexes. II. Biochemical and electron microscopic analysis of simian virus 40 virion assembly. J Virol 31:199–208.

Colomar MC, Degoumois-Sahli C, Beard P (1993): Opening and refolding of simian virus 40 and in vitro packaging of foreign DNA. J Virol 67:2779–2786.

Conti E, Uy M, Leighton L, Blobel G, Kuriyan J (1998): Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin alpha. Cell 94:193–204.

Cowie A, Tyndall C, Kamen R (1981): Sequences at the capped 5′-ends of polyoma virus late region mRNAs: An example of extreme terminal heterogeneity. Nucleic Acids Res 9:6305–6322.

Dabrowski C, Alwine JC (1988): Translational control of synthesis of simian virus 40 late proteins from polycistronic 19S late mRNA. J Virol 62:3182–3192.

Daniel AM, Frisque RJ (1993): Transcription initiation sites of prototype and variant JC virus early and late messenger RNAs. Virology 194:7–109.

Daniel AM, Swenson JJ, Mayreddy RP, Khalili K, Frisque RJ (1996): Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. Virology 216:90–101.

Dean DA, Li PP, Lee LM, Kasamatsu H (1995): Essential role of the Vp2 and Vp3 DNA-binding domain in simian virus 40 morphogenesis. J Virol 69:1115–1121.

Delos SE, Cripe TP, Leavitt AD, Greisman H, Garcea RL (1995): Expression of the polyomavirus minor capsid proteins VP2 and VP3 in *Escherichia coli*: In vitro interactions with recombinant VP1 capsomeres. J Virol 69:7734–7742.

Delos SE, Montross L, Moreland RB, Garcea RL (1993): Expression of the polyomavirus VP2 and VP3 proteins in insect cells: Coexpression with the major capsid protein VP1 alters VP2/VP3 subcellular localization. Virology 194:393–398.

Fanning E, Baumgartner I (1980): Role of fast-sedimenting SV40 nucleoprotein complexes in virus assembly. Virology 102:1–12.

Flavell AJ, Cowie A, Arrand JR, Kamen R (1980): Localization of three major capped 5′ ends of polyoma virus late mRNA's within a single tetranucleotide sequence in the viral genome. J Virol 33:902–908.

Forstova J, Krauzewicz N, Wallace S, Street AJ, Dilworth SM, Beard S, Griffin BE (1993): Cooperation of structural proteins during late events in the life cycle of polyomavirus. J Virol 67:1405–1413.

Frisque RJ, Bream GL, Cannella MT (1984): Human polyomavirus JC virus genome. J Virol 51:458–469.

Garber EA, Seidman MM, Levine AJ (1980): Intracellular SV40 nucleoprotein complexes: Synthesis to encapsidation. Virology 107:389–401.

Garcea RL, Benjamin TL (1983): Isolation and characterization of polyoma nucleoprotein complexes. Virology 130:65–75.

Gharakhanian E, Kasamatsu H (1990): Two independent signals, a nuclear localization signal and a Vp1-interactive signal, reside within the carboxy-35 amino acids of SV40 Vp3. Virology 178:62–71.

Gharakhanian E, Takahashi J, Clever J, Kasamatsu H (1988): In vitro assay for protein–protein interaction: Carboxyl-terminal 40 residues of simian virus 40 structural protein VP3 contain a determinant for interaction with VP1. Proc Natl Acad Sci USA 85:6607–6611.

Gharakhanian E, Takahashi J, Kasamatsu H (1987): The carboxyl 35 amino acids of SV40 Vp3 are essential for its nuclear accumulation. Virology 157:440–448.

Ghosh PK, Piatak M, Mertz JE, Weissman SM, Lebowitz P (1982): Altered utilization of splice sites and 5′ termini in late RNAs produced by leader region mutants of simian virus 40. J Virol 44:610–624.

Ghosh PK, Reddy VB, Swinscoe J, Lebowitz P, Weissman SM (1978): Heterogeneity and 5′-terminal structures of the late RNAs of simian virus 40. J Mol Biol 126:813–846.

Gillock ET, Rottinghaus S, Chang D, Cai X, Smiley SA, An K, Consigli RA (1997): Polyomavirus major capsid protein VP1 is capable of packaging cellular DNA when expressed in the baculovirus system. J Virol 71:2857–2865.

Gluzman Y (1981): SV40-transformed simian cells support the replication of early SV40 mutants. Cell 23:175–182.

Goldmann C, Petry H, Frye S, Ast O, Ebitsch S, Jentsch KD, Kaup FJ, Weber F, Trebst C, Nisslein T, Hunsmann G, Weber T, Luke W (1999): Molecular cloning and expression of major structural protein VP1 of the human polyomavirus JC virus: Formation of virus-like particles useful for immunological and therapeutic studies. J Virol 73:4465–4469.

Good PJ, Welch RC, Barkan A, Somasekhar MB, Mertz JE (1988a): Both VP2 and VP3 are synthesized from each of the alternative spliced late 19S RNA species of simian virus 40. J Virol 62:944–953.

Good PJ, Welch RC, Ryu WS, Mertz JE (1988b): The late spliced 19S and 16S RNAs of simian virus 40 can be synthesized from a common pool of transcripts. J Virol 62:563–571.

Grass DS, Manley JL (1986): Effects of the adenovirus 2 late promoter on simian virus 40 transcription and replication. J Virol 57:129–137.

Grass DS, Manley JL (1987): Selective translation initiation on bicistronic simian virus 40 late mRNA. J Virol 61:2331–2335.

Griffith JP, Griffith DL, Rayment I, Murakami WT, Caspar DL (1992): Inside polyomavirus at 25-Å resolution. Nature 355:652–654.

Haegeman G, Fiers W (1978): Localization of the 5′ terminus of late SV40 mRNA. Nucleic Acids Res 5:2359–2371.

Haegeman G, Iserentant D, Gheysen D, Fiers W (1979a): Characterization of the major altered leader sequence of late mRNA induced by SV40 deletion mutant d1-1811. Nucleic Acids Res 7:1799–1814.

Haegeman G, van Heuverswyn H, Gheysen D, Fiers W (1976b): Heterogeneity of the 5′ terminus of late mRNA induced by a viable simian virus 40 deletion mutant. J Virol 31:484–493.

Hay N, Skolnik-David H, Aloni Y (1982): Attenuation in the control of SV40 gene expression. Cell 29:183–193.

Hunter T, Gibson W (1978): Characterization of the mRNA's for the polyoma virus capsid proteins VP1, VP2, and VP3. J Virol 28:240–253.

Ishii N, Minami N, Chen EY, Medina AL, Chico MM, Kasamatsu H (1996): Analysis of a nuclear localization signal of simian virus 40 major capsid protein Vp1. J Virol 70:1317–1322.

Ishii N, Nakanishi A, Yamada M, Macalalad MH, Kasamatsu H (1994): Functional complementation of nuclear targeting-defective mutants of simian virus 40 structural proteins. J Virol 68:8209–8216.

Jay G, Nomura S, Anderson CW, Khoury G (1981): Identification of the SV40 agnogene product: A DNA binding protein. Nature 291:346–349.

Kamen R, Favaloro J, Parker J (1980): Topography of the three late mRNA's of polyoma virus which encode the virion proteins. J Virol 33:637–651.

Kenney S, Natarajan V, Salzman NP (1986): Mapping 5′ termini of JC virus late RNA. J Virol 58:216–219.

Kiselev NA, Klug A (1969): The structure of viruses of the papilloma-polyoma type. V. Tubular variants built of pentamers. J Mol Biol 40:155–171.

Kosukegawa A, Arisaka F, Takayama M, Yajima H, Kaidow A, Handa H (1996): Purification and characterization of virus-like particles and pentamers produced by the expression of SV40 capsid proteins in insect cells. Biochim Biophys Acta 1290:37–45.

Kozak M (1986): Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44:283–292.

Kozak M (1987): An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res 15:8125–8148.

Kozak M (1996): Interpreting cDNA sequences: Some insights from studies on translation. Mamm Genome 7:563–574.

Kozak M (1999): Initiation of translation in prokaryotes and eukaryotes. Gene 234: 187–208.

Lai CJ, Dhar R, Khoury G (1978): Mapping the spliced and unspliced late lytic SV40 RNAs. Cell 14:971–982.

Lazarides E (1980): Intermediate filaments as mechanical integrators of cellular space. Nature 283:249–256.

Legon S, Flavell AJ, Cowie A, Kamen R (1979): Amplification in the leader sequence of late polyoma virus mRNAs. Cell 16:373–388.

Li M, Lyon MK, Garcea RL (1995): In vitro phosphorylation of the polyomavirus major capsid protein VP1 on serine 66 by casein kinase II. J Biol Chem 270:26006–26011.

Liddington RC, Yan Y, Moulai J, Sahli R, Benjamin TL, Harrison SC (1991): Structure of simian virus 40 at 3.8-Å resolution. Nature 354:278–284.

Lin W, Hata T, Kasamatsu H (1984): Subcellular distribution of viral structural proteins during simian virus 40 infection. J Virol 50:363–371.

Margolskee RF, Nathans D (1983): Suppression of a VP1 mutant of simian virus 40 by missense mutations in serine codons of the viral agnogene. J Virol 48:405–409.

Matsuda M, Jona M, Yasui K, Nagashima K (1987): Genetic characterization of JC virus Tokyo-1 strain, a variant oncogenic in rodents. Virus Res 7:159–168.

Montross L, Watkins S, Moreland RB, Mamon H, Caspar DL, Garcea RL (1991): Nuclear assembly of polyomavirus capsids in insect cells expressing the major capsid protein VP1. J Virol 65:4991–4998.

Moreland RB, Garcea RL (1991): Characterization of a nuclear localization sequence in the polyomavirus capsid protein VP1. Virology 185:513–518.

Nagashima K, Yamaguchi K, Yasui K, Ogiwara H (1981): Progressive multifocal leukoencephalopathy. Neuropathology and virus isolation. Acta Pathol Jpn 31:953–961.

Nandi A, Das G, Salzman NP (1985): Characterization of a surrogate TATA box promoter that regulates in vitro transcription of the simian virus 40 major late gene. Mol Cell Biol 5:591–594.

Ng SC, Bina M (1984): Temperature-sensitive BC mutants of simian virus 40: Block in virion assembly and accumulation of capsid-chromatin complexes. J Virol 50: 471–477.

Nomura S, Khoury G, Jay G (1983): Subcellular localization of the simian virus 40 agnoprotein. J Virol 45:428–433.

Ou WC, Wang M, Fung CY, Tsai RT, Chao PC, Hseu TH, Chang D (1999): The major capsid protein, VP1, of human JC virus expressed in Escherichia coli is able to self-assemble into a capsid-like particle and deliver exogenous DNA into human kidney cells. J Gen Virol 80:39–46.

Piatak M, Ghosh PK, Norkin LC, Weissman SM (1983): Sequences locating the 5′ ends of the major simian virus 40 late mRNA forms. J Virol 48:503–520.

Piatak M, Subramanian KN, Roy P, Weissman SM (1981): Late messenger RNA production by viable simian virus 40 mutants with deletions in the leader region. J Mol Biol 153:589–618.

Rayment I, Baker TS, Caspar DL, Murakami WT (1982): Polyoma virus capsid structure at 22.5 Å resolution. Nature 295:110–115.

Resnick J, Shenk T (1986): Simian virus 40 agnoprotein facilitates normal nuclear location of the major capsid polypeptide and cell-to-cell spread of virus. J Virol 60: 1098–1106.

Rinaldo CH, Traavik T, Hey A (1998): The agnogene of the human polyomavirus BK is expressed. J Virol 72:6233–6236.

Ryu WS, Mertz JE (1989): Simian virus 40 late transcripts lacking excisable intervening sequences are defective in both stability in the nucleus and transport to the cytoplasm. J Virol 63:4386–4394.

Salunke DM, Caspar DL, Garcea RL (1986): Self-assembly of purified polyomavirus capsid protein VP1. Cell 46:895–904.

Salunke DM, Caspar DL, Garcea RL (1989): Polymorphism in the assembly of polyomavirus capsid protein VP1. Biophys J 56:887–900.

Sandalon Z, Dalyot-Herman N, Oppenheim AB, Oppenheim A (1997): In vitro assembly of SV40 virions and pseudovirions: Vector development for gene therapy. Hum Gene Ther 8:843–849.

Sandalon Z, Oppenheim A (1997): Self-assembly and protein–protein interactions between the SV40 capsid proteins produced in insect cells. Virology 237:414–421.

Sedman SA, Gelembiuk GW, Mertz JE (1990): Translation initiation at a downstream AUG occurs with increased efficiency when the upstream AUG is located very close to the 5′ cap. J Virol 64:453–457.

Sedman SA, Good PJ, Mertz JE (1989): Leader-encoded open reading frames modulate both the absolute and relative rates of synthesis of the virion proteins of simian virus 40. J Virol 63:3884–3893.

Sedman SA, Mertz JE (1988): Mechanisms of synthesis of virion proteins from the functionally bigenic late mRNAs of simian virus 40. J Virol 62:954–961.

Shishido Y, Nukuzuma S, Mukaigawa J, Morikawa S, Yasui K, Nagashima K (1997): Assembly of JC virus-like particles in COS7 cells. J Med Virol 51:265–272

Shishido-Hara Y, Hara Y, Larson T, Yasui K, Nagashima K, Stoner GL (2000): Analysis of capsid formation of human polyomavirus JC (Tokyo-1 strain) by a eukaryotic expression system: Splicing of late RNAs, translation and nuclear transport of major capsid protein VP1, and capsid assembly. J Virol 74:1840–1853.

Siddell SG, Smith AE (1978): Polyoma virus has three late mRNA's: one for each virion protein. J Virol 27:427–431.

Silverman L, Rubinstein LJ (1965): Electron microscopic observations on a case of progressive multifocal leukoencephalopathy. Acta Neuropathol (Berl) 5:215–224.

Sock E, Renner K, Feist D, Leger H, Wegner M (1996): Functional comparison of PML-type and archetype strains of JC virus. J Virol 70:1512–1520.

Somasekhar MB, Mertz JE (1985a): Exon mutations that affect the choice of splice sites used in processing the SV40 late transcripts. Nucleic Acids Res 13:5591–5609.

Somasekhar MB, Mertz JE (1985b): Sequences involved in determining the locations of the 5′ ends of the late RNAs of simian virus 40. J Virol 56:1002–1013.

Stamatos NM, Chakrabarti S, Moss B, Hare JD (1987): Expression of polyomavirus virion proteins by a vaccinia virus vector: Association of VP1 and VP2 with the nuclear framework. J Virol 61: 516–525.

Stehle T, Gamblin SJ, Yan Y, Harrison SC (1996): The structure of simian virus 40 refined at 3.1 Å resolution. Structure 4:165–182.

Stehle T, Harrison SC (1997): High-resolution structure of a polyomavirus VP1-oligosaccharide complex: Implications for assembly and receptor binding. EMBO J 16: 5139–5148.

Stehle T, Yan Y, Benjamin TL, Harrison SC (1994): Structure of murine polyomavirus complexed with an oligosaccharide receptor fragment. Nature 369:160–163.

Takebe Y, Seiki M, Fujisawa J, Hoy P, Yokota K, Arai K, Yoshida M, Arai N (1988): SR alpha promoter: An efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol 8:466–472.

Wychowski C, Benichou D, Girard M (1987): The intranuclear location of simian virus 40 polypeptides VP2 and VP3 depends on a specific amino acid sequence. J Virol 61:3862–3869.

Yan Y, Stehle T, Liddington RC, Zhao H, Harrison SC (1996): Structure determination of simian virus 40 and murine polyomavirus by a combination of 30-fold and 5-fold electron-density averaging. Structure 4:157–164.

Yogo Y, Kitamura T, Sugimoto C, Ueki T, Aso Y, Hara K, Taguchi F (1990): Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J Virol 64:3139–3143.

Zu Rhein GM, Chou SM (1965): Particles resembling papova viruses in human cerebral demyelinating disease. Science 148:1477–1479.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

9

CELLULAR RECEPTORS FOR THE POLYOMAVIRUSES

WALTER J. ATWOOD, PH.D.

1. INTRODUCTION

Polyomaviruses are widely distributed in nature and are associated with the establishment of lifelong persistent infections in their respective natural hosts. All of the polyomaviruses display a high degree of species specificity. This has generally been linked to factors involved in species-specific DNA replication. Within a given species, the host range, or cell type specificity of the polyomaviruses, can be either very broad or very specific. For example, the mouse polyoma virus (PyV), BK virus (BKV), and simian virus 40 (SV40) all infect a wide variety of cell types both in vivo and in vitro. In contrast, JC virus (JCV) and the lymphotropic papovavirus (LPV) infect a very narrow range of cell types. The presence or absence of cell type-specific transcription factors are important determinants of polyomavirus host range and tissue tropism. It is also becoming clear that the presence or absence of specific polyomavirus receptors and pseudoreceptors contributes to the tropism, spread, and pathogenicity of these viruses. This chapter focuses on past and current efforts to define a role for cellular receptors in mediating infection of cells by polyomaviruses with both wide and narrow cellular tropism. The roles of specific cellular receptors in determining virus tropism are thoroughly examined and discussed. The major focus is on viruses that infect humans; however, this cannot

be done without including the excellent and seminal work involving polyomaviruses of other species, most notably the mouse.

2. VIRUS RECEPTORS AND TROPISM

The initial step in the establishment of virus infection is the interaction between the virus and receptors present on the surfaces of cells and tissues. In general, viruses that have a very narrow host range and tissue tropism are often shown to interact with high affinity to a limited number of specific receptors present on susceptible cells (Marsh and Helenius, 1989). In some instances virus tropism is strictly determined by the presence of specific receptors that mediate binding and entry (Dalgleish et al., 1984; Klatzman et al., 1984; Mendelsohn et al., 1989; Racaniello, 1990; Tomassini et al., 1989; Weiss and Tailor, 1995). In other instances, successful entry into a cell is necessary but not sufficient for viral replication (Atwood and Norkin, 1989; Bass and Greenberg, 1992; Mei and Wadell, 1995). In these cases additional permissive factors that interact with viral regulatory elements are required.

The complexity of virus host–cell receptor interactions is apparent when one examines the role of CD4 in mediating HIV-1 infection of susceptible cells. Early observations of the selective loss of CD4-positive T cells and subsequent propagation of HIV-1 in T cells led to the suggestion that CD4 was a specific HIV-1 receptor. It was then shown that human cells lacking CD4 were resistant to infection by HIV-1 and that these cells could be rendered susceptible to infection by the introduction of CD4 (Dalgleish et al., 1984; Klatzman et al., 1984). In contrast, mouse cells expressing human CD4 could not be rendered susceptible to infection with HIV-1. In addition, several primary isolates of HIV-1 failed to infect human CD4-positive T-cell lines. These latter studies concluded that CD4 was necessary, but not sufficient, for infection (Clapham, 1991; James et al., 1996). This discrepancy was resolved when several independent groups discovered that multiple co-factors, in addition to CD4, are required for efficient entry of HIV-1. A seven transmembrane G protein–coupled receptor named CXCR-4 has been identified as a co-factor for entry of several T-cell tropic strains of HIV-1 into diverse cell types (Feng et al., 1996). The β-chemokine receptor CCR-5 functions as the co-factor for entry of many macrophage tropic isolates of HIV-1 (Deng et al., 1996; Dragic et al., 1996). Thus, successful infection of a cell with HIV-1 requires the coordinated participation of several cell surface receptors.

3. THE POLYOMAVIRUS VIRION

Polyomaviruses are small, nonenveloped, double-stranded DNA-containing viruses. The virions range in size from 40 to 50 nm, have sedimentation coefficients of 240S in sucrose gradients, and have a density of 1.34 g/ml in cesium

Table 9.1. Capsid Proteins of the Human Polyomaviruses

Virus	Host	Genome Size	Virus-Encoded Proteins (Amino Acids)		
			VP1	VP2	VP3
JCV	Human	5130	354	344	225
BKV	Human	5133	362 (78)[a]	351 (79)	232 (75)
SV40	Rhesus monkey/ human?	5243	362 (75)	352 (72)	234 (66)

[a]Percent identity with JCV.

chloride. The virions are relatively resistant to heat, surviving for up to 1 hour in water at 55°C. The polyomavirus capsids are assembled from three virus-encoded structural proteins termed VP1, VP2, and VP3. VP1, VP2, and VP3 are relatively conserved among the polyomaviruses JCV, BKV and SV40, sharing approximately 75% amino acid identity (Table 9.1). VP1 is the major capsid protein and represents 80% of the total virion protein in the capsid. VP2 and VP3 are minor components and together represent 20% of the total virion protein in the capsid. The virus particles are icosahedrally symmetric (T = 7d) and consist of 360 copies of VP1 arranged in 72 pentamers with both five- and sixfold axes of rotation (Salunke et al., 1986) (Fig. 9.1). The pentameric structures are held together by the carboxy-terminal segments of each VP1 monomer, which interact with adjoining pentamers in the icosahedral shell. The major receptor binding determinants of the polyomaviruses reside on each of the VP1 monomers, which are exposed at the surface of the virion (Stehle et al., 1994). It has been suggested that VP2 may also play a role in virus entry

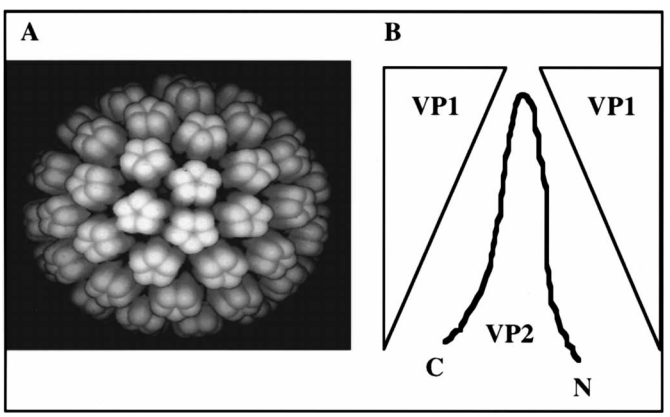


Figure 9.1. (**A**) Computer-generated image of a polyomavirus virion. (**B**) Schematic of the location of VP2 within each VP1 pentamer (only two of the five VP1 molecules are shown).

as the protein extends a loop into the cavity formed within the VP1 pentameric structure (Fig. 9.1) (Chen et al., 1998). VP2 and VP3 may also play a role in anchoring histone-associated viral DNA to VP1 in the capsid; however, virus-like particles (VLPs) consisting of only VP1 can self-assemble and package DNA (Gillock et al., 1997; Salunke et al., 1986). Currently, these VLPs are being developed as gene delivery vehicles. It is unclear whether VLPs share the same cell type specificity as native virions.

4. CELLULAR RECEPTORS FOR THE MOUSE POLYOMA VIRUS

Introduction

The PyV was originally isolated from adenocarcinomas that developed in C3H mice that had been inoculated with extracts prepared from murine leukemia virus–infected AK mice (Gross, 1953). The subsequent demonstration that this virus was capable of inducing a wide range of tumors in experimentally infected newborn mice led to the name *poly-oma* (Stewart et al., 1958). Polyomaviruses have a very broad cellular tropism, infecting more than 30 different cell types (Dawe et al., 1987). This indicates that polyomavirus receptors are either ubiquitously expressed on cells or that polyomaviruses can use more than one receptor. Recent evidence based on the inability to generate monoclonal antibodies that block polyomavirus infection suggest that the latter may be the case (Bauer et al., 1999).

Virus–Receptor Interactions

The interaction between PyV and host cells involves a direct association between the major viral capsid protein VP1 and N-linked glycoproteins bearing specific linkages of sialic acid (Table 9.2). This interaction is critical for viral hemagglutination of red blood cells, for virus infection, and for in vivo tumorigenicity (Cahan et al., 1983; Dubensky et al., 1991; Freund et al., 1991a,b; Fried et al., 1981; Ricci et al., 1992; Sahli et al., 1993).

Early work identified two sialic acid binding variants of mouse polyomavirus, one giving rise to small plaques (RA) and the other to large plaques (PTA) (Diamond and Crawford, 1964). These two strains also differed dramatically in their ability to induce tumors in mice (Dawe et al., 1987). The small plaque strain was found to be very inefficient at inducing tumors in mice, whereas the large plaque strain was highly tumorigenic (Dawe et al., 1987). Early biochemical data demonstrated that a major difference between these two virus strains involved their interaction with specific linkages of sialic acid (Cahan et al., 1983). The small plaque strain was found to recognize both $\alpha(2-3)$-linked and $\alpha(2-6)$-linked sialic acids, whereas the large plaque strain only recognized the $\alpha(2-3)$-linked sugar (Cahan et al., 1983). Subsequent work mapped the ability of these strains to discriminate between $\alpha(2-3)$-linked and

Table 9.2. Polyoma Virus Receptors

Virus[a]	Receptor	Number/ cell	Kd	Tropism	Notes
PyV (LP)	N-linked glycoprotein containing α(2–3)-linked sialic acid	25,000	1.8×10^{-11} M	Broad	Mutations in VP1 alter sialic acid recognition and tissue tropism
PyV (SP)	N-linked glycoprotein containing α(2–3)-linked sialic acid	25,000	1.8×10^{-11} M	Broad	Also binds to a branched α(2–6)-linked sialic acid pseudoreceptor
SV40	MHC class I proteins, also a role for O-linked glycans	90,000	3.76×10^{-12} M	Broad	Only family member that does not interact with sialic acids
LPV	O-linked glycoprotein containing α(2–6)-linked sialic acid	1800	2.9×10^{-12} M	Restricted to B cells	Presence of a specific receptor correlates with susceptibility to infection
BKV	Glycolipid containing α(2–3)-linked sialic acid	?	?	Broad	
JCV	N-linked glycoprotein containing α(2–6)-linked sialic acid	50,000	?	Restricted to glial cells and B cells	Does not share receptor specificity with SV40

[a]LP and SP refer to the large plaque (PTA) and small plaque (RA) isolates of PyV, respectively.

α(2–6)-linked sialic acids to a single amino acid polymorphism in the major capsid protein VP1 (Bauer et al., 1995, 1999; Mezes and Amati, 1994; Stehle et al., 1994). The small plaque strain has glycine at position 91 in VP1, which accommodates the presence of a branched α(2–6)-linked sialic acid. The large plaque strain has glutamic acid at this position, which prevents binding to the branched α(2–6)-linked sugar. It is clear that the α(2–3) linkage is critical for mediating infection of cells by both of these virus strains. The ability of the small plaque strain to recognize α(2–6)-linked sialic acids reduces its ability to spread both in vitro and in vivo (Bauer et al., 1999). The α(2–6)-linked sugar is therefore considered a pseudoreceptor for this virus.

Interestingly, a third nontumorigenic but highly lethal polyoma virus strain, LID, has glutamic acid at position 91 but has an alanine rather than a valine at position 296 (Bauer et al., 1999). This change leads to less efficient binding to the α(2–3)-linked sialic acid, which results in increased viral spread and lethality in the host (Bauer et al., 1999). It is therefore clear that polyoma virus receptor interactions are critical determinants of viral spread and pathogenesis in the host.

Early attempts to identify the proteinaceous component of the PyV receptor used chemical cross-linking and co-immunoprecipitation to identify a 120 kDa protein as a candidate polyoma virus receptor (Griffith and Consigli, 1986; Marriott et al., 1987). Antiserum raised to this protein was subsequently found to block infection of mouse kidney cells by PyV (Griffith and Consigli, 1986; Marriott et al., 1987). The identity of this protein has not been determined. An alternative approach to identifying specific polyomavirus receptors is to generate and screen monoclonal antibodies prepared against permissive cells. This approach was recently attempted, and 2000 hamster monoclonal antibodies directed against mouse 3T3 cells were screened for their ability to block polyoma virus infection (Bauer et al., 1999). Several of the monoclonals were found to bind to the 3T3 cells, but none of them inhibited virus infection. This led to the suggestion that there may be multiple receptor species on 3T3 cells that can be used by polyoma virus to infect the cell (Bauer et al., 1999).

PyV Cell Entry

Early studies examining the entry of PyV into cells found that virions and empty capsids had different receptor specificities (Bolen and Consigli, 1979; Mackay and Consigli, 1976). Virions were found to be selectively targeted to the nucleus, and empty capsids were found to be targeted to phagolysosomes. The binding of virions and empty capsids to mouse cells also differed in their sensitivity to inhibition by neuraminidase. Neuraminidase could, however, inhibit infection of mouse cells with crude virus stocks, and the inhibition could be overcome by incubating the treated cells with β-galactosidase α—2,3-sialyltransferase and CMP-NeuAc (Fried et al., 1981).

Recent data indicate that infectious entry of SV40 and JCV proceeds by different mechanisms (Fig. 9.2). SV40 was found to enter cells by caveolae-

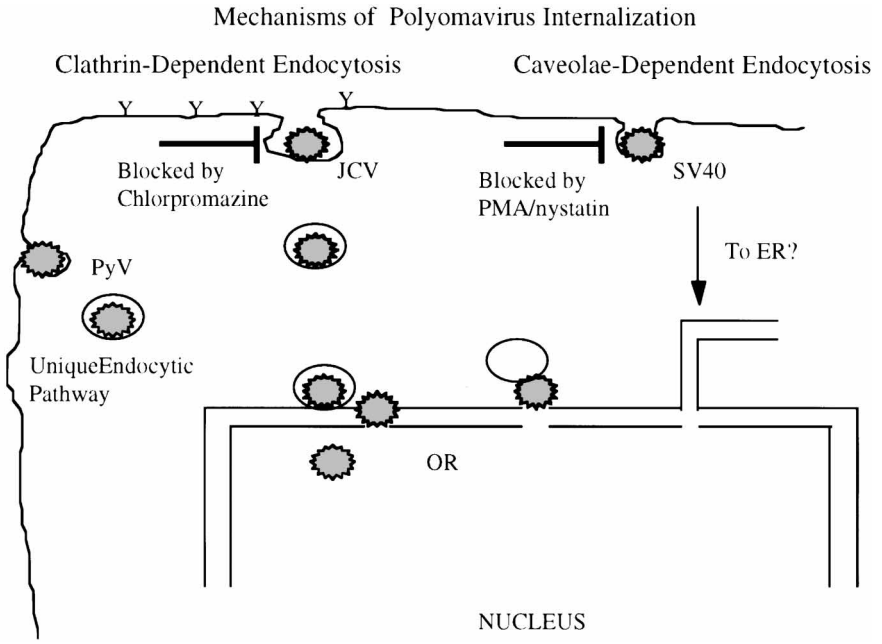


Figure 9.2. Each of the polyomaviruses shown exploits different endocytic pathways to gain access to the cell. JCV uses clathrin-dependent endocytosis, SV40 uses caveolae-dependent endocytosis, and PyV uses a unique pathway involving uncoated vessicles. All three of these viruses must then target their respective genomes to the nucleus. Evidence shows that SV40 virions are targeted at least initially to the endoplasmic reticulum (ER). The virus must then escape this compartment, gain access to the cytoplasm, and deliver the genome to the nucleus via nuclear pores. The mechanisms by which PyV and JCV accomplish this are not known.

mediated endocytosis, and JCV was found to enter cells by classic clathrin-mediated endocytosis (Anderson et al., 1996; Pho et al., 2000; Stang et al., 1997). Similar work with the small plaque isolate of PyV found that this virus uses neither of these pathways to infect baby mouse kidney cells (Gilbert and Benjamin, 2000). It thus appears that each of these related polyomaviruses uses an independent mechanism to enter cells and target their respective genomes to the nucleus (Fig. 9.2).

5. CELLULAR RECEPTORS FOR SV40

Introduction

SV40 was initially isolated from cultures of rhesus monkey kidney cells being used to propagate vaccine strains of poliovirus (Sweet and Hilleman, 1960). At the time of its isolation, several million individuals had received poliovirus

vaccines contaminated with significant amounts of this oncogenic virus (Shah and Nathanson, 1976). Fortunately, epidemiologic studies do not show increased rates of cancer among the inoculated individuals (Shah, 2000). However, recent studies suggest that SV40 may in fact circulate in the human population. SV40 has been associated with the development of a number of human cancers, including mesothelioma, osteosarcoma, and choroid plexus papillomas (Barbanti-Brodano et al., 1998; Bergsagel et al., 1992; Carbone et al., 1994, 1996; Lednicky et al., 1995).

SV40 has been the most widely studied polyomavirus, and it has been used as a model system for understanding many aspects of eukaryotic molecular biology, including DNA replication, transcription, and mRNA splicing. SV40 is also the only polyomavirus for which a specific cell surface protein (MHC class I) has been identified as a virus receptor (Atwood and Norkin, 1989; Breau et al., 1992).

Virus–Receptor Interactions

SV40 is unique among polyomaviruses as it does not interact with sialic acids and therefore lacks the ability to hemagglutinate red blood cells. Sialydase treatment of monkey kidney cells also has no effect on either SV40 binding or SV40 infection (Clayson and Compans, 1989). Similarly, treatment of monkey kidney cells with phospholipase C, phospholipase D, trypsin, chymotrypsin, endoglycosidase F, and glycopeptidase F does not inhibit SV40 binding (Clayson and Compans, 1989). The binding of SV40 to monkey kidney cells is saturable and of high avidity. The number of SV40 receptors on cells has been calculated at 9×10^4 with a dissociation constant of 3.76 pM (Table 9.2) (Clayson and Compans, 1989).

The subsequent identification of MHC class I proteins as a principal component of the SV40 receptor came about from experiments designed to determine whether SV40 infection reduced cell surface MHC class I protein expression (Atwood, 1991). It was reasoned that such a mechanism may account for the ability of SV40 to persist in its natural host, the rhesus macaque (Atwood, 1991; Norkin, 1982). During the course of these studies SV40 binding to cells was found to specifically inhibit the subsequent binding of antibodies directed at MHC class I proteins. Conversely, anti-MHC class I antibodies blocked the binding of radiolabeled SV40 to cells and inhibited infection in a dose-dependent manner (Atwood and Norkin, 1989). Subsequent experiments demonstrated that reconstitution of class I protein expression in two separate class I null cell lines could rescue virus binding to these cells but could not rescue infection (Breau et al., 1992). This indicated that MHC class I proteins were necessary but not sufficient for SV40 infection of cells. This was confirmed by a series of studies demonstrating that the distribution and levels of MHC class I protein expression on numerous human cell types did not correlate with their susceptibility to infection (Basak et al., 1992). These data indicate

that additional factors contribute to SV40 tropism. These additional factors may include transcription factors and the presence of a co-receptor.

Biochemical data also support a role for MHC class I proteins as cell surface receptors for SV40. For example, the ability of papain to cleave cell surface class I molecules correlates with its ability to inhibit SV40 binding to cells (Clayson and Compans, 1989; Wong et al., 1984). Conversely, MHC class I proteins are resistant to digestion with trypsin and chymotrypsin, and neither of these enzymes inhibits virus binding (Clayson and Compans, 1989; Wong et al., 1984).

The discovery of the SV40 receptor led others to investigate whether related polyomaviruses might also use MHC class I proteins as a receptor. Two polyomaviruses were examined, PyV and JCV, neither of which was found to use MHC class I proteins as a receptor (Liu et al., 1998; Sanjuan et al., 1992).

SV40 Cell Entry

Early work characterizing SV40 entry into cells found that virions internalized into both monopinocytotic vesicles and a larger endosomal compartment (Barbanti-Brodano et al., 1970; Hummeler et al., 1970; Maul et al., 1978). By analogy with similar work in the PyV system, it was assumed that virus in the large endosomes was degraded in phagolysosomes and that virus in monopinocytotic vesicles actually initiated a productive infection (Bolen and Consigli, 1979; Khare and Consigli, 1965; Mackay and Consigli, 1976). Subsequent work studying SV40 entry demonstrated that virus entered via uncoated membrane-bound invaginations that subsequently fused with the endoplasmic reticulum (ER) (Kartenbeck et al., 1989).

These membrane-bound invaginations were subsequently identified as caveolae (Anderson et al., 1996). Caveolae are cholesterol-rich membrane microdomains that have a high concentration of glycosylphosphatidylinositol (GPI)-anchored proteins (Anderson, 1993a,b; Anderson, 1998). They participate in the endocytosis of small-molecular-weight compounds (potocytosis) and in the trafficking of molecules across the cell (transcytosis). Caveolae also participate in the trafficking of plasma membrane components back to the ER (reverse endocytosis). Interestingly, the major cellular receptor for SV40, MHC class I proteins, are enriched in caveolae and participate in localizing SV40 to these membrane domains (Anderson et al., 1998; Stang et al., 1997). The next step in the infectious process is thought to be the release of SV40 from class I heavy chains that is either preceded by or followed by class I heavy chain cleavage by a metalloprotease (Anderson et al., 1998; Chen and Norkin, 1999). The mechanism by which SV40 then targets its genome to the nucleus is not known. The virus presumably escapes into the cytoplasm from the lumen of the endoplasmic reticulum or directly from the endosome. Evidence strongly favors entry of SV40 into the nucleus from the cytoplasm via nuclear pores (Clever et al., 1991; Yamada and Kasamatsu, 1993). It is not clear whether any components of the virion other than nucleic acid enter the nucleus.

6. CELLULAR RECEPTORS FOR THE LYMPHOTROPIC POLYOMAVIRUS

The lymphotropic papovavirus (LPV) was originally isolated from the lymph node of an African green monkey and maintained by continual passage in a B lymphocyte cell line, BJA-B (Brade et al., 1981; zur Hausen and Gissmann, 1979). The host range of LPV is limited to a very narrow subset of human B-cell lines (Takemoto et al., 1982). The restriction to infection of these cells by LPV has been shown to correlate with the presence of specific LPV receptors on permissive cells (Haun et al., 1993; Herrmann et al., 1995, 1997; Keppler et al., 1994, 1995). Binding to, and infection of, human B-cell lines was found to be dependent on the presence of O-linked glycoproteins containing terminal $\alpha(2-6)$-linked sialic acid. The proteinaceous component of LPV receptors has not been determined. The mechanism by which LPV targets its genome to the nucleus has not been studied.

7. CELLULAR RECEPTORS FOR BKV

BKV was originally isolated from the urine of a renal transplant patient and subsequently propagated in Vero cells (Gardner et al., 1971). BKV infects a wide variety of cells, including human and monkey kidney cells, embryonic lung cells, fetal brain cells, fibroblasts, and human foreskin epithelial cells (Yoshiike and Takemoto, 1986). There is little information regarding cellular receptors for BKV or the mechanisms of BKV entry into cells. BKV, like many of the other polyomaviruses, hemagglutinates red blood cells in a sialic acid–dependent manner (Seganti et al., 1981). One report has suggested a role for phospholipids in mediating hemagglutination and infection of Vero cells by BKV (Sinibaldi et al., 1992). The mechanism by which BKV targets its genome to the nucleus has not been studied.

8. CELLULAR RECEPTORS FOR JCV

Introduction

JCV was originally isolated from the brain of a patient with PML and subsequently propagated in cultures of human fetal glial cells (Padgett et al., 1971). As these cells are difficult to obtain, virus isolation and propagation was attempted in a wide variety of other cell types with little or no success (Major et al., 1992). As a result of these early studies, JCV was characterized as having a very narrow host range. In vivo, JCV infection is restricted to oligodendrocytes, astrocytes, and B lymphocytes (Houff et al., 1988; Monaco et al., 1996). This highly restricted cell type specificity is also seen in vitro as virus infection is restricted to primary cultures of human glial cells and to a few established human glial cell lines.

As JCV is the etiologic agent of a fatal central nervous system demeylinating disease, understanding the molecular events governing virus host–cell interactions, including virus–receptor interactions, is critical.

Virus–Receptor Interactions

JCV, like most other polyomaviruses, hemagglutinates red blood cells in a sialic acid–dependent manner. Until recently very little was known about JCV receptors on cells other than erythrocytes. The first suggestion that receptors played a role in tropism came from a comparison of the ability of JCV virions or JCV DNA to initiate early viral gene expression in nonpermissive HeLa cells. In these experiments, JCV virions did not infect HeLa cells, and no early viral gene expression was detected (Schweighardt and Atwood, 2000). In contrast, transfection of JCV DNA into the HeLa cells led to early viral gene expression (Schweighardt and Atwood, 2000). This suggests that one block to infection of nonpermissive cells by JCV is at an early stage in the viral life cycle, perhaps at the level of receptor binding. The nature of the glial cell receptor for JCV was then characterized biochemically. These data demonstrated that infection of glial cells by JCV can be inhibited by treating cells with tunicamycin or by enzymatic removal of $\alpha(2-3)$-linked and $\alpha(2-6)$-linked sialic acids (Liu et al., 1998). A recombinant $\alpha(2-3)$-specific neuraminidase did not inhibit infection of these cells by JCV, suggesting that the $\alpha(2-6)$-linkage is critical. Infection of cells by JCV was also not inhibited by an O-linked glycosylation inhibitor, benzylGalNac, or by trypsin, chymotrypsin, phospholipase A2, or phospholipase C.

These data, when taken together, demonstrate that the JCV receptor is a trypsin resistant N-linked glycoprotein containing $\alpha(2-6)$-linked sialic acid (Liu et al., 1998). These properties distinguish JCV from other polyomaviruses that use sialic acid as cell surface receptors (Table 9.2). It is interesting to note that treatment of glial cells with trypsin reduces virus binding to cells but, paradoxically, leads to increased infectivity (our unpublished observations). This may be due to the elimination of pseudoreceptors for JCV that act to limit accessibility to specific and productive receptor binding sites.

The Relationship Between JCV Binding and Infectivity

Recently, JCV binding to a wide variety of permissive and nonpermissive cells was studied by flow cytometry. JCV bound to all of the cell lines tested regardless of their known susceptibility to infection (Wei et al., 2000). An $\alpha(2-6)$-linked sialic acid–specific lectin, SNA, also bound to all of these cells. It is therefore likely that many cell surface glycolipids and glycoproteins are modified by $\alpha(2-6)$ sialyation but that only a minority of these molecules can serve as a specific receptors for JCV. Interestingly, when JCV binding to primary cells was examined a different story emerged. In these experiments JCV only bound to primary cells that were known to be susceptible to infection.

For example, virus bound to primary human glial cells, to primary tonsillar stromal cells, and to primary human B cells but did not bind to primary human T cells (Wei et al., 2000). This indicates that there may be specificity of JCV binding in vivo that is not apparent when one examines virus binding to tumor cell lines. The identification of a specific glial cell receptor for JCV has remained elusive.

JCV Cell Entry

Recently, the kinetics and mechanisms of JCV entry into human glial cells was compared with that of SV40. These data demonstrated that infectious entry of JCV into human glial cells proceeds more rapidly than infectious entry of SV40 (Pho et al., 2000). The majority of JCV entered into an antibody neutralization resistant compartment within 30 minutes postadsorption, whereas SV40 required 2 hours to enter cells. Specific inhibitors of either caveolae-dependent endocytosis or clathrin-dependent endocytosis were then used to study the mechanism of infectious entry. These experiments confirmed that infectious entry of SV40 is mediated by caveolae- and not by clathrin-dependent endocytosis (Pho et al., 2000). In contrast, infectious entry of JCV was not blocked by inhibitors of caveolae-dependent endocytosis but was blocked by inhibitors of the clathrin-dependent pathway (Pho et al., 2000). These data were confirmed by co-localization of labeled JCV and labeled transferrin in endosomes (Pho et al., 2000). This indicates that infectious entry of JCV proceeds via classic clathrin-dependent endocytosis. These data are also consistent with earlier work demonstrating that JCV and SV40 do not share receptor specificity on human glial cells (Liu et al., 1998).

9. CONCLUSIONS

Sialic acids are major components of cell surface receptors for several human viruses, including orthomyxoviruses, rotaviruses, and all of the polyomaviruses except SV40 (Bass et al., 1991; Chen and Benjamin, 1997; Haun et al., 1993; Ito et al., 1997; Keppler et al., 1995; Mantyjarvi et al., 1972; Willoughby et al., 1990). In each case virus infectivity is mediated by recognition of specific sialic acid linkages to the underlying glycan. In most instances the identities of the proteinaceous components of these receptors is unknown. Interestingly, each of the sialic acid–utilizing polyomaviruses recognizes different linkages of this common carbohydrate moiety.

It is also becoming clear that members of this highly related family of viruses do not share receptor specificity. For example, PyV and SV40 do not compete with each other for binding to cells and do not share MHC class I as a specific cellular receptor. Similarly, JCV and SV40 do not share receptor specificity. It is also becoming apparent that the choice of receptor dictates the endocytic pathway that each virus uses to gain entry into the cells that it infects.

For example, JCV, SV40, and PyV have different receptor specificities, and all exploit different endocytic pathways to target their genomes to the nucleus.

Direct comparisons of JCV with BKV, PyV, or LPV have not been done. This is due to the fact that these viruses all have distinct cellular tropisms that in large part are governed at the transcriptional level. Current efforts to develop polyomavirus pseudovirions that package defined nucleic acids capable of expressing reporter genes in a wide range of cells should allow for these comparisons to be made in the future. Also, efforts are underway to construct chimeric polyomaviruses that only differ with respect to the late-coding region. The identification of receptors for each of these important polyomaviruses should lead to a better understanding of the critical early events in the life cycle of these viruses. This in turn will allow a better understanding of polyomaviral tropism, spread, and pathogenicity in the host. Finally, this may make possible the development of novel therapeutic strategies to prevent infection and spread of these viruses in the immunocompromised host.

Recently, SV40 has been shown to traffic from the plasma membrane to a new subcellular organelle referred to as the caveosome. Transport to the caveosome was shown to be actin dependent, and transport out of the caveosome to smooth ER was shown to be microtubule dependent. (Pelkmans L, Kartenbeck J, Helenius A (2001) Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nat Cell Biol 3:473–483).

REFERENCES

Anderson HA, Chen Y, Norkin LC (1996): Bound simian virus 40 translocates to caveolin enriched membrane domains, and its entry is inhibited by drugs that selectively disrupt caveolae. Mol Biol Cell 7:1825–1834.

Anderson HA, Chen Y, Norkin LC (1998): MHC class I molecules are enriched in caveolae but do not enter with simian virus 40. J Gen Virol 79:1469–1477.

Anderson RGW (1993a): Caveolae: Where incoming and outgoing messengers meet. Proc Natl Acad Sci USA 90:10909–10913.

Anderson RGW (1993b): Potocytosis of small molecules and ions by caveolae. Trends Cell Biol 3:69–72.

Anderson RGW (1998): The caveolae membrane system. Annu Rev Biochem 67:199–225.

Atwood WJ (1991): Major Histocompatibility Complex Encoded HLA Class I Proteins Are Cell Surface Receptors for the Simian Virus 40. Ph.D. Dissertation, University of Massachusetts.

Atwood WJ, Norkin LC (1989): Class I major histocompatibility proteins as cell surface receptors for simian virus 40. J Virol 63:4474.

Barbanti-Brodano G, Martini F, De Mattei M, Lazzarin L, Corallini A, Tognon M (1998): BK and JC human polyomaviruses and simian virus 40: Natural history of infections in humans, experimental oncogenicity, and association with human tumors. Adv Virus Res 50:69–99.

Barbanti-Brodano G, Swetly P, Koprowski H (1970): Early events in the infection of permissive cells with simian virus 40: Adsorption, penetration, and uncoating. J Virol 6:78–86.

Basak S, Turner H, Compans RW (1992): Expression of SV40 receptors on apical surfaces of polarized epithelial cells. Virology 190:393–402.

Bass DM, Greenberg HB (1992): Strategies for the identification of icosahedral virus receptors. J Clin Invest 89:3–9.

Bass DM, Mackow E, Greenberg HB (1991): Identification and partial characterization of rhesus rotavirus binding glycoprotein on murine enterocytes. Virology 183:602–610.

Bauer PH, Bronson RT, Fung SC, Freund R, Stehle T, Harrison SC, Benjamin TL (1995): Genetic and structural analysis of a virulence determinant in polyomavirus VP1. J Virol 69:7925–7931.

Bauer PH, Cui C, Stehle T, Harrison SC, DeCaprio JA, Benjamin TL (1999): Discrimination between sialic acid containing receptors and pseudoreceptors regulates polyomavirus spread in the mouse. J Virol 73:5826–5832.

Bergsagel DJ, Finegold MJ, Butel JS, Kupsky WJ, Garcea RL (1992): DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N Engl J Med 326:988–993.

Bolen JB, Consigli RA (1979): Differential adsorption of polyoma virions and capsids to mouse kidney cells and guinea pig erythrocytes. J Virol 32:679–683.

Brade L, Vogl W, Gissman L, zur Hausen H (1981): Propagation of B-lymphotropic papovavirus (LPV) in human B-lymphoma cells and characterization of its DNA. Virology 114:228–235.

Breau WC, Atwood WJ, Norkin LC (1992): Class I major histocompatibility proteins are an essential component of the simian virus 40 receptor. J Virol 66:2037–2045.

Cahan LD, Singh R, Paulson JC (1983): Sialyloligosaccharide receptors of binding variants of polyomavirus. Virology 130:281–289.

Carbone M, Pass HI, Rizzo P, Marinetti MR, Di Muzio M, Mew DJY, Levine AS, Procopio A (1994): Simian virus 40-like sequences in human pleural mesothelioma. Oncogene 9:1781–1790.

Carbone M, Rizzo P, Procopio A, Giuliano M, Pass HI, Gebhardt MC, Mangham C, Hansen M, Malkin DF, Bushart G, Pompetti F, Picci P, Levine AS, Bergsagel JD, Garcea RL (1996): SV40-like sequences in human bone tumors. Oncogene 13:527–535.

Chen MH, Benjamin T (1997): Roles of N-glycans with alpha 2,6 as well as alpha 2,3 linked sialic acid in infection by polyoma virus. Virology 233:440–442.

Chen XS, Stehle T, Harrison SC (1998): Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry. EMBO J 17:3233–3240.

Chen Y, Norkin LC (1999): Extracellular simian virus 40 transmits a signal that promotes virus enclosure within caveolae. Exp Cell Res 246(1):83–90.

Clapham P (1991): Human immunodeficiency virus infection of nonhematopoietic cells. The role of CD4 independent entry. Rev Med Virol 1:51–58.

Clayson ET, Compans RW (1989): Characterization of simian virus 40 receptor moieties on the surfaces of Vero C1008 cells. J Virol 63:95–1100.

Clever J, Yamada M, Kasamatsu H (1991): Import of simian virus 40 virions through nuclear pore complexes. Proc Natl Acad Sci USA 88:7333–7337.

Dalgleish A, Beverly PC, Clapham P, Crawford D, Greaves M, Weiss R (1984): The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 312:763–767.

Dawe CJ, Freund R, Mandel G, Balmer-Hofer K, Talmage DA, Benjamin TL (1987): Variations in polyomavirus genotype in relation to tumor induction in mice: Characterization of wild type strains with widely differeing tumor profiles. Am J Pathol 127:243–261.

Deng H, Liu R, Ellmeier W, Choe S, Unatmaz D, Burkhart M, DiMarzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR (1996): Identification of a major co-receptor for primary isolates of HIV-1. Nature 381:661–666.

Diamond L, Crawford LV (1964): Some characteristics of large plaque and small plaque lines of polyomavirus. Virology 22:235–244.

Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA (1996): HIV-1 entry into CD4 cells is mediated by the chemokine receptor CC-CKR-5. Nature 381:667–673.

Dubensky TW, Freund R, Dawe CJ, Benjamin TL (1991): Polyomavirus replication in mice: Influences of VP1 type and route of inoculation. J Virol 65:342–349.

Feng Y, Broder CC, Kennedy PE, Berger EA (1996): HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein–coupled receptor. Science 272: 872–877.

Freund R, Calderone A, Dawe CJ, Benjamin TL (1991a): Polyomavirus tumor induction in mice, effects of polymorphisms of VP1 and large T antigen. J Virol 65:335–341.

Freund R, Garcea RL, Sahli R, Benjamin TL (1991b): A single amino acid substitution in polyomavirus VP1 correlates with plaque size and hemagglutination behavior. J Virol 65:350–355.

Fried H, Cahan LD, Paulson JC (1981): Polyoma virus recognizes specific sialyoligosaccharide receptors. Virology 109:188–192.

Gardner SD, Field AM, Coleman DV, Hulme B (1971): New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1:1253–1257.

Gilbert JM, Benjamin TL (2000): Early steps of polyomavirus entry into cells. J Virol 74:8582–8588.

Gillock ET, Rottinghaus S, Chang D, Cai X, Smiley SA, An K, Consigli RA (1997): Polyomavirus major capsid protein VP1 is capable of packaging cellular DNA when expressed in the baculovirus system. J Virol 71:2857–2865.

Griffith GR, Consigli RA (1986): Cross-linking of a polyoma attachment protein to its mouse kidney cell receptor. J Virol 58:773–781.

Gross L (1953): A filterable agent, recovered from AK leukemic extracts, causing salivary gland carcinomas in C3H mice. Proc Soc Exp Biol Med 83:414.

Haun G, Keppler OT, Bock CT, Herrmann M, Zentgraf H, Pawlita M (1993): The cell surface receptor is a major determinant restricting the host range of the B-lymphotropic papovavirus. J Virol 67:7482–7492.

Herrmann M, Oppenlander M, Pawlita M (1995): Fast and high affinity binding of B-lymphotropic papovavirus to human B-lymphoma cell lines. J Virol 69:6797–6804.

Herrmann M, Wilhelm von der Leith C, Stehling P, Reutter W, Pawlita M (1997): Consequences of subtle sialic acid modification on the murine polyomavirus receptor. J Virol 71(8):5922–5931.

Houff SA, Major EO, Katz DA, Kufta CV, Sever JL, Pittaluga S, Roberts JR, Gitt J, Saini N, Lux W (1988): Involvement of JC virus–infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. N Engl J Med 318:301–305.

Hummeler K, Tomassini N, Sokol F (1970): Morphological aspects of the uptake of simian virus 40 by permissive cells. J Virol 6:87–93.

Ito T, Suzuki Y, Mitnaul L, Vines A, Kida H, Kawaoka Y (1997): Receptor specificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species. Virology 227:493–499.

James W, Weiss RA, Simon JH (1996): The receptor for HIV: Dissection of CD4 and studies on putative accessory factors. Curr Top Microbiol Immunol 205:137–158.

Kartenbeck J, Stukenbrok H, Helenius A (1989): Endocytosis of simian virus 40 into the endoplasmic reticulum. J Cell Biol 109:2721–2729.

Keppler OT, Herrmann M, Oppenlander M, Meschede W, Pawlita M (1994): Regulation of susceptibility and cell–surface receptor for the B-lymphotropic papovavirus by N–glycosylation. J Virol 68:6933–6939.

Keppler OT, Stehling P, Herrmann M, Kayser H, Grunow D, Reutter W, Pawlita M (1995): Biosynthetic modulation of sialic acid–dependent virus-receptor interactions of two primate polyoma viruses. J Biol Chem 270:1308–1314.

Khare GP, Consigli RA (1965): Multiplication of polyoma virus: Use of selectively labeled (3H) virus to follow the course of infection. J Bacteriol 90:819–821.

Klatzman D, Champagne E, Chamaret S, Gruest J, Guetard D, Hercend T, Gluckman J, Montagnier L (1984): T lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 312:767–768.

Lednicky JA, Garcea RL, Bergsagel DJ, Butel JS (1995): Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. Virology 212:710–717.

Liu CK, Hope AP, Atwood WJ (1998a): The human polyomavirus, JCV, does not share receptor specificity with SV40 on human glial cells. J Neurovirol 4:49–58.

Liu CK, Wei G, Atwood WJ (1998b): Infection of glial cells by the human polyomavirus JC is mediated by an N-linked glycoprotein containing terminal alpha (2–6)–linked sialic acids. J Virol 72:4643–4649.

Mackay RL, Consigli RA (1976): Early events in polyoma virus infection: Attachment, penetration, and nuclear entry. J Virol 19:620–636.

Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR (1992): Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev 5:49–73.

Mantyjarvi RA, Arstila PP, Meurman OH (1972): Hemagglutination by BK virus, a tentative new member of the papovavirus group. Infect Immun 6:824–828.

Marriott SJ, Griffith GR, Consigli RA (1987): Octyl-beta-D-glucopyranoside extracts polyomavirus receptor moieties from the surfaces of mouse kidney cells. J Virol 61: 375–382.

Marsh M, Helenius A (1989): Virus entry into animal cells. Adv Virus Res 36:107–151.

Maul GG, Rovera A, Vorbrodt A, Abramczuk J (1978): Membrane fusion as a mechanism for simian virus 40 entry into different cellular compartments. J Virol 28: 936–944.

Mei YF, Wadell G (1995): Molecular determinants of adenovirus tropism. Curr Top Microbiol Immunol 199:213–228.

Mendelsohn CL, Wimmer E, Racaniello VR (1989): Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell 56:855–865.

Mezes B, Amati P (1994): Mutations of polyomavirus VP1 allow in vitro growth in undifferentiated cells and modify in vivo tissue replication specificity. J Virol 68: 1196–1199.

Monaco MGC, Atwood WJ, Gravell M, Tornatore CS, Major EO (1996): JCV infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: Implication for viral latency. J Virol 70:7004–7012.

Norkin LC (1982): Papovaviral persistent infections. Microbiol Rev 46:384–425.

Padgett BL, Walker DL, Zu Rhein G, Eckroade RJ, Dessel BH (1971): Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet 1:1257–1260.

Pho MT, Ashok A, Atwood WJ (2000): JC virus enters human glial cells by clathrin dependent receptor mediated endocytosis. J Virol 74(5):2288–2292.

Racaniello VR (1990): Cell receptors for picornaviruses. Curr Top Microbiol Immunol 161:1–22.

Ricci L, Maione R, Passananti C, Felsani A, Amati P (1992): Mutations in the VP1 coding region of polyomavirus determine differentiating stage specificity. J Virol 66: 7153–7158.

Sahli R, Freund R, Dubensky T, Garcea R, Bronson R, Benjamin T (1993): Defect in entry and altered pathogenicity of a polyoma virus mutant blocked in VP2 myristylation. Virology 192:142–153.

Salunke DM, Caspar DL, Garcea RL (1986): Self–assembly of purified polyomavirus capsid protein VP1. Cell 46:895–904.

Sanjuan N, Zijlstra M, Carroll J, Jaenisch R, Benjamin T (1992): Infection by polyomavirus of murine cells deficient in class I major histocompatibility complex expression. J Virol 66:4587–4590.

Schweighardt B, Atwood WJ (2001): Glial cells as targets of viral infection in the human central nervous system. In Progress in Brain Research, Vol. 132, Castellano Lopez B, Nieto-Sampedro M, Eds; Elsevier Science: Amsterdam, pp. 731–745.

Seganti L, Mastromarino P, Superti F, Sinibaldi L, Orsi N (1981): Receptors for BK virus on human erythrocytes. Acta Virol 25:177–181.

Shah K, Nathanson N (1976): Human exposure to SV40: Review and comment. Am J Epidemiol 103:1–12.

Shah KV (2000): Does SV40 infection contribute to the development of human cancers? Rev Med Virol 10:31–43.

Sinibaldi L, Goldoni P, Pietropaolo V, Cattani L, Peluso C, Di Taranto C (1992): Role of phospholipids in BK virus infection and haemagglutination. Microbiologica 15: 337–344.

Stang E, Kartenbeck J, Parton RG (1997): Major histocompatibility complex class I molecules mediate association of SV40 with caveolae. Mol Biol Cell 8:47–57.

Stehle T, Yan Y, Benjamin TL, Harrison SC (1994): Structure of murine polyomavirus complexed with an oligosaccharide receptor fragment. Nature 369:160–163.

Stewart SE, Eddy BE, Borgese N (1958): Neoplasms in mice inoculated with a tumor agent carried in tissue culture. J Natl Cancer Inst 20:1223.

Sweet B, Hilleman M (1960): The vacuolating virus, SV40. Proc Soc Exp Biol Med 105:420.

Takemoto KK, Furuno A, Kato K, Yoshiike K (1982): Biological and biochemical studies of African green monkey lymphotropic papovavirus. J Virol 42:502–509.

Tomassini JE, Graham D, DeWitt CM, Lineberger DW, Rodkey JA, Colonno RJ (1989): cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. Proc Natl Acad Sci USA 86:4907–4911.

Wei G, Liu CK, Atwood WJ (2000): JC virus binds to primary human glial cells, tonsillar stromal cells, and B-lymphocytes, but not to T-lymphocytes. J Neurovirol 6:127–136.

Weiss RA, Tailor CS (1995): Retrovirus receptors. Cell 82:531–533.

Willoughby RE, Yolken RH, Schnaar RL (1990): Rotaviruses specifically bind to the neutral glycosphingolipid asialo-GM1. J Virol 64:4830–4835.

Wong GH, Bartlett PF, Clark-Lewis I, Battye F, Shrader JW (1984): Inducible expression of H-2 and Ia on brain cells. Nature 310:688–691.

Yamada M, Kasamatsu H (1993): Role of nuclear pore complex in simian virus 40 nuclear targeting. J Virol 67:119–130.

Yoshiike K, Takemoto KK (1986): Studies with BK virus and monkey lymphotropic papovavirus. In The Papovaviridae, Salzman NP, Ed; Plenum Press: New York, Vol 1, pp 295–326.

zur Hausen H, Gissmann L (1979): Lymphotropic papovavirus isolated from African green monkey and human cells. Microbiol Immunol 167:137–153.

Human Polyomaviruses: Molecular and Clinical Perspectives.
Edited by Kamel Khalili, Gerald L. Stoner

ISBNs: 0-471-39009-7 (Hardback); 0-471-22194-5 (Electronic)

10

LATENT AND PERSISTENT POLYOMAVIRUS INFECTION

KRISTINA DÖRRIES, M.D.

1. AFFECTED ORGANS AND CELL TYPES IN POLYOMAVIRUS-ASSOCIATED DISEASE AND PERSISTENT VIRUS INFECTION

The two human polyomaviruses BKV and JCV are associated with persistent infection and diseases of the urogenital tract and the central nervous system (CNS). Induction of disease by the viruses is regularly linked to states of immunoincompetence. The most prominent underlying complications are AIDS and lymphoproliferative disorders. Moreover, iatrogenic immunosuppression in the course of transplantation or therapy of autoimmune disorders can contribute to polyomavirus-induced disease. Clinically overt disease usually correlates with enhanced activity of viral expression in the target organ of viral persistence. Detailed analysis of BKV- and JCV-associated diseases disclosed a variety of organs and cell types to be susceptible to virus infection. These observations fostered multiple studies on the molecular basis of polyomavirus persistence in the infected host. However, despite the availability of very sensitive techniques to discover virions and/or viral products in tissue, body fluids, or cells from infected persons, essential questions with respect to involvement of distinct organs and cell types in polyomavirus persistence are far from being answered unequivocally.

Polyomavirus Infection of the Urogenital Tract

Urogenital Diseases Associated with BKV and Asymptomatic Infection. BKV is a urotheliotropic virus, which was originally detected in the urine of a patient with ureteral stenosis after renal transplantation (RT) (Gardner et al., 1971). Nevertheless, studies from recent years suggest that interstitial tubular nephritis is the most frequent BKV-associated disease after RT (Mathur et al., 1997; Pappo et al., 1996; Purighalla et al., 1995). Clinical features may mimic graft rejection or drug toxicity (Binet et al., 1999; Randhawa et al., 1999), but histopathologic examination almost always shows interstitial infiltrates of plasma cells and lymphocytes, interstitial fibrosis, tubular atrophy, and large intranuclear inclusions in tubular epithelial cells. Cells of the transitional bladder epithelium were identified as target cells for BKV infection (Gerber et al., 1980). Virus isolation, DNA detection by polymerase chain reaction (PCR), electronmicroscopy, immunohistologic staining of BKV proteins, and in situ hybridization (ISH) suggests an etiopathologic role of the virus in about 5% of RT patients (Binet et al., 1999; Randhawa et al., 1999). With the introduction of new immunosuppressive strategies, the incidence of BKV-associated complications in RT increased further, thus confirming the close relationship between excessive virus growth and immunologic impairment.

Renal failure due to BKV infection is diagnosed in patients with underlying lymphoma, hereditary immunodeficiencies (de Silva et al., 1995; Rosen et al., 1983), in renal transplant patients (Mathur et al., 1997; Pappo et al., 1996; Purighalla et al., 1995), and with AIDS (Bratt et al., 1999; Nebuloni et al., 1999). Interstitial inflammation and focal necrosis of tubular epithelium with enlargement of tubular epithelial cells and the pelvic urothelium accompany this rare complication. Aggregates of viral particles within nuclei of renal tubular cells (Rosen et al., 1983) confirm susceptibility of the urogenital tract epithelium for BKV infection.

Hemorrhagic cystitis (HC) is a serious BKV-associated complication of bone marrow transplantation (BMT) patients. Prevalence of HC varies from 10% to 68% and leads to severe hemorrhage in about 25% of bone marrow recipients (Arthur et al., 1985; Azzi et al., 1994; Bedi et al., 1995; Chan et al., 1994; Cotterill et al., 1992). Hemorrhage and viruria most likely are due to viral activation in the uroepithelium, as virus particles can be detected in exfoliated urinary cells by means of electron microscopy (Hiraoka et al., 1991). Prolonged hematuria is associated with severe morbidity and increasing viral load in urine (Azzi et al., 1999).

In patients with AIDS, systemic BKV-associated disease involves infection of the CNS, the lung, the eye, and the kidney (Bratt et al., 1999; Hedquist et al., 1999; Nebuloni et al., 1999; Smith et al., 1998; Vallbracht et al., 1993). Concomitantly with multiple lesions in the entire nephron, an interstitial tubulonephritis is seen. Desquamated tubular cells that display focally enlarged, eosinophilic nuclei carry BKV particles and express viral products (Cubukcu-Dimopulo et al., 2000; Nebuloni et al., 1999; Vallbracht et al., 1993). Investi-

gation of tubular cells at the subcellular level revealed the presence of virions in cytoplasmic reticular cisternae (Nebuloni et al., 1999), and virus-specific antigen was located in nuclei and cytoplasm (Nebuloni et al., 1999; Smith et al., 1998). Interstitial clustering of CD68-positive cells with cytoplasmic staining of virus-specific antigens indicated uptake of the virus by phagocytotic cells (Nebuloni et al., 1999). Further analysis at the ultrastructural level detected characteristic viral particles, which occasionally formed dense crystalloid arrays, thus confirming viral presence in these cells (Smith et al., 1998).

In contrast to renal disease under severe immunoincompetence, BKV infection in other patients is most likely in an asymptomatic state. In the course of lymphoproliferative diseases a clinically silent BKV-specific involvement of the urothelial tract has been reported. Virus DNA is distributed in small foci throughout the cortex and medulla of the kidney (Heritage et al., 1981), affecting renal epithelial cells and lining cells of ureter and bladder as demonstrated by BKV-specific immunostaining. In asymptomatic tissue BKV DNA persists in episomal form, and exfoliated cells carry intranuclear inclusions containing BKV antigen (Shinohara et al., 1993; Zu Rhein and Varakis, 1974). The rate of infection varies from 13% (Chesters et al., 1983) to more than 50% (Dörries and Elsner, 1991). This may depend on the study groups, which included patients with underlying diseases ranging from carcinoma and leukemia to inflammatory diseases, coronary heart disease, and multiple injuries from traffic accidents.

Thus far, no data are available on BKV in kidney tissue in the course of pregnancy. However, there is a study addressing the question of transplacental transmission of BKV in humans using tissue specimens from aborted fetuses and placenta. BKV DNA was detected by PCR in 60% of fetal kidney and in 80% of placenta samples. In maternal tissue from a control group with normal pregnancies, BKV was detected in 50% of the samples (Pietropaolo et al., 1998). Besides the finding that BKV might be transmitted vertically, the enhanced detection rates after abortion point to an activated BKV infection and a prevalence of persistent virus infection in about 80% of the population.

Analysis of prostate biopsy specimens for BKV revealed virus DNA in 58% of asymptomatic tissue and in 87% of prostate hyperplasias sampled from prostate carcinoma patients. This was comparable to the rate in bladder tissue. In addition, 70% of cervix and vulvar tissue exhibited viral DNA, and analysis of sperm gave an incidence of 95% for the presence of BKV DNA. In contrast, DNA of the second polyomavirus JC was found in only 5% of cervical and vulvar tissue specimens. Glandular tissue yielded no JCV DNA, whereas 21% of sperm samples were positive for JCV DNA. The high rate of BKV DNA present in asymptomatic tissue and semen suggests that these sites should be considered as locations of polyomavirus persistence (Monini et al., 1995, 1996; Shinohara et al., 1993).

Activation of BKV Infection in the Urogenital Tract. Virus infection of the kidney following viruria probably occurs at the time of primary infection

in children (Di Taranto et al., 1997). Beyond childhood, viruria is more likely due to activation of persistent renal infection than to primary infection or reinfection. Virus products in the urine at different stages of immunologic incompetence and a high level of viruria in asymptomatic infection (Arthur et al., 1989; Coleman et al., 1980; Hogan et al., 1980; Kitamura et al., 1990; Reese et al., 1975) are strong indications for a relationship between immunodeficiency and polyomavirus activation.

The role of HIV-related immunodeficiency in BKV-associated viruria was analyzed in patients without BKV-associated diseases. Virus DNA was detected by PCR at a rate of 37–44% in the United Kingdom, Italy, and Tanzania (Agostini et al., 1995; Degener et al., 1997; Jin et al., 1995; Knowles et al., 1999). Lower rates of 20–24% were found in North America and Norway (Markowitz et al., 1993; Shah et al., 1997; Sundsfjord et al., 1994a). However, in all study groups an increase of BKV viruria under AIDS was reported on a basis of less than 8% in normal individuals. Staging of HIV patients according to their immune status revealed an inverse relationship of decreasing T-lymphocyte count and increasing viruria (Jin et al., 1995; Knowles et al., 1999; Markowitz et al., 1993; Shah et al., 1997; Sundsfjord et al., 1994a). From these studies it appears likely that BKV activation in the urinary tract under AIDS is correlated with the state of immunoincompetence. Contrasting reports with 6% BKV viruria in AIDS and AIDS/PML patients as detected by nested PCR technique could probably be explained by technical differences between laboratories (Brouqui et al., 1992; Ferrante et al., 1997).

Viruria in BMT patients was reported as early as 1975 (Reese et al., 1975). Although before transplantation only 1% of patients shed BKV (Arthur et al., 1985), increases have been reported to 22%, 48% (Arthur et al., 1988, 1989; Cotterill et al., 1992; Jin et al., 1993), 67% (Jin et al., 1995), and even 100% of patients in the post-transplant period if classic methods were combined (Gibson et al., 1985). With the advent of the more sensitive PCR, the prevalence of viruria was found to be continuously higher, ranging from 50% to 100%, if multiple samples from one patient were analyzed (Azzi et al., 1994, 1996; Bogdanovic et al., 1994; Flaegstad et al., 1991). BKV viruria often began 2–9 weeks after transplantation. The duration was variable and resolved spontaneously after several weeks of shedding. Virus excretion was not related to graft-versus-host disease, thus revealing that viruria after BMT is a normal asymptomatic activation event of a persistent BKV infection that often is not associated with disease. However, the involvement of BKV in hemorrhagic cystitis in about 20% of allogeneic BMT patients (Azzi et al., 1994) prompted a study of microscopic hematuria (Chan et al., 1994). Viruria in 51% of BMT patients in a 4 month period after transplantation was closely associated with hematuria in about half of the patients (Chan et al., 1994; Jin et al., 1995). Similar to asymptomatic virus shedding, most episodes of hematuria were self-limiting. From these findings it can be suggested that BKV infection after BMT is usually activated to a detectable level of virus load in the urine and might

even progress to histologic destruction before virus growth comes under control.

The first carrier of a BKV infection to be described (Gardner et al., 1971) had polyomavirus viruria after RT. Virus particles were characterized by electron microscopy and virus isolation. The virus sometimes affected epithelial cells of both the recipient and donor tissues (Coleman et al., 1973). In contrast to BMT or pregnancy, after renal transplantation primary polyomavirus infection, as defined by antibody titer rises, appears to play a role by introduction of the virus into the recipient by an infected donor (Andrews et al., 1983, 1988; Arthur et al., 1989). The prevalence of polyomavirus viruria ranged from 0% to 47% in early reports, the majority of patients being asymptomatic. Even the use of PCR did not enhance the rate of detection (Sundsfjord et al., 1994a). The discrepancies among the studies might be associated with the lower sensitivities of early methods, but highly variable therapeutic schemes may also contribute to the findings (Lecatsas et al., 1973; Shah et al., 1974). In addition, examination of multiple samples revealed an intermittent and sporadic course of virus excretion as well as a highly variable duration of viruria, ranging from periods of several weeks to years. Observations over more than 3 years revealed a prevalence of 44% viruria in all patients studied. Molecular characterization of the polyomavirus species suggested that BKV viruria was more prominent in RT patients than JCV viruria (Arthur et al., 1989; Boubenider et al., 1999; Gibson and Gardner, 1983). However, viruria often was unrelated to changes in the clinical condition or treatment or even to ureteric obstruction, demonstrating that viruria might not necessarily be associated with RT (Andrews et al., 1988; Arthur et al., 1989).

Under other immunosuppressive conditions viruria often is intermittent, with sparsely distributed infected cells in urine pointing to a rather low rate of activation. Jin et al. (1993) studied a group of patients after cardiac transplantation together with BMT patients. BKV was detected in 50% of the BMT patients and in 25% of the cardiac transplantation group. In all cases excretion was intermittent and sparse and was more often correlated with older age and more aggressive underlying disease. Activating therapeutic influences in these patients could not be stated. Besides the obvious correlation with age, the data further corroborate severe immunoimpairment as a factor for activation and urinary excretion (Hogan et al., 1983; Jin et al., 1993).

In patients with autoimmune diseases in Taiwan, about 40% were found by PCR to be excreters of polyomaviruses. Interestingly, in 15%, double infections were detected, but none was positive for BKV viruria alone (Chang et al., 1996a). Further extension of the study confirmed the lack of BKV viruria in Taiwan (Tsai et al., 1997). In contrast, patients from Scandinavia with systemic lupus erythematosus had higher levels of BK viruria than healthy control subjects, whereas JCV shedding was in the range of the normal sex-matched control group. In a follow-up study, the authors found a high prevalence of intermittent or even continuous shedding of BKV at 1 year. Immunosuppressive drugs such as corticosteroids, azathioprine, cyclophosphamide, and/or metho-

trexate did not influence the kinetics of virus shedding (Rekvig et al., 1997; Sundsfjord et al., 1999). Clearly, an autoimmune disease is able to activate a persistent BKV infection to an extent that is not observed in normal individuals.

Pregnancy is the most common condition of altered immunocompetence that has been linked to polyomavirus activation (Coleman et al., 1980; Lecatsas et al., 1981). The onset of virus excretion is related to time of gestation, most often occurring late in the second and during the third trimester. Once established, excretion continues intermittently to term and might even extend into the postpartum period (Coleman et al., 1980; Gardner and Knowles, 1994). Serologic studies revealed that excretion in pregnant women is usually the result of virus activation of a persistent infection. Although activation of infection had no clinical significance (Arthur et al., 1989), women excreting polyomaviruses had more illness before and during pregnancy and may have underlying diseases such as diabetes and sarcoidosis (Gardner and Knowles, 1994). When BKV and JCV viruria were differentiated, BKV excretion rates of 15–25% were observed (Jin et al., 1993; Markowitz et al., 1991). The incidence of JCV viruria in the same geographic regions was about 7% (Gardner and Knowles, 1994; Markowitz et al., 1991), clearly demonstrating a higher incidence of BKV than JCV activation. With more advanced PCR techniques, the incidence of BKV viruria was 47% in 40 pregnant women compared to 19 healthy adults with no viruria (Jin et al., 1995). Similar to the findings in patients with systemic lupus erythematosus, in Taiwan an incidence of about 3% of BKV excretion was reported in pregnancy on the basis of 26% JCV positive urine samples and 6.5% of samples with double infections (Chang et al., 1996b; Tsai et al., 1997). This suggests that BK viruria is generally less pronounced in Taiwan than elsewhere.

Asymptomatic immunocompetent patients and healthy individuals were often included as control groups in studies of polyomavirus excretion. In contrast to the above-mentioned basic diseases and the pregnant state, the prevalence of BKV excretion during immunocompetency ranged from 0% (Bogdanovic et al., 1994; Degener et al., 1997; Jin et al., 1993, 1995; Sundsfjord et al., 1994a; Tsai et al., 1997) to about 18% (Arthur et al., 1985, 1989; Azzi et al., 1996; Kitamura et al., 1990; Markowitz et al., 1993; Shah et al., 1997). The exception is a study by Azzi et al. (1999) on BMT and hemorrhagic cystitis patients, who found a prevalence of 40% BKV excretion in 62 immunocompetent patients. The group was not further described with respect to age or possible risk factors, and a study on JCV was not performed. However, in comparison with BMT patients, the virus load in immunocompetent individuals was low (in the range of $<1.2 \times 10^4$ genome copies in 5–10 ml urine). Although the sensitivity of the test system was not higher than that published before, the DNA amount in the urine suggests that asymptomatic BKV activation in immunocompetent adults is limited. This corresponds to a study reporting a low concentration of BKV DNA (<1 pg/ml) in general (Kitamura et al., 1990). Although a correlation between age and BKV excretion in adults was not apparent, in Japan detection

rates in those older than 60 years were higher than in younger age groups (Kitamura et al., 1990).

Because of the prevalence of renal infection and urinary excretion it appears likely that common diseases and their associated immune responses play a role in virus activation. Whereas in immunocompetent individuals the rate of infection did not exceed 18%, impairment of the immune response in pregnancy and cardiac transplantation is linked to an activation in about 25% of individuals. In RT and AIDS patients expression is further affected to a rate of 47% at maximum. The most prominent activation processes can be observed in BMT patients. BKV expression after BMT appears to be almost always activated to a high virus load in the urine combined with an asymptomatic state of infection. Due to as yet unknown factors, which may involve host genetics, differences in therapy, or influences by the donor marrow, the infection may further be activated to a stage of cytolytic infection and hematuria without clinical symptoms.

It is conceivable that expression of the virus could be limited by the immune response, or it may, depending on the state of immunoimpairment, proceed unaffected to symptomatic disease. The amount of urinary virus in healthy individuals was regularly lower than that in immunoincompetent patients. This argues for a persistent BKV infection that is progressing stepwise from the latent or attenuated basic state of persistent infection with a rather low virus load that might even be out of the limits of detection. If the virus-specific immune response is impaired, increasing virus load and dissemination could indicate further stages of activation, ultimately leading to fatal disease.

Localization of JCV in the Urinary Tract. In contrast to BKV, JCV was never described as an etiologic agent in a urogenital disorder. Evaluation of the state of renal JCV infection in PML, the only JCV-associated disease, regularly disclosed no histopathologic changes in kidney tissue (Dörries and Elsner, 1991). Nevertheless, a prevalence rate of renal JCV infection in PML patients between 50% and 100% was reported (Ault and Stoner, 1994; Dörries and terMeulen, 1983; Grinnell et al., 1983a; Newman and Frisque, 1997; White et al., 1992). JCV DNA is distributed in small foci throughout renal cortex and medulla (Chesters et al., 1983; Dörries and terMeulen, 1983; Grinnell et al., 1983b; McCance, 1983; Tominaga et al., 1992). The major site of infection is localized to the epithelial cells lining the collective tubules (Dörries and terMeulen, 1983). Isolated cells carry virions in nuclei and cytoplasm. Southern blot analyses in combination with cloning experiments revealed episomal JCV genomes in affected cells without evidence for integrated DNA (Chesters et al., 1983; Dörries and Elsner, 1991).

Compared with PML patients, randomly selected individuals and cancer patients had renal infections less often, and their viral loads in the organ were considerably lower (Chesters et al., 1983; Dörries and terMeulen, 1983; Grinnell et al., 1983a). The presence of JCV DNA in about 50% of kidney samples is contrasted with only 2% of samples being positive for JCV protein. This

most likely reflects differences in the sensitivities of the techniques applied rather than a true difference in the activation rates of the virus. Recent PCR analyses suggest that JCV DNA is regularly detected in the kidney of more than 50% of individuals (Aoki et al., 1999). This finding and the fact that primary renal infection with JCV occurs during childhood (Bordin et al., 1997; Grinnell et al., 1983a; Newman and Frisque, 1997, 1999) support the thesis that JCV persistence is most likely established during primary infection followed by an accumulation of virus in the tissue by repeated activation throughout life.

Activation of JCV Renal Infection. Like BKV, JCV DNA is more frequently detected in the kidneys of immunoincompetent individuals than in immunologically healthy persons. Under HIV infection urinary excretion was found to be a frequent event, ranging from 16% to 38% of patients in Europe, North America, and Africa (Agostini et al., 1995, 1997; Degener et al., 1997; Ferrante et al., 1997; Knowles et al., 1999; Markowitz et al., 1993; Shah et al., 1997; Sundsfjord et al., 1994a). In contrast to BKV activation, the incidence of JCV viruria parallels that in the normal population. In most reports, the pattern of JCV shedding was not influenced by the AIDS status or aggressive chemotherapy (Markowitz et al., 1993; Shah et al., 1997; Sundsfjord et al., 1994a). In general, the excretion rate was found comparable to that of normal individuals, being either stable or transient with identical genotypes shed in a period of 1–6 month. The frequency of excretion with increasing age did not differ significantly from that in HIV-negative individuals (Agostini et al., 1997). Obviously, JCV activation in the urinary tract is not influenced by HIV-induced immunoimpairment or therapeutic intervention for AIDS.

Similarly, JCV excretion is an uncommon event after BMT, occurring in about 5% of patients (Arthur et al., 1988; Gardner and Knowles, 1994; Myers et al., 1989; O'Reilly et al., 1981). Even sensitive PCR techniques did not change the basic findings (Azzi et al., 1994, 1999; Bogdanovic et al., 1994). Given the low frequency of viruria, JCV infection is even less active in BMT-associated immunoimpairment than BKV infection under comparable clinical conditions (Arthur et al., 1988; Gardner and Knowles, 1994). Compared with BMT, the frequency of JCV viruria after RT is higher, ranging from 18% to 57% (Gardner and Knowles, 1994; Hogan et al., 1980; Sundsfjord et al., 1994a; Yogo et al., 1991). Although highly variable, is seems likely that the rate of JCV activation after RT is comparable with that of BKV. Whether this is due to alteration of the JCV expression activity by factors related to the disease process or to the number of patients with activated infection is not known.

To analyze the role of CNS diseases other than PML in the activation of renal JCV infection, viruria was studied in a group of patients with multiple sclerosis (MS). PCR analysis revealed an excretion rate of 30–41% in chronic progressive MS (Agostini et al., 2000; Stoner et al., 1998). Because a control group of family members exhibited the same excretion rate, a regular influence of MS on JCV renal infection is rather unlikely. In conclusion, it can be as-

sumed that pathologic changes in the CNS do not necessarily influence JCV activation (Elsner and Dörries, 1992; Stoner et al., 1998).

Analysis of immunocompetent patients demonstrated a higher rate of renal activation with JCV than BKV. Lower polyomavirus excretion rates were in the range of 0–13% (Arthur et al., 1989; Degener et al., 1997; Jin et al., 1993, 1995; Tsai et al., 1997), whereas higher rates, between 20% and 52% (Agostini et al., 1997; Azzi et al., 1996; Bogdanovic et al., 1994; Kato et al., 1997; Kitamura et al., 1990; Markowitz et al., 1993; Shah et al., 1997; Stoner et al., 1998; Sundsfjord et al., 1994a), have also been reported. Although the broad range of excretion rates may reflect technical differences, in 1994 it became clear that urinary excretion of JCV in the normal asymptomatic population also depends on the age of the individuals analyzed (Kitamura et al., 1994). The rate was clearly higher in groups with higher mean ages (Agostini et al., 1996, 1997). Whereas younger adults shed up to $<10^2$ fg JCV DNA/ml, this amount increased with age to more than 5×10^3 fg JCV DNA/ml (Kitamura et al., 1994; Markowitz et al., 1993). The highest level of JCV DNA in urine specimens of the oldest age group reached more than 10^5 fg JCV DNA/ml (Kitamura et al., 1994). Extent and duration of excretion can be either highly variable or stable (Agostini et al., 1997). Most remarkably, the same JCV strains were identified in urine specimens from healthy persons and from patients with malignancies, cerebrovascular disease, or urologic complications over periods up to 6 years (Agostini et al., 1997; Kitamura et al., 1997). From these data it can be unequivocally concluded that JCV urinary excretion is caused by activation of a persistent virus infection.

Renal activation of JCV by diseases that are not related to severe alterations of the immune system clearly demonstrates that JCV viruria is influenced by more than the state of immunocompetence. This is consistent with the ''rule'' that in PML/non-AIDS patients concomitant JCV viruria is as frequent as in the normal population (Arthur et al., 1989; Ferrante et al., 1997; Koralnik et al., 1999a; Markowitz et al., 1993). However, the level of JCV urinary excretion in immunocompetent individuals is noticeably higher than that of BKV, which rarely exceeds a concentration of 3 fg/ml urine (500 genome copies). Although excretion rates of both BKV and JCV seem tightly linked to increasing age (Kitamura et al., 1990), shedding of BKV and JCV occurs independently (Markowitz et al., 1993). This assumption is strongly supported by the fact that urinary coactivation of both viruses is a rare event (Azzi et al., 1999; Kitamura et al., 1990). Nevertheless, although not concurrently, both viruses were repeatedly detectable in urine from the same individual, suggesting that, once polyomavirus infection is established, activation processes may be induced independently and virus multiplication might then continue intermittently throughout life or at a sustained basal level.

Polyomavirus Infection in the Central Nervous System

CNS Disease Associated with BKV. In early studies, it was reported that fetal brain cells in vitro can be permissive for BKV (Takemoto et al., 1979).

However, even after the molecular detection of BKV DNA in the brain tissue of asymptomatic patients, the neurotropism of the virus was controversial (Elsner and Dörries, 1992). Soon after, the description of BKV-associated CNS diseases, subacute meningoencephalitis, and encephalitis in HIV patients confirmed the CNS as another site of BKV infection (Bratt et al., 1999; Vallbracht et al., 1993; Voltz et al., 1996). BKV could be isolated from the CSF in tissue culture, and the specificity of BKV in autopsy tissue was proven by molecular characterization and DNA sequencing. Histopathologic examination by immunohistochemistry and ISH demonstrated BKV DNA and antigen in affected cells (Vallbracht et al., 1993). BKV infection is associated with blood vessels and fibrocytes in thickened leptomeninges. In cortex and adjoining white matter, reactive astrocytes were affected. The ventricular walls exhibited focal degeneration of the ependymal layer and affected astrocytes in subjacent brain tissue. Target cells for BKV were fibrocytes of the connective tissue, ependymal cells, endothelial and smooth muscle cells of blood vessels, infiltrating macrophages, and astrocytes, the only glial cell type involved in BKV CNS infection (Bratt et al., 1999; Vallbracht et al., 1993). It is of note that the variability of cell types involved in BKV infection is remarkably higher than that of JCV. This suggests a broader cell specificity for BKV and suggests that a large number of different cell types can be involved in BKV persistence.

Asymptomatic BKV Infection and Activation. The assumption that BKV may reach its target organs before disease suggested the presence of BKV DNA and persistent infection in the CNS of healthy and immunocompetent individuals. However, in early analyses brain tissue appeared to be free of virus (Aksamit et al., 1986; Barbanti-Brodano et al., 1987; Chesters et al., 1983; Grinnell et al., 1983a). Likewise, brain tissue from AIDS patients found positive for JCV DNA did not reveal a trace of BKV DNA (Ferrante et al., 1995; Perrons et al., 1996). In contrast, other laboratories repeatedly reported the presence of BK viral DNA in samples from the CNS (De Mattei et al., 1995; Elsner and Dörries, 1992; Vago et al., 1996). These findings were supported by sequencing of new genomic TCR subtypes and cloning of complete virus genomes from a normal brain gene library (Elsner and Dörries, 1992). Nevertheless, compared with the frequency of JCV infection, the presence of BKV DNA in asymptomatic brain infection appears to be considerably lower. Definitive evidence on the localization of BKV in the brain and the cell type involved is not yet available.

Facts about the putative activation of BKV infection in the CNS are limited. BKV is shed in the CSF of patients with BKV-associated CNS disease, and the presence of BKV in the CSF is a diagnostic marker (Bratt et al., 1999; Vallbracht et al., 1993; Voltz et al., 1996). Spinal taps from PML patients and patients at risk for PML were screened for the diagnostic significance of JCV and were found positive for BKV DNA. Co-infection of patients with BKV and JCV is a frequent event and may be detected by PCR with a common primer pair. Because these persons were free of a typical BKV CNS disease,

it is conceivable that a persistent BKV infection in the CNS might be subclinically activated under conditions comparable to those of JCV. Nevertheless, the overall frequency of such an event seems to be very low (Gibson et al., 1993; Hammarin et al., 1996; Perrons et al., 1996; Vago et al., 1996).

JCV in Progressive Multifocal Leukoencephalopathy. PML is a demyelinating disease occurring as a late complication of persistent or primary infection with JCV in the course of a basic immunosuppressive disease (Berger and Concha, 1995; Dörries, 1998; Major et al., 1992; Walker and Padgett, 1983; Weber and Major, 1997). Before the AIDS era about half of the PML cases involved lymphoproliferative diseases (Brooks and Walker, 1984). Nonlymphoproliferative malignancies made up the background in most of the residual cases. The course of PML and the cell types affected are essentially the same regardless of the immune impairment or basic disorder (Berger and Concha, 1995; Major et al., 1992; Walker and Padgett, 1983). Several years of treatment regularly precede PML in rheumatoid arthritis, chronic asthma, sarcoidosis, systemic lupus erythematosus (SLE), chronic polymyositis, and renal transplantation. These diseases are also suspected to be involved in the activation of persistent JCV infections.

The most important feature of PML is the striking alteration of cytolytically infected oligodendrocytes at the rim of defined lesions. Activated pleomorphic microglia were sometimes described in early lesions (Zu Rhein, 1969). The central demyelinated area is essentially composed of reactive astrocytes. Involvement of neurons, ependymal cells, or endothelial cells has never been confirmed. Numerous virus particles are localized in the nucleus of oligodendrocytes extending into the cytoplasm in the degenerating cell. Astrocytes may also contain virus particles or virus DNA, but the number is considerably lower than that in oligodendrocytes. From the data at present available, it cannot be determined whether astrocytes are permissive for JCV (Samorei et al., 2000). In one case a few mononuclear cells were found in Virchow-Robin spaces of several brain sections that may have contained JCV DNA and protein (Houff et al., 1988). Whether this represents infection or phagocytosis that might be followed occasionally by infection is not yet clarified.

Asymptomatic JCV Infection of the CNS. Despite the fact that JCV particles and/or viral products are easily detected in PML autopsy material, it is still a matter of controversy whether PML is the result of a cytolytic invasion of the tissue in the course of long-lasting immunosuppression or the consequence of a preceding persistent infection. Consequently, efforts were undertaken to demonstrate JCV in the CNS of patients in the absence of PML. Many attempts to detect JCV DNA in the brains of patients without signs of PML by methods like Southern blot, in situ hybridization (ISH), immunohistochemistry (Aksamit et al., 1986; Chesters et al., 1983; Heinonen et al., 1992; McCance, 1983; Stoner et al., 1988), and PCR (Buckle et al., 1992; Henson et al., 1991; Moret et al., 1993; Perrons et al., 1996; Telenti et al., 1990) failed. From these

data, it was assumed that JCV persists exclusively at peripheral sites of the body.

The first instance of JCV DNA detected by ISH in elderly individuals without neurologic diseases in Japan was judged as unspecific binding of the probe to cell structures (Heinonen et al., 1992; Mori et al., 1992). However, detection of JCV in the CNS tissue of patients without evidence of PML was confirmed by PCR or ISH. The prevalence ranged from over 30% (Caldarelli-Stefano et al., 1999; Elsner and Dörries, 1992; Ferrante et al., 1995; Mori et al., 1992; Quinlivan et al., 1992; Vago et al., 1996) to almost 70% (White et al., 1992) of randomly selected patients without PML. In contrast to PML brain samples, JCV DNA sequences were often detected only once in up to eight CNS specimens from one patient without disease (Elsner and Dörries, 1992; White et al., 1992). The identity of the cell type that is affected in persistent infection has not yet been determined. However, in brain sections of HIV patients, JCV-positive isolated nuclei were characterized as oligodendrocytes on the grounds of immunohistochemical staining. The presence of JCV DNA was not associated with myelin loss or cytopathic changes (Vago et al., 1996).

Similar to the disseminated distribution of PML lesions, JCV-specific PCR products in the asymptomatic brain could not be localized to a specific topographic region (Elsner and Dörries, 1992; Vago et al., 1996). Therefore, it may be supposed that JCV has no regional preference for particular CNS structures. Examination of virus DNA in healthy tissue resulted exclusively in free circular JCV genomes, suggesting that persistent virus infection is closely related to an episomal state of the DNA (Elsner and Dörries, 1992). The major difference between asymptomatic and diseased tissue is the amount of virus present in the samples. In brain specimens from patients with PML, thousands of genome equivalents per cell are regularly found (Dörries et al., 1979; Quinlivan et al., 1992; Walker and Padgett, 1983). In contrast, the estimated range in asymptomatic individuals is between 1 and 100 JCV genome equivalents per 20 cells (Elsner and Dörries, 1992; Quinlivan et al., 1992; White et al., 1992). Thus, the presence of JCV DNA in nondiseased brain most likely represents a persistent infection and not early stages of disease.

To date, divergent detection rates of polyomavirus in the nonaffected brain are intensively discussed. At least in part, the observed discrepancies can be explained by technical and methodologic variables. Sensitivity as well as specificity of applied technologies differ between laboratories. In addition, laboratory-specific strategies for sampling, processing, extraction, and storage of specimens might have a serious impact on the success of viral DNA detection (Ferrante et al., 1995; Mori et al., 1992; Quinlivan et al., 1992; Vago et al., 1996). A potential problem that could be associated with the detection of lymphotropic viruses in brain tissue is the contamination of tissue specimens with blood. This is especially true for studies that use the most sensitive PCR technologies. However, PCR examination of highly vascularized liver tissue was consistently negative for JCV DNA (Mori et al., 1992; Quinlivan et al., 1992), making contamination of the JCV-positive brain specimens with blood cell–

associated virus rather unlikely. Demonstration of JCV by less sensitive methods as Southern blot analysis or direct cloning without PCR adds further support to the idea that JCV DNA is present in brain tissue of non-PML persons (Elsner and Dörries, 1992). However, besides detection in bulk DNA, for an ultimate tissue-specific localization of JCV in the course of subclinical persistence, histologic association of cell nuclei with virus DNA or expression products in situ is necessary.

Investigations of the influence of basic disease or of an immunoimpaired state on the frequency of JCV DNA detection in normal brain revealed enhanced rates in patients with HIV infection or with proliferative diseases (Elsner and Dörries, 1992; Quinlivan et al., 1992; Vago et al., 1996). Analysis of multiple areas of the brain disclosed that JCV DNA is similarly distributed as typically described in PML tissue (Walker and Padgett, 1983). However, the amount of virus-specific DNA in the samples is consistently lower than in PML tissue, suggesting that persistent polyomavirus infection is restricted to a few isolated cells. In cases of severe immunosuppression, virus infection appears to be activated (Kleihues et al., 1985), an event that probably contributes to viral spread and an increase in infected cells. This is reflected in the higher prevalence of polyomavirus DNA in multiple CNS specimens of patients with malignant diseases (Hogan et al., 1983; Mori et al., 1992; Rieckmann et al., 1994) and in the higher frequency of activated JCV infection in the elderly (Kitamura et al., 1990).

Activation of Asymptomatic JCV Infection in the CNS. The presence of JCV DNA in CNS tissues of patients whose deaths were attributable to diseases other than PML supports the thesis that asymptomatic persistent JCV infection of the CNS can occur. An increased prevalence of polyomavirus DNA in multiple CNS specimens of patients with impaired immunity and the absence of JCV DNA in fetal brain further suggest that disturbances of immunocompetence are a risk factor not only for the development of CNS disease but also for a higher rate of activation (Elsner and Dörries, 1992; Mori et al., 1992; Quinlivan et al., 1992).

One of the earliest reports on activation of JCV infection in the absence of PML was published by Mori et al. (1992). They described virus-specific protein in a limited number of oligodendrocytes and astrocytes in brain sections. This points to an active expression of viral genes very likely associated with an increase in infected cells, DNA load, and transcription products. Nevertheless, persistent infection remains asymptomatic, probably indicating that the actual number of infected cells and the virus load is low and difficult to detect. In contrast, polyomavirus disease in the CNS correlates strongly with viral presence in CSF (Dörries, 1996; Dörries et al., 1998; Eggers et al., 1999; Vallbracht et al., 1993; Weber and Major, 1997). Consequently, detection of JCV DNA in CSF can serve as an indirect marker for activation of a persistent virus infection.

PCR on CSF of non-PML patients with and without neurologic symptoms was often performed as a control for the establishment of the PML diagnosis (Agostini et al., 2000; Bogdanovic et al., 1998; de Luca et al., 1996; Gibson et al., 1993; Henson et al., 1991; Moret et al., 1993; Perrons et al., 1996; Sugimoto et al., 1998; Vignoli et al., 1993; Weber et al., 1994). Based on the results of all these studies, one could conclude that JCV DNA is not present in the CSF of patients without PML. This is in agreement with the presence of persistent JCV infection in 31% of samples from HIV CNS tissue but the absence of JCV DNA in the corresponding CSF samples (Vago et al., 1996). However, longitudinal determination of the virus load in CSF samples from PML patients over a period of several months demonstrated that, even with PML, virus might be shed to the CSF only intermittently (Drews et al., 2000). Consequently, it appears likely that episodic activation of JCV infection in the absence of PML could be missed if only a single CSF sample is analyzed. Although the presence of detectable JCV DNA in CSF is probably a rare event, it emphasizes the possible activation of virus infection in the CNS of non-PML patients (Dörries, 1996, 1998).

Early evidence for the activation of JCV infection in the brain came from PCR analyses. The prevalence ranged from 0.22% to 8% of patients with neurologic symptoms and/or HIV infection (Cinque et al., 1996; Ferrante et al., 1997; Fong et al., 1995; Koralnik et al., 1999a; McGuire et al., 1995). In other studies, the rate of JCV DNA detection increased from 9% to 100% (Ciappi et al., 1999; Dörries et al., 1998; Ferrante et al., 1998; Matsiota-Bernard et al., 1997). Most of the reports concentrated on HIV infection and other diseases at risk for PML. Association of a specific neurologic disorder or type of immune impairment with an increasing prevalence could not be deduced. However, activation of JCV DNA in the CSF of patients with lymphoproliferative diseases and HIV/AIDS appears to be more frequent than in immunocompetent individuals with other neurologic symptoms. An influence of the immunosuppressive state as defined by the CD4-positive T-lymphocyte count in AIDS patients was not discovered (Dörries et al., 1998).

Due to a possible role of early virus infection for the development of MS, several studies focused on these patients (Agostini et al., 2000; Bogdanovic et al., 1998; Dörries et al., 1998; Ferrante et al., 1998; Koralnik et al., 1999a). Most did not detect virus DNA by PCR, even though a single study of patients from Italy using nested PCR found rates of 7.2% in relapsing remitting courses, 16% in primary chronic progressive MS (CPMS), and 13% in secondary CPMS (Ferrante et al., 1998). Patients with and without other neurologic diseases who were analyzed for control purposes were found negative for JCV DNA in CSF.

Although the variable results on the presence of JCV DNA in CSF cannot be simply explained, several aspects should be envisaged: (1) due to the lack of standardized procedures, the performance of PCR varies considerably from laboratory to laboratory; (2) the heterogeneity of patients is high, and epidemiologic as well as anamnestic data are often not given; (3) time of sampling in the course of basic disease may play a role; and (4) therapeutic regimens

may influence the activation of a persistent virus infection. However, cloning and sequencing of new JCV subtypes from the CSF of non-PML individuals (Ciappi et al., 1999) strongly argues for asymptomatic JCV activation in persistently infected brain tissue followed by the occasional presence of JCV DNA in the CSF. The divergence of data confirms that activation of the infection must be a rare event. It is very likely that expression is transient and virus is only infrequently present in the CSF. Whether activation is followed by lifelong accumulation of JCV in the CNS (McGuire et al., 1995) is a question that can probably only be answered in the primate animal model of SV40 and simian PML.

Polyomaviruses in the Lymphatic System

Persistent polyomavirus infection has been suspected to affect a variety of different organs. Dissemination to a wide range of body compartments and the distinct cell specificity of the human polyomaviruses led to the question of routes of viral spread within the host. Multifocal distribution of JCV in PML brain tissue opened the prospect that peripheral blood cells might be a vehicle for hematogeneous spread of polyomaviruses. This assumption was supported by an early report on polyomavirus particles in lymphocytes of immunocompetent children with measles virus infection (Lecatsas et al., 1976) and gave rise to a large number of studies.

Association of BKV with Cells of the Immune System. A study of children with respiratory diseases revealed that the tonsils were affected by BKV infection in more than 40%. Detection of BKV DNA in a throat washing (Jin et al., 1993; Sundsfjord et al., 1994b) pointed to the oropharynx, specifically the lymphoid tissues of Waldayer's ring, as the initial site of BKV infection (Goudsmit et al., 1982; Portolani et al., 1985). BKV DNA in PBLs and tonsils was exclusively in the episomal state (Dörries et al., 1994; Goudsmit et al., 1982). These findings strongly support the idea of lymphoid cell involvement in polyomavirus infection.

In early studies, the stimulatory effects of BKV on human lymphocytes in culture (Lecatsas et al., 1977) were described. BKV-specific receptors on the surface of PBLs argued for their susceptibility to infection (Possati et al., 1983). Indeed, multiplication of BKV was reported in B- and T-lymphocyte cultures from peripheral blood. The rate of infected cells in the presence of virus growth remained stable even for weeks of culture. The amount of virus yield was reported to be 100 times less than in human embryonic fibroblasts, suggesting a restricted growth of BKV in lymphocytes. In monocytes, virus attachment and penetration were observed without subsequent expression, suggesting that monocytes are involved in degradation rather than in multiplication of engulfed virus particles in vivo (Portolani et al., 1985). However, treatment of monocyte cultures with BKV-specific antiserum was followed by virus replication. Therefore, it was assumed that circulating monocytes or tissue resident mac-

rophages in the normal individual might be permissive for polyomavirus infection (Traavik et al., 1988).

In peripheral blood mononuclear cells (PBMC) of individuals after BMT, either no (Schneider and Dörries, 1993) or an increasing prevalence for BKV was reported in more than 60% of patients at later times after transplantation (Azzi et al., 1996; Bogdanovic et al., 1996). As an explanation, a two-step process was assumed. In the course of pretransplantation treatment, loss of lymphocytes and clearance of polyomavirus from the blood are followed by the gradual invasion of the virus after transplantation from persistently infected sites such as the kidney (Azzi et al., 1996). However, in HIV-infected patients the findings were similarly variable. Two laboratories did not find BKV DNA in the PBMC of any patient, including those with PML (Ferrante et al., 1997; Perrons et al., 1996). Other studies reported rates of less than 10% (Degener et al., 1997; Sundsfjord et al., 1994a, 1999). These findings are contrasted by rates of 66% (Azzi et al., 1996) and 100% (Degener et al., 1999). No virus was detected in autoimmune SLE patients (Sundsfjord et al., 1999) or in the cord blood of newborn children (Dörries et al., 1994). In healthy individuals the incidences ranged from none detected (Degener et al., 1997) to 53%, 71%, and 94%, respectively (Azzi et al., 1996; De Mattei et al., 1995; Dörries et al., 1994), whereas in immunocompetent individuals with neurologic diseases an incidence of 82% was reported (Dörries et al., 1994). Two recent reports with refined PCR techniques, including extensive precautions for contamination, demonstrated BKV DNA at a rate of 26% and 55% in blood donors and healthy individuals from Italy and the United States (Chatterjee et al., 2000; Dolei et al., 2000). These conflicting data can mean either that BKV does not persistently infect blood cells or that the amount of virus DNA and/or the number of cells affected must be exceedingly low. Most of these studies were performed by nested PCR techniques, often at the limits of detection, conditions carrying the greatest risk for contamination. The amount of BKV DNA was indeed estimated to be very low compared with that of JCV in peripheral blood cells (Chatterjee et al., 2000; Dörries et al., 1994).

A subtype-specific analysis of peripheral blood cells that may enhance the detection rate has not yet been reported. However, in a study of PBMC in adult blood donors early BKV mRNA expression was detected by RTPCR in 100% of the samples that were positive at a rate of 71% by PCR for BKV DNA (De Mattei et al., 1995). The detection rate by RTPCR is in line with an activated virus infection, thus providing further evidence for a persistent BKV infection in lymphoid cells.

At present, an influence of immunoimpairment on BKV infection in blood cells cannot be assumed on the grounds of published data. However, a study of BKV in the plasma of renal allograft recipients recently revealed the presence of BKV DNA in serum samples from a patient with persistent viral nephropathy (Nickeleit et al., 2000). Provided that lymphoid infection contributes to BKV viremia, it is likely that therapeutic immunoimpairment is a promoting factor for BKV infection in the lymphoid compartment.

The divergence of results from studies of the lymphotropism of polyomaviruses appears to be higher than for other compartments of the host. This might be linked to a variable cell type susceptibility in each blood donor. Alternatively, it may depend on the half-life of the affected cell type or the state of infection at the time of sampling. Moreover, it may also be due to the clinical specimen itself because serum may contain more inhibitors for PCR than other body fluids. Taken together, the high variability of results makes a decision on the role of BKV in blood cell infection rather difficult. However, including the data on BKV-specific ISH in PBMC (Dörries et al., 1994), the detection of mRNA in PBMC (De Mattei et al., 1995), and the indirect evidence for BKV susceptibility of monocytes (Traavik et al., 1988), there is now a wide body of evidence for a regular lymphotropism of BKV in the host.

JCV in Lymphoid Organs and Blood Cells. The pronounced cell specificity of JCV for glial cells in tissue culture was not suggestive of an interaction of the virus with lymphoid cell types. However, general searches for peripheral organs involved in persistent JCV infection rendered spleen and lymph nodes occasionally positive for JCV (Grinnell et al., 1983a). JCV DNA was detected in lymphoid tissue from two children with PML in the course of primary infection (Grinnell et al., 1983a; Newman and Frisque, 1997). Subsequent studies of an adult PML patient demonstrated virus DNA in spleen and lymph node in the persistently infected host (Newman and Frisque, 1999). JCV DNA was also found in the spleens of PML/AIDS patients (Houff et al., 1988). Although JCV DNA could not be detected in the spleens of HIV patients and controls (Caldarelli-Stefano et al., 1999), a 40% incidence in PML patients suggests that virus DNA may accumulate in lymphoid organs.

The thesis of lymphoid cells as a reservoir for JCV (Houff et al., 1988) closed a gap in the understanding of viral pathogenesis. It supported the assumption that JCV-infected lymphoid cells can act as a vector for JCV dissemination. The role of lymphoid infection was further clarified by the detection of tonsillar lymphocytes and tonsillar stromal cells as host cells for JCV (Monaco et al., 1996, 1998). These findings not only suggest the involvement of the tonsils in primary infection but also argue for a persistent polyomavirus infection in tonsillar cell types and peripheral blood cells.

Virus infection in bone marrow was demonstrated for the first time in PML patients. JCV DNA and intranuclear virus capsid protein were located in scattered mononuclear cells as detected by ISH and immunohistochemistry (Houff et al., 1988). In the CNS the characteristic lesions were accompanied by an increased density of infected glial cells in the parenchyma adjacent to blood vessels. Occasionally, infected mononuclear cells were found in the Virchow-Robin spaces, and other infected cells were located just beneath the ependymal layer. Although a possible JCV infection of ependymal cells was never examined extensively, this is reminiscent of BKV infection in HIV-associated CNS disease and suggests a possible invasion of the parenchyma by JCV via the ependymal layer of the ventricles. The mononuclear cells carrying JCV

DNA and products of viral expression were found to be B lymphocytes but not T lymphocytes. The presence of JCV DNA at a concentration of more than 200–1000 virus copies per cell suggested ongoing replication (Houff et al., 1988). A second study reported on oligodendrocytes in PML brain that may occasionally react with B-lymphocyte markers by immunohistochemical staining (Aksamit and Leypold, 1993). Whether this discrepancy is due to technical differences between laboratories, to individual differences of patients, or to course of disease remains open.

To study more precisely the role of lymphoid cells in JCV persistence and pathogenesis, it was asked whether JCV DNA is present in circulating peripheral blood cells during episodes of impaired immunocomptence in the healthy individual. A study of virus infection of bone marrow aspirates from PML patients by ISH revealed the presence of JCV in 31% of the mononuclear cells (Katz et al., 1994; Tornatore et al., 1992). In contrast, amplification by PCR detected JCV DNA in 89.5% of the peripheral lymphocytes. Interestingly, the virus was consistently demonstrable in the course of illness, and even in cells of a prolonged PML survivor JCV DNA was present 4 years after diagnosis of PML. PCR has a considerably higher sensitivity than ISH. Therefore, it cannot be determined whether the higher incidence in PBMC was due to the different methods used or represented a true difference in the amount of infected cells (Tornatore et al., 1992). In subsequent studies, most peripheral blood cell samples of PML patients were positive. Altogether, the prevalence of JCV DNA detected by PCR ranges from 30% to 100%. This indicates that lymphoid cell preparations harbor JCV DNA irrespective of the underlying disease, the affected cell type, the JCV burden, or the number of infected cells (Andreoletti et al., 1999; Dörries et al., 1994; Dubois et al., 1997, 1998; Ferrante et al., 1997; Koralnik et al., 1999a). Quantification of virus load by competitive PCR revealed an average of 35 copies per microgram of cellular DNA in the PBMC of PML patients (Koralnik et al., 1999a). This is higher than in most normal persons (Dörries et al., 1994), but a correlation between HIV immune state and copy number was not detected. In summary, the data are divergent with respect to the level of JCV DNA present. Nevertheless, there is a tendency for a regular involvement of lymphoid cells in the disease process rather than for a nonspecific association.

The question whether lymphoid cells play a role in the dissemination of virus infection in the course of persistence and prior to the induction of disease was addressed by studies of immunoincompetent patients. The detection of JCV DNA in leukocytes from bone marrow and blood of leukemia patients added further support to the idea that polyomaviruses are generally lymphotropic. Detection of JCV DNA in almost all patients before conditioning for BMT revealed that leukemia patients are at a high risk for mononuclear cell infection. The incidence of JCV infection early after BMT was about 10% in an Italian study (Azzi et al., 1996) and 12% in Japanese patients (Shimizu et al., 1999). In contrast, at 60 days post-BMT an increase to 60% was reported (Azzi et al., 1996). This corresponds to a rate of 88% when serial samples were analyzed

after BMT (Schneider and Dörries, 1993). In the same study the amount of virus DNA was found to be highly variable in serial PBMC specimens studied up to 1 year after BMT. Although an association of JCV with PBMC in leukemia and BMT patients is evident, highly variable amounts of virus in serial samples point to an intermittent rather than to a continuous interaction (Schneider and Dörries, 1993).

HIV-infected patients are the most prominent group at risk for PML. JCV infection in the peripheral blood cells of such persons was highly divergent, ranging from no trace of virus DNA (Perrons et al., 1996; Quinlivan et al., 1992; Sundsfjord et al., 1994a) to rates between 10% and 25% (Andreoletti et al., 1999; Dubois et al., 1998; Ferrante et al., 1997; Koralnik et al., 1999b; Lafon et al., 1998), to a maximum of 60% (Azzi et al., 1996; Degener et al., 1997; Dubois et al., 1996, 1997; Pietzuch et al., 1996; Tornatore et al., 1992). Interestingly, the presence of JCV DNA in blood cells is not linked to the clinical state or immunocompetence of HIV patients (Andreoletti et al., 1999; Dubois et al., 1996; Lafon et al., 1998). In contrast to HIV patients, patients with other neurologic diseases, including MS, Parkinson's, and SLE, had a prevalence well below 10% (Dörries et al., 1994; Ferrante et al., 1998; Shimizu et al., 1999; Sundsfjord et al., 1999; Tornatore et al., 1992). An increased rate of JCV in PBL can also be seen as an indicator for an enhanced virus load, thus suggesting an influence of HIV-related disease on JCV association with peripheral blood cells.

If the human polyomaviruses are lymphotropic agents and lymphocytes are sites of persistent infection, then JCV DNA should regularly be present in the blood cells of the normal immunocompetent individual. This was supported by the detection of episomal JCV DNA in PBMC from healthy laboratory staff at a rate of 30% by Southern blot analyses. PCR demonstrated JCV DNA at a rate of more than 80% in the same group, although the concentration of JCV DNA varied from case to case. Serologic evaluation of the patients revealed high antibody titers, pointing to a pronounced humoral immune response and a persistent rather than a latent virus infection (unpublished findings; Dörries et al., 1994). Based on all study results, the prevalence of virus-specific DNA ranged from 0% to 59%. This included blood donors with 39% (Azzi et al., 1996) and 4% (Lafon et al., 1998); healthy persons with 8% (Dubois et al., 1997), 10% (Ferrante et al., 1998), and 59% (Pietzuch et al. 1996); and unspecified immunocompetent control persons without JCV-specific amplification (Koralnik et al., 1999b; Schneider and Dörries, 1993).

ISH confirmed virus-specific DNA signals in close association with cell nuclei (Dörries et al., 1994). This made an entirely unspecific association of the virus with PBMC rather unlikely. The concentration of virus-specific DNA was estimated to be less than one genome equivalent in 20 cells. This probably explains the low rates of detection or even failure to amplify JCV DNA by PCR, as extraction of target DNA sequences appears to be a critical step in the detection of persisting virus genomes.

Hematopoietic Cell Subsets as Targets for JCV. The rates of polyomaviruses detected in peripheral blood cells have been highly variable over the years. Therefore, it became essential to further analyze the nature of virus–cell interactions in the lymphoid compartment (Jensen and Major, 1999). One of the most important questions concerns the cellular target of JCV in lymphoid tissue or peripheral blood. Initially, kappa light chain–carrying B lymphocytes were supposed to be the only target cell in bone marrow and spleen (Houff et al., 1988). An attempt to detect JCV by PCR in the peripheral blood of a PML patient demonstrated virus DNA in CD19-positive B lymphocytes (Monaco et al., 1996). However, in B lymphocyte–depleted PBMC from an HIV patient, JCV-specific amplification was similarly reported (Dubois et al., 1997). These findings and our own observation of JCV DNA in B- and T-lymphocyte cultures from bone marrow before transplantation suggested that JCV can be associated with different lymphocyte subsets. This was further confirmed by Koralnik et al. (1999b), who analyzed four different cell types of hematopoietic origin (CD3+, CD19+, CD14+, CD16+) from HIV patients and from a control group. The study confirmed the assumption that the population of JCV DNA–carrying persons among PML patients is higher than among HIV-infected patients alone or normal individuals. Unsorted cells from normal persons were negative for JCV, whereas virus DNA could be detected in sorted populations from the same patient. JCV DNA was detectable in B and T lymphocytes, in granulocytes, and in monocytes. Amplification of DNA was achieved either in one cell type alone or in different combinations from each patient. The finding that JCV DNA could be amplified only in sorted normal blood cells corresponds to reports of a low virus load in blood cells from healthy individuals (Dörries et al., 1994; Shimizu et al., 1999). However, JCV DNA was not quantified; therefore, it remains open whether a specific cell type is dominantly affected.

Studies of JCV infection in tissue culture revealed that cells of the CD19-positive B-lymphocyte subsets, CD34-positive hematopoietic progenitor cells including B-lymphoblastoid cell lines, and B lymphocytes from peripheral blood could serve as viral target cells. Infectious virus was produced, although no more than approximately 2% of B lymphocytes were infected (Monaco et al., 1996). Restricted virus growth in lymphoid cells strengthens the idea that viral replication is generally attenuated in lymphoid subpopulations (Atwood et al., 1992; Monaco et al., 1996). Virus specificity for hematopoietic cells at different stages of ontogeny was supported by the loss of JCV susceptibility after differentiation of the CD34-positive progenitor cell line KG-1 to a macrophage-like cell by the phorbol ester PMA. From these results, it appears likely that JCV DNA molecules are permanently present in the cells of different cell lineages in bone marrow and peripheral blood. However, more experiments are necessary to unequivocally determine the cell types susceptible to infection and those that might be able to mediate virus multiplication in vivo.

Activation of JCV Infection in Lymphoid Cells. Attempts to understand the nature of JCV infection in hematopoietic cells in vivo concentrated on

virus-specific transcription in blood cells. Due to the low amount of DNA in these cells, studies were performed by RTPCR on nucleic acids from PBMC. JCV expression as detected by cDNA was reported in lymphocytes of 52% of PML patients and at about the same rate in HIV-infected and healthy individuals. In the same study JCV-specific cDNA was demonstrated not only in B lymphocytes but also in the non-B-lymphocyte fraction after magnetic separation (Pietzuch et al., 1996). This supports our own findings of JCV-affected cells showing that virus-specific DNA load and the targeted cell type both are highly variable in blood donors (personal observations). Additionally, it was confirmed that not only B lymphocytes but also other circulating cell types can be involved in JCV infection. Studies of virus expression by analysis of mRNA in peripheral blood cells or in separated B lymphocytes were performed recently. mRNA was found by RTPCR in five of seven PML patients, whereas no mRNA could be detected in the PBLs or B lymphocytes of a large group of HIV patients and blood donors (Andreoletti et al., 1999; Dubois et al., 1997; Lafon et al., 1998). Irrespective of transient activation events, these data imply a latent state of virus infection in peripheral blood cells rather than a persistent infection with consistent attenuated expression. However, confirming studies from other laboratories are needed to unequivocally determine the state of the virus infection in peripheral blood cells.

Comparable to shedding of virus into the CSF, an activated state of the virus infection in PBLs could be reflected by the virus load in serum. PCR studies on this question were performed after lymphotropism of the polyomaviruses had been detected. As anticipated from earlier PCR studies, the rate of detection varied from no virus present to 4% in SLE patients and HIV-negative control groups, including healthy individuals and blood donors (Koralnik et al., 1999a,b; Sundsfjord et al., 1999). In HIV patients with and without other neurologic symptoms, rates from 4% to 23% were reported (Dubois et al., 1998; Koralnik et al., 1999a,b; Lafon et al., 1998). In contrast, in 32% of all HIV/PML patients studied to date, viremia was detected (Dubois et al., 1997; Koralnik et al., 1999a,b; Tornatore et al., 1992), clearly indicating a higher prevalence under PML. Based on the accumulated data, it can be proposed that the amount of JCV DNA in peripheral blood cells is limited and the virus can be detected only after enrichment of lymphoid subtypes by cell separation techniques. Similarly, expression in lymphoid cells may occur at such a low level that it is not detectable by PCR on bulk DNA.

It seems clear now that JCV recirculates cell-associated as well as cell-free in peripheral blood. The crucial question whether association of the virus with peripheral blood cells is due to productive infection, to phagocytosis, or to unspecific binding to cellular membranes is not yet answered. Although detection of mRNA is a strong argument for an activated virus infection, localization of virus DNA to the nucleus of JCV-infected lymphoid subtypes remains to be demonstrated. Moreover, it cannot be excluded that different types of virus–cell interaction may coexist in the host—each of them regulated by different control mechanisms such as cell specificity, hormonal changes, or immune

modulation. Besides the problems associated with PCR detection in blood, such a scenario would explain the extraordinary variance in virus presence detected.

Association of the Human Polyomaviruses With Other Organs

The first detection of a human polyomavirus in the eye was BKV with multifocal slowly progressing retinitis in AIDS patients (Bratt et al., 1999; Hedquist et al., 1999). BKV infection precipitates focal changes in the retinal pigment epithelium. Immunohistochemical examination revealed a diffuse virus protein pattern in necrotic lesions with distinct staining in single cells among photoreceptor remnants. Similar to the focal destruction of subependymal astrocytes and ventricular ependyma in BKV encephalitis (Vallbracht et al., 1993), in retinitis BKV was localized in nuclei adjacent to areas of retinal necrosis. However, the authors assumed that the infected cell type in the retina was more likely a photoreceptor cell than a retinal astrocyte.

In primary BKV infection mild respiratory tract disease suggests the presence of BKV-susceptible cells (Goudsmit et al., 1982; Noordaa and Wertheim-van Dillen, 1977). This corresponds to the description of virus-infected cells in an AIDS patient with interstitial pneumonitis (Vallbracht et al., 1993). Two more reports of pneumonia after umbilical cord transplantation and in the course of AIDS established BKV as a cause of respiratory disease (Cubukcu-Dimopulo et al., 2000; Sandler et al., 1997). Alterations in the lung were characterized by aggregates of desquamated pneumocytes and focal interstitial fibrosis. Pneumocytes and bronchiolar epithelium contained inclusion bodies. Virus products were detected in pneumocytes, in epithelial and smooth muscle cells of the bronchioli, and in fibrocytes. In contrast to the kidney, isolated endothelial cells in the lung occasionally carried virus protein (Vallbracht et al., 1993).

In a study of Kaposi's sarcoma (KS) and a possible interaction of BKV as a co-factor for tumor progression, skin samples from AIDS patients and immunocompetent individuals were analyzed by PCR (Monini et al., 1996). The study revealed an astonishingly high prevalence of BKV in KS skin biopsy specimens (100%) that was also reflected in 75% BKV-positive normal skin biopsies. In cases of JCV, the rate of detection decreased to 20% in classic KS and 16% in normal skin. In the same study JCV DNA was also detected in genital tissues and sperm (Monini et al., 1996). The surprisingly high level of polyomavirus in skin, in genital tissues, and in semen demands further studies to evaluate these body compartments as sites of polyomavirus infection either alone or as a co-factor with other viruses.

Other suspected sites of BKV infection include lung, pancreas, and heart tissue. All such tissues proved to be negative by molecular biologic and immunohistologic methods (Dörries and Elsner, 1991; Vallbracht et al., 1993). Occasionally BKV DNA was cloned from liver tissue (Knepper and diMayorca, 1987); however, in other studies of isolated cases the liver did not show BKV DNA (Dörries and Elsner, 1991; Vallbracht et al., 1993).

In contrast to BKV, JCV is only associated with PML, and the detection of JCV DNA in other organs remains a rare event. Specimens from liver (Bordin et al., 1997; Dörries and Elsner, 1991; Grinnell et al., 1983a; Newman and Frisque, 1997, 1999), lung (Caldarelli-Stefano et al., 1999; Dörries and Elsner, 1991; Grinnell et al., 1983a; Newman and Frisque, 1997, 1999), and cardiac muscle were occasionally found positive for JCV DNA by PCR (Newman and Frisque, 1997, 1999; Quinlivan et al., 1992). Recently, a study of colorectal mucosa revealed JCV DNA in normal epithelium and cancer tissue, differing only in the virus load (Laghi et al., 1999) and in the organization of the Mad-1 like regulatory region (Ricciardiello, et al. 2001). Most of these studies were performed on single clinical specimens, in small groups, or even in single patients who were in highly variable states of health. In addition, amplification of a small DNA fragment alone is not sufficient to determine the localization of a virus infection. Therefore, at present, it is almost impossible to differentiate between infection by JCV virus or accidental presence of virus DNA unrelated to an infectious state in all these organs.

2. POSSIBLE MECHANISMS OF ACTIVATION

Persistent polyomavirus infection is characterized by three major states of infection: the latent or attenuated state, a state of limited activation, and activated virus growth accompanied by tissue destruction. Determination of which factors are responsible for virus activation includes not only the role of the immune system but also the possible influence of other viruses that often co-infect immunoimpaired patients.

Cytomegalovirus infection is common in BMT and RT patients, and HIV infection is one of the major risks for PML. Recently, HHV-6 was found to co-localize with JCV in PML lesions. In addition, co-infection with a second polyomavirus may also have an impact on virus expression. The most important candidates for heterologous transactivation are BKV, herpesviruses, and retroviruses, which are known to have viral transactivating capacities. Heterologous transactivation of viruses can happen at different stages of virus–host interaction and virus growth. In the case of polyomaviruses, the most prominent transactivators are regulatory early proteins acting as either replicating enzymes or as transcription activators. Alternatively, the interaction of cellular proteins on the heterologous promoter can be influenced indirectly by changing the pattern of transcription factors within the cell.

Concomitant infections of BKV and JCV in the urogenital tract affect the same target cells as verified by the detection of viruses in kidney tissue and by viruria. It is a common event, frequently established in individuals after renal and bone marrow transplantation, in HIV-infected patients (Markowitz et al., 1993), in pregnant women (Arthur et al., 1989; Markowitz et al., 1991), and in immunocompetent individuals (Arthur et al., 1989; Chesters et al., 1983; Dörries and Elsner, 1991; Flaegstad et al., 1991; Grinnell et al., 1983b;

McCance, 1983; Sundsfjord et al., 1994b). Nonetheless, the influence of double infections on renal symptomatology or extent of viruria has not been reported (Arthur et al., 1989; Sundsfjord et al., 1994a). Even in BMT patients, JCV in close association with BKV viruria (Azzi et al., 1999; Chan et al., 1994) exhibited no particular differences among single excreters and co-excreting patients. Hence co-expression had no noticeable influence on either JCV or BKV viruria and associated disease (Chan et al., 1994).

The presence of virus DNA in the peripheral blood cells of both healthy and immunocompromised persons was established by PCR analyses and ISH with a radioactive virus-specific probe of genomic length. Reduced sensitivity of the in situ technique detected merely single virus-infected cells; however, the cells carried almost identical amounts of BKV and JCV DNA (Dörries et al., 1994). Because both viruses are able to replicate under restrictions in lymphocytes in vitro (Atwood et al., 1992; Portolani et al., 1985), it is conceivable that they are both periodically activated to virus growth in peripheral blood cells. Whether this has consequences or may influence each other's activity is not yet known.

BKV was not expected to invade the CNS at a high rate, and there have been only rare reports of the presence of both virus genomes in brain tissue in either the asymptomatic brain (Elsner and Dörries, 1992; Vago et al., 1996) or the PML patient (Ferrante et al., 1995; Vago et al., 1996). Cell types promoting polyomavirus persistence in the CNS are not yet defined. Nevertheless, the detection rate of genomic virus DNA in study groups from different laboratories revealed that the amount of BKV DNA in tissue specimens was considerably lower than that of JCV DNA (Elsner and Dörries, 1992; Ferrante et al., 1995; Vago et al., 1996; White et al., 1992). This corresponds to PCR analyses of the CSF demonstrating that BKV is rarely detectable in a large number of samples (Gibson et al., 1993; Hammarin et al., 1996; Perrons et al., 1996; Vago et al., 1996). Although concomitant infection with BKV and JCV can frequently be detected in tissue specimens and in all groups of polyomavirus-infected patients, a transactivating mechanism and the resulting effects remain rather unlikely.

Transactivation may also involve herpesviruses, which can act on polyomavirus DNA replication. Cytomegalovirus (CMV) is highly prevalent in the human population and can infect virtually any organ of its host (Sinzger and Jahn, 1996; Tevethia and Spector, 1989). Co-infection can occur in the kidney, lung, CNS, and lymphoid organs. Specifically, epithelial cells, fibroblasts, and endothelial cells are potential common host cells for BKV and CMV. Stromal cells and CD34-positive bone marrow progenitor cells might be cell types that can be co-targeted by JCV and CMV (Mendelson et al., 1996; Sinclair and Sissons, 1996). CMV infection is often activated in AIDS patients after RT, and there is a high incidence of CMV infection in patients with hemorrhagic cystitis (HC) after BMT (Childs et al., 1998). In AIDS patients no co-detection and no correlation between polyomavirus and CMV viruria was observed (Sundsfjord et al., 1994a). Similarly, the high incidence of CMV after kidney

transplantation (Tolkoff-Rubin and Rubin, 1997) is not matched by an enhanced activity of polyomavirus infection.

Molecular interaction of CMV with JCV is believed to affect the level of DNA replication (Heilbronn et al., 1993) or possibly transcriptional activity. In contrast, co-infection of CMV and BKV in tissue culture does not result in activation of BKV infection (Goldstein et al., 1984). Lack of interaction among CMV and the human polyomaviruses was confirmed by treatment with acyclovir being able to reduce CMV activation in HC patients, but having no influence on BKV-associated HC. Although BKV T antigen is able to induce the expression of CMV immediate early and early gene expression (Kristoffersen et al., 1997), co-infection of both viruses in the same cell in vivo has not yet been reported and analyses of polyomavirus load in lymphoid subpopulations do not point to an interaction in vivo (personal observations).

At present, the most interesting virus detected in close association with JCV is human herpesvirus type six (HHV6) in oligodendrocytes within PML lesions (Blumberg et al., 2000). Co-localization was detected by in situ PCR, and correlation of polyomavirus infection with that of HHV6 was astonishingly high. HHV6 is ubiquitous, with a high prevalence in the adult population. It establishes life-long infection in the brain, the urogenital tract, the lung, the liver, and peripheral blood cells. It is conceivable that polyomaviruses and HHV6 have a common host cell not only in the CNS but also in peripheral organs. HHV6 activation occurs frequently after transplantation and is often associated with CMV infection. In the adult, it is usually asymptomatic and not associated with severe illness unless accompanied by CMV (Stoeckle, 2000). Due to the transactivation mechanisms that come into effect by infections with other herpesviruses, a comparable interaction of HHV6 with the human polyomaviruses is conceivable. However, it is a single study exclusively using in situ PCR, which demonstrated co-localization of JCV and HHV6 in PML lesions. Moreover, in situ PCR is one of the most sensitive and most difficult methods at present available and as such is inclined to non-specific signals. Although fascinating, the findings need to be confirmed by other methods and have to be proven by further studies on possible molecular mechanisms involved.

The question of whether the retrovirus HIV-1 may transactivate JCV is important because it became clear that PML is one of the life threatening opportunistic infections in AIDS patients. Molecular studies revealed that transactivation occurs in vitro at the level of transcription by HIV–Tat induction of the JCV promoter, and BKV T antigen is able to transactivate the HIV long terminal repeat (LTR). One site that has recently been described for BKV infection is the skin in AIDS-related Kaposi's sarcoma. The detection rate of BKV in the skin appeared to be higher than at other sites (Monini et al., 1996). However, transactivation events of a persistent virus in combination with progression to tumor development could explain the exceedingly high level of BKV (Cavallaro et al., 1996; Corallini et al., 1996). CNS infection of HIV-1 in oligodendrocytes is regularly low (Bagasra et al., 1996); however, Tat protein

appears to be secreted by HIV-infected cells and might then be taken up by the JCV-infected oligodendrocyte and induce transcription on Tat-responsive genes. This thesis was examined by Valle et al. (2000), who demonstrated a localization of accumulated Tat protein to the nuclei of JCV-infected oligodendrocytes.

Recently, PML cases were reported without immunosuppression in the setting of co-infection of another retrovirus, HTLV-1 (Okada et al., 2000; Shimizu et al., 1999). This raised the question of whether HTLV-1 could activate JCV expression by interaction of the transactivating protein, Tax. Tax has been shown to interact with other viruses, such as HIV, CMV, and SV40. In vitro studies revealed a glial cell–specific interaction of Tax with the JCV promoter. Therefore, a comparable interaction to that of HIV-1 Tat protein is conceivable (Okada et al., 2000).

In summary, the detection of amplification products belonging to both polyomavirus species is strong evidence for concomitant infection in all tissues found positive for polyomavirus DNA. The number of individuals with simultaneous JCV and BKV infection is high, probably reflecting the true incidence of polyomavirus infection in the population. However, changes in virus-associated expression or the histologic picture due to co-infection have never been reported; therefore, an influence on expression activity by transactivation events appears to be rather unlikely. Similarly, co-infection with other viruses has been reported in a large number of patients and healthy individuals. Although heterologous transactivation may come into effect in individual cases, a common interaction among heterologous viral transactivators with human polyomaviruses cannot be stated. Consequently, transactivating mechanisms probably do not play a general role in the control of the polyomavirus life cycle and pathogenesis.

CONCLUDING REMARKS

Life-long human polyomavirus infection is established early in life. At present, it cannot be determined whether in the healthy individual the infection is in a latent state or is persistent with a continuous or intermittent expression involving restricted reactivation. Based on in vitro studies on virus expression, it can be assumed that promoter elements are able to mediate basic transcription. This function appears to be unrelated to cell specificity or activation processes and could therefore be responsible for a basal activity in any target cell. Limited activation obviously occurs almost always as a result of functional changes in the immune system. The exact pathways leading to expression of virus in vivo are barely defined. However, it can be assumed that impairment of immunocompetency, and hormonal changes as occur during pregnancy, older age, transient inflammatory states, malignant tumor growth, and AIDS, favor viral activation at sites of latent or persistent infection.

Some individuals may undergo an apparently sporadic activation; however, this could be related to their genotype or to accidental transactivation by heterologous viruses. Additionally, differences in the quality of activation signals apparently result in specific infection patterns that may vary according to the target organ as well as to the activity of virus growth. Host genetics, heterologous viruses present in the target organ, and the host-specific immune pattern all play a decisive role in the induction of different states of infection that precede tissue destruction and disease.

REFERENCES

Agostini HT, Brubaker GR, Shao J, Levin A, Ryschkewitsch CF, Blattner WA, Stoner GL (1995): BK virus and a new type of JC virus excreted by HIV-1 positive patients in rural Tanzania. Arch Virol 140:1919–1934.

Agostini HT, Ryschkewitsch CF, Baumhefner RW, Tourtellotte WW, Singer EJ, Komoly S, Stoner GL (2000): Influence of JC virus coding region genotype on risk of multiple sclerosis and progressive multifocal leukoencephalopathy. J Neurovirol 6: S101–108.

Agostini HT, Ryschkewitsch CF, Mory R, Singer EJ, Stoner, GL (1997): JC virus (JCV) genotypes in brain tissue from patients with progressive multifocal leukoencephalopathy (PML) and in urine from controls without PML: Increased frequency of JCV type 2 in PML. J Infect Dis 176:1–8.

Agostini HT, Ryschkewitsch CF, Stoner GL (1996): Genotype profile of human polyomavirus JC excreted in urine of immunocompetent individuals. J Clin Microbiol 34:159–164.

Aksamit AJ, Leypold B (1993): JC virus is not present in B cells in PML brain. Neurology 43:A177.

Aksamit AJ, Sever JL, Major EO (1986): Progressive multifocal leukoencephalopathy: JC virus detection by in situ hybridization compared with immunohistochemistry. Neurology 36:499–504.

Andreoletti L, Dubois V, Lescieux A, Dewilde A, Bocket L, Fleury HJ, Wattre P (1999): Human polyomavirus JC latency and reactivation status in blood of HIV-1–positive immunocompromised patients with and without progressive multifocal leukoencephalopathy. AIDS 13:1469–1475.

Andrews CA, Daniel RW, Shah KV (1983): Serologic studies of papovavirus infections in pregnant women and renal transplant recipients. Prog Clin Biol Res 105:133–141.

Andrews CA, Shah KV, Daniel RW, Hirsch MS, Rubin RH (1988): A serological investigation of BK virus and JC virus infections in recipients of renal allografts. J Infect Dis 158:176–181.

Aoki N, Kitamura T, Tominaga T, Fukumori N, Sakamoto Y, Kato K, Mori M (1999): Immunohistochemical detection of JC virus in nontumorous renal tissue of a patient with renal cancer but without progressive multifocal leukoencephalopathy. J Clin Microbiol 37:1165–1167.

Arthur RR, Beckmann AM, Li CC, Saral R, Shah KV (1985): Direct detection of the human papovavirus BK in urine of bone marrow transplant recipients: Comparison of DNA hybridization with ELISA. J Med Virol 16:29–36.

Arthur RR, Dagostin S, Shah KV (1989): Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J Clin Microbiol 27:1174–1179.

Arthur RR, Shah KV, Charache P, Saral R (1988): BK and JC virus infections in recipients of bone marrow transplants. J Infect Dis 158:563–569.

Atwood WJ, Amemiya K, Traub R, Harms J, Major EO (1992): Interaction of the human polyomavirus, JCV, with human B-lymphocytes. Virology 190:716–723.

Ault GS, Stoner GL (1994): Brain and kidney of progressive multifocal leukoencephalopathy patients contain identical rearrangements of the JC virus promoter/enhancer. J Med Virol 44:298–304.

Azzi A, Cesaro S, Laszlo D, Zakrzewska K, Ciappi S, De Santis R, Fanci R, Pesavento G, Calore E, Bosi A (1999): Human polyomavirus BK (BKV) load and haemorrhagic cystitis in bone marrow transplantation patients. J Clin Virol 14:79–86.

Azzi A, De Santis R, Ciappi S, Leoncini F, Sterrantino G, Marino N, Mazzotta F, Laszlo D, Fanci R, Bosi A (1996): Human polyomaviruses DNA detection in peripheral blood leukocytes from immunocompetent and immunocompromised individuals. J Neurovirol 2:411–416.

Azzi A, Fanci R, Bosi A, Ciappi S, Zakrzewska K, de Santis R, Laszlo D, Guidi S, Saccardi R, Vannucchi AM, et al. (1994): Monitoring of polyomavirus BK viruria in bone marrow transplantation patients by DNA hybridization assay and by polymerase chain reaction: An approach to assess the relationship between BK viruria and hemorrhagic cystitis. Bone Marrow Transplant 14:235–240.

Bagasra O, Lavi E, Bobroski L, Khalili K, Pestaner JP, Tawadros R, Pomerantz RJ (1996): Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: Identification by the combination of in situ polymerase chain reaction and immunohistochemistry. AIDS 10:573–585.

Barbanti-Brodano G, Silini E, Mottes M, Milanesi G, Pagnani M, Reschiglian P, Gerna G, Corallini A (1987): DNA probes to evaluate the possible association of papovaviruses with human tumors. In Monoclonals and DNA Probes in Diagnostic and Preventive Medicine; Gallo RC, Della Porta G, Albertini A, Eds; Raven Press: New York, pp 147–155.

Bedi A, Miller CB, Hanson JL, Goodman S, Ambinder RF, Charache P, Arthur RR, Jones RJ (1995): Association of BK virus with failure of prophylaxis against hemorrhagic cystitis following bone marrow transplantation. J Clin Oncol 13:1103–1109.

Berger JR, Concha M (1995): Progressive multifocal leukoencephalopathy: The evolution of a disease once considered rare. J Neurol Virol 1:5–18.

Binet I, Nickeleit V, Hirsch HH, Prince O, Dalquen P, Gudat F, Mihatsch MJ, Thiel G (1999): Polyomavirus disease under new immunosuppressive drugs: A cause of renal graft dysfunction and graft loss. Transplantation 67:918–922.

Blumberg BM, Mock DJ, Powers JM, Ito M, Assouline JG, Baker JV, Chen B, Goodman AD (2000): The HHV6 paradox: Ubiquitous commensal or insidious pathogen? A two-step in situ PCR approach. J Clin Virol 16:159–178.

Bogdanovic G, Brytting M, Cinque P, Grandien M, Fridell E, Ljungman P, Lönnquvist B, Hammarin AL (1994): Nested PCR for detection of BK virus and JC virus DNA. Clin Diagn Virol 2:211–220.

Bogdanovic G, Ljungman P, Wang F, Dalianis T. (1996): Presence of human polyomavirus DNA in the peripheral circulation of bone marrow transplant patients with and without hemorrhagic cystitis. Bone Marrow Transplant 17:573–576.

Bogdanovic G, Priftakis P, Hammarin AL, Soderstrom M, Samuelson A, Lewensohn Fuchs I, Dalianis T. (1998): Detection of JC virus in cerebrospinal fluid (CSF) samples from patients with progressive multifocal leukoencephalopathy but not in CSF samples from patients with herpes simplex encephalitis, enteroviral meningitis, or multiple sclerosis. J Clin Microbiol 36:1137–1138.

Bordin G, Boldorini R, Caldarelli Stefano R, Omodeo Zorini E (1997): Systemic infection by JC virus in non-HIV induced immunodeficiency without progressive multifocal leukoencephalopathy. Ann Ital Med Int 12:35–38.

Boubenider S, Hiesse C, Marchand S, Hafi A, Kriaa F, Charpentier B (1999): Post-transplantation polyomavirus infections. J Nephrol 12:24–29.

Bratt G, Hammarin AL, Grandien M, Hedquist BG, Nennesmo I, Sundelin B, Seregard S. (1999): BK virus as the cause of meningoencephalitis, retinitis and nephritis in a patient with AIDS. AIDS 13:1071–1075.

Brooks BR, Walker DL (1984): Progressive multifocal leukoencephalopathy. Neurol Clin 2:299–313.

Brouqui P, Bollet C, Delmont J, Bourgeade A (1992): Diagnosis of progressive multifocal leucoencephalopathy by PCR detection of JC virus from CSF. Lancet 339: 1182.

Buckle GJ, Godec MS, Rubi JU, Tornatore C, Major EO, Gibbs CJ, Jr, Gajdusek DC, Asher DM (1992): Lack of JC viral genomic sequences in multiple sclerosis brain tissue by polymerase chain reaction. Ann Neurol 32:829–831.

Caldarelli-Stefano R, Vago L, Omodeo-Zorini E, Mediati M, Losciale L, Nebuloni M, Costanzi G, Ferrante P (1999): Detection and typing of JC virus in autopsy brains and extraneural organs of AIDS patients and non-immunocompromised individuals. J Neurovirol 5:125–133.

Cavallaro U, Gasparini G, Soria MR, Maier JA (1996): Spindle cells isolated from Kaposi's sarcoma-like lesions of BKV/tat-transgenic mice co-express markers of different cell types. AIDS 10:1211–1219.

Chan PK, Ip KW, Shiu SY, Chiu EK, Wong MP, Yuen KY (1994): Association between polyomaviruria and microscopic haematuria in bone marrow transplant recipients. J Infect 29:139–146.

Chang D, Tsai RT, Wang M, Ou WC (1996a): Different genotypes of human polyomaviruses found in patients with autoimmune diseases in Taiwan. J Med Virol 48:204–209.

Chang D, Wang M, Ou WC, Lee MS, Ho HN, Tsai RT (1996b): Genotypes of human polyomaviruses in urine samples of pregnant women in Taiwan. J Med Virol 48: 95–101.

Chatterjee M, Weyandt TB, Frisque RJ (2000): Identification of archetype and rearranged forms of BK virus in leukocytes from healthy individuals. J Med Virol 60: 353–362.

Chesters PM, Heritage J, McCance DJ (1983): Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J Infect Dis 147: 676–684.

Childs R, Sanchez C, Engler H, Preuss J, Rosenfeld S, Dunbar C, van Rhee F, Plante M, Phang S, Barrett AJ (1998): High incidence of adeno- and polyomavirus-induced hemorrhagic cystitis in bone marrow allotransplantation for hematological malignancy following T cell depletion and cyclosporine. Bone Marrow Transplant 22: 889–893.

Ciappi S, Azzi A, De Santis R, Leoncini F, Sterrantino G, Mazzotta F, Mecocci L (1999): Archetypal and rearranged sequences of human polyomavirus JC transcription control region in peripheral blood leukocytes and in cerebrospinal fluid. J Gen Virol 80:1017–1023.

Cinque P, Vago L, Dahl H, Brytting M, Terreni MR, Fornara C, Racca S, Castagna A, Monforte AD, Wahren B, Lazzarin A, Linde A (1996): Polymerase chain reaction on cerebrospinal fluid for diagnosis of virus-associated opportunistic diseases of the central nervous system in HIV-infected patients. AIDS 10:951–958.

Coleman DV, Field AM, Gardner SD, Porter KA, Starzl TE (1973): Virus-induced obstruction of the ureteric and cystic duct in allograft recipients. Transplant Proc 5: 95–98.

Coleman DV, Wolfendale MR, Daniel RA, Dhanjal NK, Gardner SD, Gibson PE, Field AM (1980): A prospective study of human polyomavirus infection in pregnancy. J Infect Dis 142:1–8.

Corallini A, Campioni D, Rossi C, Albini A, Possati L, Rusnati M, Gazzanelli G, Benelli R, Masiello L, Sparacciari V, Presta M, Mannello F, Fontanini G, Barbanti Brodano G (1996): Promotion of tumour metastases and induction of angiogenesis by native HIV-1 Tat protein from BK virus/tat transgenic mice. AIDS 10:701–710.

Cotterill HA, Macaulay ME, Wong V (1992): Reactivation of polyomavirus in bone marrow transplant recipients. J Clin Pathol 45:445.

Cubukcu-Dimopulo O, Greco A, Kumar A, Karluk D, Mittal K, Jagirdar J (2000): BK virus infection in AIDS. Am J Surg Pathol 24:145–149.

Degener AM, Pietropaolo V, Di Taranto C, Jin L, Ameglio F, Cordiali-Fei P, Trento E, Sinibaldi L, Orsi N (1999): Identification of a new control region in the genome of the DDP strain of BK virus isolated from PBMC. J Med Virol 58:413–419.

Degener AM, Pietropaolo V, Di Taranto C, Rizzuti V, Ameglio F, Cordiali Fei P, Caprilli F, Capitanio B, Sinibaldi L, Orsi N (1997): Detection of JC and BK viral genome in specimens of HIV-1 infected subjects. New Microbiol 20:115–122.

de Luca A, Cingolani A, Linzalone A, Ammassari A, Murri R, Giancola ML, Maiuro G, Antinori A (1996): Improved detection of JC virus DNA in cerebrospinal fluid for diagnosis of AIDS-related progressive multifocal leukoencephalopathy. J Clin Microbiol 34:1343–1346.

De Mattei M, Martini F, Corallini A, Gerosa M, Scotlandi K, Carinci P, Barbanti-Brodano G, Tognon M (1995): High incidence of BK virus large-T-antigen–coding sequences in normal human tissues and tumors of different histotypes. Int J Cancer 61:756–760.

de Silva LM, Bale P, de Courcy J, Brown D, Knowles W (1995): Renal failure due to BK virus infection in an immunodeficient child. J Med Virol 45:192–196.

Di Taranto C, Pietropaolo V, Orsi GB, Jin L, Sinibaldi L, Degener AM (1997): Detection of BK polyomavirus genotypes in healthy and HIV-positive children. Eur J Epidemiol 13:653–657.

Dolei A, Pietropaolo V, Gomes E, Di Taranto C, Ziccheddu M, Spanu MA, Lavorino C, Manca M, Degener AM (2000): Polyomavirus persistence in lymphocytes: Prevalence in lymphocytes from blood donors and healthy personnel of a blood transfusion centre. J Gen Virol 81:1967–1973.

Dörries K (1996): Virus–host interactions and the diagnosis of human polyomavirus associated disease. Intervirology 39:165–175.

Dörries K (1998): Molecular biology and pathogenesis of human polyomavirus infections. Dev Biol Stand 94:71–79.

Dörries K, Arendt G, Eggers C, Roggendorf W, Dörries R (1998): Nucleic acid detection as a diagnostic tool in polyomavirus JC induced progressive multifocal leukoencephalopathy. J Med Virol 54:196–203.

Dörries K, Elsner C (1991): Persistent polyomavirus infection in kidney tissue of patients with diseases other than progressive multifocal leucoencephalopathy. Virol Adv 10:51–61.

Dörries K, Johnson RT, terMeulen V (1979): Detection of polyoma virus DNA in PML-brain tissue by (in situ) hybridization. J Gen Virol 42:49–57.

Dörries K, terMeulen V (1983): Progressive multifocal leucoencephalopathy: Detection of papovavirus JC in kidney tissue. J Med Virol 11:307–317.

Dörries K, Vogel E, Gunther S, Czub S (1994): Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. Virology 198:59–70.

Drews K, Bashir T, Dorries K (2000): Quantification of human polyomavirus JC in brain tissue and cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy by competitive PCR. J Virol Methods 84:23–36.

Dubois V, Dutronc H, Lafon ME, Poinsot V, Pellegrin JL, Ragnaud JM, Ferrer AM, Fleury HJ (1997): Latency and reactivation of JC virus in peripheral blood of human immunodeficiency virus type 1–infected patients. J Clin Microbiol 35:2288–2292.

Dubois V, Lafon ME, Ragnaud JM, Pellegrin JL, Damasio F, Baudouin C, Michaud V, Fleury HJ (1996): Detection of JC virus DNA in the peripheral blood leukocytes of HIV-infected patients. AIDS 10:353–358.

Dubois V, Moret H, Lafon ME, Janvresse CB, Dussaix E, Icart J, Karaterki A, Ruffault A, Taoufik Y, Vignoli C, Ingrand D (1998): Prevalence of JC virus viraemia in HIV-infected patients with or without neurological disorders: A prospective study. J Neurovirol 1998:539–544.

Eggers C, Stellbrink HJ, Buhk T, Dörries K (1999): Quantification of JC virus DNA in the cerebrospinal fluid of patients with human immunodeficiency virus–associated progressive multifocal leukoencephalopathy—A longitudinal study. J Infect Dis 180: 1690–1694.

Elsner C, Dörries K (1992): Evidence of human polyomavirus BK and JC infection in normal brain tissue. Virology 198:59–70.

Ferrante P, Caldarelli-Stefano R, Omodeo-Zorini E, Cagni AE, Cocchi L, Suter F, Maserati R (1997): Comprehensive investigation of the presence of JC virus in AIDS patients with and without progressive multifocal leukoencephalopathy. J Med Virol 52:235–242.

Ferrante P, Caldarelli-Stefano R, Omodeo-Zorini E, Vago L, Boldorini R, Costanzi G (1995): PCR detection of JC virus DNA in brain tissue from patients with and without progressive multifocal leukoencephalopathy. J Med Virol 47:219–225.

Ferrante P, Omodeo Zorini E, Caldarelli Stefano R, Mediati M, Fainardi E, Granieri E, Caputo D (1998): Detection of JC virus DNA in cerebrospinal fluid from multiple sclerosis patients. Mult Scler 4:49–54.

Flaegstad T, Sundsfjord A, Arthur RR, Pedersen M, Traavik T, Subramani S. (1991): Amplification and sequencing of the control regions of BK and JC virus from human urine by polymerase chain reaction. Virology 180:553–560.

Fong IW, Britton CB, Luinstra KE, Toma E, Mahony JB (1995): Diagnostic value of detecting JC virus DNA in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. J Clin Microbiol 33:484–486.

Gardner SD, Field AM, Coleman DV, Hulme B (1971): New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet I:1253–1257.

Gardner SD, Knowles WA (1994): Human polyomaviruses. In Principles and Practice of Clinical Virology; Zuckerman AJ, Banatvala JE, Pattison JR, Eds; John Wiley: Chichester, pp 635–651.

Gerber MA, Shah KV, Thung SN, Zu Rhein GM (1980): Immunohistochemical demonstration of common antigen of polyomaviruses in routine histologic tissue sections of animals and man. Am J Clin Pathol 73:795–797.

Gibson PE, Gardner SD (1983): Strain differences and some serological observations on several isolates of human polyomaviruses. Prog Clin Biol Res 105:119–132.

Gibson PE, Gardner SD, Porter AA (1985): Detection of human polyomavirus DNA in urine specimens by hybridot assay. Arch Virol 84:233–240.

Gibson PE, Knowles WA, Hand JF, Brown DW (1993): Detection of JC virus DNA in the cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. J Med Virol 39:278–281.

Goldstein SC, Tralka TS, Rabson AS (1984): Mixed infection with human cytomegalovirus and human polyomavirus (BKV): J Med Virol 13:33–40.

Goudsmit J, Wertheim van Dillen P, van Strien A, van der Noordaa J (1982): The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. J Med Virol 10:91–99.

Grinnell BW, Padgett BL, Walker DL (1983a): Distribution of nonintegrated DNA from JC papovavirus in organs of patients with progressive multifocal leukoencephalopathy. J Infect Dis 147:669–675.

Grinnell BW, Padgett BL, Walker DL (1983b): Comparison of infectious JC virus DNAs cloned from human brain. J Virol 45:299–308.

Hammarin AL, Bogdanovic G, Svedhem V, Pirskanen R, Morfeldt L, Grandien M. (1996): Analysis of PCR as a tool for detection of JC virus DNA in cerebrospinal fluid for diagnosis of progressive multifocal leukoencephalopathy. J Clin Microbiol 34:2929–2932.

Hedquist BG, Bratt G, Hammarin AL, Grandien M, Nennesmo I, Sundelin B, Seregard S. (1999): Identification of BK virus in a patient with acquired immune deficiency syndrome and bilateral atypical retinitis. Ophthalmology 106:129–132.

Heilbronn R, Albrecht I, Stephan S, Burkle A, zur Hausen H (1993): Human cytomegalovirus induces JC virus DNA replication in human fibroblasts. Proc Natl Acad Sci USA 90:11406–11410.

Heinonen O, Syrjanen S, Mantyjarvi R, Syrjanen K, Riekkinen P (1992): JC virus infection and Alzheimer's disease: Reappraisal of an in situ hybridization approach. Ann Neurol 31:439–441.

Henson J, Rosenblum M, Armstrong D, Furneaux H (1991): Amplification of JC virus DNA from brain and cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. Neurology 41:1967–1971.

Heritage J, Chesters PM, McCance DJ (1981): The persistence of papovavirus BK DNA sequences in normal human renal tissue. J Med Virol 8:143–150.

Hiraoka A, Ishikawa J, Kitayama H, Yamagami T, Teshima H, Nakamura H, Shibata H, Masaoka T, Ishigami S, Taguchi F (1991): Hemorrhagic cystitis after bone marrow transplantation: Importance of a thin sectioning technique on urinary sediments for diagnosis. Bone Marrow Transplant 7:107–111.

Hogan TF, Borden EC, McBain JA, Padgett BL, Walker DL (1980): Human polyomavirus infections with JC virus and BK virus in renal transplant patients. Ann Intern Med 92:373–378.

Hogan TF, Padgett BL, Walker DL, Borden EC, Frias Z (1983): Survey of human polyomavirus (JCV, BKV) infections in 139 patients with lung cancer, breast cancer, melanoma, or lymphoma. Prog Clin Biol Res 105:311–324.

Houff SA, Major EO, Katz DA, Kufta CV, Sever JL, Pittaluga S, Roberts JR, Gitt J, Saini N, Lux W. (1988): Involvement of JC virus–infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. N Engl J Med 318:301–305.

Jensen PN, Major EO (1999): Viral variant nucleotide sequences help expose leukocytic positioning in the JC virus pathway to the CNS. J Leukocyte Biol 65:428–438.

Jin L, Gibson PE, Booth JC, Clewley JP (1993): Genomic typing of BK virus in clinical specimens by direct sequencing of polymerase chain reaction products. J Med Virol 41:11–17.

Jin L, Pietropaolo V, Booth JC, Ward KH, Brown DW (1995): Prevalence and distribution of BK virus subtypes in healthy people and immunocompromised patients detected by PCR-restriction enzyme analysis. Clin Diagn Virol 3:285–295.

Kato A, Kitamura T, Sugimoto C, Ogawa Y, Nakazato K, Nagashima K, Hall WW, Kawabe K, Yogo Y (1997): Lack of evidence for the transmission of JC polyomavirus between human populations. Arch Virol 142:875–882.

Katz DA, Berger JR, Hamilton B, Major EO, Post MJ (1994): Progressive multifocal leukoencephalopathy complicating Wiskott-Aldrich syndrome. Report of a case and review of the literature of progressive multifocal leukoencephalopathy with other inherited immunodeficiency states. Arch Neurol 51:422–426.

Kitamura T, Aso Y, Kuniyoshi N, Hara K, Yogo Y. (1990): High incidence of urinary JC virus excretion in nonimmunosuppressed older patients. J Infect Dis 161:1128–1133.

Kitamura T, Satoh K, Tominaga T, Taguchi F, Tajima A, Suzuki K, Aso Y, Yogo Y (1994): Alteration in the JC polyomavirus genome is enhanced in immunosuppressed renal transplant patients. Virology 198:341–345.

Kitamura T, Sugimoto C, Kato A, Ebihara H, Suzuki M, Taguchi F, Kawabe K, Yogo Y (1997): Persistent JC virus (JCV) infection is demonstrated by continuous shedding of the same JCV strains. J Clin Microbiol 35:1255–1257.

Kleihues P, Lang W, Burger PC, Budka H, Vogt M, Maurer R, Luthy R, Siegenthaler W (1985): Progressive diffuse leukoencephalopathy in patients with acquired immune deficiency syndrome (AIDS): Acta Neuropathol Berl 68:333–339.

Knepper JE, diMayorca G (1987): Cloning and characterization of BK virus–related DNA sequences from normal and neoplastic human tissues. J Med Virol 21:289–299.

Knowles WA, Pillay D, Johnson MA, Hand JF, Brown DW (1999): Prevalence of long-term BK and JC excretion in HIV-infected adults and lack of correlation with serological markers. J Med Virol 59:474–479.

Koralnik IJ, Boden D, Mai VX, Lord CI, Letvin NL (1999a): JC virus DNA load in patients with and without progressive multifocal leukoencephalopathy. Neurology 52:253–260.

Koralnik IJ, Schmitz JE, Lifton MA, Forman MA, Letvin, NL (1999b): Detection of JC virus DNA in peripheral blood cell subpopulations of HIV-1–infected individuals. J Neurovirol 5:430–435.

Kristoffersen AK, Johnsen JI, Seternes OM, Rollag H, Degre M, Traavik T (1997): The human polyomavirus BK T antigen induces gene expression in human cytomegalovirus. Virus Res 52:61–71.

Lafon ME, Dutronc H, Dubois V, Pellegrin I, Barbeau P, Ragnaud JM, Pellegrin JL, Fleury HJ (1998): JC virus remains latent in peripheral blood B lymphocytes but replicates actively in urine from AIDS patients. J Infect Dis 177:1502–1505.

Laghi L, Randolph AE, Chauhan DP, Marra G, Major EO, Neel JV, Boland CR (1999): JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. Proc Natl Acad Sci USA 96:7484–7489.

Lecatsas G, Blignaut E, Schoub BD (1977): Lymphocyte stimulation by urine-derived human polyoma virus (BK): Arch Virol 55:165–167.

Lecatsas G, Boes EG, Horsthemke E (1981): Intermediate size papovavirus particles in pregnancy urine. J Gen Virol 52:359–362.

Lecatsas G, Prozesky OW, Wyk JV, Els HJ (1973): Papova virus in urine after renal transplantation. Nature 241:343–344.

Lecatsas G, Schoub BD, Rabson AR, Joffe M (1976): Papovavirus in human lymphocyte cultures. Lancet 2:907–908.

Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR (1992): Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus–induced demyelinating disease of the human brain. Clin Microbiol Rev. 5:49–73.

Markowitz RB, Eaton BA, Kubik MF, Latorra D, McGregor JA, Dynan WS (1991): BK virus and JC virus shed during pregnancy have predominantly archetypal regulatory regions. J Virol 65:4515–4519.

Markowitz RB, Thompson HC, Mueller JF, Cohen JA, Dynan WS (1993): Incidence of BK virus and JC virus viruria in human immunodeficiency virus–infected and –uninfected subjects. J Infect Dis 167:13–20.

Mathur VS, Olson JL, Darragh TM, Yen TS (1997): Polyomavirus-induced interstitial nephritis in two renal transplant recipients: Case reports and review of the literature. Am J Kidney Dis 29:754–758.

Matsiota-Bernard P, De Truchis P, Gray F, Flament-Saillour M, Voyatzakis E, Nauciel C (1997): JC virus detection in the cerebrospinal fluid of AIDS patients with progressive multifocal leucoencephalopathy and monitoring of the antiviral treatment by a PCR method. J Med Microbiol 46:256–259.

McCance DJ (1983): Persistence of animal and human papovaviruses in renal and nervous tissues. Prog Clin Biol Res 105:343–357.

McGuire D, Barhite S, Hollander H, Miles M (1995): JC virus DNA in cerebrospinal fluid of human immunodeficiency virus-infected patients: Predictive value for progressive multifocal leukoencephalopathy. Ann Neurol 37:395–399.

Mendelson M, Monard S, Sissons P, Sinclair J (1996): Detection of endogenous human cytomegalovirus in CD34+ bone marrow progenitors. J Gen Virol 77:3099–3102.

Monaco MC, Atwood WJ, Gravell M, Tornatore CS, Major EO (1996): JC virus infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: Implications for viral latency. J Virol 70:7004–7012.

Monaco MCG, Jensen PN, Hou J, Durham LC, Major EO (1998): Detection of JC virus DNA in human tonsil tissue: Evidence for site of initial viral infection. J Virol 72:9918–9923.

Monini P, Rotola A, de Lellis L, Corallini A, Secchiero P, Albini A, Benelli R, Parravicini C, Barbanti-Brodano G, Cassai E (1996): Latent BK virus infection and Kaposi's sarcoma pathogenesis. Int J Cancer 66:717–722.

Monini P, Rotola A, di Luca D, de Lellis L, Chiari E, Corallini A, Cassai E (1995): DNA rearrangements impairing BK virus productive infection in urinary tract tumors. Virology 214:273–279.

Moret H, Guichard M, Matheron S, Katlama C, Sazdovitch V, Huraux JM, Ingrand D (1993): Virological diagnosis of progressive multifocal leukoencephalopathy: Detection of JC virus DNA in cerebrospinal fluid and brain tissue of AIDS patients. J Clin Microbiol 31:3310–3313.

Mori M, Aoki N, Shimada H, Tajima M, Kato K (1992): Detection of JC virus in the brains of aged patients without progressive multifocal leukoencephalopathy by the polymerase chain reaction and Southern hybridization analysis. Neurosci Lett 141: 151–155.

Myers C, Frisque RJ, Arthur RR (1989): Direct isolation and characterization of JC virus from urine samples of renal and bone marrow transplant patients. J Virol 63: 4445–4449.

Nebuloni M, Tosoni A, Boldorini R, Monga G, Carsana L, Bonetto S, Abeli C, Caldarelli R, Vago L, Costanzi G (1999): BK virus renal infection in a patient with the acquired immunodeficiency syndrome. Arch Pathol Lab Med 123:807–811.

Newman JT, Frisque RJ (1997): Detection of archetype and rearranged variants of JC virus in multiple tissues from a pediatric PML patient. J Med Virol 52: 243–252.

Newman JT, Frisque RJ (1999): Identification of JC virus variants in multiple tissues of pediatric and adult PML patients. J Med Virol 58:79–86.

Nickeleit V, Klimkait T, Binet IF, Dalquen P, Del Zenero V, Thiel G, Mihatsch MJ, Hirsch HH (2000): Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med 342:1309–1315.

Noordaa JV, Wertheim-van Dillen P (1977): Rise in antibodies to human papova virus BK and clinical disease. BMJ 1:1471.

Okada Y, Sawa H, Tanaka S, Takada A, Suzuki S, Hasegawa H, Umemura T, Fujisawa J, Tanaka Y, Hall WW, Nagashima K (2000): Transcriptional activation of JC virus by human T-lymphotropic virus type I Tax protein in human neuronal cell lines. J Biol Chem 275:17016–17023.

O'Reilly RJ, Lee FK, Grossbard E, Kapoor N, Kirkpatrick D, Dinsmore R, Stutzer C, Shah KV, Nahmias AJ (1981): Papovavirus excretion following marrow transplantation: Incidence and association with hepatic dysfunction. Transplant Proc 13:262–266.

Pappo O, Demetris AJ, Raikow RB, Randhawa PS (1996): Human polyoma virus infection of renal allografts: Histopathologic diagnosis, clinical significance, and literature review. Mod Pathol 9:105–109.

Perrons CJ, Fox JD, Lucas SB, Brink NS, Tedder RS, Miller RF (1996): Detection of polyomaviral DNA in clinical samples from immunocompromised patients: Correlation with clinical disease. J Infect 32:205–209.

Pietropaolo V, Di Taranto C, Degener AM, Jin L, Sinibaldi L, Baiocchini A, Melis M, Orsi N (1998): Transplacental transmission of human polyomavirus BK. J Med Virol 56:372–376.

Pietzuch A, Bodemer M, Frye S, Cinque P, Trebst C, Lüke W, Weber T (1996): Expression of JCV-specific cDNA in peripheral blood lymphocytes of patients with PML, HIV-infection, multiple sclerosis and in healthy controls. J Neurovirology 2: 46.

Portolani M, Piani M, Gazzanelli G, Borgatti M, Bartoletti A, Grossi MP, Corallini A, Barbanti-Brodano G (1985): Restricted replication of BK virus in human lymphocytes. Microbiologica 8:59–66.

Possati L, Rubini C, Portolani M, Gazzanelli G, Piani M, Borgatti M (1983): Receptors for the human papovavirus BK on human lymphocytes. Arch Virol 75:131–136.

Purighalla R, Shapiro R, McCauley J, Randhawa P (1995): BK virus infection in a kidney allograft diagnosed by needle biopsy. Am J Kidney Dis 26:671–673.

Quinlivan EB, Norris M, Bouldin TW, Suzuki K, Meeker R, Smith MS, Hall C, Kenney S (1992): Subclinical central nervous system infection with JC virus in patients with AIDS. J Infect Dis 166:80–85.

Randhawa PS, Finkelstein S, Scantlebury V, Shapiro R, Vivas C, Jordan M, Picken MM, Demetris AJ (1999): Human polyoma virus–associated interstitial nephritis in the allograft kidney. Transplantation 67:103–109.

Reese JM, Reissing M, Daniel RW, Shah KV (1975): Occurrence of BK virus and BK virus–specific antibodies in the urine of patients receiving chemotherapy for malignancy. Infect Immun 11:1375–1381.

Rekvig OP, Moens U, Fredriksen K, Traavik T (1997): Human polyomavirus BK and immunogenicity of mammalian DNA: A conceptual framework. Methods 11:44–54.

Ricciardiello L, Chang DK, Laghi L, Goel A, Chang CL, Boland CR (2001): Mad-1 is the exclusive JC virus strain present in the human colon, and its transcriptional control region has a deleted 98-base pair sequence in colon cancer tissues. J Virol 75:1996–2001.

Rieckmann P, Michel U, Kehrl JH (1994): Regulation of JC virus expression in B lymphocytes. J Virol 68:217–222.

Rosen S, Harmon W, Krensky AM, Edelson PJ, Padgett BL, Grinnell BW, Rubino MJ, Walker DL (1983): Tubulo-interstitial nephritis associated with polyomavirus (BK type) infection. N Engl J Med 308:1192–1196.

Samorei IW, Schmid M, Pawlita M, Vinters HV, Diebold K, Mundt C, von Einsiedel RW (2000): High sensitivity detection of JC-virus DNA in postmortem brain tissue by in situ PCR. J Neurovirol 6:61–74.

Sandler ES, Aquino VM, Goss-Shohet E, Hinrichs S, Krisher K (1997): BK papova virus pneumonia following hematopoietic stem cell transplantation. Bone Marrow Transplant 20:163–165.

Schneider EM, Dörries K (1993): High frequency of polyomavirus infection in lymphoid cell preparations after allogeneic bone marrow transplantation. Transplant Proc 25:1271–1273.

Shah KV, Daniel RW, Strickler HD, Goedert JJ (1997): Investigation of human urine for genomic sequences of the primate polyomaviruses simian virus 40, BK virus, and JC virus. J Infect Dis 176:1618–1621.

Shah KV, Daniel RW, Zeigel RF, Murphy GP (1974): Search for BK and SV40 virus reactivation in renal transplant recipients. Transplantation 17:131–134.

Shimizu N, Imamura A, Daimaru O, Mihara H, Kato Y, Kato R, Oguri T, Fukada M, Yokochi T, Yoshikawa K, Komatsu H, Ueda R, Nitta M (1999): Distribution of JC virus DNA in peripheral blood lymphocytes of hematological disease cases. Intern Med 38:932–937.

Shinohara T, Matsuda M, Cheng SH, Marshall J, Fujita M, Nagashima K (1993): BK virus infection of the human urinary tract. J Med Virol 41:301–305.

Sinclair J, Sissons P (1996): Latent and persistent infections of monocytes and macrophages. Intervirology 39:293–301.

Sinzger C, Jahn G (1996): Human cytomegalovirus cell tropism and pathogenesis. Intervirology 39:302–319.

Smith RD, Galla JH, Skahan K, Anderson P, Linnemann CC, Jr, Ault GS, Ryschkewitsch CF, Stoner GL (1998): Tubulointerstitial nephritis due to a mutant polyomavirus BK virus strain, BKV(Cin), causing end-stage renal disease. J Clin Microbiol 36:1660–1665.

Stoeckle MY (2000): The spectrum of human herpesvirus 6 infection: From roseola infantum to adult disease. Annu Rev Med 51:423–430.

Stoner GL, Agostini HT, Ryschkewitsch CF, Komoly S (1998): JC virus excreted by multiple sclerosis patients and paired controls from Hungary. Mult Scler 4:45–48.

Stoner GL, Soffer D, Ryschkewitsch CF, Walker DL, Webster HD (1988): A double-label method detects both early (T-antigen) and late (capsid) proteins of JC virus in progressive multifocal leukoencephalopathy brain tissue from AIDS and non-AIDS patients. J Neuroimmunol 19:223–236.

Sugimoto C, Ito D, Tanaka K, Matsuda H, Saito H, Sakai H, Fujihara K, Itoyama Y, Yamada T, Kira J, Matsumoto R, Mori M, Nagashima K, Yogo Y (1998): Amplification of JC virus regulatory DNA sequences from cerebrospinal fluid: Diagnostic value for progressive multifocal leukoencephalopathy. Arch Virol 143:249–262.

Sundsfjord A, Flaegstad T, Flo R, Spein AR, Pedersen M, Permin H, Julsrud J, Traavik T (1994a): BK and JC viruses in human immunodeficiency virus type 1–infected persons: Prevalence, excretion, viremia, and viral regulatory regions. J Infect Dis 169:485–490.

Sundsfjord A, Osei A, Rosenqvist H, Van Ghelue M, Silsand Y, Haga HJ, Rekvig OP, Moens U (1999): BK and JC viruses in patients with systemic lupus erythematosus: Prevalent and persistent BK viruria, sequence stability of the viral regulatory regions, and nondetectable viremia. J Infect Dis 180:1–9.

Sundsfjord A, Spein AR, Lucht E, Flaegstad T, Seternes OM, Traavik T (1994b): Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. J Clin Microbiol 32:1390–1394.

Takemoto KK, Linke H, Miyamura T, Fareed GC (1979): Persistent BK papovavirus infection of transformed human fetal brain cells. I. Episomal viral DNA in cloned lines deficient in T-antigen expression. J Virol 29:1177–1185.

Telenti A, Aksamit A, Jr, Proper J, Smith TF (1990): Detection of JC virus DNA by polymerase chain reaction in patients with progressive multifocal leukoencephalopathy. J Infect Dis 162:858–861.

Tevethia MJ, Spector DJ (1989): Heterologous transactivation among viruses. Prog Med Virol 36:120–190.

Tolkoff-Rubin NE, Rubin RH (1997): Urinary tract infection in the immunocompromised host. Lessons from kidney transplantation and the AIDS epidemic. Infect Dis Clin North Am 11:707–717.

Tominaga T, Yogo Y, Kitamura T, Aso Y (1992): Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. Virology 186:736–741.

Tornatore C, Berger JR, Houff SA, Curfman B, Meyers K, Winfield D, Major EO (1992): Detection of JC virus DNA in peripheral lymphocytes from patients with and without progressive multifocal leukoencephalopathy. Ann Neurol 31:454–462.

Traavik T, Uhlin-Hansen L, Flaegstad T, Christie KE (1988): Antibody-mediated enhancement of BK virus infection in human monocytes and a human macrophage-like cell line. J Med Virol 24:283–297.

Tsai RT, Wang M, Ou WC, Lee YL, Li SY, Fung CY, Huang YL, Tzeng TY, Chen Y, Chang D (1997): Incidence of JC viruria is higher than that of BK viruria in Taiwan. J Med Virol 52:253–257.

Vago L, Cinque P, Sala E, Nebuloni M, Caldarelli R, Racca S, Ferrante P, Trabottoni G, Costanzi G (1996): JCV-DNA and BKV-DNA in the CNS tissue and CSF of AIDS patients and normal subjects. Study of 41 cases and review of the literature. J Acquir Immune Defic Syndr Hum Retrovirol 12:139–146.

Vallbracht A, Lohler J, Gossmann J, Gluck T, Petersen D, Gerth HJ, Gencic M, Dörries K (1993): Disseminated BK type polyomavirus infection in an AIDS patient associated with central nervous system disease. Am J Pathol 143:29–39.

Valle LD, Croul S, Morgello S, Amini S, Rappaport J, Khalili K (2000): Detection of HIV-1 Tat and JCV capsid protein, VP1, in AIDS brain with progressive multifocal leukoencephalopathy. J Neurovirol 6:221–228.

Vignoli C, De Lamballerie X, Zandotti C, Tamalet C, De Micco P (1993): Detection of JC virus by polymerase chain reaction and colorimetric DNA hybridization assay. Eur J Clin Microbiol Infect Dis 12:958–961.

Voltz R, Jager G, Seelos K, Fuhry L, Hohlfeld R (1996): BK virus encephalitis in an immunocompetent patient. Arch Neurol 53:101–103.

Walker DL, Padgett BL (1983): Progressive multifocal leukoencephalopathy. In Comprehensive Virology, Vol. 18. Virus-Host Interactions, Fraenkel-Conrat H and Wagner RR, Eds. Plenum Press: New York, pp 161–193.

Weber T, Major EO (1997): Progressive multifocal leukoencephalopathy: Molecular biology, pathogenesis and clinical impact. Intervirology 40:98–111.

Weber T, Turner RW, Frye S, Ruf B, Haas J, Schielke E, Pohle HD, Luke W, Luer W, Felgenhauer K, et al. (1994): Specific diagnosis of progressive multifocal leukoencephalopathy by polymerase chain reaction. J Infect Dis 169:1138–1141.

White FD, Ishaq M, Stoner GL, Frisque RJ (1992): JC virus DNA is present in many human brain samples from patients without progressive multifocal leukoencephalopathy. J Virol 66:5726–5734.

Yogo Y, Kitamura T, Sugimoto C, Hara K, Iida T, Taguchi F, Tajima A, Kawabe K, Aso Y (1991): Sequence rearrangement in JC virus DNAs molecularly cloned from immunosuppressed renal transplant patients. J Virol 65:2422–2428.

Zu Rhein GM (1969): Association of papova-virions with a human demyelinating disease (progressive multifocal leukoencephalopathy): Prog Med Virol 11:185–247.

Zu Rhein GM, Varakis J (1974): Letter: Papovavirions in urothelium of treated lymphoma patient. Lancet 2:783–784.