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Carlos M. Farinha

CFTR and Cystic Fibrosis

From Structure to Function

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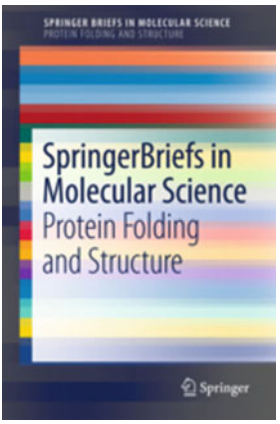

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Foreword

Protein function is tightly related to folding and structure, but proteins in the cell also need to be at the right location to carry out their biological roles. Therefore, as a protein folds, complex machineries assure, in tightly orchestrated processes, not only the quality of folded proteins but also its correct cellular trafficking and lipid bilayer insertion in the case of membrane proteins. Indeed, several human diseases result from combined folding and trafficking defects. Among these is cystic fibrosis, a hallmark loss of function protein-folding disease caused by defects in a transmembrane chloride channel called cystic fibrosis transmembrane conductance regulator (CFTR). CFTR misfolding in cystic fibrosis, which results mostly from the F508del mutation, impairs traffic and membrane insertion causing proteasomal degradation. In this fifth volume of the "Protein Folding and Structure" series of the "Springer Briefs in Molecular Science", the leading expert Carlos M. Farinha provides an updated perspective of the genetic, functional and cellular processes involving CFTR in connection with cystic fibrosis. Starting with a historical perspective on cystic fibrosis and its clinical features, the author departs into an in-depth description of the biology of the CFTR protein, ending with a discussion on the latest approaches aimed at developing corrective therapies for cystic fibrosis. Through this integrated perspective, the reader will obtain a unique insight into this fascinating membrane-bound protein and its associated disease. Enjoy reading.

Lisboa
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Cláudio M. Gomes
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Contents

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Carlos M. Farinha obtained his Ph.D. (2003) and Habilitation (2015) in Biochemistry from the University of Lisbon. He has worked at the National Institute of Health Dr. Ricardo Jorge and at the Faculty of Sciences, University of Lisbon with training periods at the Johns Hopkins University School of Medicine, Baltimore, MD, USA and at the University of Pittsburgh School of Medicine—Children's Hospital of Pittsburgh, Pittsburgh, PA, USA. Currently, he is Assistant Professor with habilitation at the Faculty of Sciences, University of Lisbon, and Principal Investigator at BioISI—Biosystems and Integrative Sciences Institute. His research focuses on the molecular mechanisms of cystic fibrosis (CF), in particular the endoplasmic reticulum retention and early degradation of CFTR's most common mutant and characterization of the role of CFTR interacting proteins. He received the 2012 ERS Romain Pauwels Research Award from the European Respiratory Society for "research into the different aspects of CFTR biology".

Abbreviations

Aa	Amino acid
ABC	ATP-binding cassette
AFT	Arginine-framed tripeptide
ASL	Airway surface liquid
ATP	Adenosine 5'Triphosphate
Bag	Bcl-2 associated atharogene
Ca^{2+}	Calcium ion
CaCC	Calcium-activated Chloride Channel
CAL	CFTR-associated ligand
cAMP	Cyclic Adenosine 5'Monophosphate
CBAVD	Congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFTR	Gene encoding CFTR
CHIP	Carboxy-terminal of Hsp70 interacting protein
CK2	Casein kinase 2
Cl^-	Chloride ion
COP	Coat protein
Dab-2	Disabled-2
DHS	DNase I hypersensitive site
ECL	Extracellular loop
EMT	Epithelial-to-mesenchymal transition
ENaC	Epithelium Sodium Channel
EPAC	Exchange protein directly activated by cAMP
ERAD	Endoplasmic reticulum-associated degradation
ER	Endoplasmic reticulum
ERQC	Endoplasmic reticulum quality control
FDA	Food and Drug Administration
GERAD	Glycoprotein endoplasmic reticulum associated degradation

GSH	Glutathione
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
Hdj	Human DnaJ
HGF	Hepatocyte growth factor
Hsp	Heat shock protein
HTS	High Throughput Screening
IBMX	3-isobutyl-1-methylxanthine
ICL	Intracellular loop
K^+	Potassium ion
LMTK2	Lemur tyrosine kinase 2
MDR	Multidrug resistance
MRP	Multidrug-related protein
MSD	Membrane-spanning domain
Na^+	Sodium ion
NBD	Nucleotide binding domain
NH3	Na^+/H^+ exchanger
NHERF1	Na^+/H^+ exchanger regulatory factor isoform-1
NKCC1	Na^+-K^+-$2Cl^-$ cotransporter
NMD	Nonsense-mediated decay
NPD	Nasal Potential Difference
ORCC	Outwardly Rectifying Chloride Channel
PCR	Polymerase chain reaction
PDE	Phosphodiesterases
PDZ	Psd-95, Disc-large and ZO-1
PGE	Prostaglandins
PI	Pancreatic Insufficiency
PK	Protein kinase
PPQC	Peripheral protein quality control
PS	Pancreatic Sufficiency
PTC	Premature termination codon
RD	Regulatory domain
RFLP	Restriction fragment length polymorphism
ROMK	Renal outer medullary K^+ channel
SLC26	Solute carrier 26 family
SNARE	Soluble NSF Attachment Protein Receptor
SRP	Signal recognition particle
SUMO	Small ubiquitin-like modifier
SYK	Spleen tyrosine kinase
TAP	Transporter associated with antigen presentation
TfR	Transferrin
TM	Transmembrane segment
Ubc	Ubiquitin-conjugating enzymes

UGGT	UDP-glucose glucosyltransferase
UPP	Ubiquitin Proteasome Pathway
WNK	With-no-lysine kinase
wt-CFTR	Wild-type CFTR

Abstract

Cystic fibrosis (CF) is a monogenic autosomal recessive disorder that affects Caucasian individuals. Clinically it is characterized by chronic pulmonary dysfunction, pancreatic insufficiency, increased saline concentration in sweat, and male infertility. The gene responsible for the disease was cloned in 1989 and encodes an ABC transporter, the 1480-amino acid protein named Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), that functions as a chloride (Cl^-) channel in the apical membrane of epithelial cells.

Since the cloning of the gene over 2,000 mutations were identified but a single one, deletion of phenylalanine 508 (F508del), accounts for about 70% of the CF chromosomes.

CFTR protein is synthesized at ER-anchored ribosomes and co-translationally inserted into the ER membrane where it is core-glycosylated. From there, the protein is exported to the Golgi where it undergoes several modifications at its glycidic moieties and after full processing finally it reaches the plasma membrane. This process is complex and a certain proportion of the wt protein and almost all the protein bearing the F508del mutation fail this export, being alternatively sent to proteasomal degradation coupled to the ER.

This brief focus on CFTR and Cystic Fibrosis, as a paradigmatic example of a loss-of-function conformational disorder, and is directed to a targeted audience whose interests span from human genetics to protein folding, protein trafficking, and physiology.

It covers the basic aspects of Cystic Fibrosis as a disorder, focusing on its genetics and mutation prevalence/incidence. Then, the major part is devoted to the CFTR protein—its structure and classification within the ABC transporter superfamily, its biogenesis with membrane insertion and chaperone-assisted folding, its glycosylation and the endoplasmic reticulum quality control mechanisms that assess CFTR folding status. Then, attention is given to post-ER trafficking and regulation

of membrane stability/anchoring and to CFTR function. This is linked to the molecular mechanisms through which different CFTR mutations cause cystic fibrosis.

The last part covers the different efforts aiming at rescuing the basic defect, most of which address CFTR dysfunction(s).

Keywords CFTR · Cystic Fibrosis · Protein folding · Protein trafficking · Ion channels · Membrane proteins · ABC transporters · Post-translational modifications

Chapter 1
CFTR and Cystic Fibrosis

1.1 Cystic Fibrosis: An Overview

1.1.1 Historical Perspective

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasians and appears mostly in childhood. The first medical reports of CF symptoms date to the seventeenth century with descriptions of the meconium ileus but the condition was known previously with some medieval references such as the one that states: *Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die.*

However, CF as a unique disease remained uncharacterized till the late 1930s. It was in 1936 that Fanconi introduced the term "mukoviszidose" (German for "thickened mucus") and then in 1938 Dorothy Anderson presented the first complete description of the pathology and named it "cystic fibrosis of the pancreas". A major finding came in the 1950s during a heat wave in New York: Paul di Sant'Agnese reported the identification of an increased salt content in the sweat of CF patients, establishing the basis for the sweat chloride test still used in CF diagnosis.

During the decade of 1980, relevant work by Paul Quinton on the chloride (Cl^-) impermeability in sweat duct cells identified the major defect in the apical membrane of CF epithelial cells with decreased Cl^- and increased sodium (Na^+) transport (Knowles et al. 1983; Quinton and Bijman 1983; Boucher et al. 1986). Finally, in 1989, the joint efforts of different laboratories resulted in the cloning of the gene responsible for CF and the identification of the protein named as Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) based on the structure inferred from the amino acid sequence (Riordan et al. 1989; Rommens et al. 1989; Kerem et al. 1989). Similarly to what happened with other genes identified by

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positional cloning, proof that this was the gene responsible for CF came from the identification of mutations in patients, accounting for the phenotypic divergence between these individuals and non-affected ones. At the time, one mutation was identified in about 70% of the alleles in CF patients. This is a 3 bp deletion in exon 10 (NBD1), resulting in the deletion of Phe-508. This mutation was called ΔF508 (Riordan et al. 1989) and more recently changed to F508del according to the guidelines proposed by Antonarakis (1998). The final proof that this was the gene responsible for CF came from experiments with cells derived from CF patients and the restoration of Cl^- transport upon transfection with the CFTR cDNA (Drumm et al. 1990; Rich et al. 1990).

1.1.2 Clinical Presentation and Diagnosis

Formerly known as "cystic fibrosis of the pancreas", this condition has increasingly been called simply as "cystic fibrosis", abbreviated as CF. Manifestations relate not only to the disruption of exocrine function of the pancreas but also to progressive lung disease which is the main cause of morbidity and mortality. Additional complications involve the intestinal glands (meconium ileus), biliary tree (biliary cirrhosis), and sweat glands (high sweat electrolyte with water depletion in a hot environment). Infertility occurs almost always in males but also in females (Zielenski and Tsui 1995).

CF is characterized by persistent *Pseudomonas aeruginosa* colonization of the conducting airways, leading to the migration of inflammatory cells, including polymorphonuclear leukocytes (PMNs), into the airways of CF patients. PMNs release a potent chemokinetic and chemoattractant, leukotriene B, during an inflammatory response, resulting in the further migration of inflammatory cells. The oxidative metabolites of arachidonic acid and the inflammatory cell-derived proteases have been implicated in the destruction and shedding of the airway epithelia observed in CF (Miele et al. 1997).

The frequency of the disease is variable among different ethnic groups and is higher in Caucasian individuals, with 1 in 2500–6000 newborns being affected. Similarly in this population, the heterozygote frequency reaches the rather remarkable value of about 1 in 25 individuals (Collins 1992).

The large clinical variability and diversity of symptoms makes CF diagnosis in infants a complex issue. Prenatal diagnosis followed by voluntary pregnancy interruption, offered to couples with a previous affected child or relative, is thus an alternative strategy to control CF in the community (Ryley et al. 1992).

The sweat test is commonly used as the main CF diagnosis tool. CF is diagnosed when chloride (Cl^-) concentration in sweat is over 60 mEq/l, in association with pancreatic insufficiency (PI) and chronic pulmonary complications (typically *Pseudomonas* colonization). The sweat test is based on Darhling's observation that,

under certain stimuli, CF patients' sweat is very saline. In a common test, sweat is stimulated by cutaneous application of pilocarpine, which creates a potential gradient towards the sweat gland. The produced sweat is collected and Cl^- and sometimes also Na^+ concentrations are determined (Ryley et al. 1992). The applicability of the test should be reduced to patients that present any clinical symptom other than respiratory dysfunction.

CF diagnosis can also be based on the observation that nasal transepithelial potential differences are increased, mostly due to enhanced apical Na^+ absorption (Ramsey and Boat 1994).

The presence of a specific intestinal obstruction, called meconial ileus, in newborns is also one of the first presentations of CF in about 15% of cases. This observation can be complemented with the determination of meconium albumin, which is increased in CF, due to PI.

Molecular analysis of DNA began a few years before the gene was cloned in 1989. Several markers, latter shown to be in strong linkage disequilibrium with the *CFTR* gene, were used to determine whether a fetus would or not be affected with CF.

Since the cloning of the gene in 1989 (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989), linkage analysis was almost completely replaced by direct mutation detection following Polymerase Chain Reaction (PCR)-based DNA amplification.

Several techniques for mutation detection are very useful in genetic counseling, prenatal diagnosis, and carrier screening, especially in complex clinical cases.

Due to the increasing number of mutations identified in the CFTR gene (more than 2000 so far—see CFTR mutation data base at www.genet.sickkids.on.ca/cftr/), this analysis is usually be restricted in each laboratory to the most frequent mutations in the area (region/country).

Consensus diagnosis criteria assume that patients should have one or more characteristic clinical features, a history of CF in a sibling or a positive newborn screening result (nowadays in place in many western countries), followed by laboratory evidence of CFTR dysfunction (Rosenstein and Cutting 1998). Abnormal CFTR function will usually be documented by two elevated sweat Cl^- concentrations obtained on separate days or by the identification of two CFTR mutations. For patients with normal or borderline sweat Cl^- concentrations and for those without two CF mutations identified, an abnormal measurement of sweat Cl^- recorded on 2 separate days can be used as evidence of CFTR dysfunction. These criteria do not exclude, however, accurate clinical judgement, particularly when there is conclusive evidence for CFTR dysfunction (Rosenstein and Cutting 1998). In the present, this evaluation can be complemented by sequencing of the complete coding sequence of the CFTR gene (and sometimes even of the intronic regions close to the splice sites). This may lead to the identification of two alterations—however, as most of the mutations are extremely rare, the simple finding of two alterations in the CFTR gene is not, on its own, a confirmation of the diagnosis.

Diagnosis criteria for CF can be summarized in the Table 1.1:

Table 1.1 Cystic fibrosis diagnosed criteria combining characteristic phenotypic features and evidence of CFTR dysfunction (adapted from Moskowitz et al. 2008)

Diagnosis criteria for cystic fibrosis	
One or more characteristic phenotypic features and evidence of CFTR dysfunction	
Phenotypic features	**CFTR dysfunction**
Chronic sinopulmonary disease	Presence of two *CFTR* disease causing mutations
Obstructive azoospermia	Two abnormal quantitative pilocarpine iontophoresis sweat Cl^- values (>60 mmol/L)
Gastrointestinal/nutritional abnormalities	NPD measurements characteristic of CF
Salt-loss syndrome (with or without metabolic alkalosis)	
Failure to thrive	
Recurrent pancreatitis	
Hepatobiliary disease	
Hypoproteinemia	
Fat-soluble vitamin deficiencies	
A history of CF in siblings	
A positive newborn screening test	

1.2 CFTR Gene and Protein

1.2.1 The Gene

For decades, CF was a puzzling enigma for physiologists and biochemists. Although it was demonstrated that Cl^- efflux in the membrane of epithelial cells was impaired in CF (Quinton 1990), this was not sufficient for the direct identification of the protein product defective in this pathology.

The CF *locus* was discovered in 1986 and this prompted the utilization of RFLPs (restriction fragment length polymorphisms) for diagnosis purposes (Ferrie et al. 1992). Among the polymorphic markers identified, two of them, XV-2c and Km-19, were found to be in strong linkage disequilibrium with the CF gene (Ferrie et al. 1992).

Cloning of the CF gene in 1989 was the result of the joint efforts of three laboratories headed by Lap-Chee Tsui in Toronto, Francis Collins in Ann Arbor and Robert Williamson in London. This concluded 5 years of hard work that began with the finding of the correct DNA markers, identified the *locus*, and ended with the isolation of the gene itself (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989).

The cloning was made using a strategy called positional cloning (Collins 1992). The gene was initially mapped to the long arm of chromosome 7, specifically in 7q31, by linkage analysis in patients and their families using a large number of DNA polymorphic markers. From this point, analysis lead to the localization of the

gene in a segment of 1.5 Mb. Using the two approaches of chromosome walking and chromosome jumping, the gene was finally identified and its transcript detected in the sweat gland, lung, and pancreas (Rommens et al. 1989).

The gene is very long (Fig. 1.1a), spanning a genomic region of approximately 189 Kb (Ellsworth et al. 2000) and was first described to have 24 exons, numbered from 1 to 24. Soon after, three of these exons (6, 14, and 17) were found to contain small introns. For this reason, the total number of exons is 27. For a while, they continued to be numbered from 1 to 24, including exons 6a and 6b, 14a and 14b, 17a and 17b, but recently the numbering 1–27 was adopted. The size of the exons varies between 50 and 250 bp, with the exception of exon 13 with 723 bp. The size of the introns is also variable, having the largest about 28 Kb (Ellsworth et al. 2000). The CF mRNA has 6.2 Kb and encodes the CFTR protein with 1480 amino acid residues (Riordan et al. 1989) (Fig. 1.1b, c).

The minimal *CFTR* promoter lies within about 226 bp upstream of the transcription start site and appears to have a typical structure of a house-keeping promoter (Yoshimura et al. 1991): it is CpG-rich, contains no TATA box, has multiple transcription start sites and has several putative binding sites for the transcription factor Sp1 (Specificity protein 1) (Yoshimura et al. 1991). As it

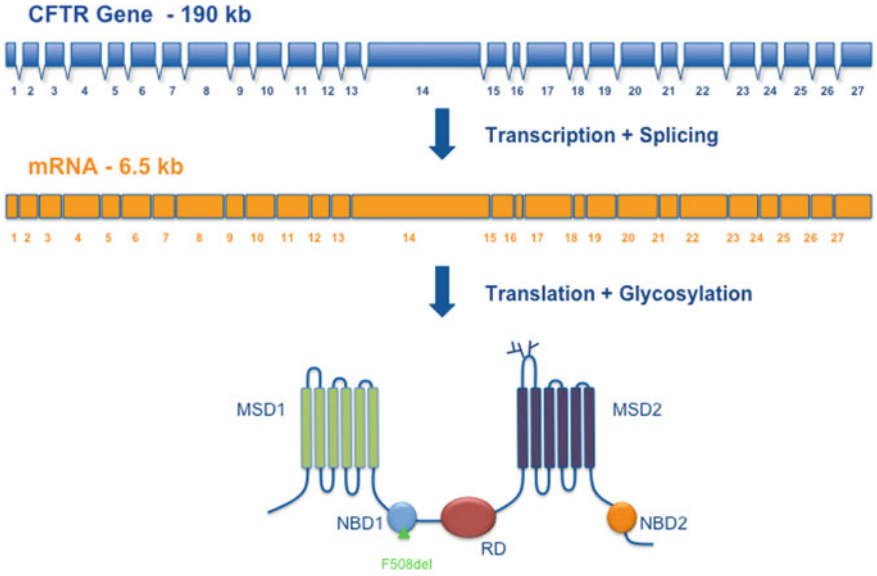

Fig. 1.1 From gene to protein. Gene, transcript and predicted structure for the CFTR gene and protein. The gene contains 27 exons and spans a region of about 190 kb in the long arm of chromosome 7. The mRNA is 6.1 kb long including the UTRs. The protein belongs to the ABC transporter superfamily, including two membrane-spanning domains (MSD1 and MSD2), two nucleotide-binding domains (NBD1 and NBD2) and a regulatory domain (RD). The site of the most common disease-causing mutation F508del (deletion of phenylalanine at position 508) is shown. Adapted from Collins (1992)

happens usually with promoters of this type, the promoter is weak and has no evident tissue-specificity, suggesting that distal regulatory elements may have a role in controlling CFTR expression. In fact, the genomic region encompassing the CFTR locus contains several DNase I hypersensitive sites (DHS), either upstream (at −20.9 Kb) or downstream of the coding region (at +5.4, +6.8, +7.0, +7.4 and +15.6 Kb) and in various introns (Phylactides et al. 2002; Smith et al. 2000).

An element that regulates positively *CFTR* expression in intestinal cells (Rowntree et al. 2001) was identified in the first intron, at 185 + 10 Kb (Smith et al. 1996). This cell-type-specific DHS functions as a classical, tissue-specific enhancer that can recruit general factors involved in transcription initiation (Smith et al. 1996; Mogayzel and Ashlock 2000; Ott et al. 2009b, c). Two enhancer-blocking insulators, also DHS, are located also upstream and downstream of the gene and exhibit distinct properties. The insulator located at −20.9 Kb is associated with a CTCF (CCCTC-binding factor)-dependent element. The second insulator, located within a DHS at +15.6 Kb, exhibits also enhancer-blocking activity although independent of CTCF binding.

Analysis of the DNA sequence of the promoter also revealed potential regulatory elements. Basal and cAMP-mediated regulation of *CFTR* transcription involves a consensus inverted CCAAT (Y-box) element in tandem with a weak cAMP response element (CRE) consensus (Romey et al. 2000).

Recent evidence proposes the existence of a *CFTR* transcriptional hub in which intronic enhancer elements are brought into close proximity to the *CFTR* promoter to activate cell-type-specific transcription (Blackledge et al. 2009; Ott et al. 2009a). The loop that is formed is detected in cells that express *CFTR* but absent from cells in which the gene is inactive. This structure allows a close interaction between the proximal promoter, sequences within the gene but 100 Kb downstream of the promoter and others located 3' to the locus and all correspond to relevant DHS, thus being involved in the recruitment of proteins that modify chromatin structure (Ott et al. 2009a).

Temporal and spatial regulation of gene expression also relies on the use of multiple transcription start sites (Yoshimura et al. 1991; Koh et al. 1993) and the recruitment of alternative upstream exons (Broackes-Carter et al. 2002; Mouchel et al. 2003; Lewandowska et al. 2010). Among these, it was reported that a cis-acting element contributes to the basal activity of the promoter in airway epithelial cells, whose regulation involves a combination of epigenetic modifications (Lewandowska et al. 2010).

The pattern of *CFTR* tissue-specific expression is complex, with CFTR mRNA transcripts being detect at low levels (one–two copies per cells) in specialized epithelial cells of gut, airways, pancreas, sweat gland ducts and the male reproductive tract in non-CF individuals (Trapnell et al. 1991; Crawford et al. 1991; Trezise and Buchwald 1991; Trezise et al. 1992; Engelhardt et al. 1992).

Even in the airways, that are responsible for most of the morbidity and mortality associated with CF, *CFTR* expression varies with the cell type—high levels are detected in serous cells of submucosal glands (Engelhardt et al. 1994) and also, in

terms of protein, at the apical membrane of ciliated cells in the airway superficial epithelium (Kreda et al. 2005).

The pattern of *CFTR* expression in the different epithelia greatly influences organ involvement in clinical CF presentation. In decreasing order of expression levels, CFTR is detected in submucosal glands of the airways, pancreatic ducts, sweat glands, bronchi, intestine, hepatic ducts, *vas deferens*, and uterus (Collins 1992).

1.2.2 The Protein—Domains and Structure

The *CFTR* gene encodes the CFTR protein, a 1480-amino acid long polytopic membrane protein, whose structure is composed of two membrane-spanning domains (MSD1 and MSD2) containing each six membrane-spanning regions or transmembrane segments (TM1 to TM12), two ATP-binding domains (NBD1 and NBD2) and a unique highly polar R domain (RD) with several phosphorylation sites (Fig. 1.2).

Based on its structure, CFTR is a member of the ABC (ATP-Binding Cassette) transporters superfamily, a large group of proteins involved in active transport of various metabolites, peptides, and ions across biological membranes in both prokaryotes and eukaryotes (Riordan et al. 1989). These proteins are also called

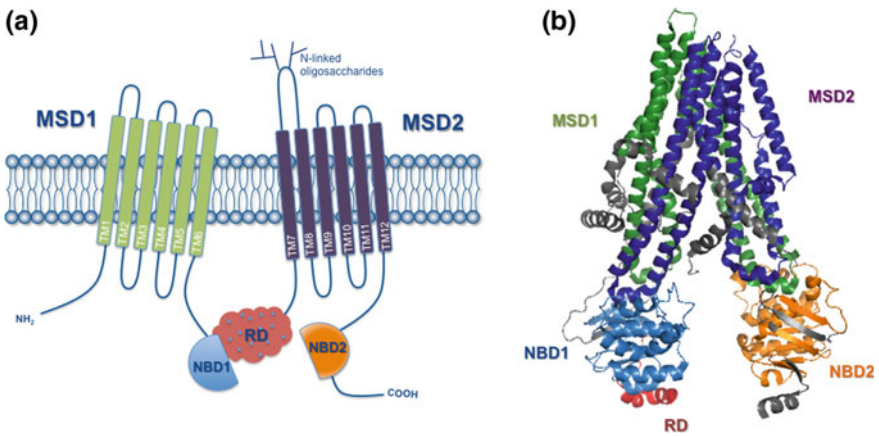

Fig. 1.2 CFTR protein and its domains. **a** The CFTR protein is composed of five domains—MSD1 and MSD2, each one composed of six transmembrane segments (TM1–6 and 7–12) forming the channel pore, two cytosolic NBD1 and NBD2 and the regulatory RD. CFTR possesses two N-linked glycans attached to asparagine residues in the fourth extracellular loop and several phosphorylation sites (mainly at the RD). **b** Assembly of the five domains involve specific intramolecular interactions leading to the compact structure of native CFTR, here depicted according to the PDB coordinates of the published cryo-EM structure of human CFTR (Liu et al. 2017). Figure prepared using the open source program PyMOL (https://www.pymol.org/)

"traffic adenosine triphosphatases" (ATPases) or TM6-NBD (according to its structure with six α-helix transmembranar segments and nucleotide-binding domains) and there are more than 50 members reported, including the yeast α-mating factor ST6; P-glycoprotein/multidrug resistance (P-gp/MDR) that confers cancer cells resistance to chemotherapy drugs; or transporter associated with antigen processing 1 and 2 (TAP1/2), involved in antigen presentation through binding to major histocompatibility class I molecules, just to mention a few. A typical ABC transporter has two domains: one MSD and one NBD (Higgins 1992a). Some, like CFTR and MDR, have this structure in each half, thus a total of two MSDs and two NBDs. CFTR sequence analysis revealed the presence of the additional domain, RD, acting as a regulatory element in protein function (Higgins 1992b).

CFTR functions mainly as a Cl^- channel, regulated by cAMP-dependent protein kinase A (PKA) and ATP at the apical membrane of epithelial cells (Higgins 1992b).

The two MSDs are highly hydrophobic and each of them possesses six transmembranar α-helix segments that correspond to the pore of the channel and determine its ionic specificity (Riordan 1993). The MSDs of ABC transporters share a similar topological organization, albeit having different primary sequences, a fact that explains why they transport such a diversity of substrates. The most conserved TM segments in CFTR are TM6 and TM12 that line the pore of the channel (Sheppard and Welsh 1999). The TM segments are linked by six extracellular (ECL) and four intracellular (ICL) loops. ECL4 possesses two consensus N-glycosylation sites (N894 and N900). The ICL sequences are involved in interdomain interactions and mediate specific contacts between the MSDs and the NBDs, being most important for CFTR processing and function (Sheppard and Welsh 1999; Serohijos et al. 2008; Thibodeau et al. 2010).

The NBDs and the RD are localized in the cytoplasmic side of the membrane and are rich in charged residues. Like the MSDs, the NBDs also exhibit significant sequence conservation throughout the ABC family and share three conserved motifs: Walker A, Walker B and the signature motif (LSGGQ), located just upstream the Walker B sequence (Schneider and Hunke 1998). These motifs are involved in NBD ability to bind ATP, coupling nucleotide hydrolysis to transporter (or channel, as in CFTR) activity. The two NBDs in CFTR assemble in a head-to-tail orientation, with the dimer presenting two ATP-binding sites located at the interface rather than at each NBD individually. These binding sites are formed by the Walker A and B of one NBD and the signature motif of the other NBD (Lewis et al. 2004). Despite the apparent symmetry in CFTR, the similarity between the two NBDs is only 29% (Riordan et al. 1989), thus creating asymmetry in the binding sites—one behaves as a stable ATP-binding site, at which hydrolysis does not occur (site I) and the other is the hydrolytic site (site II) (Riordan 2005).

The structure of the NBDs is quite similar to that of other ABCs transporters including a central α/β F1-type core containing sequences for Walker A and B, an antiparallel β-sheet (ABCβ) and an α-helical (ABCα) subdomain where the signature sequence lies (Lewis et al. 2004). CFTR NBD1 has some differences to this basic structure that include: (i) the insertion of two short α-helices between the two

β-strands (S1 and S2); (ii) an added long α-helix in the C-terminal; (iii) the deletion of a segment between β-strands S4 and S6 that typically consists of a β-strand and an α-helix; and (iv) a truncation between helices H3 and H4 (Lewis et al. 2004). The two segments, that were termed regulatory insertion (RI) and regulatory extension (RE), are quite flexible and contain serine residues that can be phosphorylated in vitro. The RE and RI are conformationally dynamic segments upon phosphorylation of certain sites by PKA, namely: 660Ser, 670Ser, and 422Ser (Pasyk et al. 2015; Dahan et al. 2001), and may be involved in the formation of the NBD1/NBD2 ATP-sandwich for channel gating (Lewis et al. 2004, 2005; Kanelis et al. 2010). Both RI and RE are mobile elements in solution that bind transiently to the ß-sheet and α/ß subdomains of NBD1 (Kanelis et al. 2010).

The role of the NBDs in CFTR activation has been determined through strategies that include the ability to function and to bind ATP in the presence of different point mutations in the sequence (Higgins 1992b). ATP is important for the opening of the channel, so even when the channel is phosphorylated at the R domain, ATP is needed to keep it in the open conformation. Regulation by ATP binding is described to be due to a conformational change after nucleotide binding (Higgins 1992b). The differences between the two NBDs suggest that, although they cooperate to regulate CFTR opening and closure, they are not functionally equivalent (Sheppard and Welsh 1999), with early suggestions that binding of ATP to NBD1 opens the CFTR channel whereas binding to NBD2 is responsible for closing it (Sheppard and Welsh 1999) (see Sect. 1.3.1 and Fig. 1.8).

Finally, the RD, as a distinctive feature between CFTR and others members of the ABC transporters superfamily, is the product of exon 13 translation and was described to account for a further regulation in CFTR function. The RD contains several phosphorylation sites, substrates of different kinases, mainly protein kinases A and C (PKA and PKC) (Riordan 1993). Phosphorylation and dephosphorylation of the RD seem to act as molecular switch by which the channel opens and closes (Tabcharani et al. 1991), although this regulation is very complex. Independent of its phosphorylation status, the RD is mostly unstructured allowing great flexibility to regulate intra- and intermolecular interactions. The RD contains multiple overlapping binding elements. The non-phosphorylated RD interacts mainly with the NBDs blocking heterodimerization and thus channel activity. Phosphorylation excludes RD from the NBD dimer formation, facilitating gating and promoting interaction with other CFTR interacting proteins (Bozoky et al. 2013).

High-resolution structures of human NBD1 have been determined, but a structure for whole CFTR was a long-awaited advance—probably due to the lack of abundant sources for its purification and also to its limited solubility (Riordan 1993; Rosenberg et al. 2004). The 3D structures for several ABC transporters that have been put forward—including those of *Staphylococcus aureus* Sav1866 (Dawson and Locher 2006), the inward-facing conformation of *Vibrio cholerae* MsbA (Ward et al. 2007), other bacterial transporters such as BtuCD and ModBC (Dawson and Locher 2006; Locher et al. 2002; Hollenstein et al. 2007) and, more recently, the structures of eukaryotic transporters such P-glycoprotein from mouse (Aller et al.

2009) and from *Caenorhabditis elegans* (Jin et al. 2012), a P-glycoprotein homolog (ABCB1) from *Cyanidyoschyzon merolae* (Kodan et al. 2014), human ABCB10 (Shintre et al. 2013), Atm1 from yeast (Srinivasan et al. 2014) or the *Thermotoga maritima* "TM287/ 288" (Hohl et al. 2012)—provided important new insights into the mechanisms by which these proteins accomplish transmembrane translocation of their substrates. Comparison of the 3D structures of ABC exporters, with those of importers, shows that in the first group the TM helices protrude largely into the cytoplasm and have longer intracellular loops (ICLs) (Mornon et al. 2015). These short coupling helices in the ICLs run parallel to membrane and provide transmission interfaces with the NBDs. Although CFTR functions as a channel rather than an exporter, sequence and biochemical similarities between some of these transporters and CFTR suggest that they are likely to be structurally related (Serohijos et al. 2008). Using these comparisons, homology models have been built for the CFTR MSD-NBD assembly. The first models that have been put forward (Serohijos et al. 2008; Mornon et al. 2008) were based in the 3D structure of Staphylococcus aureus Sav1866 (Dawson and Locher 2006), in an outward-facing conformation. In these models, ICL2 and ICL4 mediate relevant interactions with NBD2 and 1, respectively. In particular, the region in NBD1 where F508 residue is located (deleted in the most common disease-causing mutation) makes critical contacts with several aromatic amino acids in ICL4 (F1068, Y1073, and F1074) as well as with R1070 in the same loop, highlighting the role of this region in interdomain assembly of CFTR and also in the regulation of its activity through domain–domain interaction. More recent modeling studies explored the transition from the inward- to outward-facing conformation (Furukawa-Hagiya et al. 2013), based on the structure of mouse P-glycoprotein. This transition apparently involves a rapid NBD dimerization, resulting in a MsbA closed-like conformation. The most recent modeling results, based in elements from the ABC transporters whose 3D structure is available and also contain asymmetric NBDs, with one degenerate and one consensus nucleotide-binding sites, provide the structure of a full-open conformer of the CFTR protein, in which opening of large lateral tunnels will allow the passage of ions and small molecules from the cytosol to the channel (Mornon et al. 2015). The same study identifies that the onset of this full-open conformation is linked to a switch of the NBD1 region bearing F508 toward an alternative position. This full-open channel then evolves toward a closed form, after local movement of the upper part of the transmembrane helices, with the rest of the structure being essentially unchanged.

The missing high-resolution structures came finally to light very recently (Zhang and Chen 2016; Liu et al. 2017). A first publication describes the cryo-EM structure of zebrafish CFTR without ATP and fully dephosphorylated. Zebrafish CFTR shares about 55% sequence homology with the human protein, but previous studies suggested significant similarity in terms of function and mechanism of action (Aleksandrov et al. 2012). Novel features include a previously uncharacterized "lasso"-shaped domain at the N-terminus (predicted hub for interaction with protein partners) and the suggestion that the RD is at least partially ordered (Zhang and Chen 2016). The structure includes a large anion conduction pathway lined by

numerous positively charged residues. The channel is closed in the extracellular surface by a single gate.

A second publication finally described an equivalent cryo-EM structure for human CFTR, once again in the dephosphorylated, ATP-free form (Liu et al. 2017). This structure is quite similar to the one of the zebrafish ortholog, being one of the most striking differences the description of a helix belonging to the RD docked into the intracellular vestibule and precluding channel opening. The similarity between the zebrafish and the human structure suggests that the conformational flexibility, usually observed for the inward-facing conformation in many ABC transporters, is diminished in CFTR, probably due to the presence of the RD, that inserts into the cytosolic loops of the TM segments packing along NBD1. The location of a large number of positively charged amino acids throughout the MSDs suggest that selection of anions over cations in CFTR conducting pore does not come from individual residues but from the overall positive charge. Comparison with the structure of other ABC transporters, namely that of multidrug resistance protein MRP1 (Johnson and Chen 2017), shows that in CFTR TM8 breaks into three short helices, displacing TM7 from its normal position, with this helix-loop transition likely being a structural basis for CFTR being a channel (and not a transporter) (Liu et al. 2017).

These recent published structures also highlight also why many disease-causing mutations may lead to defects either in folding, ion conduction, or gating, providing a novel molecular understanding and is for sure a further step towards innovative therapeutic intervention.

There has been some controversy about CFTR quaternary structure. Some studies on cellular and purified CFTR defend that the CFTR pore assembles as a monomer (Bear et al. 1992). Others also support the notion that monomer CFTR is the minimal functional unit, based on low-resolution studies and biochemical and electrophysiological readings at the single channel level (Rosenberg et al. 2004; Ramjeesingh et al. 2001). However, several other studies have been published supporting the idea that CFTR assembles in vivo as a homodimer (Zerhusen et al. 1999; Schillers et al. 2004). According to these studies, dimerization would occur possibly through the C-terminal PDZ motif and would be regulated by PKA phosphorylation (Schillers et al. 2004; Ramjeesingh et al. 2003). Most of these data come from distinct techniques and may thus lead to different interpretations—detergent solubilization, or crystallization procedures may affect the result. Using single molecule fluorescence imaging in intact membranes of live cells, Haggie and Verkman provided direct evidence that CFTR occurs exclusively as a monomer (Haggie and Verkman 2008).

1.2.3 CFTR Dysfunction and Disease-Causing Mutations

More than 2000 mutations have already been reported to the CFTR Mutation Database (The CFTR mutation database 2017). According to the effect that a

specific DNA alteration causes upon gene expression, mutations can be classified as missense (causing a change of an amino acid residue to a different residue), nonsense/stop (causing the introduction of a premature stop codon in mRNA sequence), frameshift (commonly deletions or insertions that cause a change in the open-reading frame) plus small deletions, insertions or duplications. There are also mutations that affect mRNA processing—the so-called splicing mutations, i.e., mutations that cause alterations to the splicing process—although these mutations will also produce one of the above described effects upon the protein product. In order to know the exact effect that a certain DNA mutation has upon the respective protein product, expression studies should be carried out in patient-derived materials and/or in heterologous systems (Rubenstein and Zeitlin 1998). The *CFTR* gene alterations that have been described consist of missense (39.6%), frameshift (15.6%), splicing (11.4%), and nonsense (8.3%) mutations; large (2.6%) and in-frame (2.0%) deletions or insertions; promoter mutations (0.7%); and presumed non-pathological variants (15.0%) (Amaral 2015).

Although all disease-causing mutations described in the *CFTR* gene result in decreased Cl^- secretion in the epithelia where the gene is expressed, different cellular phenotypes occur. The number of mutations for which direct relation between mutation effect and CF disease has been shown is increasing (Sosnay et al. 2013), which is quite relevant not only to understand disease mechanisms but also to propose novel protein-directed therapies aimed at correcting the basic defect.

Several classifications of *CFTR* mutations were proposed since the cloning of the gene, mostly based on the functional consequences at the protein level (Welsh and Smith 1993; Welsh et al. 2001; Wilschanski et al. 1995; Rowe et al. 2005; De Boeck and Amaral 2016). The original classification grouped mutations into four classes, that were complemented during the years, leading to recent classifications describing seven classes.

CFTR gene mutations are therefore grouped into the following seven classes:

Class I—Absence of protein production; in this class there are mainly nonsense mutations associated with severe clinical phenotypes, such as G542X, R553X, Q637X, or W1282X, frameshift mutations (leading to premature stop codons) as 394delTT or 3905insT, and splicing mutations as 621+1G > T.
Class II—Deficient intracellular trafficking resulting in the absence or reduction in the amount of protein present in the plasma membrane; this class includes mutations characterized by deletion of an amino acid residue, such as F508del (the most common disease-causing mutation) or I507del, and missense mutations such as S549T, A561E, N1303K, R1066C, generally associated with several clinical phenotype and pancreatic insufficiency (PI).
Class III—Protein present at the membrane but with defective response to cAMP stimulation (defective regulation); mutations in this class are mainly missense mutations, such as G551D, G551S, G1244E, S1255P, or G1349D, also generally associated with a severe clinical phenotype.

Class IV—Protein present at the membrane and responsive to cAMP but with reduced conductance; this class encompasses mutations causing a mild clinical phenotype, generally associated with pancreatic sufficiency (PS), such as R334W, R347P, or A455E.
Class V—Reduction in protein production; this class includes mutations that lead to the concomitant presence of abnormal and normal CFTR (due to alternatively splicing, for example). In these cases, there is therefore production of normal CFTR but in reduced levels, such as in the splicing mutations 3849-10kbCT or 3272-26A>G, and so these are in general associated with a mild phenotype.
Class VI—Reduced stability of protein at the plasma membrane; this class includes mutations in which CFTR folds, traffics, and functions (almost) properly, but has a decreased half-life at the membrane. This accelerated turnover can be due to either an increase in endocytosis or a decrease in recycling. 120del23, a mutation in which CFTR lacks its N-terminal (Ramalho et al. 2009) and F508del when rescued to the membrane present this defect.
Class VII—This class has been introduced to account for mutations in which there is not even production of CFTR mRNA—such as in large deletions. Although these mutations can be included in class I, the definition of a novel class accounts for the fact that they are un-rescuable through any pharmacological approach (De Boeck and Amaral 2016). Mutations such as dele2,3(21 kb) and 1717-1G>A have been included in this class.

Figure 1.3 summarizes the basic defect characteristic of each mutation class and Table 1.2 gives examples of mutations that have been included in each group.

Classifying a mutation however is often not a straightforward task (Welsh et al. 2001), as sometimes studies in heterologous expression systems are contradictory to

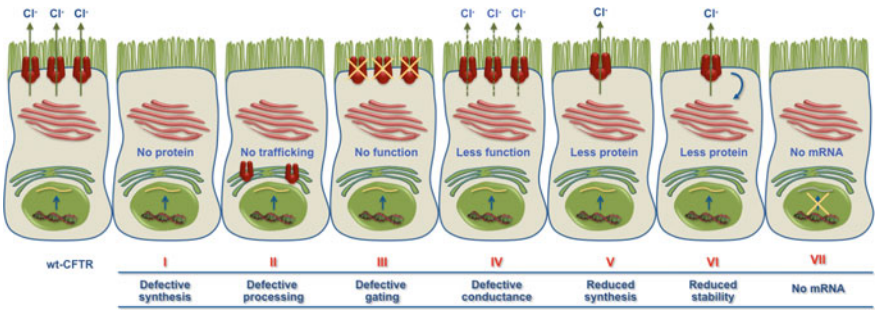

Fig. 1.3 Classification of CFTR mutations into seven different classes, accordingly to the functional defect at the biochemical and cellular levels: class I—absence of synthesis of protein; class II—defective intracellular trafficking; class III—defective activation/regulation; class IV—defective conduction; class V—reduced synthesis of protein; class VI—decreased membrane stability; class VII—absence of CFTR mRNA (adapted from Welsh and Smith 1993; Rowe et al. 2005; De Boeck and Amaral 2016)

Table 1.2 Examples of mutations identified in the seven different mutation classes (Welsh et al. 2001; De Boeck and Amaral 2016)

I	II	III	IV	V	VI	VII
No protein synthesis	Defective processing	Defective regulation	Defective conduction	Reduced synthesis	Decreased stability	No mRNA
Nonsense G542X R553X Q637X Y1092X W1282X 394delTT 3905insT	S549T A559T N1303 K R1066C A561E F508del I507del	G551D G551S G1244E S1255P G1349D	R334 W R347P A455E	IVS8(5T) 2789+5G > A 3272-26A > G 3849 + 10 kb C > T	120del23 rF508del	dele2,3 (21 kb) 1717-1G > A

the observations in native tissues. One of the most striking examples is the most common mutation F508del. Immunocytochemical localization of wt- and F508del-CFTR in epithelial cells of sweat glands showed that this mutant protein does not reach the apical membrane, remaining intracellularly localized (Dalemans et al. 1992). This abnormal localization was confirmed in primary airway epithelial cells from homozygous F508del patients (Kartner et al. 1992), leading to its inclusion in class II. However, soon after F508del-CFTR was detected at the apical membrane in native tissues (Kalin et al. 1999). It was later shown that in fact F508del-CFTR is at the apical region of only a small proportion of cells (Penque et al. 2000) and thus still consistent with a trafficking defect. A certain mutation can cause dysfunction at distinct levels and thus be included in more than one class. Thus, although mislocalization can be identified as the major dysfunction associated with F508del, additional defects were described namely: reduced function (Drumm et al. 1991), increased membrane instability (Lukacs et al. 1993), deficient recruitment from subapical membranes (Bradbury et al. 1992) and also, more recently, reduced levels of transcripts (Ramalho et al. 2002)—for which reasons, F508del-CFTR is frequently associated with classes III and VI (Farinha and Matos 2016). Increasing knowledge of the effects of individual mutations was greatly improved by the CFTR2 project that originally characterized the disease liability of ~160 variants (Sosnay et al. 2013) and then proceeded with a gradually increasing number of mutations (available at http://www.cftr2.org).

The difficulty in classifying each individual mutation (due to the specific defects that can be found, and whose combination is probably unique for each one) lead to an alternative classification approach in which variants are grouped into *theratypes* — defects can be divided in those that affect CFTR quantity (covering transcription, splicing, folding, trafficking, and stability) and those that affect CFTR function (covering conductance and activity) (Cutting 2015). More complex systems have also been proposed according to which every mutation will be classified with a combination of numbers reflecting all the different categories that its basic defect would fit into (Veit et al. 2016).

1.3 CFTR in the Cell

1.3.1 Biosynthesis, Processing, and Degradation of CFTR

The final step in gene expression is the biosynthesis of a functional protein. Being a membrane protein, CFTR follows the secretory pathway. This functions as a general route for the export of proteins from the ER to the Golgi and from there, within secretory vesicles, to their final destination in the cell—lysosomes, the extracellular medium or (as it occurs for CFTR) the plasma membrane. CFTR biogenesis events are summarized in Fig. 1.4.

CFTR biogenesis initiates by its co-translational insertion into the ER membrane (Lu et al. 1998). Proteins that are co-translationally inserted into the ER membrane possess a signal sequence of around 20 amino acid residues responsible for this insertion. This sequence is recognized by the signal recognition particle (SRP) and promotes the ribosome coupling to the ER membrane. SRP binds to a specific

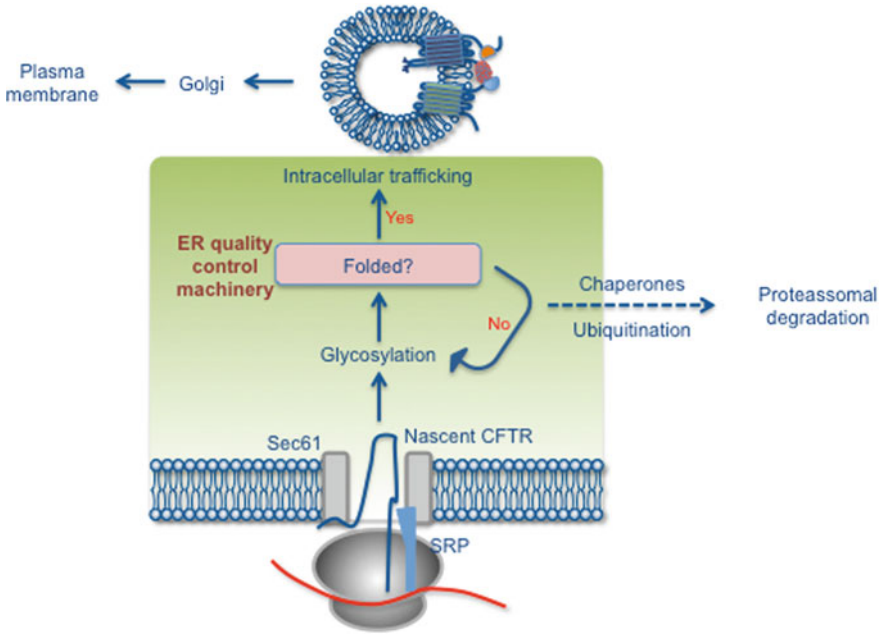

Fig. 1.4 CFTR biogenesis and degradation. Synthesis of CFTR begins on cytosolic ribosomes that are targeted to the translocon in the ER membrane. CFTR topology is established in the ER membrane and folding of domains is facilitated by molecular chaperones. For unclear reasons, only a certain percentage of wt-CFTR is trafficked out of the ER. This varies, however, with the cell type. A certain amount of the wt-protein fails to be exported from the ER to the Golgi, undergoes retrotranslocation and ubiquitination and is degraded at the proteasome. For many mutants, specially the most common F508del, degradation is almost total (adapted from Skach 2000)

receptor in the ER membrane that anchors the ribosome to a channel called translocon, composed by several subunits, being the most important the Sec61 complex. The signal sequence enters the channel and allows co-translational insertion of the nascent polypeptide into the ER membrane.

Multidomain proteins are inserted through this mechanism, although for many of them the actual transmembrane segments function as signal sequence to promote membrane insertion. These sequences are then aided by the presence of stop transfer sequences, that terminate ongoing translocation, disrupt the ribosome membrane junction, close the translocon and direct hydrophobic transmembrane helices out of the translocon and into the lipid bilayer. Insertion of polytopic proteins is thus composed of a large number of molecular events that must be precisely coordinated (Lu et al. 1998).

For CFTR insertion (Fig. 1.5), the topology of the first two segments TM1 and TM2 is established by two possible pathways—initiated by either one of the segments. Most of the times, a posttranslational mechanism in which TM2 insertion drags TM1 into the translocon is followed. This occurs because TM1 lacks a strong translocation signal thus requesting additional information to promote insertion. It was described that TM2 acts as a signal sequence with translocation specificity

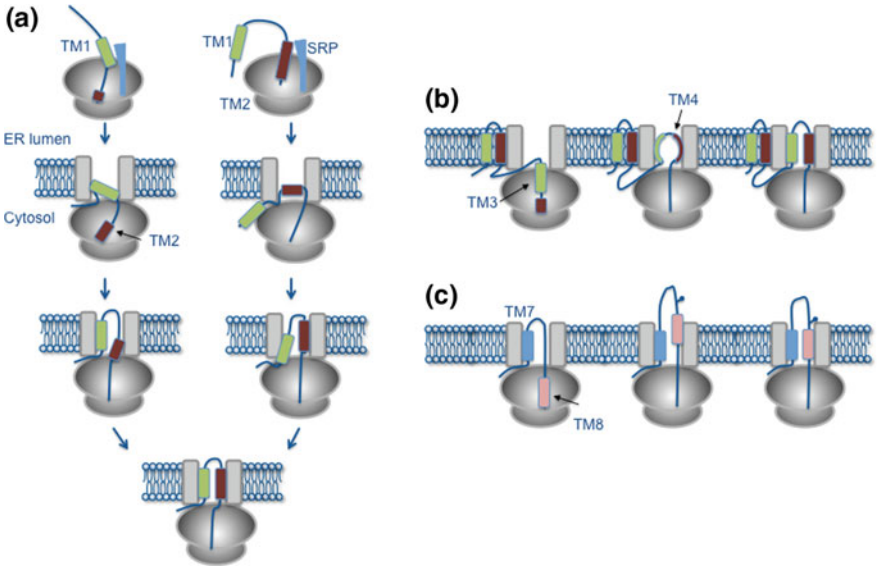

Fig. 1.5 CFTR insertion into the ER membrane. **a** In a conventional co-translational pathway, CFTR topology is established through the action of TM1 signal sequence and TM2 stop transfer sequence. The posttranslational alternative pathway is utilized by more than 60% of wt chains. In this model, TM2 acts as the initial signal sequence to start translocation. In both pathways, the final topology is equivalent: TM1 and TM2 span the membrane and intervening peptide loop is located in the ER lumen (Lu et al. 1998). **b** TM3 and TM4 are inserted together. **c** TM7 and TM8 follow a complex integration pattern with transient exposure of TM8 in the ER lumen (adapted from Kim and Skach 2012)

complementary to TM1 and therefore, is capable of posttranslationally directing the insertion not only of itself but also of TM1 and ECL1 (Lu et al. 1998). The low efficiency of TM1 as a signal sequence is due to the presence of two charged amino acid residues in this hydrophobic segment, namely E92 and K95 (Lu et al. 1998).

Mutations in the transmembrane segments have pleiotropic effects—G85E and G91R (in TM1) change the folding efficiency without disturbing the protein global topology, whereas G216D (in TM2) and D924V (in TM8) seem to alter the frontiers of their respective TMs (Skach 2000).

TM3 and TM4 insert together as a helical hairpin, probably driven by the existence of a very small loop linking these two segments, the same occurring for the pairs TM5–6, TM9–10, and TM11–12. The pair TM7–8 also follows a complex integration pattern, in which the stop transfer activity of TM8 is weak due to the presence of the charged residue D924—this results in partial exposure of TM8 in the lumen of the ER before proper membrane insertion (Carveth et al. 2002; Enquist et al. 2009; Kim and Skach 2012).

After insertion into the ER membrane, the newly synthesized CFTR, either wild-type (wt) or mutant (e.g. F508del), is glycosylated at the asparagine residues 894 and 900, located in the fourth extracellular loop (ECL4). N-glycosylation occurs by the addition of a glyconjugate with 14 osidic residues. This oligosaccharide contains two N-acetylglucosamine (GlcNAc), three glucose (Gluc) and nine mannose (Man) residues and is added to these asparagine residues located as they occur within the consensus sequences Asn-X-Ser/Thr. This structure is synthesized within the ER and transferred from the membrane lipidic precursor dolichol phosphate to the protein in a reaction catalyzed by a specific oligosaccharyltransferase.

Glycosylation events at the ER originate a core-glycosylated (immature) form of the protein, the so-called band B. From the ER, wt-CFTR traffics to the Golgi complex, where it undergoes fully glycosylation, originating the mature form, also termed band C (Cheng et al. 1990).

The first evidence as to how the F508del mutation compromises CFTR function was provided by Cheng et al. from studies on the biosynthesis of F508del-CFTR in transfected COS cells, suggesting that this mutant is substantially retained within ER (Cheng et al. 1990). Indeed, although complete core-glycosylated CFTR chains are formed in the ER, they are unable to mature conformationally and hence are precluded from exiting the ER into the Golgi, going instead to the proteasome for degradation (Lukacs et al. 1994). The F508del mutation, however, does not result in a total loss of Cl^- transport, since upon heterologous expression in *Xenopus* (Drumm et al. 1991) or insect cells (Sarkadi et al. 1992), or at reduced temperatures (Denning et al. 1992), as well as in lipid bilayer reconstitution experiments (Li et al. 1993), or even in the presence of chemical chaperones, such as glycerol (Sato et al. 1996), deoxyspergualin (Jiang et al. 1998) or 4-phenylbutyrate (Rubenstein et al. 1997), F508del-CFTR has been shown to display at least some Cl^- channel activity. The high prevalence of the F508del mutation explains the primary importance of studying its molecular and cellular defect.

Core-glycosylated forms of both wt- and F508del-CFTR turn over rapidly with a half-life of about 30 min and conversion of the wt-protein to the mature form does

not occur with 100% efficiency (Lukacs et al. 1994). A variable proportion of the wt precursor and most of the F508del variant are rapidly degraded before exiting from the ER. CFTR folding and processing is much less efficient than other ABC transporters, like multidrug resistance (MDR or P-gp) protein or multidrug-related protein (MRP) (Chang et al. 1997) at least in heterologous expression systems. This process is however cell-type dependent, as it was shown that endogenously expressed CFTR processing is very efficient in human epithelial cells.

An ER quality control system (ERQC) that rapidly targets for degradation abnormal membrane and secretory proteins is stringently applied to CFTR. Of the repertoire of proteins available to assist and "police" protein biosynthesis in the ER, molecular chaperones have been the most implicated in the retention of misfolded proteins or unassembled subunits (Ohtsuka and Hata 2000). Molecular chaperones bind to and stabilize an otherwise unstable conformer of another protein and, by controlled binding and release of their substrate, facilitate its correct folding in vivo (Ohtsuka and Hata 2000).

Molecular chaperones typically recognize and bind to hydrophobic amino acid residues exposed at the outer surface of unfolded polypeptides and release their substrates in a controlled manner, thereby preventing unproductive aggregation and promoting proper folding. The molecular chaperone system is thought to be a defence mechanism against proteotoxic stresses such as heat and chemicals that challenge the cellular environment. Although substrates of molecular chaperone are usually normal or wt-proteins, the machinery can also deal with mutant proteins, correcting their folding defects or targeting them for degradation (Ohtsuka and Hata 2000). Chaperones are essential players in promoting CFTR domain assembly and in controlling how its glycosylation also affects its trafficking.

CF belongs to a wide variety of inherited diseases known to result from the inability of the mutant or abnormal protein to achieve its functional conformation, usually called protein folding or conformational disorders (Taubes 1996).

In vitro studies by Thomas et al. using just isolated NBD1 of CFTR suggest that the efficiency of F508del-NBD1 folding is severely impaired compared with that of the wt-NBD1 (Strickland et al. 1997). The same authors showed that a synthetic peptide corresponding to the sequence around residue 508 of CFTR has less β-structure when the Phe residue is absent in that position (Strickland et al. 1997). It was also shown that the mutant polypeptide is unable to undergo an ATP-dependent conformational change in the ER (Qu et al. 1997). Altered protease sensitivity provided additional in vivo evidence that in the ER, two distinct immature and mature forms of CFTR exist and have different conformations and that F508del-CFTR is unable to achieve the latter (Zhang et al. 1998).

Molecular chaperones acting on both sides of the ER membrane may have a role in modulating the conversion between these two CFTR conformations. Specifically, cytoplasmic Hsc70/Hsp70 (Yang et al. 1993), Hsp90 (Loo et al. 1998), the luminal ER chaperone calnexin (Pind et al. 1994), the co-chaperone Hdj-2 (cytoplasmic Hsp40) (Meacham et al. 1999) and the cytosolic Hsc70-interacting CHIP (Meacham et al. 2001) have been shown to interact with CFTR.

Hsc70/Hsp70 (human heat shock protein of 72/73 kDa) are members of one of the most important chaperone classes—the Hsp70 family. Their intrinsic properties make these proteins ideally suited to aid unfolded proteins en route to their native conformations by limiting protein aggregation (Freeman and Morimoto 1996).

Yang et al. have shown that both normal (wt) and mutated forms of CFTR complex with Hsc70/Hsp70. The wt protein dissociates from Hsc70/Hsp70 before its transport to the Golgi and the membrane. The protein would be subsequently degraded at the lysosome pathway. By contrast, the complex formed between F508del-CFTR and the Hsc70/Hsp70 chaperone is retained in the ER and mutant CFTR is rapidly degraded by the ubiquitin-proteasome pathway. It thus seems that Hsc70/Hsp70 discriminates between the normal and the most common mutant variant of CFTR (Yang et al. 1993). The ER Hsp70 family member—BiP, also known as Grp78, glucose-related protein 78—does not play a major role in the folding of CFTR probably because the largest ECL of CFTR has only 31 amino acid residues (Yang et al. 1993). These results were later confirmed by in vitro studies that identified increased binding affinity of Hsp70 to purified F508del-NBD1 (Scott-Ward and Amaral 2009).

Meacham et al. investigated whether Hdj-2 (human DnaJ 2) directs Hsc70/Hsp70 to facilitate the assembly of cytosolic regions on CFTR (Meacham et al. 1999). Hdj-2 belongs to the Hsp40 (DnaJ-related) family, the major class of Hsp70 co-chaperones, of which the *Escherichia coli* DnaJ was first characterized. They are known to stimulate the Hsp70 ATP hydrolytic cycle (Cyr et al. 1994). Hdj-2 was found in complexes with either wt- or F508del-CFTR. Levels of complex formation between F508del-CFTR and the chaperone pair Hsc70/Hdj-2 were found to be approximately two fold higher than those with wt-CFTR. The earliest stage at which this chaperone pair binds CFTR is just after the synthesis of NBD1 and complex formation is greatly reduced after the RD is synthesized. In vitro experiments confirmed that Hdj-2 and Hsc70 act synergistically to suppress NBD1 aggregation. Hdj-2 thus seems to act together with Hsc70 to facilitate early steps in CFTR assembly, probably helping in the formation of an intramolecular NBD1-RD complex (Meacham et al. 1999).

Other studies highlighted the major role of the Hsp70 chaperone systems in wt- and F508del-CFTR folding. Overexpression of Hsp70 with its co-chaperone Hdj-1/Hsp40 was shown to stabilize the immature form of wt-CFTR, but not of F508del-CFTR, suggesting that these chaperones act on a wt-specific conformation. As the efficiency of conversion into the fully glycosylated form was not altered, the lack of these two chaperones does not seem to be critical for the maturation of wt-CFTR (Farinha et al. 2002).

Hsp90 was also described to interact with CFTR (Loo et al. 1998). Members of Hsp90 family play a major role both in the prevention of protein aggregation and the facilitation of protein folding or degradation. In particular, Hsp90 was shown to stabilize steroid receptors at the plasma membrane and to have an active role in the transport of receptor-ligand complexes to the nucleus (Kimmins and MacRae 2000). Loo et al. have shown that cytoplasmic Hsp90 binds to both wt- and F508del nascent CFTR, while Grp94, the ER luminal member of this chaperone family, does

not (Loo et al. 1998). The Hsp90 association with CFTR can be disrupted by drugs such as geldanamycin or herbimycin A, and this inhibition prevents CFTR maturation and strongly accelerates its proteasomal degradation (Loo et al. 1998). These findings are consistent with previously documented antagonistic actions of Hsp90 and the proteasome in the turnover of other substrates and provided the first example of the involvement of this chaperone in the biosynthetic processing of a nascent integral membrane protein at the ER (Loo et al. 1998). Involvement of the Hsp90 is also relevant in the folding of F508del-CFTR, as knockdown of Aha1—a co-chaperone of Hsp90—promotes partial rescue of the mutant protein (Wang et al. 2006).

Altogether, the Hsp70/Hsp40/Hsp90 chaperone systems form a chaperone trap (Coppinger et al. 2012) that constitutes a first checkpoint in the pathway (Fig. 1.6) that scrutinizes between folded and misfolded CFTR, targeting the latter (usually mutant protein, as it happens for F508del-CFTR) to proteasomal degradation (Farinha and Amaral 2005; Farinha and Canato 2017). Prolonged retention of immature CFTR at this first checkpoint allows another Hsc70-interacting factor, CHIP (carboxy-terminus of Hsc70 interacting protein) to facilitate its degradation. CHIP is a cytosolic U-box protein that interacts with Hsc70 through a set of tetratricorepeat motifs (Meacham et al. 2001; Jiang et al. 2001). CHIP functions with Hsc70 to sense the folded state of CFTR and targets aberrant forms for proteasomal degradation by promoting their ubiquitination. CHIP is a co-chaperone that converts Hsc70 from a protein-folding machine into a degradation factor that functions in the ER quality control, being thus responsible at least in part for targeting CFTR for degradation (Meacham et al. 2001). More recently, CHIP was described as having the activity of a U-box containing E3 ubiquitin ligase activity (Jiang et al. 2001) and to work together with E2 enzyme UbcH5 (Younger et al. 2004).

Other co-chaperones of Hsp70 have been shown to play also a role in CFTR targeting to degradation. Bag-1 (Bcl-2 associated atharogene) has a dual role on F508del-CFTR stabilization: it contributes to the release of the mutant protein from the Hsp70 machinery (Meacham et al. 2001) but also competes with ubiquitin for binding to F508del-CFTR, thus preventing its proteasomal proteolysis (Mendes et al. 2012). Additionally, overexpression of cysteine string proteins (Csp)—that are also J-domain proteins like Hsp40—blocks CFTR maturation, suggesting a role in regulating CFTR release from the Hsp70 machinery (Zhang et al. 2002).

When the Hsp70 checkpoint is successfully overcome (as occurs to most wt-CFTR), CFTR folding continues to be scrutinized by a mechanism dependent on its N-linked glycans—the calnexin(-calreticulin) cycle. Calnexin, a Ca^{2+}-binding chaperone that is an intrinsic 88KDa-protein of the ER membrane, has been shown to bind transiently to a large number of newly synthesized proteins, including both transmembrane and secretory proteins (Bergeron et al. 1994). In addition, calnexin displays a prolonged binding to certain misfolded proteins and to incompletely assembled forms of oligomeric proteins and was demonstrated to be involved in the intracellular retention of incompletely folded or assembled protein complexes (Tatu and Helenius 1997).

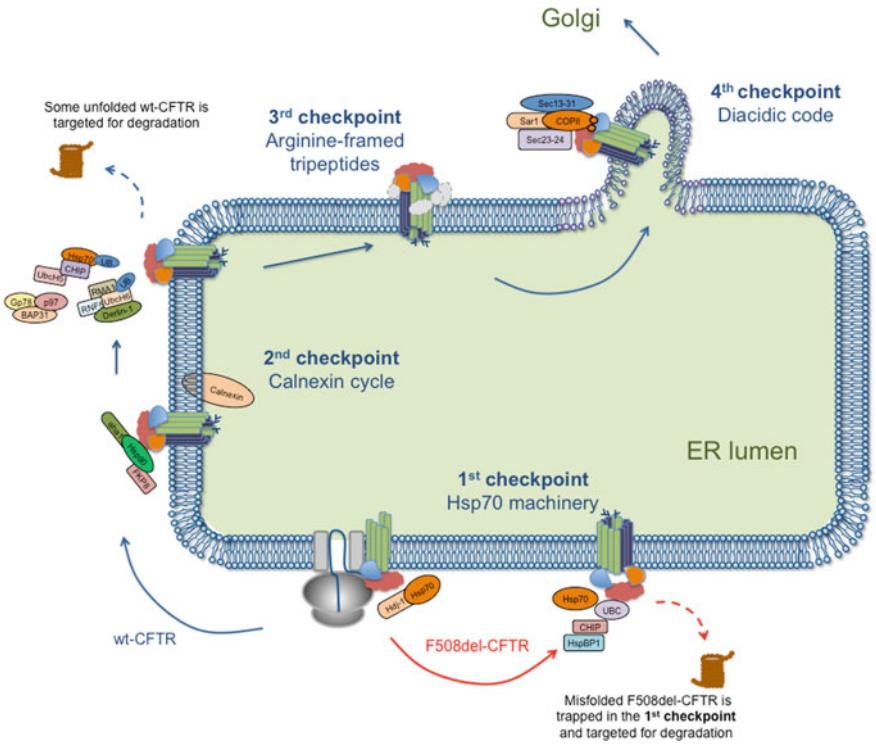

Fig. 1.6 The ER quality control for CFTR. When CFTR emerges from the ribosome it interacts with chaperones/co-chaperones that promote its folding (first checkpoint). The glycosylation process is co-translational and allows the protein to undergo repeated interactions with the lectin calnexin (second checkpoint). CFTR exit from the ER is regulated through the exposure of retention/retrieval motifs that form a third checkpoint. As correct folding is achieved, a diacidic code mediates inclusion into COPII-vesicles (4th checkpoint). Misfolded F508del-CFTR is trapped by the chaperone machinery (mainly the Hsp70 system) and prematurely targeted to proteasomal degradation (adapted from Farinha and Amaral 2005; Farinha et al. 2013b; Farinha and Canato 2017)

The processing of the 14-unit oligosaccharyl glycan in the ER plays a major role in the protein quality control and exemplifies one mechanism by which misfolded proteins may be retained in the ER (Hammond and Helenius 1995). Glycoproteins with the attached $Glc_3Man_9GlcNAc_2$ moiety undergo rapid trimming in which the two external glucose residues are sequentially removed through the actions of glucosidase I and II, respectively. The monoglucosylated 12-unit glycan is recognized by calnexin (or for ER lumenal proteins, its soluble homolog calreticulin), which binds the glycoprotein and facilitates its folding. Removal of the terminal glucose, again by glucosidase II, triggers dissociation of the calnexin-glycoprotein complex. If the glycoprotein is correctly folded it exits the ER. However, misfolded proteins are recognized by UDP-glucose glucosyltransferase (UGGT) and re-glucosylated, leading to re-association with calnexin (or calreticulin) and

allowing another chance at folding (Cabral et al. 2001). UGGT is a soluble ER lumenal enzyme that recognizes only misfolded proteins and serves as a folding sensor in the calnexin cycle (Hebert et al. 1995).

Pind et al. showed that newly synthesized wt- and F508del-CFTR associate specifically with calnexin (Pind et al. 1994). This association was shown to be specific of the immature forms of CFTR but only wt-CFTR was able to escape from this association and exit the ER. Release from calnexin-containing complexes corresponds to a step in the maturation of wt-CFTR that is not attained by F508del-CFTR. This second checkpoint (Fig. 1.6) seems however to be a minor step in F508del folding pathway (mainly retained at the Hsp checkpoint)—wt-CFTR in contrast would undergo successive rounds of release-deglucosylation and reglucosylation-rebinding to the ER membrane chaperone/lectin calnexin (Farinha and Amaral 2005). A fraction of wt-CFTR unable to acquire a folded conformation, and possibly a small amount of F508del-CFTR escaping degradation at the first ERQC checkpoint, undergo proteolytic glycan-mediated degradation at this second (calnexin-dependent) checkpoint (Farinha and Amaral 2005).

These quality control mechanisms in the ER ensure that nascent proteins that are unable to fold correctly are rapidly degraded. Because these degradation phenomena are lysosome-independent and occur close to the ER, it has been named ER-associated degradation or ERAD (Werner et al. 1996) or more recently, GERAD, for glycoproteins ERAD (Cabral et al. 2001). The acronym GERAD is a commonly used descriptive term that refers to the fate of many structurally flawed glycoproteins and orphan subunits that are restricted from traversing the entire secretory pathway. From a mechanistic standpoint, GERAD is the endpoint of a complex multistep disposal process composed of distinct signal formation and recognition steps, all of which provide a global and selective mechanism for recruiting misfolded proteins to the intracellular proteolytic machinery (Cabral et al. 2001). Proteins that are retained for long periods in this folding cycle undergo degradation. Signals that target these proteins for proteolysis include removal of the outermost mannose unit from the middle branch (α1-3 structure) by ER α1-2 mannosidase I, generating the Man8B isomer, that functions as a proximal ER degradation signal (Cabral et al. 2001). This modification by ER mannosidase I was described to function as a molecular clock in which the prolonged accessibility of glycans to the enzyme preferentially tags misfolded glycoproteins for degradation, whereas nascent folding intermediates are spared from destruction as they have not encountered the processing enzyme (Cabral et al. 2001). Unlike modification of glycosyl groups in the Golgi, which creates protein diversity, modification of the basic glycosylation structure in the ER appears to be used primarily by the quality control machinery to distinguish folded from misfolded proteins.

One of the first recognized needs for a quality control system is towards the retention of misassembled protein complexes; polypeptides that normally associate with other polypeptides cannot progress through the secretory pathway until they are bound to their partners or until they acquire their native conformation. A molecular basis for this observation was suggested by studies on the trafficking of subunits of a mammalian ATP-sensitive potassium (K^+) channel (Zerangue et al.

1999). The channel is formed by four regulatory subunits and four K^+ ion channel subunits, and proper function requires co-expression of all eight subunits. Sequence analysis combined with mutagenesis studies identified the motif RXR (arginine—any amino acid—arginine) that when present, blocks the trafficking of channel subunits to the cell surface, causing their retention in the ER (Zerangue et al. 1999).

Unlike sorting motifs typical of ER resident proteins (such as KDEL or KKXX mnotifs), the use of RXR-motifs as retrieval signals has only been documented for proteins with a final location other than the ER. The RXR motif (usually referred to as arginine-framed tripeptide AFT) only acts as retrieval signal when present in misassembled or misfolded proteins, suggesting that the motifs are buried in assembled proteins. The masking of RXR-motifs in the mature state of proteins appears to be the mechanism that makes these motifs acting as retrieval signals in the quality control and not as constitutive retention signals (Zerangue et al. 1999).

Chang et al. showed that export-incompetent CFTR displays multiple AFT sequences. Simultaneous inactivation of four of these motifs by replacement of arginine residues at positions R29, R516, R555, and R766 with lysine residues simultaneously causes mutant F508del-CFTR protein to escape ER quality control and function at the cell surface. R29K alone can also induce some effect, although at lower levels. These findings suggest that interference with recognition of these signals may be helpful for the management of CF (Chang et al. 1999). Later, it was shown that abrogation of the four AFTs does not correct F508del-CFTR folding (with the protein albeit localized at the plasma membrane still evidencing a gating defect indicative of its misfolding), only allows the bypassing of this so-called third ER checkpoint (Roxo-Rosa et al. 2006; Farinha et al. 2013a) (Fig. 1.6).

Detection of AFTs as sensors for the presence of aberrant structures may also involve the COPII coat-protein complex, which coats vesicles that are destined for delivery to the ER-**G**olgi **i**ntermediary **c**ompartment (ERGIC). COPII proteins interact with positive export signals on nascent CFTR. The better characterized motif for wt-CFTR exit from the ER is the diacidic code—$D_{565}AD_{567}$ sequence located in NBD1—which acts in fact as a positive cargo signal necessary for Sec24-mediated COPII packing (Nishimura and Balch 1997) and whose disruption reduces both Sec24-CFTR association and ER exit (Wang et al. 2004), forming a fourth checkpoint for CFTR ERQC. Similarly, association with COPI vesicles that are responsible for retrograde retrieval may be mediated by putative negative signals (Chang et al. 1999) (Fig. 1.6).

Large-scale interactomics studies have assessed the overall proteostasis environment controlling the folding of CFTR (Pankow et al. 2015). These studies identified interactors whose loss is critical for CFTR biogenesis, demonstrating that global remodeling of the interactions of the mutant protein is crucial for its rescue.

As folded CFTR exits the ER and traffics through the secretory pathway, misfolded CFTR is, as stated above, degraded by the ERAD/GERAD that involves three key steps: (a) recognition of the aberrant polypeptide; (b) export of soluble proteins to the cytoplasm back through the translocation pore (retrotranslocation); and (c) degradation by the proteasome (Cabral et al. 2001).

Recognition of the aberrant proteins has been described above. Retrotranslocation of transmembrane proteins may proceed through the Sec61 channel although these might be exceptions. CFTR is believed to be retrotransported through the Sec61 trimeric complex back to the cytosol, escorted by the subunit of Sec61 and from there made available to the proteasome (Xiong et al. 1999).

Multiple studies indicate that GERAD substrates are degraded in the cytoplasm by the ubiquitin-proteasome pathway (Ciechanover 1998). Substrates are first modified by covalent addition of multiple ubiquitin moieties through the action of cytosolic- or membrane-bound ubiquitin activating (E1), conjugating (E2), and ligating (E3) enzymes. Ubiquitinated proteins are then recognized by the cytosolic 26S proteasome complex that removes ubiquitin chains and digests the substrate into small peptide fragments (Ciechanover 1998). The 26S proteasome is a complex proteolytic machine consisting of a catalytic 20S cylindrical core particle and two copies of the 19S (PA700) regulatory particle that "caps" the 20S subunit (Ciechanover 1998). This assembly creates a central chamber into which the proteolytic active site is segregated from the bulk solution. From these observations, it is easy to conclude that the cytosolic degradation machinery has access to proteins in the ER lumen thus suggesting that translocation across the ER membrane is a bidirectional process regulated by the folded state of a given protein (Ciechanover 1998).

Several studies have demonstrated that the ubiquitin-proteasome pathway plays a key role in ER-associated degradation of CFTR being responsible for pre-Golgi lysosome-independent elimination of misfolded forms of the protein (Jensen et al. 1995; Ward et al. 1995). Jensen et al. demonstrated that CFTR and presumably other intrinsic membrane proteins are substrates for proteosomal degradation during their maturation within the endoplasmic reticulum. Inhibition of proteasome activity stabilizes the immature form (band B), but does not alter the efficiency of its conversion into the mature form (band C) (Jensen et al. 1995).

Elucidation on how CFTR is recognized for degradation and delivered to the proteasome may involve ubiquitination prior to completion of protein synthesis (Sato et al. 1998). Xiong et al. have also shown that full-length membrane integrated CFTR is also a substrate for polyubiquitination (Xiong et al. 1999).

Inhibiting the proteasome activity or overexpressing CFTR leads to the accumulation of stable, high molecular weight, detergent-insoluble, multi-ubiquitinated forms of CFTR, into a structure which was termed the aggresome (Johnston et al. 1998). The formation of a similar structure including CFTR in which, besides ubiquitin, the molecular chaperones Hsp70 and Hsp90 also concentrate was also described (Wigley et al. 1999). Many human neurodegenerative diseases are characterized by the presence of inclusions which are morphologically and biochemically similar to the experimentally induced aggresomes described for CFTR—although for CFTR this accumulation seems to occur mainly in overexpressing systems and not so frequently in endogenously expressing systems.

Sensing of CFTR misfolding also involves Hsp27—a member of the small Hsp family highly expressed in airway epithelial cells and with a described role in protein-folding and aggregation processes (Ahner et al. 2013). Hsp27 selectively

binds and targets F508del-CFTR for degradation, through a process that depends on conjugation with small ubiquitin-like modifier (SUMO). The SUMO conjugation cascade resembles that of ubiquitin conjugation and Ubc9, the only known SUMO E2 enzyme, is essential for conjugation with CFTR (Ahner et al. 2013). This modification involves the SUMO-2/3 paralogs that are able to form poly-SUMO chains and to recruit the SUMO-targeted ubiquitin ligase RFN4 to target CFTR to proteasome-mediated degradation. The process occurs by recognition of a non-native conformer of the F508del NBD1 (Gong et al. 2016).

1.3.2 CFTR Trafficking and Membrane Anchoring

CFTR exits the ER in COPII-vesicles and proceeds to the Golgi. As it traffics through the different cisternae of the organelle in COPI-vesicles, its glycidic residues undergo several modification catalyzed by different Golgi-resident glycosyl-transferases. This processing modifies the ER-characteristic high-mannose structures into complex structures (in vitro resistant to the activity of endoglycosidase H)—including removal of glycan units and addition of new ones, such as the trans-Golgi characteristic fucose, neuraminic or sialic acid. These modifications increase CFTR molecular weight (see above) (Amaral et al. 2016).

When trafficking out of the trans-Golgi, CFTR is delivered to the apical membrane by a process that can follow three different routes: (i) direct transport from the trans-Golgi to the membrane via transport vesicles; (ii) initial transport from the trans-Golgi to the basolateral membrane followed by transcytosis to the apical membrane; (iii) initial traffic from the trans-Golgi to apical recycling endosomes (AREs) and from there to the apical membrane. As CFTR arrives at the membrane, its levels are finely as a result of a balance from the anterograde trafficking, endocytosis, and recycling (Fig. 1.7).

Besides the conventional trafficking pathway, evidence has appeared suggesting that CFTR can follow also alternative routes from the ER to the membrane. In situ morphological observations suggested that CFTR is largely absent from the Golgi (Bannykh et al. 2000), raising the possibility of a novel trafficking pathway through the early secretory pathway. In a cell type-specific manner, processing of CFTR from the core-glycosylated (band B) ER form to the complex-glycosylated (band C) isoform followed a non-conventional pathway that was insensitive to dominant negative Arf1, Rab1a/Rab2 GTPases, or the SNAP Receptor (SNARE) component syntaxin 5, all of which block the conventional trafficking pathway from the ER to the Golgi. Moreover, CFTR transport through the non-conventional pathway was potently blocked by overexpression of the late endosomal target-SNARE syntaxin 13, suggesting that recycling through a late Golgi/endosomal system was a prerequisite for CFTR maturation. CFTR transport in the early secretory pathway can thus involve a novel pathway between the ER and late Golgi/endosomal compartments that may influence developmental expression of CFTR on the cell surface in polarized epithelial cells (Yoo et al. 2002). More recent studies proposed Golgi

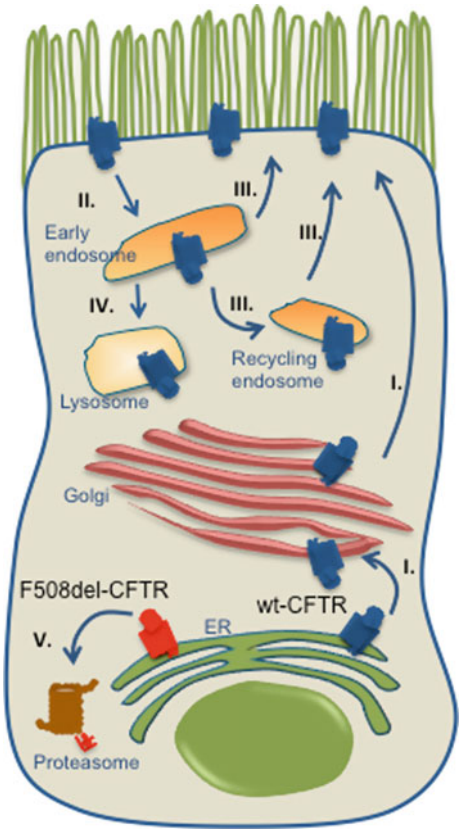

Fig. 1.7 Trafficking pathways for wt- and F508del-CFTR. CFTR is synthesized in ER-associated ribosomes (I). F508del-CFTR is mostly retained in the ER due to misfolding and degraded at the proteasome (V). wt-CFTR traffics to the Golgi and from there to the plasma membrane (I). CFTR is endocytosed in clathrin coated vesicles (II), from where it recycles back to the cell surface (directly or through recycling endosomes (III). Endocytosed CFTR can alternatively be sent to lysosomes and degraded (IV). Adapted from Ameen et al. (2007)

reassembly stacking proteins (GRASPs) as mediators of unconventional CFTR trafficking (Gee et al. 2011). The process involves GRASPs monomerization and ER relocalization allowing them to access CFTR in the ER (Kim et al. 2016). This Golgi bypass of CFTR is still unclear but it may involve a peri-centriolar intermediate compartment where CFTR would accumulate in case of blockage of the conventional pathway (Grieve and Rabouille 2011).

The population of wt-CFTR that reaches Golgi and post-Golgi compartments is quite stable. Moreover, CFTR pool in the cell membrane is rapidly internalized, at a rate of 10% per minute. CFTR molecules that are internalized follow the clathrin-coated vesicle endocytic pathway and are recycled or sent to lysosomal degradation (Riordan 2008; Sharma et al. 2004). Although it is not possible to fully ignore the hypothesis of very low levels of CFTR entrance in the caveolae, the data argue that if such caveolar CFTR exists, it is a minor component of the total cell surface CFTR pool (Ameen et al. 2007).

Both wt- and mutant CFTR that reach the cell surface are endocytosed, the later much more rapidly than the former. The biochemical half-life of plasma membrane F508del-CFTR is about 4 h whereas the biochemical half-life of plasma membrane

wt-CFTR exceeds 48 h. This instability is due to more rapid internalization of mutant protein, its selective targeting for rapid degradation and its decreased recycling (Heda et al. 2001; Sharma et al. 2004; Swiatecka-Urban et al. 2005).

CFTR endocytosis relies on the presence of endocytic signals—usually short linear arrays of four to seven amino acids in the cytoplasmic domains of integral membrane proteins. The critical amino acids are bulky and hydrophobic. Two major classes of endocytic motifs have been identified: tyrosine-based (NPXY or YXXΦ, where X stands for a variable amino acid and Φ for a bulky hydrophobic amino acid) and dileucine-based (D/EXXXLL/I and DXXLL). The alignment of the amino acid sequences of CFTR C-termini from a variety of species reveals the presence of a conserved YXXL motif—^{1424}YDSI, which was shown to be a real tyrosine internalization motif (Ameen et al. 2007; Hu et al. 2001). Hu et al. demonstrated that the C-terminus of CFTR contains three endocytic signals, ^{1424}YDSI, ^{1413}FVLI and ^{1430}LL. Disruption of these signals with alanine mutations (^{1424}YDSI → ^{1424}ADSI; ^{1413}FVLI → ^{1413}AVLI and ^{1430}LL → ^{1430}AL) largely reduced endocytosis and induced accumulation of CFTR at the cell surface (Hu et al. 2001).

The endocytic signals are recognized by specific adaptors that promote CFTR internalization in clathrin-coated vesicles. Early work showed that full-length CFTR binds to the endocytic adaptor complex AP2 (Weixel and Bradbury 2000). Substitution of an alanine residue for tyrosine at position 1424 significantly reduced the ability of AP2 to bind the C-terminus of CFTR. However, mutation to a phenylalanine residue, which is normally found in dogfish CFTR at this position, did not perturb AP2 binding. Taken together, this data suggests that the C-terminus of CFTR contains a tyrosine-based internalization signal that interacts with the endocytic adaptor complex AP2 to facilitate efficient entry of CFTR into clathrin-coated vesicles (Weixel and Bradbury 2000). In fact, in non-epithelial cells, AP2 is necessary for efficient CFTR endocytosis, and the μ2 adaptin interacts directly with the CFTR YDSI motif (Prince et al. 1999; Weixel and Bradbury 2000, 2001). However, the process is more complex in airway epithelial cells with efficient μ2 knockdown resulting in a not-so-dramatic reduction in CFTR endocytosis when compared with the dramatic reduction seen for purely YXXΦ-mediated uptake (Fu et al. 2012). The fact that several endocytic signals exist in the C-terminal tail of CFTR suggests that although initially described as an essential adaptor in CFTR endocytosis, AP2 may not be obligatory for CFTR recruitment to CCVs in airway epithelia (Hu et al. 2001).

The clathrin-associated sorting protein (CLASP) Disabled-2 (Dab-2) has also been implicated in CFTR endocytosis. Dab-2 can sustain endocytosis of NPXY-containing proteins and can also work together with AP2. Dab-2 was shown to co-precipitate with CFTR and myosin VI in human airway epithelial cells (Swiatecka-Urban et al. 2004) and to colocalize with AP2 and myosin VI in rat enterocytes (Collaco et al. 2010). As CFTR does not have a canonical NPXY motif, it has been proposed that the role for Dab-2 is dependent on AP2 and/or myosin VI. More recent studies reported that in human airway epithelial cells Dab-2 recruits CFTR to CCVs and mediates CFTR endocytosis by an AP2 independent

mechanism that requires the Dab-2 DH domain. The same study showed that AP2 is not essential for CFTR recruitment to clathrin-coated vesicles. This mechanism was proposed as a putative pharmacological approach to stabilize F508del-CFTR at the plasma membrane (Cihil et al. 2012).

Several GTPases have also been described to regulate the trafficking of CFTR (Ameen et al. 2007). Using external epitope-tagged constructs, Gentzsch et al. revealed that surface CFTR enters several different routes: including a Rab5-dependent initial step to early endosomes, then either Rab11-dependent recycling back to the surface or Rab7-regulated movement to late endosomes or alternatively Rab9-mediated transit to the *trans*-Golgi network. Moreover, it was shown that F508del-CFTR can be rescued to the plasma membrane by Rab11 overexpression, proteasome inhibitors or inhibition of Rab-5 dependent endocytosis (Gentzsch et al. 2004). The exit of CFTR from early endosomes can also be mediated by Rab4 which directly targets CFTR back to the plasma membrane. Moreover, RME-1 facilitates exit of CFTR from the recycling endosome (Ameen et al. 2007) (Fig. 1.7).

Myosin motors and actin filaments play also vital roles in membrane trafficking. Myosin Vb and myosin VI are part of the myosin superfamily which consists of 18 different classes of myosin motors capable of using ATP hydrolysis to move on actin filaments (Swiatecka-Urban et al. 2004). Myosin VI is most likely to participate in the apical membrane endocytosis in epithelial cells because it moves toward the F-actin minus end, which is oriented away from the plasma membrane. Myosin VI regulates early steps of transferrin receptor (TfR) endocytosis, including uptake of TfR into clathrin-coated pits and formation of clathrin-coated vesicles, as well as the later stages of TfR endocytosis, including movement of uncoated vesicles toward the early endosomes on actin filaments (Swiatecka-Urban et al. 2004). Myosin VI is relevant at early endocytic events such as clustering of CFTR in clathrin-coated pits and formation of clathrin-coated vesicles, in the complex involving Dab-2 (Swiatecka-Urban et al. 2004).

Myosin Vb, which specifically interacts with Rab11a, regulates CFTR-mediated chloride secretion across human airway epithelial cells by facilitating the apical membrane recycling of both wt- and F508del-CFTR (Swiatecka-Urban et al. 2007). Interestingly, it has been shown recently that Rab11b also regulates the apical recycling of CFTR in polarized intestinal epithelial cells (T84). These observations highlight a possible tissue-specific regulation of CFTR trafficking by Rab11 isoforms (Silvis et al. 2009).

In addition to trafficking events at the membrane, CFTR membrane anchoring relies on multiple protein–protein interactions, among which those with PDZ proteins are the most relevant. Proteins with PDZ domains (named for 3 proteins in which this domain was first described namely PSD-95, Disc-large and ZO-1) play an essential role in determining cell polarity (Fanning and Anderson 1996, 1998). PDZ domains are modular 70–90 amino acid domains in proteins that bind to short peptide sequences at the C-terminus of other proteins, called PDZ-interacting domains (Fanning and Anderson 1996; Cushing et al. 2008; Ranganathan and Ross

1997). A PDZ-interacting domain was first identified in CFTR and described to act as an apical localization signal (Short et al. 1998). In fact, the C-terminus of CFTR (residues DTRL) is a consensus PDZ-binding motif (C-terminal X-[S/T]-X-[V/I/L]) that binds several PDZ proteins (Haggie et al. 2006; Wang et al. 1998), including Na^+/H^+-exchanger regulatory factor isoform-1 (NHERF-1, also known as EBP50, ezrin-binding protein, 50 kDa), NHERF-2, NHERF-3 (also known as CFTR-associated protein 70KDa, or CAP70), NHERF-4 and CAL (CFTR-associated ligand) (Guggino and Stanton 2006). Recent results show that besides the C-terminus motif a sequence further upstream also modulates interaction with NHERF-1 (Sharma et al. 2016). Initially, CFTR interaction with these proteins was believed to be important in determining the magnitude of cAMP-stimulated CFTR Cl^- channel activity (Short et al. 1998) but the role seems to be mainly in the regulation of membrane trafficking events—reviewed in (Farinha et al. 2013b).

NHERF-1 tethers CFTR to filamentous actin and is also important to target the protein to exosomes and endosomes in polarized cells (Haggie et al. 2006; Short et al. 1998; Swiatecka-Urban et al. 2002). NHERF-1 interaction with ezrin anchors CFTR to the apical actin cytoskeleton, whose integrity is critical for CFTR membrane retention (Ganeshan et al. 2007). This process is regulated by small GTPases of the Rho family—the Rho GTPase Rac1 is a key regulator of ezrin-mediated anchoring of CFTR to F-actin in airway epithelial cells. Interestingly, hepatocyte growth factor (HGF)-induced activation of endogenous Rac1 signaling, is able to improve the apical retention of rescued F508del-CFTR in human airway epithelial cells (Moniz et al. 2013). This effect occurs through activation of ezrin by phosphorylation, promoting its interaction with F-actin and NHERF-1 (Favia et al. 2010; Loureiro et al. 2015; Abbattiscianni et al. 2016).

The retention of the mutated protein at the cell surface after rescue is target for a peripheral protein quality control (PPQC) system (Sharma et al. 2004; Loureiro et al. 2015). The general folding machinery is a major player in the PPQC and co-chaperones Bag-1, Hsp40, HOP, and Aha1 facilitate nonnative CFTR internalization and lysosomal targeting through the interaction with ubiquitination machinery (Okiyoneda et al. 2010).

Whereas NHERF-1 and also NHERF-2 are CFTR stabilizers, overexpression of CAL shortens the half-life of mature CFTR by promoting its lysosomal degradation following endocytosis (Cheng et al. 2004). Therefore, cell surface levels of F508del-CFTR can be enhanced by overexpression of NHERF1/2, or by suppression of endogenous CAL. Interesting, as the affinity of CAL for the CFTR C-terminus is much lower than those of NHERF1/2, this has prompted the identification of pharmaceutical inhibitors than can displace CFTR from CAL but not from NHERF1/2 (Cushing et al. 2008).

Several other mechanisms have been reported to regulate CFTR membrane anchoring and trafficking. According to an old model, CFTR-containing recycling endosomes were proposed to migrate the cell membrane in response to stimulation to an intracellular increase in levels of cAMP—suggesting that the cAMP effect upon CFTR would not just be through stimulation of its activity as a Cl^- channel

(see Sect. 4) but also through an increase in the number of CFTR molecules present at the cell surface. This mechanism was recently shown to involve the activation of the cAMP sensor EPAC1 (exchange protein directly activated by cAMP) whose interaction with CFTR through NHERF1 stabilizes CFTR at the membrane by decreasing its endocytosis (Lobo et al. 2016).

CFTR phosphorylation has also been implicated in the regulation of its trafficking (reviewed in Farinha et al. 2016). Casein Kinase 2 (CK2), Spleen Tyrosine (SYK), Lemur Tyrosine Kinase 2 (LMTK2) and With-No-Lysine Kinase 4 (WNK4) all regulate CFTR stability and trafficking. Phosphorylation of CFTR at Tyr^{512} by SYK decreases its PM stability through a process that involves SYK regulation by WNK4 (Luz et al. 2011; Mendes et al. 2011). The membrane-anchored Serine/Threonine (Ser/Thr) kinase LMTK2 (Lemur tyrosine kinase-2) phosphorylates CFTR at Ser^{737}, and facilitates its endocytosis (Luz et al. 2014; Wang and Brautigan 2006). LMTK2 is a myosin VI-binding partner, most likely promoting CFTR inclusion into early endosomes.

CFTR trafficking and stability are also dependent on the regulatory mechanisms of the epithelial tissue. The protein is expressed at the apical membrane only in well-differentiated epithelial cells (Puchelle et al. 1992). In basal cells during remodeling or in non-differentiated epithelium, even wt-CFTR is mostly intracellular (Dupuit et al. 1995). Interestingly, epithelial remodeling is also affected in CF—wound healing is slower in cells from CF patients, although these studies were performed with cells grown in plastic, thus hardly recapitulating a well-differentiated and polarized airway epithelium (Trinh et al. 2012). Taken together, these observations suggest that functional CFTR has a critical role in wound repair but also that differentiation/polarization is apparently related to CFTR trafficking (Trinh et al. 2012) (see Sect. 1.4.3).

1.4 CFTR Function

1.4.1 CFTR as a Chloride Channel

Even before the cloning of the *CFTR* gene in 1989, CF was associated with a defective Cl^- and water transport in epithelial secretory and absorptive cells (Quinton 1990). Following gene cloning, several studies were initiated in order to elucidate the real function of CFTR protein as a Cl^- channel or as a regulator of other channels, raising different hypotheses. One of these postulated that CFTR was an ATP-driven transporter, as it belongs to the ABC transporter superfamily. A second hypothesis held that CFTR functioned as a regulator of a separate channel molecule, and finally a third hypothesis proposed that CFTR was itself a regulated Cl^- channel. The final proof that CFTR exhibited regulated Cl^- channel activity was obtained through reconstitution of the protein in a planar lipid bilayer (Bear et al. 1992). Today, there is enough data to identify the Cl^- channel activity at the apical

membrane of epithelial cells as the main function of CFTR. However, data provided through the years indicates that CFTR has several alternative/secondary functions.

Similar to other ionic channels, CFTR inserted in the membrane allows regulated ion transport through a conduction pore that facilitates rapid ionic movement, acts as a selective filter responsible for the channel selectivity, and provides a gating mechanism that regulates the opening probability of the channel (Devidas and Guggino 1997).

CFTR channel activity is regulated mainly by a mechanism evolving the action of cellular secondary messengers namely nucleotide binding to the two NBDs and phosphorylation events in the RD (see Sect. 1.2.2). In the model currently accepted for the mechanism of channel function and regulation (Fig. 1.8), phosphorylation of the RD by cAMP-dependent protein kinase A (PKA, and also PKC), allows ATP binding to the NBDs, which then dimerize (Hwang and Sheppard 1999, 2009; Sheppard and Welsh 1999). Consequently, the MSDs alter their conformation, allowing the opening of the channel pore and passive flow of ions.

Although the mechanism of the CFTR channel gating is not fully understood, opening and closing of this Cl^- channel is tightly regulated by the cellular balanced activities of kinases and phosphatases and by ATP levels. Furthermore, the open probability of the channel is controlled by the extent of RD phosphorylation at multiple sites (Hwang and Sheppard 2009).

The function and cooperation of the various domains of CFTR confer to the channel several distinctive characteristics: (i) a small single-channel conductance (6–10 pS) (Welsh et al. 1992); (ii) a linear current-voltage (I–V) relationship; (iii) selectivity for anions over cations; (iv) an anion permeability sequence of $Br^- > Cl^- > I^-$; (v) time- and voltage-independent gating behavior; and (vi) activity regulated by cAMP-dependent phosphorylation and by intracellular nucleotides (Sheppard and Welsh 1999).

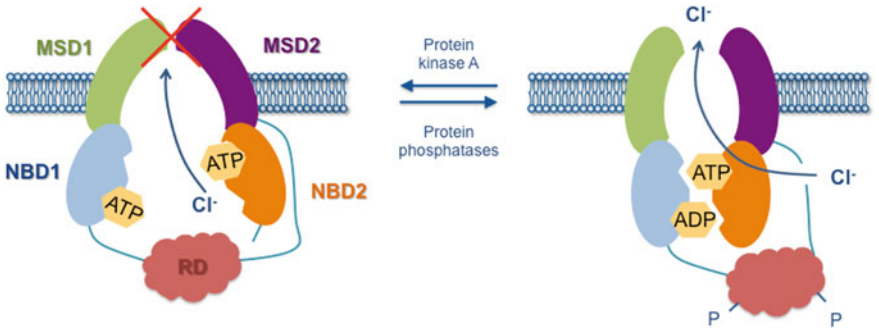

Fig. 1.8 Changes in CFTR conformation leading to channel gating. CFTR alternates between the inactive (*left*) and activated (*right*) conformation. Phosphorylation of the RD allows heterodimerization of NBD1 and NBD2 involving ATP binding and hydrolysis. Adapted from Hwang and Sheppard (2009)

1.4.2 CFTR as Regulator of Other Channels and of Epithelial Ion Transport

Besides functioning as Cl^- channel, CFTR has been described to regulate other channels and transporters, with this contributing to the overall regulation of ion transport in epithelia. As stated above, CFTR is expressed in the luminal membranes of secretory and absorptive epithelia, playing a role in cAMP—but also Ca^{2+}-activated-secretion of electrolytes (Fig. 1.9).

In secretory epithelia, Cl^- is absorbed on the basolateral side by the Na^+–K^+–$2Cl^-$ (NKCC1) cotransporter, accumulating inside the cell. Secretion through CFTR can occur upon stimulation from the apical membrane (Fig. 1.9). CFTR also regulates reabsorption of electrolytes by controlling the activity of the amiloride-sensitive epithelial Na^+ channel (ENaC). In CF patients, although the main dysfunction is a defective Cl^- secretion, abnormalities in the transport of other ionic species, particularly Na^+, are usually detected.

Increased Na^+ absorption was first suggested by Knowles and collaborators in 1983. Increased potential difference in CF patients' epithelia was then suggested to be due to this increase in Na^+ transport (Knowles et al. 1983).

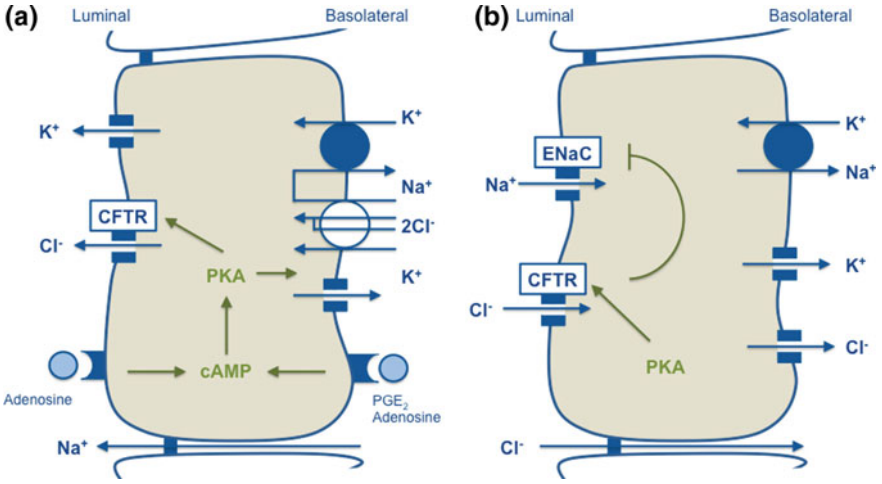

Fig. 1.9 Electrolyte secretion and absorption in the airway and intestinal epithelia. **a**. In secretory cells, Cl^- enters the cell from the basolateral side by the Na^+–K^+–$2Cl^-$ (NKCC1) cotransporter. K^+ exits via basolateral K^+ channels, and Na^+ pumped through the Na^+–K^+-ATPase. Cl^- exits by the apically located CFTR. Na^+ is secreted via the paracellular shunt and K^+ is via luminal K^+ channels. Activation occurs through an increase in cAMP triggered by either adenosine (airways) or prostaglandin E_2 (PGE_2, intestine). **b**. In absorptive cells, Na^+ enters the cell from the luminal side via ENaC. Cl^- passes through a basolateral and probably via CFTR. Na^+ is also pumped out via the basolateral Na^+–K^+-ATPase. Cl^- and K^+ exit through basolateral Cl^- and K^+ channels. CFTR activity and/or expression inhibits ENaC. Adapted from Kunzelmann and Mall (2001)

Stutts et al. in 1995 described a reduction in Na^+ currents when CFTR was stimulated by cAMP and concluded that CFTR negatively regulates ENaC through a cAMP-dependent mechanism. These findings explain the increased Na^+ absorption in CF airways (Stutts et al. 1995).

When activated via the PKA-dependent pathway, it is believed that CFTR inhibits ENaC, thus reducing Na^+ absorption (Kunzelmann et al. 2001). In CF epithelia, both the secretion and absorption of electrolytes are found to be impaired.

In absorptive epithelia, like the sweat gland, the transport direction is reverted, being both Cl^- and Na^+ absorbed at the apical side and then secreted basolaterally upon stimulation. CFTR is thus required for upregulation of ENaC, causing a decreased Na^+ conductance in CF sweat ducts (Reddy et al. 1999).

In the respiratory and intestinal epithelia, CFTR can function either in the absorptive or the secretory mode. The regulation of Airway Surface Liquid (ASL) in the respiratory epithelia is ensured by electrolyte absorption that occurs at the surface epithelium, and secretion, in the submucosal glands (Kunzelmann and Mall 2001). Cl^- secretion by the submucosal glands prompts the creation of a lumen-negative transepithelial voltage, that will force Na^+ and water to exit towards the lumen, allowing its hydration. The concomitant absorption process of Cl^- and water proceeds through both cellular and paracellular pathways. Two competing theories have been put forward to explain the abnormalities in the CF airway epithelium: (i) the "High Salt" model, where dysfunction of CFTR leads to diminished absorption of counter-ions, favoring the accumulation of salt in the ASL; and (ii) the "Low Volume" model, where dysfunction of CFTR leads to hyperabsorption of mainly Na^+, decreasing osmotic pressure and consequent dey-dration of ASL (Rowe et al. 2005).

CFTR has been also linked to the control of oxidative stress in the airways. Glutathione (GSH) is reduced in ASL of CF patients (Roum et al. 1993). Interestingly, CFTR was observed to mediate GSH transport in its reduced form, providing an explanation for the augmented basal state of inflammation found in CF lungs (Kogan et al. 2003; Linsdell and Hanrahan 1998).

The colonic epithelium has also absorptive and secretory functions, being characterized by a net absorption of NaCl, short-chain fatty acids and water, resulting in a very low content of water and salt in feces. Colonic cells also secret mucus, bicarbonate, and KCl. These features point to a dual function of CFTR in the mammalian colon partly enabling the switch from absorption to secretion when stimulated by secretagogues (Kunzelmann and Mall 2001).

CFTR is essential for Cl^- secretion in the lower crypts, whereas in the upper crypts (especially in the surface epithelium), it may regulate other transport proteins such as ENaC and Na^+/H^+ exchanger NHE3, playing a role in absorption. There is a strong correlation between expression of wt-CFTR and the stimulation of colonic Cl^- secretion by increasing both intracellular cAMP and Ca^{2+}.

Besides Cl^- transport, CFTR has also been implicated in HCO_3^- transport in the lungs, gastrointestinal tract and pancreas (Park et al. 2010; Hug et al. 2003). CFTR

exhibits a low permeability to HCO_3^-, but this permeation has important implications (Poulsen et al. 1994). HCO_3^- is involved in several cellular functions, including acting as a pH buffer and thus enhancing the solubility of many proteins. The role of CFTR in HCO_3^- secretion may also provide an explanation for the abnormally viscous mucus that is characteristic of CF. Whether this mucus results from an aberrant intracellular synthetic process (Mohapatra et al. 1995) or to extracellular mechanisms that compromise the mucins upon their secretion (Garcia et al. 2009; Coakley et al. 2003) is a long standing controversy.

Some studies have proposed that the abnormal mucus release in CF is a consequence of the defective HCO_3^- secretion through the channel, promoting the "mucoviscidosis" defect (Chen et al. 2010). The defective permeability would thus contribute to a change in the pH of the lumen, affecting mucus swelling. Mucins are tightly packed when secreted and at the lowered pH remain compact in the extracellular matrix in CF tissues (Kuver et al. 2006; Kreda et al. 2007).

The Outwardly Rectifying Chloride Channel (ORCC), also called Intermediate Conductance Outwardly Rectifying channel (ICOR), is among the most abundant Cl^- channels in epithelial cells (Schwiebert et al. 1999). Due to its abundance, it was initially thought to be the Cl^- transporter defective in CF. This hypothesis was discarded because most of this channel is not directly stimulated by cAMP, contrary to CFTR. CFTR and ORCC are distinct channels but are linked functionally (Schwiebert et al. 1999). Schwiebert et al. presented results from whole-cell and single-channel patch-clamp recordings, short-circuit current recordings, and ATP-release assays of wild-type and mutant CFTR-transfected CF airway cultured epithelial cells indicating that CFTR regulates ORCCs by triggering the transport of the potent agonist, ATP, out of the cell (Schwiebert et al. 1999). The results suggested that CFTR functions to regulate other Cl^- secretory pathways in addition to conducting Cl^- itself. Accordingly to this hypothesis, ATP secreted by CFTR to the apical side would bind to purinergic receptors coupled to ORCC, thus activated by ATP transport through CFTR. This model is, however, controversial, mainly because it is not generally accepted that CFTR can act as an ATP transporter. Hyperabsorption of NaCl in CF epithelia, in special of Cl^-, can be explained by the presence of ORCC channels (Kunzelmann and Schreiber 1999).

It was also demonstrated that the potassium (K^+) channels ROMK (originally detected in renal epithelia and called Kir1.2) have an increased opening probability when CFTR is expressed and co-activated in the same cells (McNicholas et al. 1997). KvLQT-1, a different category of basolateral K^+channels expressed in intestinal and respiratory epithelia, seems to be activated by cAMP being this effect not detected in CF epithelia (Mall et al. 2000). CFTR was thus also implicated in the co-activation of this channel. Regulation of K^+ channels by CFTR is very important, because basolateral K^+ conductance is essential to ensure the driving force for Cl^- secretion in the apical side of epithelia (Mall et al. 2000).

Among the channels controlled by CFTR is also the anoctamin family, better known as calcium (Ca^{2+})-activated Cl^- channels (CaCCs). CaCCs mediate Ca^{2+}-

dependent Cl^- secretion in glands and flat epithelia and modify cellular responses to adequate stimuli in muscle, nerve and receptors (Kunzelmann and Mehta 2013; Tian et al. 2012). Some studies already reported a functional relationship between these two entities, however the mechanism behind this crosstalk is still not completely understood (Ousingsawat et al. 2011). Nevertheless such relation also points to a link between cAMP (involved in CFTR activation) and Ca^{2+}-dependent secretion, two very important signaling pathways, that were considered independent entities for a long time.

1.4.3 Other Functions

The regulation of other channels and of epithelial ion transport by CFTR relies on its role as an "anchoring platform" at the cell membrane. Specialized "microdomains" anchored to CFTR may group together a number of proteins that are dynamically regulated by molecular switches, that include signaling molecules, kinases, transport proteins, PDZ-domain-containing proteins, myosin motors, Rab GTPases, and SNAREs (Guggino and Stanton 2006).

CFTR was also proposed to regulate intracellular pH, namely in endosomes. It was suggested that when CFTR is present in the endosomes, during its trafficking to the plasma membrane, it functions as a Cl^- channel in the organelle membrane and, therefore, internal pH of the organelle would result from a balance between CFTR and the H^+-dependent ATPase (proton pump) activities (Barasch et al. 1991; Lukacs et al. 1992). It was additionally demonstrated that endosome acidification is defective in CF cells (Barasch et al. 1991). This pH change would lead to an alteration of the activity of enzymes responsible for specific glycosylations, namely the sialyltransferases, that are inactive if proper acidification of the endosome is not achieved. In CF patients, membrane glycoproteins (other than CFTR) present a decrease in their sialylation levels and increased presence of sulfate groups, probably having consequences to the adherence of pathogenic agents namely *Pseudomonas* (Barasch et al. 1991).

Finally, CFTR has also been demonstrated to play a role in processes such as cellular differentiation, epithelium regeneration and organization. Evidence has accumulated supporting such a role and include the delay in epithelial differentiation processes detected in CF versus non-CF, the need for functional CFTR for the rapid regeneration of human airway surface epithelium after injury, the differential expression of a significant set of differentiation genes in CF versus non-CF tissues, the abnormal tracheal development in neonatal mice, pigs, and children caused by loss of CFTR, the triggering of Epithelial-to-Mesenchymal Transition (EMT) and the associated poor prognosis of cancer caused by CFTR downregulation and the increased risk of digestive tract and other cancer forms in CF patients (Meyerholz et al. 2010; Leigh et al. 1995; Clarke et al. 2015).

1.5 Therapies Aimed at Correcting the Basic Defect

1.5.1 CFTR-Based Approaches

CF has been the paradigm for other genetic diseases in various aspects, namely for the therapeutic approaches. Being a monogenic disorder, CF is an obvious candidate to gene therapy, that in principle has the big advantage of its success not being dependent on a complete understanding of the function of the gene product (Rubenstein and Zeitlin 1998). Although some early reports showed that delivery of CFTR to a small percentage of cells was able to restore CFTR-dependent Cl^- transport (Chu et al. 1992), technical difficulties, relating largely to gene delivery and host response were a major hurdle throughout the years. Several vectors were tested for delivery of the *CFTR* gene to respiratory epithelial cells—viral vectors, including adenoviruses, adeno-associated viruses, and retroviruses were mostly unsuccessful (inefficient delivery coupled to trigger of the immune response) and nonviral ones have been only marginally successful. Up to this date, the work performed by the UK CF gene therapy consortium succeeded in evidencing a modest, although significant, improvement of the lung function in CF patients after monthly repeated application of a plasmid DNA-liposome formulation for 1 year (Alton et al. 2015).

Due to the limited success of gene therapy approaches, much effort has been put in understanding the molecular mechanisms that lead to CFTR dysfunction to unravel new strategies for the pharmacological restoration of CFTR function. Several attempts to overcome the molecular/cellular defects of mutant CFTR are in the basis of this flourishing field in CF therapy. The main goal of these assays is the repair of mutant CFTR function according to the class of the respective mutant. So it is usually referred to as protein repair therapy (Zeitlin 1999; Rubenstein and Zeitlin 1998).

The early finding that F508del-CFTR defect is temperature sensitive (Denning et al. 1992) gave the first evidence that the protein is rescuable. Recent evidence showed that in fact, low temperature is allowing the mutant protein to escape the ERQC without a fully corrected folding, thus highlighting the need for different combinatorial approaches to correct the multiple basic defects on this most common disease-causing mutation (Amaral and Farinha 2013; Farinha et al. 2013a; Farinha and Matos 2016).

Correction of F508del-CFTR by a genetic approach through introduction of second site mutations (called suppressors or revertants) has also been extensively reported and studied in order to identify mechanisms that are amenable to modulation in order to rescue the protein (Mendoza et al. 2012; He et al. 2010, 2013).

The attempts that have been followed in recent years are summarized in Fig. 1.10 and follow a class-specific approach.

Nonsense class I mutations, that create premature stop codons, impair CFTR production. Early reports showed that some aminoglycosidic compounds such as G418, a neomycin analog, and gentamycin can suppress the termination effect of

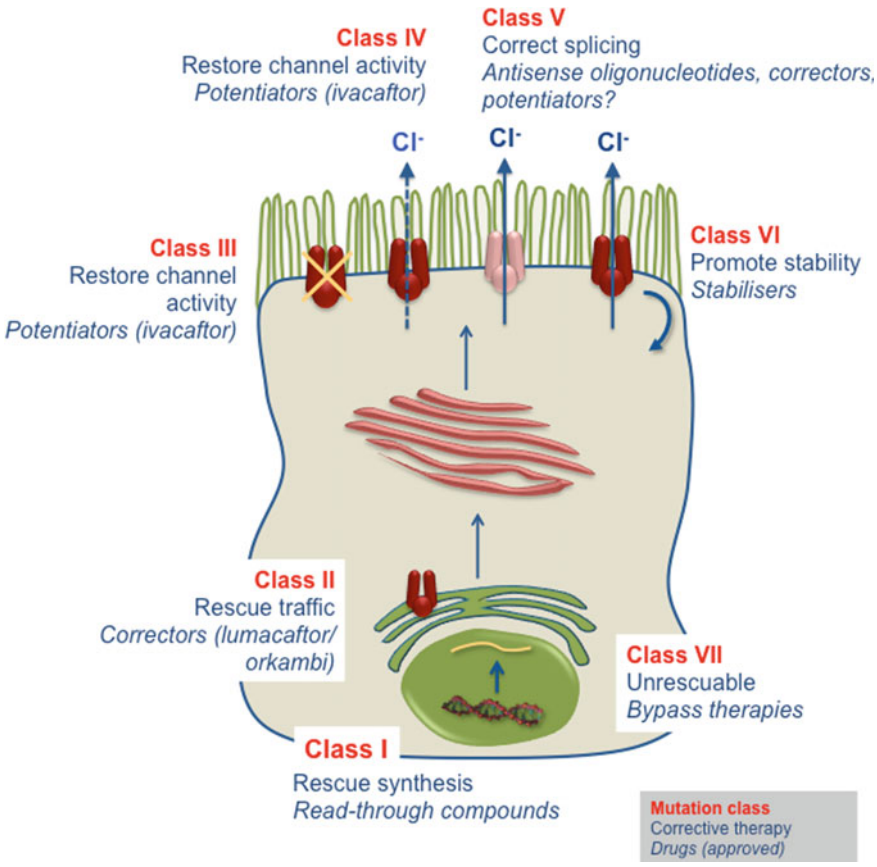

Fig. 1.10 CFTR repairing strategies aimed at the distinct mutation classes. For each class, the corrective strategy is mentioned, the type of drug under use or trial and any already approved and available to patients. Adapted from De Boeck and Amaral (2016) and Zeitlin (1999)

the mutations R553X and W1282X (Clancy et al. 2001; Shoshani et al. 1994). Due to the success of these in vitro studies, gentamycin was used in clinical trials in USA and Israel, in CF patients with at least one stop mutation (Clancy et al. 2001). Although evidencing some success, the response was variable and some potential toxic effects were reported (Linde et al. 2007).

Using high-throughput screening approaches, the company PTC Therapeutics identified the compound PTC124 or Ataluren as able to induce ribosomal read-through of premature stop codons but not normal stop ones. This compound was used in clinical trials and the most recent results show that the compound was found to be beneficial for CF patients that were not under aminoglycoside antibiotic therapy (Kerem et al. 2014). In the meanwhile, Ataluren was approved for use in Duchenne Muscular Dystrophy patients under the trade name Translarna (Ryan 2014).

Class I mutations that lead to aberrant splicing can be targeted by modulators of splice site usage by cellular and/or viral splicing factors. With such aim, antisense oligonucleotides have been described to successfully correct aberrant splicing in specific CFTR mutations (Igreja et al. 2016), an approach that can be used also for class V splicing mutations.

Protein repair therapy for class II mutations aims at correcting the folding defect of the mutant protein that is recognized by the ERQC machinery preventing its trafficking. Mimicking molecular chaperones activity towards promoting protein folding has been attempted by the use of small organic molecules termed "chemical chaperones" (Welch and Brown 1996). Like molecular chaperones, they do not provide any direct information to the folding process but seem to positively influence the rate and/or fidelity of the folding, probably by stabilizing the properly folded form or an intermediate of the polypeptide (Welch and Brown 1996). Most of the molecules with chemical chaperoning activity play this role naturally inside living cells to deal with adverse changes in their environment that may lead to protein denaturation/aggregation. Some of these cellular compounds are also called "compatible osmolytes" (Welch and Brown 1996) due to the fact that they accumulate within the cell to rather high concentrations without disturbing protein function, in fact with a protective role towards native protein conformations (Welch and Brown 1996). Glycerol is included in this group.

It was demonstrated by Welch and collaborators (Brown et al. 1996) and by Kopito and collaborators (Sato et al. 1996) that F508del-CFTR expressing cells incubated with glycerol express the mature form of CFTR (i.e., post-Golgi) and also display cAMP-activated chloride transport. Glycerol was also described to facilitate the in vitro folding of NBD1 by preventing the appearance of off-pathway intermediates (Qu et al. 1997). For glycerol, like for other polyhydric alcohols (polyols) in general, a mechanism has been described according to which the polyol is excluded from the immediate vicinity of a polypeptide and as a result, at high concentrations, it will increase the relative hydration around the polypeptide (Welch and Brown 1996). In response to this effect, the protein will tend to decrease its relative surface area by an increase in self-association or tighter packing. This hydrophobic effect enhances the stability of the protein reducing its potential to unfold in response to thermal or chemical treatments (Brown et al. 1996).

Repair of proteins bearing class II mutations can also be achieved with drugs that modulate interactions with molecular chaperones. In particular, deoxyspergualin (DSG), commonly used as an anti-rejection compound in transplantations, was described to bind Hsc70 thus diminishing the chaperone levels in the cell and rescuing F508del-CFTR trafficking and function (Jiang et al. 1998).

Several high-throughput (HTS) efforts have identified some lead "correctors" (Van Goor et al. 2006), i.e., compounds that rescue the trafficking defect of F508del-CFTR. These compounds have become increasingly potent, more specific and less toxic. The most efficient ones include laboratory reagents like the quinazoline derivative VRT-325 (C3) (Van Goor et al. 2006), or compound 4a (C4), or (pre-) clinical correctors like VX-809 (Lumacaftor) or VX-661. The investigational drug VX-809 reported in 2011 by Vertex Pharmaceuticals improved

F508del-CFTR processing in primary cultures of epithelial cells from individuals homozygous for F508del (Van Goor et al. 2011). Although more efficacious and selective than all previously reported CFTR correctors, results of Phase IIa clinical trials had only limited success (Clancy et al. 2011) leading to its testing in combination with potentiator VX-770 (ivacaftor). This combination led to a modest, albeit significant, increase of lung function in F508del homozygous patients (Boyle et al. 2014), prompting its approval by the FDA and the EMA (with the brand name Orkambi®).

The limited efficacy of this combination pushed forward continued efforts to find better correctors, some of which could be used in combination with VX-809—aimed at correcting the different molecular defects in the F508del variant.

These efforts have been directed to F508del-CFTR, but several laboratories have been using personalized medicine approaches to test whether approved drugs are effective in rescuing CFTR bearing rare class II mutations (Awatade et al. 2015; Dekkers et al. 2016).

Mutations that originate proteins with deficient activation or regulation (class III) or low conductance (class IV) can be corrected with compounds that have specific channel activation properties (potentiators).

Early attempts have been made using genistein or related flavonoids (e.g., quercetin) (Lehrich et al. 1998). This class of flavonoids was described to stimulate the Cl^- transport activity of CFTR bearing the G551D mutation (Illek et al. 1999). Another approach to deal with these mutants was the usage of phosphodiesterase inhibitors, as isobutylmethylxanthine (IBMX), in order to increase intracellular levels of cAMP. Alternatively, phosphatase inhibitors were used to inhibit CFTR dephosphorylation. These two strategies led to encouraging results in vitro (Schultz et al. 1999) and in mice (Smith et al. 1998). Stimulation of CFTR conductance in class IV mutants was also tried using xanthine-derivatives such as 8-cyclopentyl-1, 3-dimethylxanthine, flavonoids like genistein, or other compounds like milrinone, 1-ethyl-2-benzimidazolone (EBIO), and trimethylamine oxide (TMAO).

HTS approaches have also aimed at these mutations and identified potentiators that restore CFTR gating (Pedemonte et al. 2005), including laboratory reagents such as VRT-532 and the first approved CFTR modulator VX-770/Ivacaftor (brand name Kalydeco®) (Pedemonte et al. 2005; Van Goor et al. 2006, 2009, 2011).

VX-770 was described to promote CFTR-mediated Cl^- transport in cells bearing the class III mutation G551D. After successful trials, the drug was approved by both the FDA and the EMA for patients bearing G551D, and later for patients bearing several other less frequent mutations belonging to class III/IV (Van Goor et al. 2009; Yu et al. 2012; Cutting 2015). However, some mutations belonging to these two classes do not respond to this drug, evidencing the need for continued search for alternative potentiators.

Class V includes mainly splicing mutations that generate both aberrant and correctly spliced mRNAs in variable amounts among patients. The major aim for therapy in these cases is to increase the levels of correctly spliced transcripts. Although modulating the splicing process is very complex, it has been successfully achieved (see above). A feasible approach for these mutations could also be the

combined use of CFTR correctors, such as VX-809, and potentiators, such as VX-770, to further increase trafficking of CFTR to the plasma membrane and channel activity.

Class VI includes mutations that cause reduced stability of CFTR at the membrane. Several approaches have been suggested based on specific mechanisms that regulate CFTR membrane anchoring (see Sect. 1.3.2) and are amenable to be used therapeutically—these include Rac1 activation with Hepatocyte Growth Factor (HGF) (Moniz et al. 2013), inhibition of Spleen Tyrosine Kinase (SYK) (Luz et al. 2011; Mendes et al. 2011), a strategy that is under trial for different pathologies, and possibly also additional pathways [such as LMTK2 inhibition (Luz et al. 2014)].

Finally, class VII mutations cannot be pharmacologically rescued—due to the absence of CFTR (as it happens in large deletions). The only CFTR-based approach possible would be gene therapy or an alternative strategy (independent of the mutation class and usually called a bypass approach) (De Boeck and Amaral 2016).

1.5.2 CFTR-Independent Approaches

Correction of the CF-characteristic defect has been also attempted by modulation of the quantity and function of other ion channels that are regulated by or may replace CFTR. As stated above (see Sect. 1.4.2), CFTR regulates several other channels and transporters and thus ion transport in epithelia (Kunzelmann and Schreiber 1999).

Modulation of ENaC activity is a major therapeutic target in CF. Additionally to modulation of ENaC, recent focus is also being given to alternative Cl^- channels, particularly anoctamins (ANO1 and probably other family members) and SLC26A9. These alternative strategies of targeting other membrane transporters are supported by evidence showing that the presence of CFTR (even the mutant protein) at the membrane seems to be enough to affect the activity of these other proteins (Suaud et al. 2007) (reviewed in De Boeck and Amaral 2016; Farinha and Matos 2016).

1.6 Conclusion

An impressive amount of work in the last decades has increased our knowledge on all the basic mechanisms related to this lethal recessive disorder—the structure and expression of the gene, the complex structure of the protein, its biogenesis, processing, trafficking and membrane stability, and its several functions. Understanding the molecular basis of the disease paved the way to the introduction of therapeutic approaches targeting the basic defects that are improving the quality of life of an increasing number of patients with CF. Hopefully, this will continue to flourish to the day that there is a cure for every patient with this disease.

References

Abbattiscianni AC, Favia M, Mancini MT, Cardone RA, Guerra L, Monterisi S, Castellani S, Laselva O, Di Sole F, Conese M, Zaccolo M, Casavola V (2016) Correctors of mutant CFTR enhance subcortical cAMP-PKA signaling through modulating ezrin phosphorylation and cytoskeleton organization. J Cell Sci 129(6):1128–1140. doi:10.1242/jcs.177907

Ahner A, Gong X, Schmidt BZ, Peters KW, Rabeh WM, Thibodeau PH, Lukacs GL, Frizzell RA (2013) Small heat shock proteins target mutant cystic fibrosis transmembrane conductance regulator for degradation via a small ubiquitin-like modifier-dependent pathway. Mol Biol Cell 24(2):74–84. doi:10.1091/mbc.E12-09-0678

Aleksandrov AA, Kota P, Cui L, Jensen T, Alekseev AE, Reyes S, He L, Gentzsch M, Aleksandrov LA, Dokholyan NV, Riordan JR (2012) Allosteric modulation balances thermodynamic stability and restores function of ΔF508 CFTR. J Mol Biol 419(1–2):41–60. doi:10.1016/j.jmb.2012.03.001

Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G (2009) Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science 323(5922):1718–1722. doi:10.1126/science.1168750

Alton EW, Armstrong DK, Ashby D, Bayfield KJ, Bilton D, Bloomfield EV, Boyd AC, Brand J, Buchan R, Calcedo R, Carvelli P, Chan M, Cheng SH, Collie DD, Cunningham S, Davidson HE, Davies G, Davies JC, Davies LA, Dewar MH, Doherty A, Donovan J, Dwyer NS, Elgmati HI, Featherstone RF, Gavino J, Gea-Sorli S, Geddes DM, Gibson JS, Gill DR, Greening AP, Griesenbach U, Hansell DM, Harman K, Higgins TE, Hodges SL, Hyde SC, Hyndman L, Innes JA, Jacob J, Jones N, Keogh BF, Limberis MP, Lloyd-Evans P, Maclean AW, Manvell MC, McCormick D, McGovern M, McLachlan G, Meng C, Montero MA, Milligan H, Moyce LJ, Murray GD, Nicholson AG, Osadolor T, Parra-Leiton J, Porteous DJ, Pringle IA, Punch EK, Pytel KM, Quittner AL, Rivellini G, Saunders CJ, Scheule RK, Sheard S, Simmonds NJ, Smith K, Smith SN, Soussi N, Soussi S, Spearing EJ, Stevenson BJ, Sumner-Jones SG, Turkkila M, Ureta RP, Waller MD, Wasowicz MY, Wilson JM, Wolstenholme-Hogg P, Consortium UKCFGT (2015) Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. doi:10.1016/S2213-2600(15)00245-3

Amaral MD (2015) Novel personalized therapies for cystic fibrosis: treating the basic defect in all patients. J Intern Med 277(2):155–166. doi:10.1111/joim.12314

Amaral MD, Farinha CM (2013) Rescuing mutant CFTR: a multi-task approach to a better outcome in treating cystic fibrosis. Curr Pharm Des 19(19):3497–3508

Amaral MD, Farinha CM, Matos P, Botelho HM (2016) Investigating alternative transport of integral plasma membrane proteins from the ER to the golgi: lessons from the cystic fibrosis transmembrane conductance regulator (CFTR). Methods Mol Biol 1459:105–126. doi:10.1007/978-1-4939-3804-9_7

Ameen N, Silvis M, Bradbury NA (2007) Endocytic trafficking of CFTR in health and disease. J Cyst Fibr 6(1):1–14. doi:10.1016/j.jcf.2006.09.002

Antonarakis SE (1998) Recommendations for a nomenclature system for human gene mutations. Nomenclature working group. Hum Mutat 11(1):1–3. doi:10.1002/(SICI)1098-1004(1998) 11:1

Awatade NT, Uliyakina I, Farinha CM, Clarke LA, Mendes K, Sole A, Pastor J, Ramos MM, Amaral MD (2015) Measurements of Functional responses in human primary lung cells as a basis for personalized therapy for cystic fibrosis. EBioMedicine 2(2):147–153. doi:10.1016/j.ebiom.2014.12.005

Bannykh SI, Bannykh GI, Fish KN, Moyer BD, Riordan JR, Balch WE (2000) Traffic pattern of cystic fibrosis transmembrane regulator through the early exocytic pathway. Traffic 1(11):852–870

Barasch J, Kiss B, Prince A, Saiman L, Gruenert D, al-Awqati Q (1991) Defective acidification of intracellular organelles in cystic fibrosis. Nature 352(6330):70–73

Bear CE, Li CH, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, Riordan JR (1992) Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell 68:809–818

Bergeron JJ, Brenner MB, Thomas DY, Williams DB (1994) Calnexin: a membrane-bound chaperone of the endoplasmic reticulum. Trends Biochem Sci 19(3):124–128

Blackledge NP, Ott CJ, Gillen AE, Harris A (2009) An insulator element 3' to the CFTR gene binds CTCF and reveals an active chromatin hub in primary cells. Nucleic Acids Res 37 (4):1086–1094. doi:10.1093/nar/gkn1056

Boucher RC, Stutts MJ, Knowles MR, Cantley L, Gatzy JT (1986) Na+ transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. J Clin Invest 78:1245–1252

Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E, Huang X, Waltz D, Patel NR, Rodman D (2014) A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. Lancet Respir Med 2(7):527–538. doi:10.1016/S2213-2600(14)70132-8

Bozoky Z, Krzeminski M, Muhandiram R, Birtley JR, Al-Zahrani A, Thomas PJ, Frizzell RA, Ford RC, Forman-Kay JD (2013) Regulatory R region of the CFTR chloride channel is a dynamic integrator of phospho-dependent intra- and intermolecular interactions. Proc Natl Acad Sci U S A 110(47):E4427–E4436. doi:10.1073/pnas.1315104110

Bradbury NA, Jilling T, Berta G, Sorscher EJ, Bridges RJ, Kirk KL (1992) Regulation of plasma membrane recycling by CFTR. Science 256(5056):530–532

Broackes-Carter FC, Mouchel N, Gill D, Hyde S, Bassett J, Harris A (2002) Temporal regulation of CFTR expression during ovine lung development: implications for CF gene therapy. Hum Mol Genet 11(2):125–131

Brown CR, Hong-Brown LQ, Biwersi J, Verkman AS, Welch WJ (1996) Chemical chaperones correct the mutant phenotype of the ΔF508 cystic fibrosis transmembrane conductance regulator protein. Cell Stress Chaperones 1(2):117–125

Cabral CM, Liu Y, Sifers RN (2001) Dissecting glycoprotein quality control in the secretory pathway. Trends Biochem Sci 26(10):619–624

Carveth K, Buck T, Anthony V, Skach WR (2002) Cooperativity and flexibility of cystic fibrosis transmembrane conductance regulator transmembrane segments participate in membrane localization of a charged residue. J Biol Chem 277(42):39507–39514. doi:10.1074/jbc.M205759200

Chang XB, Hou YX, Riordan JR (1997) ATPase activity of purified multidrug resistance-associated protein [published erratum appears in J Biol Chem 1998 Mar 27; 273(13):7782]. J Biol Chem 272:30962–30968

Chang X, Cui L, Hou Y, Jensen TJ, Aleksandrov AA, Mengos A, Riordan JR (1999) Removal of multiple arginine-framed trafficking signals overcomes misprocessing of ΔF508 CFTR present in most patients with cystic fibrosis. Mol Cell 4(1):137–142

Chen EY, Yang N, Quinton PM, Chin WC (2010) A new role for bicarbonate in mucus formation. Am J Physiol Lung Cell Mol Physiol 299(4):L542–L549. doi:10.1152/ajplung.00180.2010

Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, Smith AE (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63(4):827–834

Cheng J, Wang H, Guggino WB (2004) Modulation of mature cystic fibrosis transmembrane regulator protein by the PDZ domain protein CAL. J Biol Chem 279(3):1892–1898. doi:10.1074/jbc.M308640200

Chu CS, Trapnell BC, Curristin SM, Cutting GR, Crystal RG (1992) Extensive posttranscriptional deletion of the coding sequences for part of nucleotide-binding fold 1 in respiratory epithelial mRNA transcripts of the cystic fibrosis transmembrane conductance regulator gene is not associated with the clinical manifestations of cystic fibrosis. J Clin Invest 90(3):785–790

Ciechanover A (1998) The ubiquitin-proteasome pathway: on protein death and cell life. EMBO J 17(24):7151–7160. doi:10.1093/emboj/17.24.7151

Cihil KM, Ellinger P, Fellows A, Stolz DB, Madden DR, Swiatecka-Urban A (2012) Disabled-2 protein facilitates assembly polypeptide-2-independent recruitment of cystic fibrosis transmembrane conductance regulator to endocytic vesicles in polarized human airway epithelial cells. J Biol Chem 287(18):15087–15099. doi:10.1074/jbc.M112.341875

Clancy JP, Bebok Z, Ruiz F, King C, Jones J, Walker L, Greer H, Hong J, Wing L, Macaluso M, Lyrene R, Sorscher EJ, Bedwell DM (2001) Evidence that systemic gentamicin suppresses premature stop mutations in patients with cystic fibrosis. Am J Respir Crit Care Med 163 (7):1683–1692

Clancy JP, Rowe SM, Accurso FJ, Aitken ML, Amin RS, Ashlock MA, Ballmann M, Boyle MP, Bronsveld I, Campbell PW, Deboeck K, Donaldson SH, Dorkin HL, Dunitz JM, Durie PR, Jain M, Leonard A, McCoy KS, Moss RB, Pilewski JM, Rosenbluth DB, Rubenstein RC, Schechter MS, Botfield M, Ordonez CL, Spencer-Green GT, Vernillet L, Wisseh S, Yen K, Konstan MW (2011) Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. Thorax. doi:10.1136/thoraxjnl-2011-200393

Clarke LA, Botelho HM, Sousa L, Falcao AO, Amaral MD (2015) Transcriptome meta-analysis reveals common differential and global gene expression profiles in cystic fibrosis and other respiratory disorders and identifies CFTR regulators. Genomics 106(5):268–277. doi:10.1016/j.ygeno.2015.07.005

Coakley RD, Grubb BR, Paradiso AM, Gatzy JT, Johnson LG, Kreda SM, O'Neal WK, Boucher RC (2003) Abnormal surface liquid pH regulation by cultured cystic fibrosis bronchial epithelium. Proc Natl Acad Sci U S A 100(26):16083–16088. doi:10.1073/pnas.2634339100

Collaco A, Jakab R, Hegan P, Mooseker M, Ameen N (2010) Alpha-AP-2 directs myosin VI-dependent endocytosis of cystic fibrosis transmembrane conductance regulator chloride channels in the intestine. J Biol Chem 285(22):17177–17187. doi:10.1074/jbc.M110.127613

Collins FS (1992) Cystic fibrosis: molecular biology and therapeutic implications. Science 256:774–779

Coppinger JA, Hutt DM, Razvi A, Koulov AV, Pankow S, Yates JR 3rd, Balch WE (2012) A chaperone trap contributes to the onset of cystic fibrosis. PLoS One 7(5):e37682. doi:10.1371/journal.pone.0037682

Crawford I, Maloney PC, Zeitlin PL, Guggino WB, Hyde SC, Turley H, Gatter KC, Harris A, Higgins CF (1991) Immunocytochemical localization of the cystic fibrosis gene product CFTR. Proc Natl Acad Sci U S A 88(20):9262–9266

Cushing PR, Fellows A, Villone D, Boisguerin P, Madden DR (2008) The relative binding affinities of PDZ partners for CFTR: a biochemical basis for efficient endocytic recycling. Biochemistry 47(38):10084–10098. doi:10.1021/bi8003928

Cutting GR (2015) Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet 16(1):45–56. doi:10.1038/nrg3849

Cyr DM, Langer T, Douglas MG (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. Trends Biochem Sci 19(4):176–181

Dahan D, Evagelidis A, Hanrahan JW, Hinkson DA, Jia Y, Luo J, Zhu T (2001) Regulation of the CFTR channel by phosphorylation. Pflugers Arch 443(Suppl 1):S92–S96. doi:10.1007/s004240100652

Dalemans W, Hinnrasky J, Slos P, Dreyer D, Fuchey C, Pavirani A, Puchelle E (1992) Immunocytochemical analysis reveals differences between the subcellular localization of normal and ΔPhe508 recombinant cystic fibrosis transmembrane conductance regulator. Exp Cell Res 201:235–240

Dawson RJ, Locher KP (2006) Structure of a bacterial multidrug ABC transporter. Nature 443 (7108):180–185

De Boeck K, Amaral MD (2016) Progress in therapies for cystic fibrosis. Lancet Respir Med 4 (8):662–674. doi:10.1016/S2213-2600(16)00023-0

Dekkers JF, Berkers G, Kruisselbrink E, Vonk A, de Jonge HR, Janssens HM, Bronsveld I, van de Graaf EA, Nieuwenhuis EE, Houwen RH, Vleggaar FP, Escher JC, de Rijke YB, Majoor CJ, Heijerman HG, de Winter-de Groot KM, Clevers H, van der Ent CK, Beekman JM (2016)

Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. Science Transl Med 8(344):344ra384. doi:10.1126/scitranslmed.aad8278

Denning GM, Anderson MP, Amara JF, Marshall J, Smith AE, Welsh MJ (1992) Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. Nature 358(6389):761–764

Devidas S, Guggino WB (1997) CFTR: domains, structure, and function. J Bioenerg Biomembr 29:443–451

Drumm ML, Pope HA, Cliff WH, Rommens JM, Marvin SA, Tsui LC, Collins FS, Frizzell RA, Wilson JM (1990) Correction of the cystic fibrosis defect in vitro by retrovirus- mediated gene transfer [published erratum appears in Cell 1993 Jun 16;74(1):215]. Cell 62:1227–1233

Drumm ML, Wilkinson DJ, Smit LS, Worrell RT, Strong TV, Frizzell RA, Dawson DC, Collins FS (1991) Chloride conductance expressed by ΔF508 and other mutant CFTRs in Xenopus oocytes. Science 254:1797–1799

Dupuit F, Kalin N, Brezillon S, Hinnrasky J, TÅmmler B, Puchelle E (1995) CFTR and differentiation markers expression in non-CF and ΔF508 homozygous CF nasal epithelium. J Clin Invest 96:1601–1611

Ellsworth RE, Jamison DC, Touchman JW, Chissoe SL, Braden MV, Bouffard GG, Dietrich NL, Beckstrom-Sternberg SM, Iyer LM, Weintraub LA, Cotton M, Courtney L, Edwards J, Maupin R, Ozersky P, Rohlfing T, Wohldmann P, Miner T, Kemp K, Kramer J, Korf I, Pepin K, Antonacci-Fulton L, Fulton RS, Green D (2000) Comparative genomic sequence analysis of the human and mouse cystic fibrosis transmembrane conductance regulator genes. Proc Natl Acad Sci U S A 97(3):1172–1177

Engelhardt JF, Yankaskas JR, Ernst SA, Yang Y, Marino CR, Boucher RC, Cohn JA, Wilson JM (1992) Submucosal glands are the predominant site of CFTR expression in the human bronchus. Nat Genet 2:240–248

Engelhardt JF, Zepeda M, Cohn JA, Yankaskas JR, Wilson JM (1994) Expression of the cystic fibrosis gene in adult human lung. J Clin Invest 93:737–749

Enquist K, Fransson M, Boekel C, Bengtsson I, Geiger K, Lang L, Pettersson A, Johansson S, von Heijne G, Nilsson I (2009) Membrane-integration characteristics of two ABC transporters, CFTR and P-glycoprotein. J Mol Biol 387(5):1153–1164. doi:10.1016/j.jmb.2009.02.035

Fanning AS, Anderson JM (1996) Protein-protein interactions: PDZ domain networks. Curr Biol 6:1385–1388

Fanning AS, Anderson JM (1998) PDZ domains and the formation of protein networks at the plasma membrane. Curr Top Microbiol Immunol 228:209–233

Farinha CM, Amaral MD (2005) Most F508del-CFTR is targeted to degradation at an early folding checkpoint and independently of calnexin. Mol Cell Biol 25(12):5242–5252. doi:10.1128/MCB.25.12.5242-5252.2005

Farinha CM, Canato S (2017) From the endoplasmic reticulum to the plasma membrane: mechanisms of CFTR folding and trafficking. Cell Mol Life Sci 74(1):39–55. doi:10.1007/s00018-016-2387-7

Farinha CM, Matos P (2016) Repairing the basic defect in cystic fibrosis—one approach is not enough. FEBS J 283(2):246–264. doi:10.1111/febs.13531

Farinha CM, Nogueira P, Mendes F, Penque D, Amaral MD (2002) The human DnaJ homologue (Hdj)-1/heat-shock protein (Hsp) 40 co-chaperone is required for the in vivo stabilization of the cystic fibrosis transmembrane conductance regulator by Hsp70. Biochem J 366(Pt 3):797–806

Farinha CM, King-Underwood J, Sousa M, Correia AR, Henriques BJ, Roxo-Rosa M, Da Paula AC, Williams J, Hirst S, Gomes CM, Amaral MD (2013a) Revertants, low temperature, and correctors reveal the mechanism of F508del-CFTR rescue by VX-809 and suggest multiple agents for full correction. Chem Biol 20(7):943–955. doi:10.1016/j.chembiol.2013.06.004

Farinha CM, Matos P, Amaral MD (2013b) Control of CFTR membrane trafficking: not just from the ER to the golgi. FEBS J 280(18):4396–4406. doi:10.1111/febs.12392

Farinha CM, Swiatecka-Urban A, Brautigan DL, Jordan P (2016) Regulatory crosstalk by protein kinases on CFTR trafficking and activity. Front Chem 4:1. doi:10.3389/fchem.2016.00001

Favia M, Guerra L, Fanelli T, Cardone RA, Monterisi S, Di Sole F, Castellani S, Chen M, Seidler U, Reshkin SJ, Conese M, Casavola V (2010) Na+/H+ exchanger regulatory factor 1 overexpression-dependent increase of cytoskeleton organization is fundamental in the rescue of F508del cystic fibrosis transmembrane conductance regulator in human airway CFBE41o- cells. Mol Biol Cell 21(1):73–86. doi:10.1091/mbc.E09-03-0185

Ferrie RM, Schwarz MJ, Robertson NH, Vaudin S, Super M, Malone G, Little S (1992) Development, multiplexing, and application of ARMS tests for common mutations in the CFTR gene. Am J Hum Genet 51(2):251–262

Freeman BC, Morimoto RI (1996) The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. EMBO J 15(12):2969–2979

Fu L, Rab A, Tang LP, Rowe SM, Bebok Z, Collawn JF (2012) Dab2 is a key regulator of endocytosis and post-endocytic trafficking of the cystic fibrosis transmembrane conductance regulator. Biochem J 441(2):633–643. doi:10.1042/BJ20111566

Furukawa-Hagiya T, Furuta T, Chiba S, Sohma Y, Sakurai M (2013) The power stroke driven by ATP binding in CFTR as studied by molecular dynamics simulations. J Phys Chem B 117 (1):83–93. doi:10.1021/jp308315w

Ganeshan R, Nowotarski K, Di A, Nelson DJ, Kirk KL (2007) CFTR surface expression and chloride currents are decreased by inhibitors of N-WASP and actin polymerization. Biochim Biophys Acta 1773(2):192–200. doi:10.1016/j.bbamcr.2006.09.031

Garcia MA, Yang N, Quinton PM (2009) Normal mouse intestinal mucus release requires cystic fibrosis transmembrane regulator-dependent bicarbonate secretion. J Clin Invest 119(9): 2613–2622. doi:10.1172/JCI38662

Gee HY, Noh SH, Tang BL, Kim KH, Lee MG (2011) Rescue of ΔF508-CFTR trafficking via a GRASP-dependent unconventional secretion pathway. Cell 146(5):746–760. doi:10.1016/j.cell.2011.07.021

Gentzsch M, Chang XB, Cui L, Wu Y, Ozols VV, Choudhury A, Pagano RE, Riordan JR (2004) Endocytic trafficking routes of wild type and ΔF508 cystic fibrosis transmembrane conductance regulator. Mol Biol Cell 15(6):2684–2696. doi:10.1091/mbc.E04-03-0176 [pii]

Gong X, Ahner A, Roldan A, Lukacs GL, Thibodeau PH, Frizzell RA (2016) Non-native conformers of cystic fibrosis transmembrane conductance regulator NBD1 are recognized by Hsp27 and conjugated to SUMO-2 for degradation. J Biol Chem 291(4):2004–2017. doi:10.1074/jbc.M115.685628

Grieve AG, Rabouille C (2011) Golgi bypass: skirting around the heart of classical secretion. Cold Spring Harb Perspect Biol 3(4). doi:10.1101/cshperspect.a005298

Guggino WB, Stanton BA (2006) New insights into cystic fibrosis: molecular switches that regulate CFTR. Nat Rev Mol Cell Biol 7(6):426–436. doi:10.1038/nrm1949

Haggie PM, Verkman AS (2008) Monomeric CFTR in plasma membranes in live cells revealed by single molecule fluorescence imaging. J Biol Chem 283(35):23510–23513. doi:10.1074/jbc.C800100200

Haggie PM, Kim JK, Lukacs GL, Verkman AS (2006) Tracking of quantum dot-labeled CFTR shows near immobilization by C-terminal PDZ interactions. Mol Biol Cell 17(12):4937–4945. doi:10.1091/mbc.E06-08-0670

Hammond C, Helenius A (1995) Quality control in the secretory pathway. Curr Opin Cell Biol 7 (4):523–529

He L, Aleksandrov LA, Cui L, Jensen TJ, Nesbitt KL, Riordan JR (2010) Restoration of domain folding and interdomain assembly by second-site suppressors of the ΔF508 mutation in CFTR. FASEB J 24(8):3103–3112. doi:10.1096/fj.09-141788

He L, Kota P, Aleksandrov AA, Cui L, Jensen T, Dokholyan NV, Riordan JR (2013) Correctors of ΔF508 CFTR restore global conformational maturation without thermally stabilizing the mutant protein. FASEB J 27(2):536–545. doi:10.1096/fj.12-216119

Hebert DN, Foellmer B, Helenius A (1995) Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. Cell 81(3):425–433

Heda GD, Tanwani M, Marino CR (2001) The ΔF508 mutation shortens the biochemical half-life of plasma membrane CFTR in polarized epithelial cells. Am J Physiol Cell Physiol 280(1): C166–C174

Higgins CF (1992a) ABC transporters: from microorganisms to man. Annu Rev Cell Biol 8: 67–113

Higgins CF (1992b) Cystic fibrosis transmembrane conductance regulator (CFTR). Br Med Bull 48:754–765

Hohl M, Briand C, Grutter MG, Seeger MA (2012) Crystal structure of a heterodimeric ABC transporter in its inward-facing conformation. Nat Struct Mol Biol 19(4):395–402. doi:10.1038/nsmb.2267

Hollenstein K, Frei DC, Locher KP (2007) Structure of an ABC transporter in complex with its binding protein. Nature 446(7132):213–216

Hu W, Howard M, Lukacs GL (2001) Multiple endocytic signals in the C-terminal tail of the cystic fibrosis transmembrane conductance regulator. Biochem J 354(Pt 3):561–572

Hug MJ, Tamada T, Bridges RJ (2003) CFTR and bicarbonate secretion by epithelial cells. News Physiol Sci 18:38–42

Hwang TC, Sheppard DN (1999) Molecular pharmacology of the CFTR Cl- channel. Trends Pharmacol Sci 20(11):448–453

Hwang TC, Sheppard DN (2009) Gating of the CFTR Cl- channel by ATP-driven nucleotide-binding domain dimerisation. J Physiol 587(Pt 10):2151–2161. doi:10.1113/jphysiol.2009.171595

Igreja S, Clarke LA, Botelho HM, Marques L, Amaral MD (2016) Correction of a cystic fibrosis splicing mutation by antisense oligonucleotides. Hum Mutat 37(2):209–215. doi:10.1002/humu.22931

Illek B, Zhang L, Lewis NC, Moss RB, Dong JY, Fischer H (1999) Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein. Am J Physiol 277 (4 Pt 1):C833–C839

Jensen TJ, Loo MA, Pind S, Williams DB, Goldberg AL, Riordan JR (1995) Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. Cell 83:129–135

Jiang C, Fang SL, Xiao YF, O'Connor SP, Nadler SG, Lee DW, Jefferson DM, Kaplan JM, Smith AE, Cheng SH (1998) Partial restoration of cAMP-stimulated CFTR chloride channel activity in ΔF508 cells by deoxyspergualin. Am J Physiol 275(1 Pt 1):C171–C178

Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, Patterson C (2001) CHIP is a U-box-dependent E3 ubiquitin ligase: Identification of Hsc70 as a target for ubiquitylation. J Biol Chem 276(46):42938–42944

Jin MS, Oldham ML, Zhang Q, Chen J (2012) Crystal structure of the multidrug transporter P-glycoprotein from Caenorhabditis elegans. Nature 490(7421):566–569. doi:10.1038/nature11448

Johnson ZL, Chen J (2017) Structural basis of substrate recognition by the multidrug resistance protein MRP1. Cell 168(6):1075–1085. doi:10.1016/j.cell.2017.01.041 (e1079)

Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. J Cell Biol 143(7):1883–1898

Kalin N, Claass A, Sommer M, Puchelle E, Tummler B (1999) ΔF508 CFTR protein expression in tissues from patients with cystic fibrosis. J Clin Invest 103(10):1379–1389

Kanelis V, Hudson RP, Thibodeau PH, Thomas PJ, Forman-Kay JD (2010) NMR evidence for differential phosphorylation-dependent interactions in WT and ΔF508 CFTR. EMBO J 29 (1):263–277. doi:10.1038/emboj.2009.329

Kartner N, Augustinas O, Jensen TJ, Naismith AL, Riordan JR (1992) Mislocalization of ΔF508 CFTR in cystic fibrosis sweat gland. Nat Genet 1:321–327

Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245: 1073–1080

Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, Elborn JS, Melotti P, Bronsveld I, Fajac I, Malfroot A, Rosenbluth DB, Walker PA, McColley SA,

Knoop C, Quattrucci S, Rietschel E, Zeitlin PL, Barth J, Elfring GL, Welch EM, Branstrom A, Spiegel RJ, Peltz SW, Ajayi T, Rowe SM, Cystic Fibrosis Ataluren Study G (2014) Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Respir Med 2(7):539–547. doi:10.1016/S2213-2600(14)70100-6

Kim SJ, Skach WR (2012) Mechanisms of CFTR folding at the Endoplasmic Reticulum. Front Pharmacol 3:201. doi:10.3389/fphar.2012.00201

Kim J, Noh SH, Piao H, Kim DH, Kim K, Cha JS, Chung WY, Cho HS, Kim JY, Lee MG (2016) Monomerization and ER relocalization of GRASP Is a requisite for unconventional secretion of CFTR. Traffic 17(7):733–753. doi:10.1111/tra.12403

Kimmins S, MacRae TH (2000) Maturation of steroid receptors: an example of functional cooperation among molecular chaperones and their associated proteins. Cell Stress Chaperones 5(2):76–86

Knowles MR, Stutts MJ, Spock A, Fischer N, Gatzy JT, Boucher RC (1983) Abnormal ion permeation through cystic fibrosis respiratory epithelium. Science 221:1067–1070

Kodan A, Yamaguchi T, Nakatsu T, Sakiyama K, Hipolito CJ, Fujioka A, Hirokane R, Ikeguchi K, Watanabe B, Hiratake J, Kimura Y, Suga H, Ueda K, Kato H (2014) Structural basis for gating mechanisms of a eukaryotic P-glycoprotein homolog. Proc Natl Acad Sci U S A 111(11):4049–4054. doi:10.1073/pnas.1321562111

Kogan I, Ramjeesingh M, Li C, Kidd JF, Wang Y, Leslie EM, Cole SP, Bear CE (2003) CFTR directly mediates nucleotide-regulated glutathione flux. EMBO J. 22(9):1981–1989

Koh J, Sferra TJ, Collins FS (1993) Characterization of the cystic fibrosis transmembrane conductance regulator promoter region. Chromatin context and tissue-specificity. J Biol Chem 268:15912–15921

Kreda SM, Mall M, Mengos A, Rochelle L, Yankaskas J, Riordan JR, Boucher RC (2005) Characterization of wild-type and ΔF508 cystic fibrosis transmembrane regulator in human respiratory epithelia. Mol Biol Cell 16(5):2154–2167. doi:10.1091/mbc.E04-11-1010

Kreda SM, Okada SF, van Heusden CA, O'Neal W, Gabriel S, Abdullah L, Davis CW, Boucher RC, Lazarowski ER (2007) Coordinated release of nucleotides and mucin from human airway epithelial Calu-3 cells. J Physiol 584(Pt 1):245–259. doi:10.1113/jphysiol.2007.139840

Kunzelmann K, Mall M (2001) Pharmacotherapy of the ion transport defect in cystic fibrosis. Clin Exp Pharmacol Physiol 28(11):857–867

Kunzelmann K, Mehta A (2013) CFTR: a hub for kinases and cross-talk of cAMP and Ca. FEBS J. 280(18):4417–4429. doi:10.1111/febs.12457

Kunzelmann K, Schreiber R (1999) CFTR, a regulator of channels. J Membr Biol 168(1):1–8

Kunzelmann K, Schreiber R, Boucherot A (2001) Mechanisms of the inhibition of epithelial Na(+) channels by CFTR and purinergic stimulation. Kidney Int 60(2):455–461. doi:10.1046/j.1523-1755.2001.060002455.x

Kuver R, Wong T, Klinkspoor JH, Lee SP (2006) Absence of CFTR is associated with pleiotropic effects on mucins in mouse gallbladder epithelial cells. Am J Physiol Gastrointest Liver Physiol 291(6):G1148–G1154. doi:10.1152/ajpgi.00547.2005

Lehrich RW, Aller SG, Webster P, Marino CR, Forrest JN Jr (1998) Vasoactive intestinal peptide, forskolin, and genistein increase apical CFTR trafficking in the rectal gland of the spiny dogfish, Squalus acanthias. Acute regulation of CFTR trafficking in an intact epithelium. J Clin Invest 101(4):737–745

Leigh MW, Kylander JE, Yankaskas JR, Boucher RC (1995) Cell proliferation in bronchial epithelium and submucosal glands of cystic fibrosis patients. Am J Respir Cell Mol Biol 12:605–612

Lewandowska MA, Costa FF, Bischof JM, Williams SH, Soares MB, Harris A (2010) Multiple mechanisms influence regulation of the cystic fibrosis transmembrane conductance regulator gene promoter. Am J Respir Cell Mol Biol 43(3):334–341. doi:10.1165/rcmb.2009-0149OC

Lewis HA, Buchanan SG, Burley SK, Conners K, Dickey M, Dorwart M, Fowler R, Gao X, Guggino WB, Hendrickson WA, Hunt JF, Kearins MC, Lorimer D, Maloney PC, Post KW, Rajashankar KR, Rutter ME, Sauder JM, Shriver S, Thibodeau PH, Thomas PJ, Zhang M,

Zhao X, Emtage S (2004) Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator. EMBO J 23(2):282–293

Lewis HA, Zhao X, Wang C, Sauder JM, Rooney I, Noland BW, Lorimer D, Kearins MC, Conners K, Condon B, Maloney PC, Guggino WB, Hunt JF, Emtage S (2005) Impact of the ΔF508 mutation in first nucleotide-binding domain of human cystic fibrosis transmembrane conductance regulator on domain folding and structure. J Biol Chem 280(2):1346–1353. doi:10.1074/jbc.M410968200

Li C, Ramjeesingh M, Reyes E, Jensen T, Chang X, Rommens JM, Bear CE (1993) The cystic fibrosis mutation (ΔF508) does not influence the chloride channel activity of CFTR. Nat Genet 3(4):311–316

Linde L, Boelz S, Nissim-Rafinia M, Oren YS, Wilschanski M, Yaacov Y, Virgilis D, Neu-Yilik G, Kulozik AE, Kerem E, Kerem B (2007) Nonsense-mediated mRNA decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin. J Clin Invest 117(3):683–692. doi:10.1172/JCI28523

Linsdell P, Hanrahan JW (1998) Glutathione permeability of CFTR. Am J Physiol. 275:C323–6

Liu F, Zhang Z, Csanady L, Gadsby DC, Chen J (2017) Molecular structure of the human CFTR Ion channel. Cell 169(1):85–95 e88. doi:10.1016/j.cell.2017.02.024

Lobo MJ, Amaral MD, Zaccolo M, Farinha CM (2016) EPAC1 activation by cAMP stabilizes CFTR at the membrane by promoting its interaction with NHERF1. J Cell Sci 129(13): 2599–2612. doi:10.1242/jcs.185629

Locher KP, Lee AT, Rees DC (2002) The E. coli BtuCD structure: a framework for ABC transporter architecture and mechanism. Science 296(5570):1091–1098. doi:10.1126/science.1071142

Loo MA, Jensen TJ, Cui L, Hou Y, Chang XB, Riordan JR (1998) Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome. EMBO J 17(23):6879–6887

Loureiro CA, Matos AM, Dias-Alves A, Pereira JF, Uliyakina I, Barros P, Amaral MD, Matos P (2015) A molecular switch in the scaffold NHERF1 enables misfolded CFTR to evade the peripheral quality control checkpoint. Sci Signal 8(377):ra48. doi:10.1126/scisignal.aaa1580

Lu Y, Xiong X, Helm A, Kimani K, Bragin A, Skach WR (1998) Co- and posttranslational translocation mechanisms direct cystic fibrosis transmembrane conductance regulator N terminus transmembrane assembly. J Biol Chem 273(1):568–576

Lukacs GL, Chang XB, Kartner N, Rotstein OD, Riordan JR, Grinstein S (1992) The cystic fibrosis transmembrane regulator is present and functional in endosomes. Role as a determinant of endosomal pH. J Biol Chem 267(21):14568–14572

Lukacs GL, Chang XB, Bear C, Kartner N, Mohamed A, Riordan JR, Grinstein S (1993) The ΔF508 mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells. J Biol Chem 268(29):21592–21598

Lukacs GL, Mohamed A, Kartner N, Chang XB, Riordan JR, Grinstein S (1994) Conformational maturation of CFTR but not its mutant counterpart (ΔF508) occurs in the endoplasmic reticulum and requires ATP. EMBO J 13(24):6076–6086

Luz S, Kongsuphol P, Mendes AI, Romeiras F, Sousa M, Schreiber R, Matos P, Jordan P, Mehta A, Amaral MD, Kunzelmann K, Farinha CM (2011) Contribution of casein kinase 2 and spleen tyrosine kinase to CFTR trafficking and protein kinase A-induced activity. Molecular and cellular biology 31(22):4392–4404. doi:10.1128/MCB.05517-11

Luz S, Cihil KM, Brautigan DL, Amaral MD, Farinha CM, Swiatecka-Urban A (2014) LMTK2 mediated phosphorylation regulates CFTR endocytosis in human airway epithelial cells. J Biol Chem 289(21):15080–15093. doi:10.1074/jbc.M114.563742

Mall M, Wissner A, Schreiber R, Kuehr J, Seydewitz HH, Brandis M, Greger R, Kunzelmann K (2000) Role of K(V)LQT1 in cyclic adenosine monophosphate-mediated Cl(-) secretion in human airway epithelia. Am J Respir Cell Mol Biol 23(3):283–289

McNicholas CM, Nason MW Jr, Guggino WB, Schwiebert EM, Hebert SC, Giebisch G, Egan ME (1997) A functional CFTR-NBF1 is required for ROMK2-CFTR interaction. Am J Physiol 273 (5 Pt 2):F843–F848

Meacham GC, Lu Z, King S, Sorscher E, Tousson A, Cyr DM (1999) The Hdj-2/Hsc70 chaperone pair facilitates early steps in CFTR biogenesis. EMBO J 18(6):1492–1505

Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nat Cell Biol 3(1):100–105

Mendes AI, Matos P, Moniz S, Luz S, Amaral MD, Farinha CM, Jordan P (2011) Antagonistic Regulation of CFTR cell surface expression by protein kinases WNK4 and spleen tyrosine kinase. Mol Cell Biol 31(19):4076–4086. doi:10.1128/MCB.05152-11

Mendes F, Farinha CM, Felicio V, Alves PC, Vieira I, Amaral MD (2012) BAG-1 stabilizes mutant F508del-CFTR in a ubiquitin-like-domain-dependent manner. Cell Physiol Biochem 30 (5):1120–1133. doi:10.1159/000343303

Mendoza JL, Schmidt A, Li Q, Nuvaga E, Barrett T, Bridges RJ, Feranchak AP, Brautigam CA, Thomas PJ (2012) Requirements for efficient correction of ΔF508 CFTR revealed by analyses of evolved sequences. Cell 148(1–2):164–174. doi:10.1016/j.cell.2011.11.023

Meyerholz DK, Stoltz DA, Namati E, Ramachandran S, Pezzulo AA, Smith AR, Rector MV, Suter MJ, Kao S, McLennan G, Tearney GJ, Zabner J, McCray PB Jr, Welsh MJ (2010) Loss of cystic fibrosis transmembrane conductance regulator function produces abnormalities in tracheal development in neonatal pigs and young children. Am J Respir Crit Care Med 182 (10):1251–1261. doi:10.1164/rccm.201004-0643OC

Miele L, Cordella-Miele E, Xing M, Frizzell R, Mukherjee AB (1997) Cystic fibrosis gene mutation (ΔF508) is associated with an intrinsic abnormality in Ca2+-induced arachidonic acid release by epithelial cells. DNA Cell Biol 16(6):749–759

Mogayzel PJ Jr, Ashlock MA (2000) CFTR intron 1 increases luciferase expression driven by CFTR 5'-flanking DNA in a yeast artificial chromosome. Genomics 64(2):211–215. doi:10.1006/geno.2000.6119

Mohapatra NK, Cheng PW, Parker JC, Paradiso AM, Yankaskas JR, Boucher RC, Boat TF (1995) Alteration of sulfation of glycoconjugates, but not sulfate transport and intracellular inorganic sulfate content in cystic fibrosis airway epithelial cells. Pediatr Res 38:42–48

Moniz S, Sousa M, Moraes BJ, Mendes AI, Palma M, Barreto C, Fragata JI, Amaral MD, Matos P (2013) HGF stimulation of Rac1 signaling enhances pharmacological correction of the most prevalent cystic fibrosis mutant F508del-CFTR. ACS Chem Biol 8(2):432–442. doi:10.1021/cb300484r

Mornon JP, Lehn P, Callebaut I (2008) Atomic model of human cystic fibrosis transmembrane conductance regulator: membrane-spanning domains and coupling interfaces. Cell Mol Life Sci 65(16):2594–2612. doi:10.1007/s00018-008-8249-1

Mornon JP, Hoffmann B, Jonic S, Lehn P, Callebaut I (2015) Full-open and closed CFTR channels, with lateral tunnels from the cytoplasm and an alternative position of the F508 region, as revealed by molecular dynamics. Cell Mol Life Sci 72(7):1377–1403. doi:10.1007/s00018-014-1749-2

Moskowitz SM, Chmiel JF, Sternen DL, Cheng E, Gibson RL, Marshall SG, Cutting GR (2008) Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. Genet Med 10(12):851–868. doi:10.1097/GIM.0b013e31818e55a2

Mouchel N, Broackes-Carter F, Harris A (2003) Alternative 5' exons of the CFTR gene show developmental regulation. Hum Mol Genet 12(7):759–769

Nishimura N, Balch WE (1997) A di-acidic signal required for selective export from the endoplasmic reticulum. Science 277(5325):556–558

Ohtsuka K, Hata M (2000) Molecular chaperone function of mammalian Hsp70 and Hsp40—a review. Int J Hyperthermia 16(3):231–245

Okiyoneda T, Barriere H, Bagdany M, Rabeh WM, Du K, Hohfeld J, Young JC, Lukacs GL (2010) Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science 329(5993):805–810. doi:10.1126/science.1191542

Ott CJ, Blackledge NP, Kerschner JL, Leir SH, Crawford GE, Cotton CU, Harris A (2009a) Intronic enhancers coordinate epithelial-specific looping of the active CFTR locus. Proc Natl Acad Sci U S A 106(47):19934–19939. doi:10.1073/pnas.0900946106

Ott CJ, Blackledge NP, Leir SH, Harris A (2009b) Novel regulatory mechanisms for the CFTR gene. Biochem Soc Trans 37(Pt 4):843–848. doi:10.1042/BST0370843

Ott CJ, Suszko M, Blackledge NP, Wright JE, Crawford GE, Harris A (2009c) A complex intronic enhancer regulates expression of the CFTR gene by direct interaction with the promoter. J Cell Mol Med 13(4):680–692

Ousingsawat J, Kongsuphol P, Schreiber R, Kunzelmann K (2011) CFTR and TMEM16A are separate but functionally related Cl- channels. Cell Physiol Biochem 28(4):715–724. doi:10.1159/000335765

Pankow S, Bamberger C, Calzolari D, Martinez-Bartolome S, Lavallee-Adam M, Balch WE, Yates JR 3rd (2015) F508 CFTR interactome remodelling promotes rescue of cystic fibrosis. Nature 528(7583):510–516. doi:10.1038/nature15729

Park HW, Nam JH, Kim JY, Namkung W, Yoon JS, Lee JS, Kim KS, Venglovecz V, Gray MA, Kim KH, Lee MG (2010) Dynamic regulation of CFTR bicarbonate permeability by [Cl-]i and its role in pancreatic bicarbonate secretion. Gastroenterology 139(2):620–631. doi:10.1053/j.gastro.2010.04.004

Pasyk S, Molinski S, Ahmadi S, Ramjeesingh M, Huan LJ, Chin S, Du K, Yeger H, Taylor P, Moran MF, Bear CE (2015) The major cystic fibrosis causing mutation exhibits defective propensity for phosphorylation. Proteomics 15(2–3):447–461. doi:10.1002/pmic.201400218

Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta LJ, Verkman AS (2005) Small-molecule correctors of defective ΔF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest 115(9):2564–2571. doi:10.1172/JCI24898

Penque D, Mendes F, Beck S, Farinha C, Pacheco P, Nogueira P, Lavinha J, Malhȩ R, Amaral MD (2000) Cystic fibrosis F508del patients have apically localized CFTR in a reduced number of airway cells. Lab Invest 80(6):857–868

Phylactides M, Rowntree R, Nuthall H, Ussery D, Wheeler A, Harris A (2002) Evaluation of potential regulatory elements identified as DNase I hypersensitive sites in the CFTR gene. Eur J Biochem 269(2):553–559

Pind S, Riordan JR, Williams DB (1994) Participation of the endoplasmic reticulum chaperone calnexin (p88, IP90) in the biogenesis of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 269:12784–12788

Poulsen JH, Fischer H, Illek B, Machen TE (1994) Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci U S A 91(12):5340–5344

Prince LS, Peter K, Hatton SR, Zaliauskiene L, Cotlin LF, Clancy JP, Marchase RB, Collawn JF (1999) Efficient endocytosis of the cystic fibrosis transmembrane conductance regulator requires a tyrosine-based signal. J Biol Chem 274(6):3602–3609

Puchelle E, Gaillard D, Ploton D, Hinnrasky J, Fuchey C, Boutterin MC, Jacquot J, Dreyer D, Pavirani A, Dalemans W (1992) Differential localization of the cystic fibrosis transmembrane conductance regulator in normal and cystic fibrosis airway epithelium. Am J Respir Cell Mol Biol 7:485–491

Qu BH, Strickland EH, Thomas PJ (1997) Localization and suppression of a kinetic defect in cystic fibrosis transmembrane conductance regulator folding. J Biol Chem 272(25):15739–15744

Quinton PM (1990) Cystic fibrosis: a disease in electrolyte transport. FASEB J 4(10):2709–2717

Quinton PM, Bijman J (1983) Higher bioelectric potentials due to decreased chloride absorption in the sweat glands of patients with cystic fibrosis. N Engl J Med 308(20):1185–1189. doi:10.1056/NEJM198305193082002

Ramalho AS, Beck S, Meyer M, Penque D, Cutting G, Amaral MD (2002) 5% of normal CFTR mRNA ameliorates the severity of pulmonary disease in cystic fibrosis. Am J Respir Cell Mol Biol 27(5):619

Ramalho AS, Lewandowska MA, Farinha CM, Mendes F, Goncalves J, Barreto C, Harris A, Amaral MD (2009) Deletion of CFTR translation start site reveals functional isoforms of the protein in CF patients. Cell Physiol Biochem 24(5–6):335–346. doi:10.1159/000257426

Ramjeesingh M, Li C, Kogan I, Wang Y, Huan LJ, Bear CE (2001) A monomer is the minimum functional unit required for channel and ATPase activity of the cystic fibrosis transmembrane conductance regulator. Biochemistry 40(35):10700–10706

Ramjeesingh M, Ugwu F, Li C, Dhani S, Huan LJ, Wang Y, Bear CE (2003) Stable dimeric assembly of the second membrane-spanning domain of CFTR (cystic fibrosis transmembrane conductance regulator) reconstitutes a chloride-selective pore. Biochem J 375(Pt 3):633–641

Ramsey BW, Boat TF (1994) Outcome measures for clinical trials in cystic fibrosis. Summary of a cystic fibrosis foundation consensus conference. J Pediatr 124(2):177–192

Ranganathan R, Ross EM (1997) PDZ domain proteins: scaffolds for signaling complexes. CurrBiol 7:R770–R773

Reddy MM, Light MJ, Quinton PM (1999) Activation of the epithelial Na+ channel (ENaC) requires CFTR Cl- channel function. Nature 402(6759):301–304. doi:10.1038/46297

Rich DP, Anderson MP, Gregory RJ, Cheng SH, Paul S, Jefferson DM, McCann JD, Klinger KW, Smith AE, Welsh MJ (1990) Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature 347:358–363

Riordan JR (1993) The cystic fibrosis transmembrane conductance regulator. Annu Rev Physiol 55:609–630

Riordan JR (2005) Assembly of functional CFTR chloride channels. Annu Rev Physiol 67: 701–718. doi:10.1146/annurev.physiol.67.032003.154107

Riordan JR (2008) CFTR function and prospects for therapy. Annu Rev Biochem 77:701–726. doi:10.1146/annurev.biochem.75.103004.142532

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245:1066–1073

Romey MC, Pallares-Ruiz N, Mange A, Mettling C, Peytavi R, Demaille J, Claustres M (2000) A naturally occurring sequence variation that creates a YY1 element is associated with increased cystic fibrosis transmembrane conductance regulator gene expression. J Biol Chem 275 (5):3561–3567

Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Zsiga M, Buchwald M, Riordan JR, Tsui LC, Collins FS (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245 (4922):1059–1065

Rosenberg MF, Kamis AB, Aleksandrov LA, Ford RC, Riordan JR (2004) Purification and crystallization of the cystic fibrosis transmembrane conductance regulator (CFTR). J Biol Chem 279(37):39051–39057. doi:10.1074/jbc.M407434200

Rosenstein BJ, Cutting GR (1998) The diagnosis of cystic fibrosis: a consensus statement. Cystic fibrosis foundation consensus panel. J Pediatr 132(4):589–595

Rowe SM, Miller S, Sorscher EJ (2005) Cystic fibrosis. N Engl J Med 352(19):1992–2001. doi:10.1056/NEJMra043184

Rowntree RK, Vassaux G, McDowell TL, Howe S, McGuigan A, Phylactides M, Huxley C, Harris A (2001) An element in intron 1 of the CFTR gene augments intestinal expression in vivo. Hum Mol Genet 10(14):1455–1464

Roxo-Rosa M, Xu Z, Schmidt A, Neto M, Cai Z, Soares CM, Sheppard DN, Amaral MD (2006) Revertant mutants G550E and 4RK rescue cystic fibrosis mutants in the first nucleotide-binding domain of CFTR by different mechanisms. Proc Natl Acad Sci U S A 103(47):17891–17896. doi:10.1073/pnas.0608312103

Rubenstein RC, Zeitlin PL (1998) Use of protein repair therapy in the treatment of cystic fibrosis. Curr Opin Pediatr 10(3):250–255

Rubenstein RC, Egan ME, Zeitlin PL (1997) In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing ΔF508-CFTR. J Clin Invest 100(10):2457–2465

Ryan NJ (2014) Ataluren: first global approval. Drugs 74(14):1709–1714. doi:10.1007/s40265-014-0287-4

Ryley HC, Goodchild MC, Dodge JA (1992) Screening for cystic fibrosis. Br Med Bull 48 (4):805–822

Sarkadi B, Bauzon D, Huckle WR, Earp HS, Berry A, Suchindran H, Price EM, Olson JC, Boucher RC, Scarborough GA (1992) Biochemical characterization of the cystic fibrosis transmembrane conductance regulator in normal and cystic fibrosis epithelial cells. J Biol Chem 267:2087–2095

Sato S, Ward CL, Krouse ME, Wine JJ, Kopito RR (1996) Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. J Biol Chem 271(2):635–638

Sato S, Ward CL, Kopito RR (1998) Cotranslational ubiquitination of cystic fibrosis transmembrane conductance regulator in vitro. J Biol Chem 273(13):7189–7192

Schillers H, Shahin V, Albermann L, Schafer C, Oberleithner H (2004) Imaging CFTR: a tail to tail dimer with a central pore. Cell Physiol Biochem 14(1–2):1–10

Schneider E, Hunke S (1998) ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. FEMS Microbiol Rev 22(1):1–20

Schultz BD, Frizzell RA, Bridges RJ (1999) Rescue of dysfunctional ΔF508-CFTR chloride channel activity by IBMX. J Membr Biol 170(1):51–66

Schwiebert EM, Benos DJ, Egan ME, Stutts MJ, Guggino WB (1999) CFTR is a conductance regulator as well as a chloride channel. Physiol Rev 79(1 Suppl):S145–S166

Scott-Ward TS, Amaral MD (2009) Deletion of Phe508 in the first nucleotide-binding domain of the cystic fibrosis transmembrane conductance regulator increases its affinity for the heat shock cognate 70 chaperone. FEBS J 276(23):7097–7109. doi:10.1111/j.1742-4658.2009.07421.x

Serohijos AW, Hegedus T, Aleksandrov AA, He L, Cui L, Dokholyan NV, Riordan JR (2008) Phenylalanine-508 mediates a cytoplasmic-membrane domain contact in the CFTR 3D structure crucial to assembly and channel function. Proc Natl Acad Sci U S A 105(9): 3256–3261. doi:10.1073/pnas.0800254105

Sharma M, Pampinella F, Nemes C, Benharouga M, So J, Du K, Bache KG, Papsin B, Zerangue N, Stenmark H, Lukacs GL (2004) Misfolding diverts CFTR from recycling to degradation: quality control at early endosomes. J Cell Biol 164(6):923–933

Sharma N, LaRusch J, Sosnay PR, Gottschalk LB, Lopez AP, Pellicore MJ, Evans T, Davis E, Atalar M, Na CH, Rosson GD, Belchis D, Milewski M, Pandey A, Cutting GR (2016) A sequence upstream of canonical PDZ-binding motif within CFTR COOH-terminus enhances NHERF1 interaction. Am J Physiol Lung Cell Mol Physiol 311(6):L1170–L1182. doi:10.1152/ajplung.00363.2016

Sheppard DN, Welsh MJ (1999) Structure and function of the CFTR chloride channel. Physiol Rev 79(1 Suppl):S23–S45

Shintre CA, Pike AC, Li Q, Kim JI, Barr AJ, Goubin S, Shrestha L, Yang J, Berridge G, Ross J, Stansfeld PJ, Sansom MS, Edwards AM, Bountra C, Marsden BD, von Delft F, Bullock AN, Gileadi O, Burgess-Brown NA, Carpenter EP (2013) Structures of ABCB10, a human ATP-binding cassette transporter in apo- and nucleotide-bound states. Proc Natl Acad Sci U S A 110(24):9710–9715. doi:10.1073/pnas.1217042110

Short DB, Trotter KW, Reczek D, Kreda SM, Bretscher A, Boucher RC, Stutts MJ, Milgram SL (1998) An Apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. J Biol Chem 273:19797–19801

Shoshani T, Augarten A, Yahav J, Gazit E, Kerem B (1994) Two novel mutations in the CFTR gene: W1089X in exon 17B and 4010delTATT in exon 21. Hum Mol Genet 3:657–658

Silvis MR, Bertrand CA, Ameen N, Golin-Bisello F, Butterworth MB, Frizzell RA, Bradbury NA (2009) Rab11b regulates the apical recycling of the cystic fibrosis transmembrane conductance regulator in polarized intestinal epithelial cells. Mol Biol Cell 20(8):2337–2350. doi:10.1091/mbc.E08-01-0084

Skach WR (2000) Defects in processing and trafficking of the cystic fibrosis transmembrane conductance regulator. Kidney Int 57(3):825–831

Smith AN, Barth ML, McDowell TL, Moulin DS, Nuthall HN, Hollingsworth MA, Harris A (1996) A regulatory element in intron 1 of the cystic fibrosis transmembrane conductance regulator gene. JBiolChem 271(17):9947–9954

Smith SN, Delaney SJ, Dorin JR, Farley R, Geddes DM, Porteous DJ, Wainwright BJ, Alton EW (1998) Effect of IBMX and alkaline phosphatase inhibitors on Cl- secretion in G551D cystic fibrosis mutant mice. Am J Physiol 274(2 Pt 1):C492–C499

Smith DJ, Nuthall HN, Majetti ME, Harris A (2000) Multiple potential intragenic regulatory elements in the CFTR gene. Genomics 64(1):90–96. doi:10.1006/geno.1999.6086

Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho AS, Amaral MD, Dorfman R, Zielenski J, Masica DL, Karchin R, Millen L, Thomas PJ, Patrinos GP, Corey M, Lewis MH, Rommens JM, Castellani C, Penland CM, Cutting GR (2013) Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet 45(10):1160–1167. doi:10.1038/ng.2745

Srinivasan V, Pierik AJ, Lill R (2014) Crystal structures of nucleotide-free and glutathione-bound mitochondrial ABC transporter Atm1. Science 343(6175):1137–1140. doi:10.1126/science.1246729

Strickland E, Qu BH, Millen L, Thomas PJ (1997) The molecular chaperone Hsc70 assists the in vitro folding of the N-terminal nucleotide-binding domain of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 272:25421–25424

Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC (1995) CFTR as a cAMP-dependent regulator of sodium channels. Science 269:847–850

Suaud L, Yan W, Carattino MD, Robay A, Kleyman TR, Rubenstein RC (2007) Regulatory interactions of N1303K-CFTR and ENaC in Xenopus oocytes: evidence that chloride transport is not necessary for inhibition of ENaC. Am J Physiol Cell Physiol 292(4):C1553–C1561. doi:10.1152/ajpcell.00064.2006

Swiatecka-Urban A, Duhaime M, Coutermarsh B, Karlson KH, Collawn J, Milewski M, Cutting GR, Guggino WB, Langford G, Stanton BA (2002) PDZ domain interaction controls the endocytic recycling of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 277(42):40099–40105. doi:10.1074/jbc.M206964200

Swiatecka-Urban A, Boyd C, Coutermarsh B, Karlson KH, Barnaby R, Aschenbrenner L, Langford GM, Hasson T, Stanton BA (2004) Myosin VI regulates endocytosis of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 279(36):38025–38031. doi:10.1074/jbc.M403141200

Swiatecka-Urban A, Brown A, Moreau-Marquis S, Renuka J, Coutermarsh B, Barnaby R, Karlson KH, Flotte TR, Fukuda M, Langford GM, Stanton BA (2005) The short apical membrane half-life of rescued ΔF508-cystic fibrosis transmembrane conductance regulator (CFTR) results from accelerated endocytosis of ΔF508-CFTR in polarized human airway epithelial cells. J Biol Chem 280(44):36762–36772. doi:10.1074/jbc.M508944200

Swiatecka-Urban A, Talebian L, Kanno E, Moreau-Marquis S, Coutermarsh B, Hansen K, Karlson KH, Barnaby R, Cheney RE, Langford GM, Fukuda M, Stanton BA (2007) Myosin Vb is required for trafficking of the cystic fibrosis transmembrane conductance regulator in Rab11a-specific apical recycling endosomes in polarized human airway epithelial cells. J Biol Chem 282(32):23725–23736. doi:10.1074/jbc.M608531200

Tabcharani JA, Chang XB, Riordan JR, Hanrahan JW (1991) Phosphorylation-regulated Cl- channel in CHO cells stably expressing the cystic fibrosis gene. Nature 352:628–631

Tatu U, Helenius A (1997) Interactions between newly synthesized glycoproteins, calnexin and a network of resident chaperones in the endoplasmic reticulum. J Cell Biol 136(3):555–565

Taubes G (1996) Misfolding the way to disease. Science 271(5255):1493–1495

The CFTR mutation database (2017). http://www.genet.sickkids.on.ca/

Thibodeau PH, Richardson JM, Wang W, Millen L, Watson JM, Mendoza JL, Du K, Fischman S, Senderowitz H, Lukacs GL, Kirk K, Thomas PJ (2010) The cystic fibrosis-causing mutation

ΔF508 affects multiple steps in cystic fibrosis transmembrane conductance regulator biogenesis. J Biol Chem 285(46):35825–35835

Tian Y, Schreiber R, Kunzelmann K (2012) Anoctamins are a family of Ca2+-activated Cl-channels. J Cell Sci 125(Pt 21):4991–4998. doi:10.1242/jcs.109553

Trapnell BC, Zeitlin PL, Chu CS, Yoshimura K, Nakamura H, Guggino WB, Bargon J, Banks TC, Dalemans W, Pavirani A (1991) Down-regulation of cystic fibrosis gene mRNA transcript levels and induction of the cystic fibrosis chloride secretory phenotype in epithelial cells by phorbol ester. J Biol Chem 266(16):10319–10323

Trezise AE, Buchwald M (1991) In vivo cell-specific expression of the cystic fibrosis transmembrane conductance regulator. Nature 353(6343):434–437

Trezise AE, Romano PR, Gill DR, Hyde SC, Sepulveda FV, Buchwald M, Higgins CF (1992) The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. EMBO J 11:4291–4303

Trinh NT, Bardou O, Prive A, Maille E, Adam D, Lingee S, Ferraro P, Desrosiers MY, Coraux C, Brochiero E (2012) Improvement of defective cystic fibrosis airway epithelial wound repair after CFTR rescue. Eur Respir J 40(6):1390–1400. doi:10.1183/09031936.00221711

Van Goor F, Straley KS, Cao D, Gonzalez J, Hadida S, Hazlewood A, Joubran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuis PD, Negulescu P (2006) Rescue of ΔF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am J Physiol Lung Cell Mol Physiol 290(6):L1117–L1130. doi:10.1152/ajplung.00169.2005

Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, Zhou J, McCartney J, Arumugam V, Decker C, Yang J, Young C, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu P (2009) Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci U S A 106 (44):18825–18830. doi:10.1073/pnas.0904709106

Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, Decker CJ, Miller M, McCartney J, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu PA (2011) Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci U S A 108(46):18843–18848. doi:10.1073/pnas.1105787108

Veit G, Avramescu RG, Chiang AN, Houck SA, Cai Z, Peters KW, Hong JS, Pollard HB, Guggino WB, Balch WE, Skach WR, Cutting GR, Frizzell RA, Sheppard DN, Cyr DM, Sorscher EJ, Brodsky JL, Lukacs GL (2016) From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. Mol Biol Cell 27 (3):424–433. doi:10.1091/mbc.E14-04-0935

Wang H, Brautigan DL (2006) Peptide microarray analysis of substrate specificity of the transmembrane Ser/Thr kinase KPI-2 reveals reactivity with cystic fibrosis transmembrane conductance regulator and phosphorylase. Mol Cell Proteomics 5(11):2124–2130. doi:10.1074/mcp.M600188-MCP200

Wang S, Raab RW, Schatz PJ, Guggino WB, Li M (1998) Peptide binding consensus of the NHE-RF-PDZ1 domain matches the C- terminal sequence of cystic fibrosis transmembrane conductance regulator (CFTR). FEBS Lett 427:103–108

Wang X, Matteson J, An Y, Moyer B, Yoo JS, Bannykh S, Wilson IA, Riordan JR, Balch WE (2004) COPII-dependent export of cystic fibrosis transmembrane conductance regulator from the ER uses a di-acidic exit code. J Cell Biol 167(1):65–74. doi:10.1083/jcb.200401035

Wang X, Venable J, LaPointe P, Hutt DM, Koulov AV, Coppinger J, Gurkan C, Kellner W, Matteson J, Plutner H, Riordan JR, Kelly JW, Yates JR 3rd, Balch WE (2006) Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell 127 (4):803–815. doi:10.1016/j.cell.2006.09.043

Ward CL, Omura S, Kopito RR (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. Cell 83:121–127

Ward A, Reyes CL, Yu J, Roth CB, Chang G (2007) Flexibility in the ABC transporter MsbA: Alternating access with a twist. Proc Natl Acad Sci U S A 104(48):19005–19010. doi:10.1073/pnas.0709388104

Weixel KM, Bradbury NA (2000) The carboxyl terminus of the cystic fibrosis transmembrane conductance regulator binds to AP-2 clathrin adaptors. J Biol Chem 275(5):3655–3660
Weixel KM, Bradbury NA (2001) Mu 2 binding directs the cystic fibrosis transmembrane conductance regulator to the clathrin-mediated endocytic pathway. J Biol Chem 276 (49):46251–46259. doi:10.1074/jbc.M104545200
Welch WJ, Brown CR (1996) Influence of molecular and chemical chaperones on protein folding [published erratum appears in Cell Stress Chaperones 1996 Sep; 1(3):207]. Cell Stress Chaperones 1(2):109–115
Welsh MJ, Smith AE (1993) Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 73(7):1251–1254
Welsh MJ, Anderson MP, Rich DP, Berger HA, Denning GM, Ostedgaard LS, Sheppard DN, Cheng SH, Gregory RJ, Smith AE (1992) Cystic fibrosis transmembrane conductance regulator: a chloride channel with novel regulation. Neuron 8:821–829
Welsh MJ, Ramsey BW, Accurso F, Cutting GR, Scriver CL, Beaudet AL, Sly WS, Valle D (2001) Cystic Fibrosis. The molecular and metabolic basis of inherited disease. McGraw-Hill, New York, pp 5121–5188
Werner ED, Brodsky JL, McCracken AA (1996) Proteasome-dependent endoplasmic reticulum-associated protein degradation: an unconventional route to a familiar fate. Proc Natl Acad Sci U S A 93(24):13797–13801
Wigley WC, Fabunmi RP, Lee MG, Marino CR, Muallem S, DeMartino GN, Thomas PJ (1999) Dynamic association of proteasomal machinery with the centrosome. J Cell Biol 145(3): 481–490
Wilschanski M, Zielenski J, Markiewicz D, Tsui LC, Corey M, Levison H, Durie PR (1995) Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J Pediatr 127:705–710
Xiong X, Chong E, Skach WR (1999) Evidence that endoplasmic reticulum (ER)-associated degradation of cystic fibrosis transmembrane conductance regulator is linked to retrograde translocation from the ER membrane. J Biol Chem 274(5):2616–2624
Yang Y, Janich S, Cohn JA, Wilson JM (1993) The common variant of cystic fibrosis transmembrane conductance regulator is recognized by hsp70 and degraded in a pre-Golgi nonlysosomal compartment. Proc Natl Acad Sci U S A 90(20):9480–9484
Yoo JS, Moyer BD, Bannykh S, Yoo HM, Riordan JR, Balch WE (2002) Non-conventional trafficking of the cystic fibrosis transmembrane conductance regulator through the early secretory pathway. J Biol Chem 277(13):11401–11409
Yoshimura K, Nakamura H, Trapnell BC, Dalemans W, Pavirani A, Lecocq JP, Crystal RG (1991) The cystic fibrosis gene has a "housekeeping"-type promoter and is expressed at low levels in cells of epithelial origin. J Biol Chem 266:9140–9144
Younger JM, Ren HY, Chen L, Fan CY, Fields A, Patterson C, Cyr DM (2004) A foldable CFTRΔF508 biogenic intermediate accumulates upon inhibition of the Hsc70-CHIP E3 ubiquitin ligase. J Cell Biol 167(6):1075–1085
Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP Jr, Urrutia A, Joubran J, Seepersaud S, Sussky K, Hoffman BJ, Van Goor F (2012) Ivacaftor potentiation of multiple CFTR channels with gating mutations. J Cyst Fibr 11(3):237–245. doi:10.1016/j.jcf.2011.12.005
Zeitlin PL (1999) Novel pharmacologic therapies for cystic fibrosis. J Clin Invest 103(4):447–452
Zerangue N, Schwappach B, Jan YN, Jan LY (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron 22(3):537–548
Zerhusen B, Zhao J, Xie J, Davis PB, Ma J (1999) A single conductance pore for chloride ions formed by two cystic fibrosis transmembrane conductance regulator molecules. J Biol Chem 274(12):7627–7630
Zhang Z, Chen J (2016) Atomic structure of the cystic fibrosis transmembrane conductance regulator. Cell 167(6):1586–1597. doi:10.1016/j.cell.2016.11.014 (e1589)
Zhang F, Kartner N, Lukacs GL (1998) Limited proteolysis as a probe for arrested conformational maturation of ΔF508 CFTR. NatStructBiol 5(3):180–183

Zhang H, Peters KW, Sun F, Marino CR, Lang J, Burgoyne RD, Frizzell RA (2002) Cysteine string protein interacts with and modulates the maturation of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 277(32):28948–28958
Zielenski J, Tsui LC (1995) Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 29:777–807