Cystic Fibrosis as a Disease of Misprocessing of the Cystic Fibrosis Transmembrane Conductance Regulator Glycoprotein

John R. Riordan

Mayo Foundation and S.C. Johnson Medical Research Center, Mayo Clinic, Scottsdale, AZ

More than 800 different mutations in the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene have been identified in cystic fibrosis (CF) patients. Despite this diversity, most individuals with CF carry at least one *CFTR* allele whose product is synthesized but fails to mature or to proceed beyond the endoplasmic reticulum (ER) within the cell. These CFTR disease variants, like the products of many other mutated genes, are not so much broken as bent. The common ΔF508 variant, as well as various missense mutations in nearly all CFTR domains, cause local misfolding and prevent the protein from attaining its native global conformation. Cells detect such improperly folded or assembled proteins and target them for degradation through a set of processes described as biosynthetic quality control.

The consequences of failed quality control can be severe. Intra- or extracellular protein aggregates accumulate in several pathological conditions, such as amyloid diseases and severe α1-antitrypsin deficiency, and disrupt cellular and tissue function. However, in the case of CF, the normal functioning of the ER-retention and -degradation pathways prevents variant CFTR molecules from reaching the plasma membranes of secreting and reabsorbing epithelial cells, where CFTR is required as a regulated chloride channel. The ΔF508 variant of CFTR and the products of some other short deletion, insertion, or missense alleles are mildly temperature sensitive; in cell-culture systems, growth at permissive temperatures and some other experimental manipulations promote maturation and transport of variant proteins to the cell surface.

How the ER quality-control apparatus distinguishes a normal conformation from an abnormal one is unknown, but, as discussed below, several key interactions have come to light. Discrete primary-structure motifs common to CFTR and other related transporter proteins may be involved. Nascent CFTR—both in wild-type form and in disease variants such as ΔF508—interacts with multiple molecular chaperones on both sides of the ER membrane—and probably with the coat proteins of the secretory vesicles that shuttle cargo proteins from the ER to the Golgi apparatus and beyond. Because ΔF508 and other CFTR variants are potentially functional if they can reach the cell surface, these interactions are of more than theoretical interest. Treatments that promote the maturation of misfolded secretory proteins could prove useful in the management of CF and of many other heritable diseases.

CFTR Biosynthesis and Intracellular Transport

A defect in the apical chloride permeability of CF epithelial cells was known before the discovery of CFTR, so the localization of the mature glycoprotein to this site came as no surprise. Like other mammalian N-linked glycoproteins, CFTR is synthesized from membrane-bound ribosomes and is glycosylated cotranslationally (fig. 1). Following conformational maturation, which involves the completion of secondary-, tertiary-, and (in the case of multisubunit proteins) quaternary-structure formation, these proteins become the cargo of COPII vesicles, which pinch off from the ER (see Nichols and Ginsburg 1999 [in this issue]). The disease-associated processing mutants of CFTR fail to complete this ER export step. Wild-type CFTR molecules, like other plasma-membrane glycoproteins, continue through the Golgi apparatus, where they acquire complex oligosaccharide chains before transiting to the plasma membrane through another series of vesicular transport steps. Mature molecules at the cell surface are much more long-lived than their precursors ($T_{\frac{1}{2}}$ ∼ 16 h compared with ∼ $\frac{1}{2}$ h), but they undergo further rounds of vesicular trafficking in an endocytotic-exocytotic cycle (Prince et al. 1994) and eventually reach the lysosome, where they are degraded. Some CFTR missense mutations may affect passage through later stages of the secretory pathway or through endocytosis and recycling. However, because ΔF508 and other mutants investigated to date are arrested at the early ER stage, most attention is focused there. The ER-to-Golgi transit step is most commonly

Received April 7, 1999; accepted for publication April 16, 1999; electronically published May 5, 1999

Correspondence should be addressed to: Dr. John R. Riordan, Mayo Clinic Scottsdale, S. C. Johnson Medical Research Center, 13400 East Shea Boulevard, Scottsdale, AZ 85259. E-mail: riordan@mayo.edu

This article represents the opinion of the author and has not been peer reviewed.

© 1999 by The American Society of Human Genetics. All rights reserved.

0002-9297/99/6406-0003$02.00

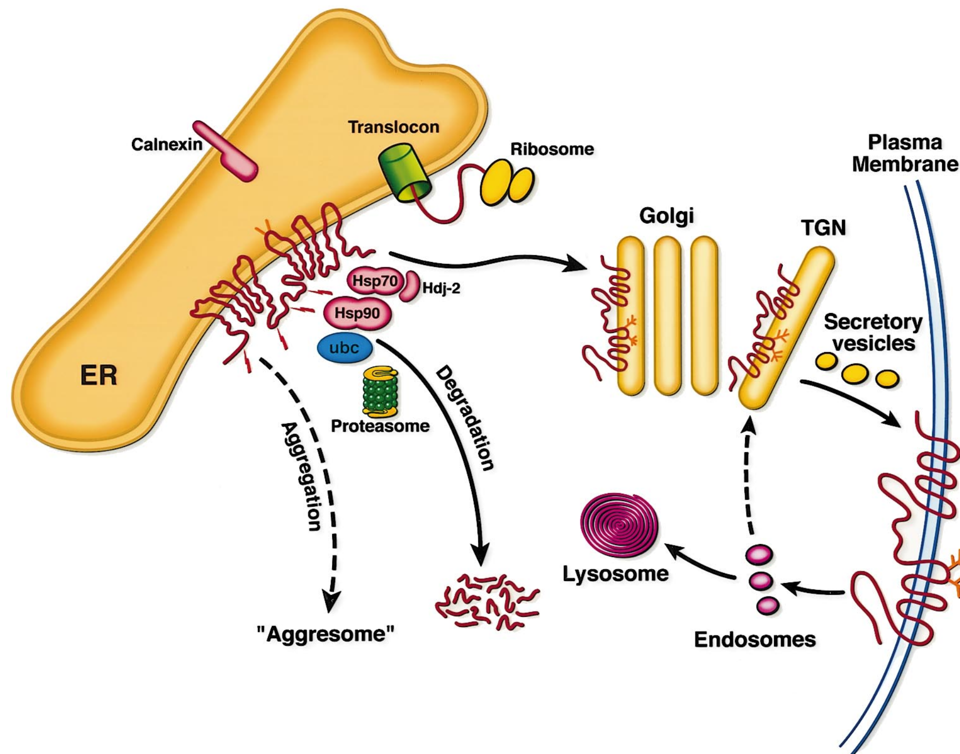

Figure 1 Fate of CFTR molecules synthesized on ER-associated ribosomes. As the primary structure is formed, the polypeptide is incorporated into the ER membrane. Core oligosaccharide chains are attached, to which calnexin binds. In addition, the cytosolic chaperones Hsp70, Hdj-2, and Hsp90 bind, and ubiquitination may occur. To be exported from the ER compartment in a productive manner via COPII-coated vesicles (not shown), a degree of higher structure must be achieved. This ATP-dependent conformational maturation is accompanied by dissociation of calnexin and the cytosolic chaperones. Fully folded CFTR is protected from degradation, but molecules that do not attain this conformation (~75% of wild-type molecules and ~100% of DF508 molecules) are substrates of ubiquitinating enzymes (ubc) and are degraded by the proteasome. As-yet-unidentified proteases may also be involved, since degradation is not completely prevented by proteasome inhibition. When degradation is blocked or saturated, extensive aggregation of export-incompetent molecules occurs. The export-competent population travel from the ER through an ERGIC (not shown) to the Golgi apparatus, where complex oligosaccharide chains are completed. Vesicles then carry the completed molecule from the trans-Golgi network (TGN) to the plasma membrane. Endocytic recycling of this population and degradation of some internalized protein by lysosomal proteases accounts for the turnover of surface-expressed CFTR.

monitored by SDS-PAGE, by following the addition of complex oligosaccharide chains to CFTR. The kinetics of the conversion may also be studied by fluorescence microscopy in real time by use of CFTR-GFP fusions. Although most evidence indicates that ΔF508 CFTR remains in the ER, Gilbert et al. (1998) have recently reported that it reaches the intermediate compartment (ERGIC) between ER and Golgi (see Nichols and Ginsburg 1999). When its proteolysis is inhibited, highly aggregated forms follow a separate terminal pathway into discrete subcellular entities, which have been termed “aggresomes” (Johnston et al. 1998).

ΔF508 and Other Processing Mutants

In a seminal paper, Cheng et al. (1990) reported that ΔF508 CFTR and several other mutants failed to be fully glycosylated in COS cells but remained in the ER, where they were degraded. However, others found that, despite its defective processing, expression of the ΔF508 CFTR molecule in *Xenopus* oocytes (Drumm et al. 1991), at reduced temperature or extreme overexpression in mammalian cells (Dalemans et al. 1991), confers some measurable regulated chloride-channel activity on transfected cells. Assuming that these observations are not just artifacts of the expression systems employed, these studies indicated that mechanisms that overcome misprocessing could provide an entrée for molecular therapeutics.

Immunocytochemical localization of wild-type and ΔF508 CFTR in epithelial cells of sweat glands showed that the mutant protein did not reach the apical membrane but remained intracellular (Kartner et al. 1992). Because the sweat gland is the only readily accessible affected tissue in CF patients that is not altered by the secondary pathological manifestations, it has been difficult to confirm this clear-cut change in subcellular

localization in other tissues. However, this aberrant localization has been observed in primary cultures of airway epithelial cells from homozygous ΔF508 patients (Yang et al. 1993). Thus, the inability of the ΔF508 CFTR nascent chain to mature and to be exported from the ER is almost certainly the basis of much of the disease.

As was initially noted by Cheng et al. (1990), the immature core-glycosylated forms of both wild-type and ΔF508 CFTR turn over rapidly ($T_{\frac{1}{2}} \sim \frac{1}{2}$ h), and conversion of the wild-type protein to the mature form occurs quite inefficiently. Thus, only ~25% conversion is seen in pulse-chase experiments (Ward and Kopito 1994). This proportion of nascent chains achieves a transport-competent conformation by an ATP-dependent mechanism. The remainder succumbs to degradation by a pathway insensitive to inhibitors of lysosomal proteases (Yang et al. 1993; Lukacs et al. 1994). Subsequently, nascent CFTR was the first mammalian-membrane glycoprotein found to be a substrate for the cytosolic proteasome (Jensen et al. 1995; Ward et al. 1995). Since then, the ubiquitination-proteasome system has been demonstrated to be an integral part of the ER quality-control apparatus that scrutinizes membrane and secretory proteins (Sommer and Wolf 1997).

Like other aberrant proteins in the early secretory pathway, nascent CFTR chains that have failed to mature conformationally may be removed from the ER membrane before or during proteasomal degradation (Xiong et al. 1999). The degradation of wild-type CFTR indicates that it has difficulty achieving a native configuration. The advantage of this seemingly wasteful biosynthetic process is unclear. Wild-type CFTR is normally kept unphosphorylated and, hence, inactive in the ER by phosphatases, but treatment with phosphatase inhibitors may allow it to be active and thereby introduce an anion conductance into the ER membrane. Perhaps the acute sensitivity to the ubiquitin-proteasome pathway helps to avoid an uncontrolled ion permeability in the ER membrane, by providing a backup to the phosphatases.

The behavior of the ΔF508 mutant is of primary importance to the disease because it is present on at least one allele in ~90% of patients. However, many other disease-associated mutations, as well as others produced in vitro for experimental purposes, cause similar misprocessing. For example, R1066C in the fourth cytoplasmic loop of CFTR has been studied extensively in both the laboratory (Seibert et al. 1996*a*) and the clinic (Casals et al. 1997). Like ΔF508, this variant fails to mature and causes severe disease. Of 30 disease-associated mutations in the four cytoplasmic loops, 18 were similarly misprocessed (Seibert et al. 1996*a*, 1996*b*, 1997), suggesting that missense mutations at virtually any location in the protein can result in misprocessing. Other examples are found in cytoplasmic domains and in membrane-spanning segments. The extracytoplasmic loops, most of which are very short, may be less crucial for efficient folding. Nine disease-associated mutations in these domains have been heterologously expressed, and all were capable of maturing (M. Hämmerle and J.R. Riordan, unpublished data).

How Are "Good" and "Bad" Nascent Chains Distinguished?

Because so many different single-amino-acid substitutions throughout the molecule can cause misprocessing, it is unlikely that discrete sequence motifs that interact with the quality-control machinery have been altered by each of these mutations. More likely, these mutations perturb the folding of the polypeptides into higher-order structures, as suggested by the temperature sensitivity of ΔF508 CFTR (Denning et al. 1992). Altered protease sensitivity provides the only direct evidence that immature and mature forms of the molecule have different conformations (Zhang et al. 1998) and that ΔF508 is unable to achieve the latter. The folding alteration caused by the absence of phe508 has been extensively studied in an isolated domain from CFTR, called "NBD1," and short peptides from within it (Qu et al. 1997). Thomas et al. (1992) originally found that a synthetic peptide corresponding to the sequence around phe508 has less β-structure when that residue is absent. However, spectroscopic measurements with a complete isolated recombinant NBD1 do not detect secondary-structure differences at steady state that can be ascribed to the absence of phe508. Rather, the kinetics of refolding of denatured NBD1 appears to be reduced by this deletion (Qu and Thomas 1996; Qu et al. 1997). Altered equilibria among competing folding pathways may account for the primary effect of the phe508 deletion on NBD1 and may explain the failure of the whole multidomain protein to reach its native global configuration. Similar local disturbances of folding of NBD1 or of other domains, due to different misprocessing mutations (see, e.g., Seibert et al. 1996*a*), probably also perturb the overall assembly.

The distinct proteolysis patterns of CFTR's immature and mature forms and the fact that ΔF508 cannot convert from the former to the latter (Zhang et al. 1998) raise the central question of how the "correct" overall conformation of the wild-type nascent chain is distinguished from an "incorrect" mutant within the cell. Perhaps a non-native conformation exposes primary-structure motifs that are specifically recognized by constituents of quality control or by vesicular transport systems. Such motifs would be analogous to the KDEL and KKXX sequences, which are responsible for the localization of resident ER proteins (Teasdale and Jackson

1996), or to the recently identified RKR triplet that prevents individual subunits of the K_{ATP} channel from leaving the ER separately (Zerangue et al. 1999). Thus, a negative regulatory mechanism may govern the quality-control process. Alternatively, positive signals may become exposed when the mature conformations are achieved. A short diacidic ER export signal has been identified in VSV-G glycoprotein (Nishimura and Balch 1997), and related sequences could be employed by other secretory and membrane proteins leaving the ER. It will be crucial to determine whether (*a*) mutant nascent CFTR fails to present a necessary positive signal required for departure or (*b*) a negative signal responsible for retention or retrieval is exposed on the immature polypeptide.

Which Molecules Recognize Signals Distinguishing "Good" from "Bad"?

No matter how nascent CFTR identifies itself as competent or incompetent for ER export, it is essential to identify the molecules involved in their differential recognition. Logical candidates include molecular chaperones, as well as mediators of vesicular transport or of ER degradation, which disposes of proteins that are incompetent for export. Ubiquitination ultimately marks such proteins as substrates for the proteasome (Ward et al. 1995) and, possibly, other proteinases (Jensen et al. 1995), but earlier steps in the recognition of these target proteins are unclear. Molecular chaperones act both to retain unfolded proteins and to assist their folding, and nascent CFTR is accessible to chaperones on both sides of the ER membrane (fig. 1). Calnexin, an ER-membrane chaperone, interacts with oligosaccharide chains on the nascent glycoprotein (Pind et al. 1994). On the cytoplasmic side, Hsp70 (Yang et al. 1993) and its cochaperone, Hdj-2 (Meacham et al. 1999), and Hsp90 (Loo et al. 1998) bind to immature, but not to mature, CFTR. Other chaperones may also be present in the large multimolecular complexes with which nascent CFTR is associated (Pind et al. 1994). However, all of these interactions occur with both wild-type and mutant nascent chains. No dramatic differences in the stoichiometry or the kinetics of chaperone-CFTR interactions have yet been found. This similarity may reflect only the intrinsically inefficient maturation of wild-type CFTR, or it may suggest that chaperone binding is not central to the cell's ability to distinguish normal from variant CFTR.

The COPII coat-protein complex, which coats vesicles that are destined for delivery to the ERGIC (Aridor et al. 1998), represents another possible point at which aberrant structures on secreted proteins may be detected. We are currently exploring the idea that COPII proteins interact with positive export signals on nascent CFTR. Similarly, association with COPI vesicles that are responsible for retrograde retrieval (Cosson and Letourneur 1997) and are mediated by putative negative signals is also being examined. The relation between the folding and degradation mechanisms and the export and retrieval pathways is not understood for any secretory molecule, but CFTR is serving to focus research efforts on this issue.

Misprocessing of Other ATP-binding cassette (ABC) Transporters

The susceptibility of CFTR to misprocessing raises the possibility that mutations in other ABC proteins might also cause them to transit the ER inefficiently. If so, several clinically important predictions might follow. If a drug-exporting transporter, such as the P-glycoprotein (P-gp) could be trapped intracellularly, this ABC protein would not render the tumor cells drug resistant. Unlike CFTR, wild-type P-gp matures efficiently (Jensen et al. 1995), but missense mutations in many regions of the protein cause it to arrest in the ER (Loo and Clarke 1997). Hence, one promising strategy for combating multidrug resistance might be to promote misprocessing of the wild-type molecule in tumors. However, somewhat ironically, some drugs that are transported by P-gp promote the maturation of misprocessed mutants (Loo and Clarke 1997), perhaps by stabilizing the native conformation of the transporter. A similar interaction between CFTR and some ligand might well provide therapeutic benefit to CF patients. Unfortunately, few reagents bind to CFTR with suitable affinity; of those that do, such as genistein, none have improved the processing of ΔF508 CFTR.

The multidrug resistance–related protein (MRP), another transporter protein of the ABC family, also reaches the surface of some cell types inefficiently. However, in cells that process CFTR poorly, we have found that wild-type MRP is efficiently processed (Chang et al. 1997). Deletion of phe728 in MRP, which corresponds to phe508 in CFTR, causes MRP to behave essentially the same as ΔF508 CFTR; other missense mutations have similar effects (X.-B. Chang and J.R. Riordan, unpublished observations). The discovery of agents that disturb the maturation of wild-type MRP might have a major impact in combating resistance to cancer chemotherapy, because there are as yet no good "reversers" of MRP-mediated multidrug resistance, whereas some such drugs are available to combat the effects of P-gp.

The type 1 sulfonylurea receptor (SUR1), another ABC protein, combines with a small inwardly rectifying potassium channel (Kir 6.2, the product of the *KCNJ11* gene) to form the oligomeric K_{ATP} channel of pancreatic β-cells (Clement et al. 1997). K_{ATP} regulates insulin secretion in response to altered intracellular ATP and ADP levels. Mutations in both the *SUR1* and *KCNJ11* genes

have been found in patients with familial persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (Agular-Bryan and Bryan 1996). As with other multisubunit membrane proteins, assembly of the two subunits to form the K_{ATP} octamer must occur in the ER for either to be able to proceed to the cell surface. Recently, a short sequence motif (RKR) common to the two subunits has been implicated in the retention of K_{ATP} subunits that are not appropriately coassembled (Zerangue et al. 1999). Failure to form the native quaternary structure is believed to expose this motif so that it is recognized by some component responsible for ER retention. The concept of exposure, in incompleted or imperfectly assembled multisubunit or multidomain proteins, of primary-structure signals normally masked in completed-native structures is of fundamental importance in the understanding of and, potentially, in the modulation of protein transport through the ER. Missense mutations in each of these ABC proteins most likely block their exit from the ER, an effect that may be relevant not just to CF and PHHI but to various other genetic diseases as well. A prime candidate is the gene for the retinal rod transporter, ABC-R, in which many different mutations have been found in patients with different retinal disorders, including Stargardt disease and some forms of macular dystrophy (Sun et al. 1999). Since >40 different ABC protein genes have already been recognized in humans, it is probable that misprocessing will be a prominent feature of other monogenic or polygenic disorders.

Means of Promotion of Maturation of ΔF508 CFTR

The most direct means of overcoming the inability of ΔF508 CFTR to mature would be to prevent the misfolding from occurring. Several agents are known to influence protein folding, including glycerol and other osmolytes (Brown et al. 1996) as well as reduced temperature (Denning et al. 1992). Unfortunately, these treatments only partially restore maturation and are not amenable to use in vivo. Other rational approaches that have been explored include inhibition of ER proteolysis and modulation of chaperone interactions. Proteasome inhibitors do slow degradation, but maturation does not increase concomitantly (Jensen et al. 1995), indicating that degradation and maturation are not in equilibrium. Binding to calnexin can be prevented by expression of an unglycosylated version of CFTR. Similarly, treating cells with castanosperimine, a glucosidase blocker that interferes with the processing of N-linked sugars on the glycoprotein, also completely abrogates the interaction with calnexin (author's unpublished data). In neither case, however, do these treatments increase maturation of ΔF508 CFTR. Geldanamycin, a member of the most specific class of chaperone drugs, prevents binding of Hsp90 to nascent CFTR, but this treatment strongly accelerates the degradation of the protein, suggesting that this chaperone normally promotes maturation rather than contributing to retention (Loo et al. 1998). On the other hand, deoxyspergualin, which influences Hsp70 binding, induces a very-low-level chloride-channel function at the surface of ΔF508 CFTR–expressing cells (Jiang et al. 1998).

There may still be hope that CFTR maturation may be promoted by manipulation of other chaperones, but it also appears that the ER quality-control system that limits the surface expression of CFTR may not be mediated primarily by this class of molecules. Any therapeutic approach based on augmention of ER export will require specificity. The most fruitful means of achieving this may come from identification of positive export signals or negative retention motifs. It may then become possible to prevent their recognition, by competitive mimicry of their structures by small molecules.

Acknowledgments

This work was supported by the National Institute of Diabetes and Digestive and Kidney diseases of the National Institute of Health. The author is the Richard O. Jacobson Professor in Molecular Medicine at the Mayo Foundation.

References

Agular-Bryan L, Bryan J (1996) ATP-sensitive potassium channels, sulfonylurea receptors and persistent hyperinvulinemic hypoglycemia of infancy. Diabetes Rev 4:336–346

Aridor M, Weissman J, Bannykh S, Nuoffer C, Balch WE (1998) Cargo selection by the COPII budding machinery during export from the ER. J Cell Biol 141:61–70

Brown CR, Hong-Brown LQ, Biwersi J, Verkman AS, Welch WJ (1996) Chemical chaperones correct the mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein. Cell Stress Chaperones 1: 117–125

Casals T, Pacheco P, Barreto C, Gimenez J, Ramos MD, Pereira S, Pinheiro JA, et al (1997) Missense mutation R1066C in the second transmembrane domain of CFTR causes a severe cystic fibrosis phenotype: study of 19 heterozygous and 2 homozygous patients. Hum Mutat 10:387–392

Chang XB, Hou YX, Riordan JR (1997) ATPase activity of purified multidrug resistance-associated protein. J Biol Chem 272:30962–30968

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

Clement JP IV, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar-Bryan L, Bryan J (1997) Association and stoichiometry of K(ATP) channel subunits. Neuron 18: 827–838

Cosson P, Letourneur F (1997) Coatomer (COPI)-coated vesicles: role in intracellular transport and protein sorting. Curr Opin Cell Biol 9:484–487

Dalemans W, Barbry P, Champigny G, Jallat S, Dott K, Dreyer D, Crystal RG, et al (1991) Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. Nature 354:526–528

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 358:761–764

Drumm ML, Wilkinson DJ, Smit LS, Worrell RT, Strong TV, Frizzell RA, Dawson DC, et al (1991) Chloride conductance expressed by delta F508 and other mutant CFTRs in *Xenopus* oocytes. Science 254:1797–1799

Gilbert A, Jadot M, Leontieva E, Wattiaux-De Coninck S, Wattiaux R (1998) Delta F508 CFTR localizes in the endplasmic reticulum-Golgi intermediate compartment in cystic fibrosis cells. Exp Cell Res 242:144–152

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, et al (1998) Partial restoration of cAMP-stimulated CFTR chloride channel activity in DeltaF508 cells by deoxyspergualin. Am J Physiol 275:C171–C178

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

Kartner N, Augustinas O, Jensen TJ, Naismith AL, Riordan JR (1992) Mislocalization of delta F508 CFTR in cystic fibrosis sweat gland. Nat Genet 1:321–327

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

Loo TW, Clarke DM (1997) Correction of defective protein kinesis of human P-glycoprotein mutants by substrates and modulators. J Biol Chem 272:709–712

Lukacs GL, Mohamed A, Kartner N, Chang XB, Riordan JR, Grinstein S (1994) Conformational maturation of CFTR but not its mutant counterpart (delta F508) occurs in the endoplasmic reticulum and requires ATP. EMBO J 13:6076–6086

Meacham GC, Lu Z, King S, Sorscher E, Tousson A, Cyr DM (1999) The Hdj-2/Hsc 70 chaperone pair facilitates early steps in CFTR biogenesis. EMBO J 18:1492–1505

Nichols WC, Ginsburg D (1999) From the ER to the Golgi: insights from the study of combined factors V and VIII deficiency. Am J Hum Genet 64:1493–1498 (in this issue)

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

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

Prince LS, Workman RB Jr, Marchase RB (1994) Rapid endocytosis of the cystic fibrosis transmembrane conductance regulator chloride channel. Proc Natl Acad Sci U S A 91:5192–5196

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

Qu BH, Thomas PJ (1996) Alteration of the cystic fibrosis transmembrane conductance regulator folding pathway. J Biol Chem 271:7261–7264

Seibert FS, Linsdell P, Loo TW, Hanrahan JW, Clarke DM, Riordan JR (1996*a*) Disease-associated mutations in the fourth cytoplasmic loop of CFTR compromise biosynthetic processing and chloride channel activity. J Biol Chem 271:15139–15143

Seibert FS, Linsdell P, Loo TW, Hanrahan JW, Riordan JR, Clarke DM (1996*b*) Cytoplasmic loop three of cystic fibrosis transmembrane conductance regulator contributes to regulation of chloride channel activity. J Biol Chem 271:27493–27499

Seibert FS, Loo TW, Clarke DM, Riordan JR (1997) Cystic fibrosis: channel, catalytic, and folding properties of the CFTR protein. J Bioenerg Biomembr 29:429–442

Sommer T, Wolf DH (1997) Endoplasmic reticulum degradation: reverse protein flow of no return. FASEB J 11:1227–1233

Sun H, Molday RS, Nathans J (1999) Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. J Biol Chem 274:8269–8281

Teasdale RD, Jackson MR (1996) Signal-mediated sorting of membrane proteins between the endoplasmic reticulum and the golgi apparatus. Annu Rev Cell Dev Biol 12:27–54

Thomas PJ, Shenbagamurthi P, Sondek J, Hullihen JM, Pedersen PL (1992) The cystic fibrosis transmembrane conductance regulator: effects of the most common cystic fibrosis-causing mutation on the secondary structure and stability of a synthetic peptide. J Biol Chem 267:5727-5730

Ward CL, Kopito RR (1994) Intracellular turnover of cystic fibrosis transmembrane conductance regulator. Inefficient processing and rapid degradation of wild-type and mutant proteins. J Biol Chem 269:25710–25718

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

Xiong XM, 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 274:2616–2624

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 USA 90:9480–9484

Zerangue N, Schwappach B, Jan YN, Jan LY (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane KATP channels. Neuron 22:537–548

Zhang F, Kartner N, Lukacs GL (1998) Limited proteolysis as a probe for arrested conformational maturation of delta F508 CFTR. Nat Struct Biol 5:180–183