INTRODUCTION

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) Cl\(^{-}\) channel cause cystic fibrosis (CF) (Welsh et al., 1995; Riordan, 1993; Collins, 1992; Tsui, 1995). The most common CF-associated mutation, ΔF508, results in the deletion of a phenylalanine in the first nucleotide-binding domain (NBD1) of CFTR. By preventing normal folding of CFTR, the ΔF508 mutation prevents exit of mutant protein from the endoplasmic reticulum (ER) (Cheng et al., 1990; Denning et al., 1992a; Lukacs et al., 1994; Ward and Kopito, 1994; Qu and Thomas, 1996; Qu et al., 1997; Welsh and Ostedgaard, 1998). As a result, the protein does not traffic to the Golgi complex or cell surface and fails to form functional CFTR Cl\(^{-}\) channels at the apical membrane of epithelia.

The biosynthesis and maturation of CFTR can be monitored by assessing its pattern of glycosylation electrophoretically (Cheng et al., 1990). In the ER, the initial attachment of carbohydrate to CFTR produces a core-glycosylated form of the protein called band B. The addition of glycosylation in the Golgi complex results in a mature protein called band C which migrates more slowly during electrophoresis. Maturation of CFTR through the Golgi complex is an inefficient process even for wild-type; only 20% of wild-type CFTR which is originally synthesized eventually matures to the fully glycosylated state with Hsp70. We used brefeldin A (BFA) to determine which processing step(s) was altered by reduced temperature. Unlike wild-type CFTR, which was converted into an intermediate that was stable in the presence of BFA at 37°C, ΔF508 and P574H produced the intermediate only when the temperature was reduced to 26°C. Furthermore the wild-type intermediate was not associated with Hsp70. These data suggest that formation of the stable intermediate is a key temperature-sensitive step and appears to be coincident with release of the wild-type protein from Hsp70.

Key words: Cystic fibrosis, Hsp70, Protein biosynthesis

SUMMARY

Cystic fibrosis transmembrane conductance regulator (CFTR) containing the ΔF508 mutation is retained in the endoplasmic reticulum (ER). This defect can be partially overcome by a reduction in temperature which allows some of the ΔF508 protein to exit the ER and move to the cell surface. Earlier studies showed that the CF-associated mutants, P574H and A455E, were also misprocessed. In this study, we found that processing of P574H and A455E was also temperature-sensitive; at 26°C, some of the protein matured. In contrast to other CFTR mutants, P574H accumulated in punctate cytoplasmic bodies that colocalized with endoplasmic reticulum (ER) markers. At 26°C, these bodies were no longer present. P574H showed a prolonged association with Hsp70 and also colocalized with Hsp70. We used brefeldin A (BFA) to determine which processing step(s) was altered by reduced temperature. Unlike wild-type CFTR, which was converted into an intermediate that was stable in the presence of BFA at 37°C, ΔF508 and P574H produced the intermediate only when the temperature was reduced to 26°C. Furthermore the wild-type intermediate was not associated with Hsp70. These data suggest that formation of the stable intermediate is a key temperature-sensitive step and appears to be coincident with release of the wild-type protein from Hsp70.

Key words: Cystic fibrosis, Hsp70, Protein biosynthesis
in NBD1 and throughout the protein (Tsui, 1995). We earlier studied two other CF-associated mutations located in NBD1, A455E and P574H (Sheppard et al., 1995). These variants form regulated Cl– channels with properties similar to those of wild-type CFTR. However, A455E and P574H generate reduced net epithelial current because the proteins are misprocessed and few functional channels reach the plasma membrane (Sheppard et al., 1995; Champigny et al., 1995). Nevertheless, the processing defect of A455E and P574H is less pronounced than that of ΔF508 and the resulting clinical phenotype is less severe (Kristidis et al., 1992; Kerem et al., 1990a; Veeze et al., 1994; Gan et al., 1995). These observations suggest differences in the processing defects of these NBD1 mutants. In this study, we compared the processing of P574H and A455E mutants to that of ΔF508. By studying mutants with different degrees of misprocessing, we hope to gain further insight into the biosynthesis of both normal and mutant CFTR which may help design interventions to improve the processing of ΔF508, P574H, and possibly other CF-associated mutants.

MATERIALS AND METHODS

Site-directed mutagenesis

The CFTR coding sequence was excised from pTM1-CFTR using the NotI and SpeI restriction sites and inserted into the pcDNA3 vector between the NotI and XhoI sites. We inserted a 6-His coding sequence between amino acids 2 and 3 of CFTR. The inclusion of 6 histidines does not affect the processing nor the channel function of the expressed protein. Using the pcDNA3-6His-CFTR as a backbone, we made the constructs A455E, P574H and ΔF508 (Kunkel, 1985) and confirmed the mutations by DNA sequencing in both directions.

Protein expression

DNA was electroporated into COS-7 cells (Bio-Rad Gene-Pulser; 960 μF, 0.32 V, time constant of 24) in a buffer that resembles the intracellular ionic composition (in mM: 120 KCl, 0.15 CaCl2, 10 K2HPO4/KH2PO4, 25 Hepes, 2 EGTA, and 5 MgCl2 titrated to 7.6 with KOH, 2 mM ATP and 2 mM glutathione) (van den Hoff et al., 1992). Cells were left on ice for 10 minutes before and after electroporation. Cells were plated in duplicate and incubated at 37°C and at 26°C. Cells incubated at 26°C were allowed to attach for 12-19 hours at 37°C before moving to a modular incubator chamber and at 26°C. Cells incubated at 26°C were allowed to attach for 12-1992). Cells were left on ice for 10 minutes before and after

with KOH, 2 mM ATP and 2 mM glutathione) (van den Hoff et al., 1995; Champigny et al., 1995). Nevertheless, the processing defect of A455E and P574H is less pronounced than that of ΔF508 and the resulting clinical phenotype is less severe (Kristidis et al., 1992; Kerem et al., 1990a; Veeze et al., 1994; Gan et al., 1995). These observations suggest differences in the processing defects of these NBD1 mutants. In this study, we compared the processing of P574H and A455E mutants to that of ΔF508. By studying mutants with different degrees of misprocessing, we hope to gain further insight into the biosynthesis of both normal and mutant CFTR which may help design interventions to improve the processing of ΔF508, P574H, and possibly other CF-associated mutants.

 RESULTS

Because earlier studies suggested that lowering the temperature allowed ΔF508 to fold correctly and exit the ER (Denning et al., 1992a; Lukacs et al., 1993; Sato et al., 1996), we first examined the temperature-sensitivity of A455E and P574H processing. We expressed these mutants in COS-7 cells at either 26°C or 37°C and then detected CFTR by immunoprecipitation, phosphorylation, and electrophoresis. Fig. 1 shows a representative time course of wild-type and each mutant. The core-glycosylated protein present in the ER (band B) migrates at ~140 kDa and the mature protein which has been glycosylated in the Golgi (band C) migrates at ~170 kDa (Cheng et al., 1990). For wild-type CFTR (A), band B and band C were present at both temperatures. For the NBD1 mutants, ΔF508 (B), A455E (C) and P574H (D), band B was the primary
form detected at 37°C. After 54 hours at 26°C, band C was also detected in the NBD1 mutants. To assess these changes in protein processing, we quantitated the amount of band C relative to total CFTR at each temperature and time point (shown below each autoradiograph in Fig. 1). For wild-type CFTR (A), the majority of protein was mature band C and was unaffected by temperature. For ∆F508 (B) and A455E (C), the relative amount of band C was minimal at each time at 37°C. Although the relative amount of band C in P574H (D) was also low, it increased slowly with time at 37°C. When the temperature was reduced to 26°C, the relative amount of band C for ∆F508 and A455E increased modestly, while the amount of P574H band C relative to total P574H increased. Although lowering the temperature caused an increase in the relative amount of P574H band C, the total amount of P574H band C was not as high as that in wild-type CFTR. These results indicate that, like ∆F508, both A455E and P574H are temperature-sensitive processing mutants. Moreover, P574H makes relatively more band C at both 37°C and 26°C than either ∆F508 or A455E.

We asked which step in the processing pathway was altered by lowering the temperature. The band B form of CFTR must adopt a more mature protein conformation before it can exit the ER and undergo further glycosylation in the Golgi (Lukacs et al., 1994; Ward and Kopito, 1994). We will refer to the folded form of the protein that can exit the ER as the

![Fig. 1. P574H and A455E are temperature-sensitive mutants. COS-7 cells were electroporated with pcDNA3 vectors encoding wild-type CFTR (A), ∆F508 (B), A455E (C), and P574H (D). After 18 hours at 37°C, half the cells were shifted to 26°C. Cells were harvested 6, 30, and 54 hours after temperature shift (equivalent to 24, 48 and 72 hours after electroporation). Proteins were solubilized, immunoprecipitated and phosphorylated. Immunoprecipitates were electrophoresed and autoradiographed. Representative autoradiographs are shown in the top of each panel. Band B (core-glycosylated) and band C (fully glycosylated) forms of CFTR are indicated by arrows. Radioactivity in bands B and C was quantitated. Representative autoradiographs are shown in the top of each panel. Band B (core-glycosylated) and band C (fully glycosylated) forms of CFTR are indicated by arrows. Radioactivity in bands B and C was quantitated.](image1)

![Fig. 2. Effect of brefeldin A on the relative amount of band B protein at 37°C or 26°C. HeLa cells infected with recombinant vaccinia virus encoding P574H-CFTR, ∆F508-CFTR, and wild-type CFTR were pulsed (P574H-CFTR and ∆F508-CFTR for 30 minutes; wild-type-CFTR for 15 minutes) with [35S]methionine at 5 hours post-infection and chased for the indicated times with 10 mM cold methionine in the presence or absence of (5 μg/ml) brefeldin A (BFA). Chase was performed at 37°C (A) or at 26°C (B). Chase was continued for 30 hours for P574H and ∆F508 to detect the stable B form. Proteins were solubilized and immunoprecipitated. After electrophoresis, radioactivity in band B and C was quantitated. Data are expressed as counts in band B at the indicated time relative to the number of counts in band B at beginning of chase (time=0). Each data point represents the average of 3 experiments ± s.e.m. Repeated measures analysis with multiple means comparison using Supernova software (Abacus Concepts, Berkeley, CA) indicates that P574H + BFA is different from ∆F508 + BFA from 7.5 hours through 30 hours (P=0.013). Wild-type immunoprecipitated with anti-CFTR antibodies (∆BFA, ○; +BFA, ●); ∆F508 (∆BFA, △; +BFA, ▲); P574H (–BFA, △; + BFA, ●).](image2)
Formation of the intermediate form of band B and then band C is an inefficient process; only 20-25% of wild-type CFTR forms this intermediate and eventually matures. Under normal experimental conditions, the intermediate band B cannot be distinguished from the population of immature band B which eventually degrades. Once the intermediate conformation of band B is formed, it rapidly leaves the ER and enters the Golgi. However, if cells are treated with brefeldin A (BFA), vesicular traffic between the ER and the Golgi is effectively inhibited (Misumi et al., 1986), and the intermediate B accumulates. As previously reported (Lukacs et al., 1994; Ward and Kopito, 1994), we found that for wild-type CFTR, accumulation of intermediate B was detectable by 2 hours at 37°C (Fig. 2A). Unlike wild-type CFTR, neither ΔF508 nor P574H formed detectable intermediate B after BFA treatment at 37°C (Fig. 2A). However, when the temperature was lowered to 26°C, the intermediate B form of P574H was detectable after 7.5 hours of BFA treatment (Fig. 2B), a time that correlates with the production of P574H band C in the absence of BFA (Fig. 1D). Likewise, a small amount of intermediate B accumulated after incubation of ΔF508-expressing cells at 26°C, but this accumulation of ΔF508 was slower than the accumulation of P574H intermediate B at 26°C (Fig. 2). These data suggest that the temperature-sensitive maturation defect of ΔF508 and P574H occurs at or prior to generation of intermediate band B protein and that P574H responds more readily to lowered temperature than ΔF508.

We used immunocytochemistry to determine if the cellular distribution of ΔF508, A455E and P574H was consistent with the quantitative biochemical differences we had observed. Fig. 3 shows the immunostaining pattern of CFTR in COS-7 cells at 37°C (left panels) and at 26°C (right panels). The immunofluorescence pattern of wild-type CFTR is characterized by abundant cytoplasmic and membrane staining at both temperatures (A and B). The fluorescence pattern of ΔF508 (C) and A455E (E) at 37°C resembles the reticular pattern characteristic of ER with an absence of plasma membrane staining. When the cells were incubated at 26°C, cytoplasmic staining became more diffuse and the outline of the cell membrane was occasionally detectable in ΔF508 (D) and A455E (F). More interestingly, in addition to a reticular
pattern, P574H presented an unusual immunofluorescence pattern of prominent punctate bodies distributed within the cytoplasm when cells were grown at 37°C (G). When the temperature was reduced to 26°C, the reticular pattern decreased, cell membrane staining became more prominent, and the punctate cytoplasmic bodies disappeared (H). These temperature-dependent changes were consistent with the increased amounts of band C produced at lower temperatures.

We asked whether the cytoplasmic bodies produced by P574H colocalized with a known intracellular organelle. If the bodies were part of the Golgi complex, we expected that they would disappear when the cells were treated with BFA. However, as shown in Fig. 4, the cytoplasmic bodies were unaltered by BFA treatment (compare Fig. 4B to Fig. 4A). In separate experiments, we found that this BFA treatment disrupted the Golgi complex as assessed by immunostaining for Golgi markers (not shown). Moreover, in cells stained with both anti-CFTR and anti-Golgi antibodies, the bodies which contain P574H (Fig. 4C) did not colocalize with the Golgi markers, p58 (Bloom and Brashear, 1989) (Fig. 4D) or β-COP (Duden et al., 1991) (not shown). These data suggested the P574H cytoplasmic bodies were not part of the Golgi complex.

We used cAMP agonists, which have been shown by others to influence membrane insertion and retrieval of endosomal CFTR (Bradbury et al., 1992; Lehrich et al., 1998), to determine if the P574H bodies were part of endocytic or exocytic vesicles. However, cAMP treatment did not alter the pattern of cytoplasmic bodies (not shown). Although CFTR has also been reported to be contained in clathrin-coated vesicles (Bradbury et al., 1994), the punctate P574H bodies did not colocalize with the more disperse network of clathrin, a component of the trans-Golgi network and the membrane coat of endocytic vesicles and lysosomes (Brodsky, 1988) (not shown).

To determine if P574H was present in the ER, we examined the staining pattern of the ER-resident protein, protein disulphide isomerase (PDI) (Kaetzel et al., 1987). In identical cells, both anti-CFTR antibody (Fig. 5A) and anti-PDI antibody (Fig. 5B) exposed a similar reticular network pattern characteristic of ER. Thus P574H, like ΔF508, is retained within the reticular network of the ER. In addition, the punctate P574H cytoplasmic bodies stained with both anti-CFTR and anti-PDI antibodies. These results suggest not only that P574H is localized in the ER, but that the punctate cytoplasmic bodies may represent a subdomain of the ER which is only detectable in cells expressing P574H. Because previous work showed that ΔF508 was associated with the cytoplasmic chaperone Hsp70 (Yang et al., 1993), we examined the possibility that the P574H in the punctate bodies might associate with Hsp70. When the same cells are stained with both anti-CFTR and anti-Hsp70 antibodies, P574H (Fig. 5C) and Hsp70 (Fig. 5D) show a striking colocalization in both the reticular ER pattern and in the cytoplasmic bodies.

Colocalization of P574H and Hsp70 suggested a physical association of P574H with Hsp70. To test this, we used anti-Hsp70 antibodies to coimmunoprecipitate P574H that was bound to Hsp70 and evaluated the time course of the retention in a pulse-chase experiment. Fig. 6 shows that band B, but not band C, of wild-type CFTR, ΔF508 and P574H were all initially associated with Hsp70. The association between wild-type CFTR and Hsp70 decreased rapidly. However, band B of both ΔF508 and P574H retain their association with Hsp70 for

Fig. 4. P574H cytoplasmic bodies are not part of the Golgi complex. COS-7 cells expressing P574H and grown at 37°C were either treated with brefeldin A (BFA) (5 μg/ml) (B) or the vehicle control (A) for 30 minutes before staining with anti-CFTR antibody. Cells expressing P574H grown at 37°C were stained with anti-CFTR antibody (C) and anti-Golgi (p58) antibody (D).

Fig. 5. P574H cytoplasmic bodies colocalize with ER markers and with Hsp70. Cells expressing P574H grown at 37°C were stained with anti-CFTR antibody (A) and with protein-disulphide isomerase antibody (PDI) (B). Cells expressing P574H grown at 37°C were stained with anti-CFTR antibody (C) and with anti-Hsp70 antibody (D).
The association of P574H and ΔF508 with Hsp70 is prolonged. HeLa cells infected with recombinant vaccinia virus encoding wild-type-CFTR, ΔF508 and P574H were pulsed for 15 minutes with [35S]methionine at 5 hours post-infection and chased for the indicated times with 10 mM cold methionine at 37°C. Proteins were solubilized, precleared, divided in half and immunoprecipitated. (A) Example of immunoprecipitation with anti-CFTR antibodies (left) and anti-Hsp70 antibody (right). Band B is indicated by arrow. (B) Data from 3 experiments. Data are counts in band B immunoprecipitated by anti-Hsp70 antibody relative to counts in band B immunoprecipitated by anti-CFTR antibody, at each time point. Wild-type-CFTR (○); P574H (△); ΔF508 (▲).

Fig. 6. The association of P574H and ΔF508 with Hsp70 is prolonged. HeLa cells infected with recombinant vaccinia virus encoding wild-type-CFTR were pulsed for 15 minutes with [35S]methionine at 5 hours post-infection and chased for the indicated times with 10 mM cold methionine in the presence or absence of brefeldin A (BFA) at 37°C. Proteins were solubilized then preabsorbed with Protein A-agarose. Precleared lysates were divided: half was immunoprecipitated with anti-Hsp70 antibody and half was immunoprecipitated with anti-CFTR. Immunoprecipitates were electrophoresed and radioactivity in band B quantitated. Data is expressed as counts in band B at the indicated time relative to the number of counts in band B at beginning of chase (time=0). n = 4. Wild-type-CFTR immunoprecipitated with anti-Hsp70 antibodies (–BFA, ○); (+BFA, ●); wild-type CFTR immunoprecipitated with anti-CFTR antibody (–BFA, □); (+BFA, ■). The initial increase in the relative amount of band B has been observed previously (Ward and Kopito, 1994) and may represent completion of nascent chain synthesis.

Fig. 7. The intermediate form of band B of wild-type CFTR is not associated with Hsp70. HeLa cells infected with recombinant vaccinia virus encoding wild-type-CFTR were pulsed for 15 minutes with [35S]methionine at 5 hours post-infection and chased for the indicated times with 10 mM cold methionine in the presence or absence of brefeldin A (BFA) at 37°C. Proteins were solubilized then preabsorbed with Protein A-agarose. Precleared lysates were divided: half was immunoprecipitated with anti-Hsp70 antibody and half was immunoprecipitated with anti-CFTR. Immunoprecipitates were electrophoresed and radioactivity in band B quantitated. Data is expressed as counts in band B at the indicated time relative to the number of counts in band B at beginning of chase (time=0). n = 4. Wild-type-CFTR immunoprecipitated with anti-Hsp70 antibodies (–BFA, ○); (+BFA, ●); wild-type CFTR immunoprecipitated with anti-CFTR antibody (–BFA, □); (+BFA, ■). The initial increase in the relative amount of band B has been observed previously (Ward and Kopito, 1994) and may represent completion of nascent chain synthesis.

DISCUSSION

Earlier work showed that CFTR containing the CF-associated mutations ΔF508, A455E, or P574H decreases cell membrane Cl− current primarily because the mutant proteins fail to fold correctly and therefore do not traffic out of the ER (Cheng et al., 1990; Lukacs et al., 1994; Ward and Kopito, 1994; Sheppard et al., 1995; Qu and Thomas, 1996; Qu et al., 1997; Yang et al., 1993; Zhang et al., 1998). The defect in ΔF508

the conformational maturation of P574H may be delayed or actually inhibited, consistent with the diminished ability of P574H to adopt the intermediate B conformation at 37°C. We used wild-type CFTR in a pulse chase experiment to test whether release of CFTR from the chaperone is coincident with formation of the intermediate B conformation (Fig. 7). We immunoprecipitated wild-type CFTR with either anti-CFTR antibodies or with anti-Hsp70 antibodies. To detect the intermediate B form of CFTR, we repeated the experiments in the presence of BFA. Fig. 7 shows the accumulation of the intermediate B form of CFTR in the presence of BFA. However, under the same conditions, this accumulated form recognized by anti-CFTR antibodies was not coimmunoprecipitated by anti-Hsp70 antibodies. These results indicate that the intermediate B was not associated with Hsp70 and suggests the release of CFTR from Hsp70 occurs at or about the same time as the protein adopts a more mature conformation.
processing can be partially corrected by incubation at a lower temperature (Denning et al., 1992a; Lukacs et al., 1993; Sato et al., 1996). We found that, like ΔF508, the processing of P574H and A455E is temperature-sensitive. Moreover, unlike ΔF508 and A455E, P574H formed some mature protein at 37°C, and at 26°C, P574H generated relatively more mature protein than ΔF508. Thus the processing defect is less severe for P574H, consistent with functional studies (Sheppard et al., 1995). These studies demonstrate that misprocessing is not an all-or-none phenomenon, but rather a continuum, with wild-type P574H >A455E>ΔF508. This is consistent with the clinical phenotype in CF patients: P574H and A455E are associated with a milder, pancreatic-sufficient phenotype and ΔF508 is associated with a severe, pancreatic-insufficient clinical phenotype (Kerem et al., 1990a,b; Kristidis et al., 1992; Veeze et al., 1994; Gan et al., 1995).

Our data suggest that the temperature-sensitive step in maturation occurs prior to or at the generation of the intermediate B form of CFTR. The marked differences in the degree of temperature-sensitive correction of processing corresponded to the efficiency of formation of the intermediate B. This was especially clear for the P574H mutant: the appearance of the intermediate B form in cells treated with BFA occurred at the same time as the maturation of band B to band C in the cells not treated with BFA, suggesting, as has been shown for wild-type, that intermediate B goes on to become the mature band C form of the protein.

At 37°C, P574H resides in the ER; P574H showed both the characteristic reticular pattern of immunocytochemical staining typical of ER as well as a unique punctate pattern of cytoplasmic bodies which colocalized with an ER marker. More interestingly, P574H also colocalized with the chaperone Hsp70 in both the reticular ER and in the punctate bodies and was associated with Hsp70 by coimmunoprecipitation. When the temperature was reduced to 26°C, the cytoplasmic bodies containing P574H and Hsp70 were no longer observed, suggested they had dissipated and P574H proceeded to make both the intermediate B and the band C forms of protein.

The punctate bodies appeared only in cells expressing P574H. These inclusions were not present in wild-type, ΔF508, A455E or any other mutant we have studied. This morphological pattern is not simply due to the level of protein expression, because the absolute amount of recombinant protein expressed is low and protein expression was no greater for P574H than for any other forms of CFTR. Nor was it dependent on cell type; we observed the same punctate cytoplasmic bodies in both COS-7 cells and in HeLa cells. Interestingly, the appearance of subcellular structures reminiscent of the P574H bodies have been reported following expression of other endogenous and recombinant membrane proteins. For example, overexpression of HMG-CoA reductase (Chin et al., 1982; Anderson et al., 1983) or microsomal alcohol dehydrogenase (Yamamoto et al., 1996) induces formation of crystallloid ER, an organelle of hexagonally stacked ER. In Saccharomyces cerevisiae, expression of the yeast form of HMG-CoA reductase produces not crystallloid arrays, but ‘karmellae’, stacks of paired ER membranes (Wright et al., 1988; Parrish et al., 1995). Other examples include the accumulation of cytochrome P-450 within perinuclear membranous bodies which also appear to be derived from the ER (Ishihara et al., 1995), as well as the accumulation of the rubella virus El membrane glycoprotein (Hobman et al., 1992) and HLA-B27 (Raposo et al., 1995) within the network of tubular membranes between the ER and Golgi complex. Thus, expanded ER compartments of various morphologies have been observed in several types of cells.

We do not know the functional significance of the P574H accumulated within the punctate bodies; it may represent a pool of protein which can convert to the intermediate B form when the temperature is lowered. The late onset of modest amounts of mature P574H band C at 37°C may represent a slow conversion or ‘leak’ from this protected pool to intermediate B. We speculate that we do not see the bodies in ΔF508 because the extent of misfolding of ΔF508 prevents it from reaching this compartment. We may not see formation of the bodies in wild-type CFTR because it exits the ER readily without accumulating. Alternatively, we cannot exclude the possibility that the punctate bodies in cells expressing P574H could represent an ER subcompartment en-route to degradation.

Finally, our results suggest that formation of the intermediate B form of wild-type CFTR and release of CFTR from Hsp70 are coincident. The inability of P574H to form intermediate B and the prolonged retention of P574H by Hsp70 are consistent with this observation. Such results coupled with the observation that the formation of intermediate B is temperature-sensitive suggest that interventions that promote formation of intermediate B may prove to be of value in designing treatments for cystic fibrosis.

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REFERENCES


transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63, 827-834.


