Post-translational Disruption of the ΔF508 Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-Molecular Chaperone Complex with Geldanamycin Stabilizes ΔF508 CFTR in the Rabbit Reticulocyte Lysate*

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The ΔF508 mutation of cystic fibrosis transmembrane conductance regulator (CFTR) is a trafficking mutant, which is retained and degraded in the endoplasmic reticulum by the ubiquitin-proteasome pathway. The mutant protein fails to reach a completely folded conformation that is no longer a substrate for ubiquitination (“stable B”). Wild type protein reaches this state with 25% efficiency. In this study the rabbit reticulocyte lysate with added microsomal membranes has been used to reproduce the post-translational events in the folding of wild type and ΔF508 CFTR. In this system wild type CFTR does not reach the stable B form if the post-translational temperature is 37 °C, whereas at 30 °C the behavior of both wild type and mutant proteins mimics that observed in the cell. Geldanamycin stabilizes ΔF508 CFTR with respect to ubiquitination only when added post-translationally. The interaction of wild type and mutant CFTR with the molecular chaperones heat shock cognate 70 (hsc70) and heat shock protein 90 (hsp90) has been assessed. Release of wild type protein from hsc70 coincides with the cessation of ubiquitination and formation of stable B. Geldanamycin immediately prevents the binding of hsp90 to ΔF508 CFTR, and after a delay releases it from hsc70. Release of mutant protein from hsc70 also coincides with the formation of stable B ΔF508 CFTR.

Mutations in the CFTR1 gene cause cystic fibrosis. The CFTR protein consists of two hydrophobic domains, with ATP binding folds at the carboxyl-terminal end of each (1). These are approximately 150 residues in length, and the first is the site of the most common mutation: ΔF508, the loss of a single phenylalanine residue from position 508 in the polypeptide chain (2).

Like most cystic fibrosis-causing mutations, expression of ΔF508 CFTR in mammalian cell culture systems does not result in the formation of a cAMP activated chloride channel at the cell surface (3, 4). However, the primary defect has been identified as intracellular processing of the mutant rather than a lack of chloride channel activity (5, 6). Wild type CFTR reaches the Golgi apparatus from the ER with approximately 25% efficiency by reaching a degradation-resistant form referred to as “stable B” (5, 7, 8). ΔF508 CFTR is functional if it reaches the cell surface, but it fails to do so in cellular systems, because it cannot reach stable B and is therefore not processed from the ER to the Golgi apparatus. The molecular chaperone hsc70 has been implicated in retaining misfolded ΔF508 CFTR in the ER (9). In addition, a role has been proposed for hsp90 in the biogenesis of the wild type protein (10). Wild type and mutant CFTR failing to leave the ER are degraded by the ubiquitin-proteasome pathway (11, 12).

The stress-90 molecular chaperones constitute a ubiquitous, major family of stress proteins. Mammalian cytosolic hsp90 is probably the most abundant molecular chaperone, being present at 1–2% of total extractable cellular protein (13). Although the roles of hsp90 in controlling the cell cycle and various DNA-binding proteins are well defined (for review see Ref. 13), the involvement of hsp90 in the folding of newly synthesized polypeptides has not been investigated extensively. At least two proteins are identified complexed with hsp90 when newly synthesized: p56

* The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; hsc70, heat shock cognate 70; hsp90, heat shock protein 90; RRL, rabbit reticulocyte lysate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

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pears to occupy the ATP binding site on the chaperone (24). Hence it stops ATP binding and prevents the normal function of the amino-terminal substrate binding site of hsp90.

Here evidence is presented that interference with the normal function of hsp90 in the rabbit reticulocyte lysate (RRL) with geldanamycin reduces degradation of ΔF508 CFTR and disrupts a chaperone complex such that the protein is no longer presented for ubiquitination. A model for the role of hsp90 and hsc70 in mediating ubiquitination of CFTR is proposed.

**EXPERIMENTAL PROCEDURES**

**Materials**—The TNT T7 coupled transcription/translation RRL and canine pancreatic microsomal membranes were obtained from Promega (Madison, WI) and used according to the manufacturer’s instructions. Antibodies against hsc70 (clone IB5), hsp90 (clone 16F1), and ubiquitin were supplied by StressGen Biotechnologies and a carboxyl-terminal-specific antibody to CFTR was supplied by Genzyme (Cambridge, MA). Protein G-Sepharose was obtained from Amersham Pharmacia Biotech. [35S]Methionine was purchased from PerkinElmer Life Sciences. Geldanamycin was a generous gift from Dr. David Newman at the United States National Cancer Institute. Wild type and ΔF508 CFTR cDNAs were supplied by Dr. Deborah Gill at the University of Oxford. The cDNA fragment from the NheI site at the 5’ end of the CFTR cDNA to the SpeI site at the 3’ end after the translation stop codon was cloned into the pSI vector from Promega. All other chemicals were purchased from Sigma.

**Expression of CFTR in the RRL**—At the chosen translation temperature of 24 °C, optimal expression was achieved using 0.5 μg of DNA, 3 units of canine pancreatic microsomal membranes, and 1 μl of [35S]methionine in a 25-μl reaction. Transcription and translation occurred in the same reaction over 120 min. Post-translational ubiquitination of CFTR and ΔF508 CFTR were measured following the addition of cycloheximide at a final concentration of 100 μg/ml.

**Immunoprecipitation of Proteins from the RRL**—For the immunoprecipitation of hsc70, aliquots of lysate were adjusted to 0.1% Triton X-100, 5 mM MgCl₂, and 10 units/ml aprotinin and agitated for 15 min at 4 °C. Sample composition was adjusted to 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS; samples were pre-cleared, and then 1 μg of anti-hsc70 antibody was added. Following exposure for 2 h at 4 °C, immune complexes were harvested with protein G-Sepharose for 2 h at 4 °C, after which the beads were washed twice for 5 min each with radioimmune precipitation buffer (50 mM Tris-Cl, pH 7.5, 10 mM MgCl₂, 0.1% sodium deoxycholate, 0.1% SDS) and once for 20 min in 10 mM Tris-Cl, pH 7.5, 0.1% Nonidet P-40. Proteins were eluted from the Sepharose beads using SDS sample buffer, and the CFTR content of the bead fraction and immuno-depleted supernatant was assessed by SDS-PAGE. Hence the proportions of wild type and ΔF508 CFTR bound to and free from molecular chaperones were assessed.

CFTR was immunoprecipitated with a carboxyl-terminal-specific antibody under identical conditions but without the preliminary depletion of ATP from the reaction. hsp90 was immunoprecipitated in a buffer consisting of 50 mM NaCl, 20 mM Tris-Cl, pH 7.5, 10 mM sodium molybdate, 0.25% Tween 20. Immune complexes on protein G-Sepharose beads were washed three times with 10 mM Tris-Cl, pH 7.5, 10 mM sodium molybdate, 0.1% Nonidet P-40. Ubiquitin was immunoprecipitated under the same conditions as hsp90, with the exclusion of sodium molybdate from the reaction.

**SDS-PAGE**—Electrophoresis was performed according to the method of Laemmli (25) using the Bio-Rad mini protean II system. 8% polyacrylamide minigels were fixed in 25% isopropyl alcohol, 10% acetic acid and dried under vacuum.

**Quantification of CFTR Expression**—Dried gels were exposed to Molecular Dynamics PhosphorImager, and densitometric analysis was undertaken using the ImageQuant software.

**RESULTS**

**Translation of CFTR in the RRL at 24 °C Produces a Full-length Protein That Is Core-glycosylated in the Presence of Microsomal Membranes**—Panel B of Fig. 1 indicates that the CFTR produced in the RRL is a full-length protein, because it is immunoprecipitated by a carboxyl-terminal-specific antibody. Panel A shows the effect of the addition of microsomes to the translation mix. The molecular mass increase observed (5–6 kDa) is consistent with two N-linked glycosylation events occurring. There are two adjacent N-linked glycosylation sites in the fourth extracellular loop of CFTR (26).

**Increasing the Post-translational Temperature to 37 °C Causes Rapid Ubiquitination of Wild Type and ΔF508 CFTR**—Following translation at 24 °C and addition of 100 μg/ml cycloheximide, the incubation temperature was increased to 37 °C (time zero). Samples were taken and analyzed by SDS-PAGE as shown in Fig. 2, panel A. Both wild type and ΔF508 CFTRs are ubiquitinated with indistinguishable kinetics. The observed half-lives of the core glycosylated bands (28.7 min for the wild type protein and 29.0 min for the mutant protein) are similar to those reported in cellular systems (7, 8).

Panel B of Fig. 2 indicates that the high molecular mass material observed following incubation of CFTR is immunoprecipitated by an anti-ubiquitin antibody. The addition of multiple ubiquitin chains retards the progress of the CFTR in the gel such that it accumulates at the top. This does not necessarily reflect the true molecular mass of the polyubiquitinated protein; its mobility through the gel suggests a molecular mass of several hundred if not thousands of kilodaltons, which would be caused by the addition of many more ubiquitin chains than has previously been reported (27, 28). The high molecular mass could be due to the polyubiquitination at a single lysine residue or multiple lysine residues on the CFTR protein. The data cannot discount the possibility that ubiquitination caused aggregation of the CFTR protein. Other investigators examining the ubiquitination of CFTR in the cell and the RRL report an
identical effect of ubiquitination on its electrophoretic mobility (11, 12, 29).

On continued incubation of core glycosylated wild type CFTR at 37 °C for several hours after synthesis is halted, there is no apparent diminution in the intensity of the polyubiquitinated CFTR band (panel C of Fig. 2). In the cell, this polyubiquitinated CFTR would be degraded by the proteasome (11, 12). However, the RRL contains hemin (20 μM), which is required for optimal translation (30). Hemin is also a potent inhibitor of proteasomal activity (31); hence proteins expressed in the RRL can be ubiquitinated but not degraded by the proteasome. Introduction of core-glycosylated CFTR into hemin-free lysate restores proteasomal degradation of this high molecular mass polyubiquitinated CFTR by the RRL.

**Wild Type CFTR Is Stabilized Compared with ΔF508 CFTR at 30 °C**—Following translation at 24 °C approximately 20% of wild type protein is stable with respect to ubiquitination when incubated at 30 °C, whereas all ΔF508 CFTR is ubiquitinated at this temperature (Fig. 3). This stable wild type protein may be analogous to the stable B form observed in cells (5, 7, 8). It is not a result of the ability of the RRL to ubiquitinate substrates being compromised; when presented with a similar amount of mutant protein it is all ubiquitinated over the same time.

**Geldanamycin Restores the Ubiquitination of ΔF508 CFTR to Wild Type Kinetics Only When Added Post-translationally**—The effect of 11 μg/ml geldanamycin on the stability of ΔF508 CFTR in the RRL at 30 °C is shown in Fig. 4. This concentration reliably gives full inhibition of hsp90 function in the RRL (17). Co-translational addition of the drug (panel A) is without effect; however, when the drug is added post-translationally (panel B), approximately 25% of the mutant protein is stabilized with respect to ubiquitination. On treatment with geldanamycin there is no change in the rate of ubiquitination of the mutant protein; there is no significant difference between the amounts of protein remaining in the presence or absence of geldanamycin after up to 6 h of incubation. However, in the presence of geldanamycin little or no further ubiquitination
mean (hours after inhibition of translation) above each lane. Graph shows from the reaction and analyzed by SDS-PAGE at the times indicated ubiquitinated over 18 h at 30 °C in the RRL. Samples were removed type CFTR, D
F508 CFTR, restoring its profile of degradation to a wild  
ence of 11 hsc70 or hsp90 (data not shown). Hence without effect on the efficiency of immunoprecipitation of ei-
to the chaperones was assessed. Geldanamycin treatment was assessed over the first 6 h of incubation at 30 °C. This was achieved through immunoprecipitation of the chaperone under conditions favoring substrate association (depletion of ATP in the case of hsc70; 10 mM sodium molybdate for hsp90). Hence the relative proportion of wild type and mutant protein bound to the chaperones was assessed. Geldanamycin treatment was without effect on the efficiency of immunoprecipitation of either hsc70 or hsp90 (data not shown).

Panel A of Fig. 5 shows the interaction of hsp90 with wild type CFTR, ΔF508 CFTR alone, and ΔF508 CFTR in the presence of 11 μg/ml geldanamycin. Panel B shows the interactions of the same three groups with hsc70. Note that whereas association of wild type protein with these chaperones is transient, ΔF508 CFTR forms a long lasting complex with hsc70 (as previously reported (9)) and hsp90. Geldanamycin rapidly prevents the association of hsp90 with ΔF508 CFTR and gradually reduces binding of hsc70 over a 4-h period. It is noteworthy that the loss of binding of hsc70 to both wild type and ΔF508 CFTR coincides with the cessation of ubiquitination of these proteins.

**DISCUSSION**

In this study, a cell-free system has been described that reproduces the differences between wild type and ΔF508 CFTR observed in the cell. Following translation, 20% of wild type protein is stable with respect to ubiquitination at 30 °C, but all occurs after 4 h of incubation at 30 °C, such that approximately 25% of the mutant protein persists on overnight incubation. Hence, 11 μg/ml geldanamycin has a stabilizing effect on ΔF508 CFTR, restoring its profile of degradation to a wild type-like appearance.

**Geldanamycin Prevents the Interaction of hsp90 and hsc70 with ΔF508 CFTR**—The interaction of wild type and ΔF508 CFTR with the molecular chaperones hsp90 and hsc70 was assessed over the first 6 h of incubation at 30 °C. This was achieved through immunoprecipitation of the chaperone under conditions favoring substrate association (depletion of ATP in the case of hsc70; 10 mM sodium molybdate for hsp90). Hence the relative proportion of wild type and mutant protein bound to the chaperones was assessed. Geldanamycin treatment was without effect on the efficiency of immunoprecipitation of either hsc70 or hsp90 (data not shown).

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**ΔF508 CFTR and Geldanamycin**

ΔF508 CFTR is ubiquitinated. The stable wild type protein observed may be analogous to the stable B form described in cells (5, 7, 8). This is addressed below. Whatever the nature of this stable form of the wild type protein, a clear difference is observed between the handling of this and the mutant protein, and pharmacological correction of this difference in the RRL such that mutant protein is stable over an overnight incubation at 30 °C may be sufficient to correct the ΔF508 trafficking defect in the intact cell.

The RRL with microosomal membranes added has been presumed to essentially provide cytosolic and ER compartments in which folding and degradation events are unaltered from the way in which they proceed in the intact cell. However, the failure of the lysate to support formation of stable B CFTR at 37 °C casts doubt on such an assumption and calls into question the validity of using the RRL as a model for the cell at this temperature (29). The fact that stable B CFTR may be achieved at 30 °C goes some way to reaffirming that the lysate is indeed a relevant cellular model. Little is known about the properties of the stable B form of the protein. Its well identified characteristics are its lack of susceptibility to ubiquitination in the cell and its ability to traffic from the ER to the Golgi apparatus. Whereas the former property has been established in the RRL, the latter cannot be tested here because trafficking out of the ER is not possible in this system. The failure of ΔF508 CFTR to achieve this state is further evidence that the observed cellular difference between wild type and ΔF508 CFTR is reproduced here; the specific lesion of ΔF508 CFTR is the conversion from
Fig. 5. Association of ΔF508 CFTR with hsp90 and hsc70 is abolished by geldanamycin. Wild type CFTR associates with both chaperones transiently, A, immunoprecipitation of ΔF508 CFTR with an hsp90 antibody indicates that the amount of mutant protein in association with the chaperone is relatively high throughout the 6-h period studied. Wild type CFTR is associated with hsp90 at a low level throughout. Addition of 11 μg/ml geldanamycin immediately abolishes the association of hsp90 with ΔF508 CFTR (graphs show mean ± S.E.; n = 6). B, association of hsc70 with ΔF508 CFTR is also high throughout its lifetime. Wild type CFTR is also highly associated with the chaperone initially but dissociates over the 6-h period examined. 11 μg/ml geldanamycin is at first without effect on the binding of hsc70; however, after 4 h of incubation the chaperone is dissociated from ΔF508 CFTR (graphs show mean ± S.E.; n = 6). In the case of the latter two groups, dissociation from hsc70 coincides with the cessation of ubiquitination observed in Fig. 3.

The fact that the RRL supports formation of stable B wild type CFTR means the effects of drugs on formation of stable B ΔF508 CFTR may be assessed. Any drug showing positive effects may facilitate the release of the mutant protein from its ER processing block in vivo. The effect of geldanamycin on ΔF508 CFTR is described in Fig. 4. This drug supports production of stable B ΔF508 CFTR in the RRL. Stabilization is only observed on post-translational addition of the drug. If binding of hsp90 to the mutant protein were simply post-translational, then geldanamycin would have an identical effect on ΔF508 CFTR whether it was present throughout translation or after it was complete. That geldanamycin is without effect on ΔF508 CFTR stability if present co-translationally implies that co-translational binding of the chaperone is required if stable B is to be achieved. Hence, the following model is put forward. Co-translational binding of ΔF508 CFTR by hsp90 mediates the formation of stable B protein; post-translational binding is involved with ubiquitination of the protein. In the cell, this model predicts that only protein already translated upon addition of geldanamycin will be stabilized with respect to ubiquitination.

However, the role of hsp90 cannot be considered alone. Blockage of hsc70 binding by treatment with an hsp90 binding agent indicates that the functions of these two proteins are linked. The effect of geldanamycin on hsc70 binding is delayed, but its effect on hsp90 binding is immediate. This delay perhaps implies that whereas the effect of this hsp90 binding agent in preventing the association of the chaperone with its substrate is mediated directly, its effect on hsc70 interactions with proteins is indirect. It also suggests that the effect of geldanamycin on hsc70 binding to ΔF508 CFTR is not a result of an interaction between geldanamycin and hsc70; such an

unstable to stable band B. It is extremely unlikely that the RRL may be unable to ubiquitinate wild type but not ΔF508 CFTR as a result of being compromised metabolically. Both proteins are synthesized from identical plasmids, save for the presence of the mutation, and similar (very small) quantities of protein are presented to the lysate for ubiquitination. It is therefore unlikely that the lysate could be competent to fully ubiquitate one but not the other unless the wild type protein is no longer a substrate for ubiquitination. The replication of the cellular behavior of nascent wild type and ΔF508 CFTR in the RRL at 30 °C validates the use of this system to study events leading to the formation of stable B protein.

Given that the RRL supports formation of stable B CFTR at 30 °C, an important question must be why it does so at this temperature but not at higher ones. The recommended (and most commonly used) temperature at which the RRL is operated is 30 °C. It is our experience that translation of other proteins in the RRL at higher temperatures produces little full-length protein; the RRL does not operate efficiently at 37 °C. The differences between the lysate at 37 °C and 30 °C cannot be readily identified. It may be that the lower temperature allows some protein complex such as the foldosome (16) to assemble and operate efficiently whereas it cannot at 37 °C. It is also possible that at 30 °C some amount of ordering may be possible in the lysate, the added microsomal membranes, the molecular chaperone system, or the polypeptide chain of CFTR that leads to stable B being favored. In the cell, such order may be imposed by the cytoskeleton. An important prediction from the observation that stable B CFTR can be formed in the RRL is that neither an intact cytoskeleton nor membrane traffic from the ER is required to support formation of stable B in the cell.
effect would be immediate. Association of hsc70 with substrate may rely on an interaction with hsp90 and other members of the foldsome complex. Dissociation of this complex following inhibition of hsp90 function may take time, such that hsc70 interactions with ΔF508 CFTR are still observed after some hours of exposure to the drug. Whatever the mechanism by which these events occur, important conclusions to be drawn are that hsp90 and hsc70 operate together in the RRL (in agreement with other results (33)) and that hsp90 and hsc70 operate cooperatively in the RRL (as suggested in Ref. 34).

The findings of Loo et al. (10) must be considered in respect of the discussion above. These authors describe a destabilization of wild type CFTR in a cellular system as a result of geldanamycin treatment blocking hsp90 function. Geldanamycin prevents the formation of stable B CFTR and targets the protein for ubiquitination and degradation by the proteasome. Blocking hsp90 interactions with wild type CFTR increases its association with hsc70 in this system. This apparently contradicts the results obtained in this study and the model described above. However, the post-translational effect of geldanamycin on ΔF508 CFTR in the RRL is unlikely to be observed in a cellular system because almost all protein examined in such a system will be translated in the presence of the drug. This may explain many of the differences observed between this study and previous work, in which authors usually observe a destabilization of an hsp90 substrate by treatment with geldanamycin (for example, Refs. 18, 22, 35–38). It is perhaps unsurprising result that blocking molecular chaperone interactions with proteins emerging from the ribosome prevents them from following the correct folding pathway. Using the methods of Loo et al. (10), it would be impossible to detect the small amount of CFTR present in the ER when the drug is applied to the cells that would escape ubiquitination, because geldanamycin is added before the [35S]methionine pulse. As a result, all labeled protein is translated in the presence of the drug, and all observations regarding the effect of geldanamycin on CFTR are made on a protein that has been translated in the presence of geldanamycin. The advantage of the RRL as the model system is that the separation of co- and post-translational events is made easy. According to our model, translation of wild type or ΔF508 CFTR in the absence of hsp90 will indeed prevent the formation of stable B protein (Fig. 4, panel A). It is only post-translational disruption of the ΔF508 CFTR-hsp90 complex that rescues the protein from ubiquitination (Fig. 4, panel B). Therefore, the results in Ref. 10 do not contradict our model and indeed in some respects confirm the observations made in this study. Specifically, the authors also observe that hsp90 is involved in the biogenesis of CFTR in another in vitro system.

According to Figs. 2–4, ubiquitination of wild type and ΔF508 CFTR in the RRL only occurs when they are interacting with hsc70. Although no causal link has been established between hsc70 and ubiquitination of CFTR by this work, others have found that hsc70 is required for ubiquitination of certain protein substrates (38–40); so our results do suggest a role for hsc70 in mediating the ubiquitination of CFTR in vitro. Release of wild type CFTR from hsc70 coincides with its stabilization in the RRL, and the same result is seen for ΔF508 CFTR when interactions with hsc70 are prevented with geldanamycin. Hence, although the link between hsc70 binding and ubiquitination has not been definitively established, it is clear that this chaperone cannot simply be regarded as a protein folder. Nor indeed can hsp90, because it is this protein whose targeting has affected the ubiquitination of ΔF508 CFTR in this system. hsc70 should be regarded as an agent that promotes protein folding and degradation equally. This is entirely in line with its function in protein quality control. The dual functions of deter-

mining the progress toward the folded state of a protein and presenting misfolded proteins to the degradation apparatus of the cell must co-exist for any protein to effectively carry out quality control in the cell. hsc70 appears to fulfil both these functions in the case of controlling the folding of wild type and ΔF508 CFTR.

It is important to appreciate that our model cannot be applied in all situations. When geldanamycin is present co-translationally, ΔF508 CFTR is not stabilized with respect to ubiquitination. According to the model, preventing hsp90 interactions with the protein should prevent ubiquitination of the protein in the RRL. This does not occur probably because the perturbations to the folding pathway are so great when co-translational chaperone binding is prevented that ubiquitination will occur regardless. Whether this effect is mediated by enhanced hsc70 binding to the misfolded protein in some hsp90-independent function (as observed in Ref. 10) will not be speculated upon. However it is achieved, ubiquitination of all ΔF508 CFTR synthesized in the presence of geldanamycin does occur.

The results presented must be considered alongside the observed correction of ΔF508 CFTR trafficking on treatment of cells with the hsc70 binding agent deoxyspergualin (41). Given the data presented here, such a result is perhaps unsurprising. Blockage of binding of the major agent involved in mediating the ubiquitination of ΔF508 CFTR restores its traffic to the plasma membrane.

The identification of the ATP binding site on hsp90 as the site of interaction of geldanamycin (24) raises the possibility that geldanamycin might also bind directly to CFTR at the first or second ATP binding fold. The first ATP binding fold is the site of ΔF508, and it could be argued that geldanamycin is able to stabilize ΔF508 CFTR through binding at the site of the mutation. We do not favor such an explanation, because there is no evidence that geldanamycin is specific for any ATP binding sites other than that on hsp90. Moreover, this cannot explain the requirement that geldanamycin be added post-translationally for its stabilizing effect to be observed in the RRL.

In summary, the molecular chaperones hsc70 and hsp90 have a role in both the folding and ubiquitination of wild type and ΔF508 CFTR. During translation, these chaperones assist the formation of correctly folded protein. Following cessation of translation, hsc70 and hsp90 appear to move into “ubiquitination mode”; blocking the binding of hsp90 and hsc70 to ΔF508 CFTR rescues the protein from ubiquitination. However, the use of chaperone binding drugs in the treatment of cystic fibrosis is unfeasible. Disruption of chaperone function by systemic administration of a drug such as geldanamycin has profound effects throughout an organism (42, 43). Any cystic fibrosis treatment must carry relatively few side effects, because a patient must use such a therapy throughout life. Therefore, disruption of chaperone interactions with nascent ΔF508 CFTR in vivo must be achieved through means other than targeting the molecular chaperones themselves, unless drugs can be targeted directly to epithelia.

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