The ability of newly synthesized proteins, particularly oligomeric or multidomain proteins, to fold into unique, three-dimensional structures in vivo is dependent on their amino acid sequence and the activities of enzymes and molecular chaperone proteins that catalyze folding (9). Evidence is accumulating that many human diseases are caused by improper folding of nascent polypeptides as they achieve a final three-dimensional structure. Such proteins are either inactive or have altered activity as a result of inappropriate folding, as is the case in Marfan syndrome and familial hypercholesterolemia, or are mislocalized due to trafficking defects arising from abnormal folding, as is the case for Tay-Sachs disease and α-1-antitrypsin deficiency.

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) give rise to cystic fibrosis (CF), the most common lethal autosomal genetic disease of Caucasians. Deletion of the codon for phenylalanine-508 (ΔF508) accounts for ~70% of all disease causing CF alleles (11) and yields a protein that is unable to exit from the endoplasmic reticulum (ER) and traffic to the plasma membrane where it functions normally as an ion channel. Available evidence suggests that nascent ΔF508-CFTR is a temperature-sensitive protein (4), which adopts a slightly altered protein conformation. As such, it is recognized by ER quality control mechanisms as abnormal, leading to ER retention (3) (but see also Ref. 6) and ultimately to degradation by the ubiquitin-proteasome pathway (7). Such retention appears, at least in part, to be due to the cellular chaperones, heat-shock protein Hsc70 and calnexin (10, 17). The question of how to shield or dissociate mutant CFTR molecules from the ER quality control machinery and allow its exit to the plasma membrane has been the focus of research in several CF laboratories. Because ΔF508-CFTR retains some regulated chloride channel activity (16), correcting the folding and/or preventing the ER retention of mutant CFTR by chaperones may have therapeutic benefit for the treatment of CF.

Attempts to pharmacologically manipulate the folding of ΔF508-CFTR have included the use of protein stabilizing reagents, or chemical chaperones, such as glycerol and trimethylamine N-oxide, as well as deuterated water (2, 14). Indeed, evidence suggests that such approaches can facilitate exit of ΔF508-CFTR from the ER and insertion into the apical plasma membrane in vivo. A previous publication by Rubenstein and colleagues (12) has shown that sodium 4-phenylbutyrate (4PBA) similarly facilitates exit of ΔF508-CFTR from the ER, though the mechanism of action has remained elusive. Previous work has shown that ΔF508-CFTR remains associated with the cellular chaperone Hsc70 to a much greater extent than wild-type CFTR (17), an association that may direct mutant CFTR to the ubiquitin-dependent degradation pathway (1). In the current article in focus (Ref. 13, see page C259 in this issue), Rubenstein and Zeitlin demonstrate that association of mutant CFTR with the heat-shock protein Hsc70 can be reduced by exposing cells to 4PBA. Mechanistically, it appears that 4PBA inhibits the interaction of ΔF508-CFTR with Hsc70, allowing mutant CFTR to escape targeting for degradation and facilitating its exit from the ER. 4PBA appears to do so by downregulating the expression of Hsc70 at the protein and mRNA level, thereby limiting the amount of Hsc70 that is available to bind to CFTR. Such a hypothesis is consistent with the observations of Cheng and colleagues (5), who utilized deoxyspergualin, a compound that does not affect Hsc70 protein levels but competitively inhibits peptide binding to Hsc70, thereby limiting the amount of Hsc70 that is available to bind to CFTR. It would appear, however, that the efficacious effects of inhibiting CFTR chaperone interactions are limited to Hsc70, since perturbation of CFTR-Hsc90 interactions have been shown to accelerate CFTR degradation (8).

Manipulating CFTR folding by “chemical chaperones” does not appear to be readily feasible in the context of a CF patient. However, preventing the recognition of misfolded CFTR as abnormal may be amenable to pharmacological strategies. Thus the current article by Rubenstein and Zeitlin offers exciting insight into how one might be able to disrupt cellular chaperone-dependent retention of mutant CFTR molecules in the ER, allowing their insertion into the plasma membrane. Moreover, both 4PBA and deoxyspergualin...
gualin are approved drugs for urea cycle disorders and antitumor/immunosuppression, respectively. Indeed, clinical trials using 4PBA have suggested a small but significant improvement in nasal epithelial chloride transport in ΔF508-homozygous CF patients. However, before such observations can be fully realized as therapies, several questions must be addressed. For example, just how much mutant CFTR must escape from the ER quality control machinery to effect clinical benefit? The studies of Cheng and colleagues (5) would argue that the amount of mutant CFTR required to reach the plasma membrane to provide a measurable cAMP-sensitive chloride conductive pathway is less than which can be detected as mature CFTR protein by biochemical methods. However, since ΔF508-CFTR does not retain full wild-type function, even after reaching the plasma membrane, further pharmacological activation of plasmalemmal resident mutant CFTR may be still required (15, 16). It remains to be determined what impact perturbation of the ER quality control machinery will have on protein folding and processing pathways for other proteins expressed in the cell. In addition, Hsc70 has other cellular functions, including the ATP-dependent uncoating of dithrin-coated vesicles in the endocytic pathway and targeting of proteins for lysosomal degradation. It is unclear what effects alterations in Hsc70 activity would have on these intracellular processes. Nevertheless, the ability to screen large libraries of compounds for drugs that disrupt CFTR-Hsc70 interactions may be fruitful in providing a pharmacological therapy for CF.

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