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Errors in protein folding represent the underlying basis for a large number of genetically inherited diseases. Point mutations, deletions, and in some cases, expanded sequences of amino acids give rise to protein products that fail to reach their native folded state. Examples of such diseases are numerous and include cystic fibrosis, chronic liver disease, emphysema, hypercholesterolemia, and, finally, a whole host of neurological pathologies (for review see ref. 1, 2). In many cases the particular mutation results in a protein product that misfolds and/or fails to reach its proper locale inside the cell. The corresponding loss of protein function leads to a cellular abnormality and the eventual development of the diseased phenotype. Hence, identifying ways to correct diseases of protein folding is at the forefront of clinical research today. Whereas gene therapy has been a favored approach, other methods now are beginning to be considered. Herein we discuss the potential value of small molecules for treating diseases of abnormal protein folding.

In many cases, mutations in a peptide are not so severe as to render the affected protein totally inactive. Oftentimes the mutation is relatively minor, resulting in a polypeptide product that still retains some semblance of wild-type function. A relevant example is the  $\alpha 1$  anti-trypsin inhibitor protein. Here, different mutations of the protein are associated with early onset of emphysema and chronic liver disease. For example, a single amino acid substitution (Glu<sup>342</sup> to Lys, the so-called “Z variant”) causes the newly synthesized trypsin inhibitor to adopt a slightly altered conformation. As a result, only about 15% of the newly synthesized protein is secreted out of the cell, with the remainder of the protein retained within the endoplasmic reticulum (ER). Interestingly, that portion of the mutant protein that is secreted func-

tions nearly as well as its wild-type counterpart in inhibiting trypsin/elastase activities (for review see ref. 3).

Another example of an apparent minor mutation that leads to a major pathologic phenotype is cystic fibrosis (CF) (4). The most common mutation observed in CF patients is a single amino acid deletion (phenylalanine at position 508) within a 1480 amino acid protein (termed the  $\Delta F508$  CFTR protein). As a consequence, the mutant protein is believed to fold improperly during the early stages of its biosynthesis and, subsequently, is degraded. Cells expressing  $\Delta F508$  CFTR, unlike those expressing the wild-type protein, are unable to regulate chloride transport activities in response to  $\beta$ -agonists. Failure of the  $\Delta F508$  CFTR protein to fold properly is temperature-dependent: simply lowering the incubation temperature of the cells by 7–10°C results in a population of the mutant protein that folds properly and moves to its correct destination at the plasma membrane. These cells now exhibit forskolin-dependent chloride movement (5).

Such observations indicate that some mutations are rather subtle and lead only to minor changes in the structure and function of the protein. In many instances, alterations of the folding environment (e.g., temperature) can lead to the mutant protein adopting a conformation that is similar, if not identical, to its wild-type counterpart. Hence, designing ways to influence the folding pathway of these mutant proteins and/or to elicit their movement through the cell, at least in theory, might prove useful for treating the diseased state.

One intriguing approach in this regard has been the use of so-called “chemical chaperones.” Here low-molecular-weight compounds including polyols (glycerol), trimethyl amines (trimethyl amine N-oxide), and certain

amino acid derivatives (taurine), when added to cells in culture, help rescue the folding of mutated proteins including  $\alpha 1$  anti-trypsin inhibitor (6) and  $\Delta F508$  CFTR (7, 8). These types of compounds, better known as cellular osmolytes, have long been known to accumulate within cells subjected to osmotic shock and to influence protein-folding pathways. For example, in bacteria and yeast osmotic shock-induced accumulation of cellular osmolytes has been shown to be effective in reverting the effects of different missense mutations. More recently, cellular osmolytes were reported to correct the folding of a number of different temperature-sensitive protein-folding mutants in animal cells (9). Although their mode of action is still not entirely understood, these different compounds, at least at high concentrations (10–1000 mM), are known to stabilize the native state of most any polypeptide (for review see ref. 10).

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The use of cellular osmolytes in the clinical setting, however, seems impractical. Achieving high enough concentrations in vivo to affect the protein-folding environment is unlikely and would certainly lead to other problems. In this issue of the *JCI*, however, Morello et al (11), describe a related, but perhaps more promising approach for correcting the misfolding of another clinically relevant protein, the V2 vasopressin receptor. As with the  $\Delta F508$

CFTR mutation, various deletions/substitutions in the vasopressin receptor result in a protein-trafficking defect. Failure of the newly synthesized vasopressin receptor protein to move from the ER to the plasma membrane disrupts the normal regulation of water transport in the cell (12). Patients suffering from this malady, referred to as nephrogenic diabetes insipidus, are unable to properly reabsorb water in the kidney and develop a number of metabolic disorders.

In a clever twist, Morello and colleagues (11) examined the effects of a receptor antagonist (SR121463A) on the maturation of a trafficking-defective vasopressin receptor mutant protein. Treatment of the cells for about 16 hours with this membrane-permeable antagonist resulted in a portion of the newly synthesized vasopressin receptor to exit the ER and move to the plasma membrane. After removal of the compound, these cells bound vasopressin peptide hormone and activated adenylate cyclase, similar to that observed for cells expressing the wild-type receptor. The increases in the amount of the mutant receptor that reached the plasma membrane were both dose and time-dependent.

Antagonist concentrations of around 100 nM were effective (about 1000 times less than the concentration of cellular osmolytes needed to correct the  $\Delta F508$  CFTR folding mutant). Moreover, incubating cells with a membrane-impermeable peptide failed to rescue the folding of the mutant vasopressin receptor. Finally, 7 other trafficking-defective vasopressin mutants also were observed to move to the plasma membrane in response to either added SR121463A, or another cell-permeable receptor antagonist.

Presumably these compounds act by diffusing into the cell, binding to the newly synthesized mutant receptor (either cotranslationally or posttranslationally) and thereby stabilizing a critical intermediate that is rate limiting for the folding/trafficking of the protein. Alternatively, these small molecules may block an "off-pathway" protein-folding event, thereby accelerating acquisition of the native state. Whatever the exact mechanism, the newly synthesized mutant receptor protein, once properly folded, exits the ER, moves to its final destination at the plasma membrane, and functions in a manner similar to that of the wild-type protein.

Similar results using small molecules have been shown to be effective for rescuing the folding of other trafficking-defective mutant proteins including P-glycoprotein, a plasma membrane component involved in drug transport. Here again, addition of cell-permeable P-glycoprotein substrates led to the appearance of a functional protein at the cell surface (13). The P-glycoprotein substrates acted as specific chemical chaperones: they were ineffective, for example, in rescuing the maturation of  $\Delta F508$  CFTR. These findings with both the P-glycoprotein and vasopressin receptor mutants are consistent with what biochemists and crystallographers have known for some time: that ligand or substrate binding can stabilize the native state of nearly any protein.

Taken together, these observations demonstrate the promise of the small-molecule approach and suggest new methods to combat diseases involving abnormal protein folding and/or defective intracellular trafficking. Small molecules that activate or inhibit the wild-type protein (e.g., agonists or antagonists) may prove to be the same molecules that help rescue the

folding of the mutant protein. Should such compounds work at low concentrations and have a relatively short half-life in vivo, they might prove useful in the clinical setting. Whatever the case, a multidisciplinary approach employing molecular, biochemical, and chemical methods clearly is our best bet for finding new agents to treat diseases involving protein misfolding.

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