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Hypothesis

Altered protein folding may be the molecular basis of most cases of cystic fibrosis

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Experiments have demonstrated that the cystic fibrosis transmembrane conductance regulator protein (CFTR), containing the most common cystic fibrosis (CF)-causing mutation (ΔF508), reaches the plasma membrane in reduced amounts. Studies of a peptide model of CFTR indicate that the ΔF508 mutated region is more sensitive to denaturating conditions. This paper proposes that altered protein folding accounts for these findings, and, thus, most cases of CF. Significantly, the hypothesis makes specific predictions about the effect of stabilizing conditions on mutant CFTR, and, further, suggests a new class of pharmaceuticals that may prove effective in the treatment of this important genetic disease.

Cystic fibrosis; Cystic fibrosis transmembrane conductance regulator (CFTR); Protein folding; Genetic disease

1. INTRODUCTION

Cystic fibrosis (CF) is a common fatal autosomal recessive disease arising from mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1,2]. Although nearly 200 disease-causing mutations have been identified to date, a three base pair deletion, resulting in an in frame loss of a phenylalanine residue at position 508 (ΔF508), particularly prevalent in northern European populations, accounts for approximately 70% of the disease-causing alleles [1,2]. Transfection of several heterologous cell types (including cultured airway epithelial cells, CFPAC-1, Sf9, HeLa, CHO, NIH 3T3, and *Xenopus* oocytes) with vectors directing expression of the CFTR cDNA result in the appearance of a cAMP-dependent chloride conductance in the plasma membrane [3–7]. Conversely, expression of the ΔF508 CFTR cDNA in some of these cell types [3,6] did not produce this anion conductance, thereby explaining the altered chloride conductance of epithelial cells in CF patients which underlies the ultimately fatal pulmonary dysfunction. Cheng et al. [8] demonstrated that, in COS-7 cells expressing the ΔF508 CFTR, the protein did not reach the plasma membrane, and, thus, proposed that defective targeting of the mutant protein was responsible for the lack of functional expression. Recent studies of a synthetic peptide model of the ΔF508 mutant [9,10] reveal that defective protein folding may be the molecular basis of this aberrant pattern of protein maturation.

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2. DEFECTIVE PROTEIN FOLDING AS THE MOLECULAR BASIS OF CF

In contrast to earlier results [3,6], Dalemans et al. [11] and Drumm et al. [12] provided evidence that the ΔF508 mutant form of CFTR reaches the plasma membrane in functional amounts when expressed in fibroblasts and oocytes, respectively. However, although the single channel anion fluxes of the cells expressing ΔF508 were similar to those for the wild-type, whole- cell measurements revealed a substantial decrease in anion currents [11,12]. In agreement with the reduced functional activity, ΔF508 CFTR could not be detected at the fibroblast cell surface [11]. Therefore, it appears that, although a greatly reduced amount of ΔF508 CFTR reaches the plasma membrane at 37°C in these systems, the fraction that is properly processed has the wild-type function. These results support the hypothesis that most cases of CF result from defective targeting of the mutant CFTR to the apical membrane of epithelial cells [8]. However, the molecular basis for the inability of ΔF508 CFTR to reach the membrane remains obscure.

The basis of the defective localization of mutant CFTR may be best explained by ΔF508 being less stable than the wild-type protein, i.e. a temperature-sensitive mutant. Improperly folded CFTR would be incapable of continuing on the appropriate pathway to the membrane. Evidence that the molecular basis for the misprocessing of ΔF508 CFTR may be due to destabil-

ization have come from studies of synthetic peptides in our laboratory [9,10]. In agreement with the results of Dalemans et al. [11] and Drumm et al. [12] the mutant peptide retained the wild-type nucleotide binding activity at 25°C. However, the mutant peptide was more sensitive to denaturation than was the wild-type, and this reduced stability was reflected as reduced nucleotide binding by the mutant peptide [10]. Thus, we propose that a reduction in stability provides a simple explanation for the decreased amounts of ΔF508 CFTR observed in the membranes of affected cells [3,6,11,12].

The fact that the mutant CFTR is functional when it reaches the membrane [11,12] can be understood within the context of the protein folding hypothesis in two ways (Fig. 1). Above we considered the possibility that a simple two-state equilibrium between native and denatured states is shifted toward the denatured state by the ΔF508 mutation. Thus, the fact that small but functional amounts of ΔF508 CFTR reach the membrane, at least in some cases [11,12], would require that conditions in these cells are near the transition between folded and denatured CFTR, that is, where the free energy of stabilization is near zero.

A second possibility is that ΔF508 CF is caused by a defect in the folding process itself, rather than in destabilization of the native state. This proposal is illustrated schematically in Fig. 1. Central to this hypothesis is the idea that an intermediate in the folding–maturation pathway is altered by the deletion mutation. The mutant intermediate would be potentially more sensitive to intracellular proteases or to the formation of aggregates which would trap CFTR in a form off the normal folding pathway, thereby reducing the amount of functional, native CFTR in the membrane. In this regard, Sturtevant et al. [13] have shown that many temperature- sensitive mutants of otherwise stable proteins are due to thermally labile folding intermediates. Furthermore, aggregation of these intermediates may be specific [14], perhaps accounting for the homogeneity of inclusion bodies formed in bacterial expression systems and of plaques formed in amyloid diseases. Finally, it is important to note that the peptide model of cystic fibrosis [9,10] may be particularly revealing, in the sense that synthetic peptides often share characteristics with molten globule, or folding intermediate, states, such as a significant amount of secondary structure, increased conformational entropy, and exposed hydrophobic surfaces.

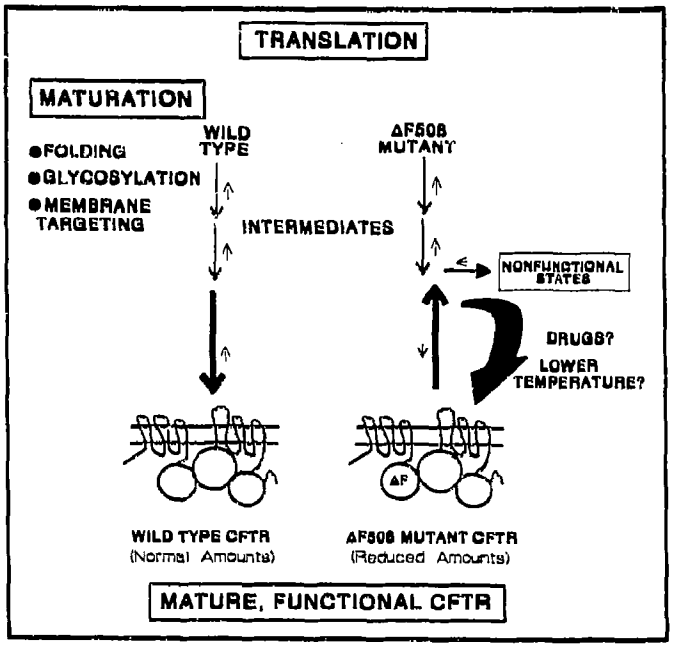

Fig. 1. Schematic representation of the maturation of CFTR and the effect of the ΔF508 mutation on the process. Maturation of the CFTR membrane protein involves three processes: folding of the nascent polypeptide chain, glycosylation, and targeting to the appropriate membrane location. Protein folding of the denatured polypeptide into a final native structure proceeds through a series of intermediate states. Because of exposed hydrophobic surfaces and other non-native attributes, some of these intermediates may be susceptible to aggregation. Formation of such off-pathway species may prevent appreciable amounts of the denatured nascent protein from reaching the native, functional structure in the membrane. One can envision that folding of the ΔF508 CFTR to the final 'native' structure proceeds through a different set of intermediates, some of which may be kinetically trapped, and, therefore, particularly susceptible to aggregation or proteolysis. Agents and conditions which prevent formation of the aggregates or push the folding equilibrium of ΔF508 CFTR toward the native structure may increase the amount of functional protein, and, thus, ameliorate the effects of the CF-causing mutation.

3. GLOBAL AND DOMAIN DESTABILIZATION

The altered protein folding hypothesis so far has considered only global destabilization of CFTR. However, the peptide results can not distinguish between global unfolding of CFTR and unfolding of the N-terminal nucleotide binding domain or a portion thereof. While the results with COS-7 [8], *Xenopus* oocytes [12] and fibroblasts [11] indicate that ΔF508 is a processing mutant, global destabilization of CFTR may not necessarily be required. Localized destabilization of the protein can not be ruled out, as at least two scenarios might explain the observed effects of the deletion mutation. First, as has been previously suggested, the sequence around F508 may be part of a putative membrane-spanning helical domain [15]. If so, the F508 region may be involved in membrane targeting and/or the development of protein topology in the lipid bilayer. Localized unfolding could, thus, disrupt a targeting function of the region. Secondly, the sequence near F508 has been suggested to be involved in inter-domain interactions [9]. It remains possible that these interactions may be involved in the maturation of CFTR.

4. DISCUSSION

The evidence that the ΔF508 CFTR is functionally active [11,12] when it is appropriately processed offers new hope for the treatment of CF. Therapies directed at correcting the folding defect, in addition to those directed at CFTR replacement, can now be envisioned. The proposal that ΔF508 is a temperature-sensitive folding mutant predicts that decreased temperature would facilitate appropriate trafficking of the mutant protein to the plasma membrane. While lowering a patient's body temperature may not be a treatment of choice, perhaps the most intriguing aspect of this proposal is the potential for new pharmacologies in the treatment of cystic fibrosis. Pharmaceuticals that raise the permissive temperature by stabilizing ΔF508 CFTR during the folding process may be sufficient to allow the mutant protein to reach the membrane where it can perform its biological function. Finally, it has not escaped our attention that other pathological states may be caused by similar mechanisms, for example, plaques of aggregated protein are formed in amyloid diseases such as Alzheimer's [16] and familial amyloidotic polyneuropathy [17], as well as in scrapie [18] and, Z α_1-antitrypsin deficiency [19]. In closing, in light of the widespread occurrence of temperature-sensitive mutants in bacterial and yeast systems, perhaps it would not be surprising if many human genetic diseases turn out to be defects of protein folding.

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Note added in proof

In a report supporting earlier peptide results [10] and some aspects of the hypothesis presented here, G.M. Denning et al. [(1992) Nature 358, 761] demonstrated that reduced temperature facilitates the processing of the ΔF508 CFTR, in 3T3 fibroblasts and C127 cells, resulting in the appearance of cAMP-regulated Cl^- channels in the plasma membrane.