

What happens to $\Delta F508$ in vivo?

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Commentary

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The protein has chloride ion channel activity, and there are over 800 mutations found in the CFTR-encoding gene that appear to cause CF. The predominant allele is a phenylalanine deletion termed $\Delta F508$. These are the few facts regarding CF and CFTR that seem clear. Most models of the physiologic processes leading to CF pulmonary disease incorporate dysfunction of ion transport and the consequent impact on transepithelial fluid secretion and absorption. Heterologous expression studies have suggested that $\Delta F508$, which is found in approximately 90% of CF patients in either the heterozygous or homozygous state, exerts its effects because of a failure to reach the plasma membrane. A small amount of the $\Delta F508$ protein appears to make it to the membrane, and it has been suggested that this pool could be increased by elevating the expression of $\Delta F508$ CFTR or by altering the intracellular environment to improve folding and processing. Such an increase in mutant CFTR might have therapeutic impact since $\Delta F508$ clearly has ion channel activity. In other words, if $\Delta F508$ can reach the membrane, it may be able to do its job. The report by Kälin et al. (1) offers surprising evidence that the degree to which $\Delta F508$ [...]

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The protein has chloride ion channel activity, and there are over 800 mutations found in the CFTR-encoding gene that appear to cause CF. The predominant allele is a phenylalanine deletion termed $\Delta F508$. These are the few facts regarding CF and CFTR that seem clear. Most models of the physiologic processes leading to CF pulmonary disease incorporate dysfunction of ion transport and the consequent impact on transepithelial fluid secretion and absorption. Heterologous expression studies have suggested that $\Delta F508$, which is found in approximately 90% of CF patients in either the heterozygous or homozygous state, exerts its effects because of a failure to reach the plasma membrane. A small amount of the $\Delta F508$ protein appears to make it to the membrane, and it has been suggested that this pool could be increased by elevating the expression of $\Delta F508$ CFTR or by altering the intracellular environment to improve folding and processing. Such an increase in mutant CFTR might have therapeutic impact since $\Delta F508$ clearly has ion channel activity. In other words, if $\Delta F508$ can reach the membrane, it may be able to do its job.

The report by Kälin et al. (1) offers surprising evidence that the degree to which $\Delta F508$ CFTR processing is impaired is tissue specific. Using several antibodies, each recognizing different sites in CFTR, they have found that protein levels and localization are similar between $\Delta F508$ and wild-type CFTR when expressed in airway and gut, but not when expressed in sweat gland. The sweat gland findings are consistent with findings in vivo by Kartner et al. (2) and with those in heterologous systems, but the airway findings are surprising, contradicting a previous report showing that high level expression in the bronchial submucosal glands is undetectable in $\Delta F508$ airways (3). Despite the technical reasons for the

discrepant results, the report by Kälin et al. has important implications.

The study of CFTR and its role in cell physiology has mostly been elucidated from cell culture systems, in which CFTR is heterologously expressed in non-epithelial cells. While the results are applicable to the cells being studied, Kälin et al. bring into question the ability to extrapolate these results to epithelial cells. The interactions of CFTR with different cytoskeletal components, other channels, etc., may or may not be relevant to native epithelial cells. Similarly, the effects of different agents on altering CFTR processing may be cell type dependent.

The clinical implications are great as well. The 800-plus CF mutations fall into 5 categories (4): (a) those that make no protein; (b) those that don't efficiently reach the membrane; (c) those that reach the membrane but don't

respond to stimulus; (d) those that reach the membrane and respond to stimulus but don't conduct chloride efficiently; and (e) those that make very little protein. Genotype/phenotype studies suggest that the level of CFTR channel activity associated with a particular mutation is a predictor for the health of patients (5). Consequently, there is interest in increasing CFTR activity as a therapeutic strategy (6). Because of the different effects of mutations, these schemes would necessarily be genotype dependent. For instance, the only way to correct a class 1 (loss of expression) mutation at the CFTR level would be gene replacement or, in some cases, suppression of premature stop codons; class 2 mutations would require methods to increase plasma membrane concentration of CFTR; and classes 3, 4, and 5 could require increased activation. The results of

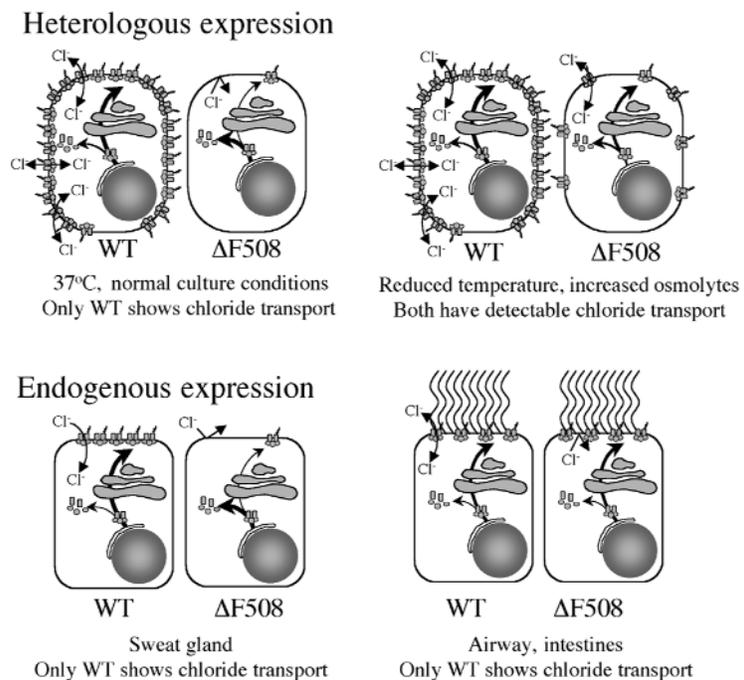


Figure 1

Under heterologous cell culture conditions (top), only wild-type (WT) CFTR shows cAMP-stimulated channel activity. Only when manipulated to induce trafficking to the membrane (top right) does $\Delta F508$ show any detectable channel activity. Tissue-specific differences in CFTR localization described by Kälin et al. are seen between epithelia of the sweat gland and epithelia of the airway and intestines (bottom). Whereas $\Delta F508$ in the membrane of transfected cells responds to cAMP (top right), $\Delta F508$ in endogenously expressing airway and intestinal cells (bottom right) does not.

Kälin et al. indicate that genotype-specific therapy may not only apply to the patient but also to the tissue to be treated. That is, $\Delta F508$ is a class 2 mutation in the sweat gland but a class 3 mutation in the airway and gut, so a therapy effective in one tissue might not apply to another.

Disease of the airway is the major cause of mortality in CF, so understanding the effect of $\Delta F508$ on the respiratory epithelium is of major importance. If there is not a deficit of CFTR protein in the majority of CF patients, as Kälin et al. has shown, then schemes that focus on restoring ion channel activity may prove more useful than previously thought. On the other hand, it raises a perplexing question: If there is not a deficit of CFTR in the membrane, then why is there so little detectable function of the $\Delta F508$ protein in the airway? In vitro patch-clamp and lipid-bilayer studies indicate $\Delta F508$ function to be 30–100% that of wild-type when the mutant protein is coerced to the plasma membrane (7, 8), but in vivo studies show no detectable channel activity from the airways of $\Delta F508$

patients (9). Furthermore, mutations that reduce the amount of CFTR mRNA to only a few percent of wild-type are associated with no symptoms, or relatively mild pulmonary disease, indicating that if $\Delta F508$ is found in normal amounts, there is another level of impairment. Perhaps molecules involved in activation of CFTR, supplied artificially for in vitro experiments, have altered stoichiometry in CF tissues in vivo, so that the cellular machinery is different between CF and non-CF in some way. Alternatively, perhaps the conditions used to activate CFTR in vitro are well beyond the physiologic range, and the amount of $\Delta F508$ CFTR activity capable in vivo is much less than that predicted from the in vitro systems.

Unfortunately, much of what is known about CFTR is derived from systems that could be manipulated, so the choice of cell line for study was based on practicality rather than relevance. Clearly, there is room for more studies in which in vitro models are compared with in vivo situations where they reflect polarized epithelia. Through these comparisons, it is hopeful we will

gain a better sense of how the mutant protein gives rise to the pathophysiology of the affected tissues and, with it, a better idea of how to fix the problem.

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