ΔF508 in cystic fibrosis: willing but not able

Kevin W Southern

The membrane protein, cystic fibrosis transmembrane conductance regulator (CFTR), functions as an ion channel.\(^1\) It is located primarily in the apical plasma membrane of epithelial cells, where it acts as a ‘gateway’ for chloride ions to leave the cell after a rise in intracellular cAMP. In addition CFTR has a regulatory role over other ion channels in the cell membrane.\(^3\) CFTR is the product of a gene on the long arm of chromosome seven, the CFTR gene.

Cystic fibrosis is an autosomal recessive condition, caused by mutation of both CFTR gene alleles. Over 600 mutations of the CFTR gene have been identified, however one mutation, ΔF508, accounts for the vast majority of cystic fibrosis. Why does this unusual mutation, a codon deletion, have such high prevalence? Greater understanding of the molecular consequences of ΔF508 may answer this question and, more importantly, lead to therapeutic opportunities. This article will review the molecular biology of the ΔF508 mutation with particular reference to clinical implications.

Identification of the CFTR gene

Identification of the ‘cystic fibrosis gene’ was achieved by examining ‘informative families’ (with two or more affected children) for genetic linkage with a large number of genomic probes (short sequences of DNA that match one area of the human genome). This strategy relied on two assumptions; that adjacent genes tend to be inherited together through generations and that one mutation would predominate in this condition. In 1985 linkage of the ‘cystic fibrosis gene’ to markers on chromosome seven was reported.\(^5\) Calculations from the inheritance patterns indicated that two of the markers were relatively close and on either side of the gene. However there was still a lot of chromosomal ground to cover. It was four years until Tsui and his colleagues, using a technique that combined the mundane with the extraordinary (walking and jumping along the chromosome), were able to publish the sequence of the gene.\(^8\)

CFTR PROTEIN

The CFTR gene codes for a protein which contains 1480 amino acids and is a membrane ion channel. From the amino acid sequence a theoretical model of the molecular structure was developed. The molecule has several distinct regions, the majority of which span the cell membrane (see fig 1). On the inside of the cell, connected to the membrane spanning domains, are two tightly folded regions that bind nucleotides. Between the nucleotide binding folds is situated a larger ‘R’ domain, a region with multiple sites for phosphorylation. The R domain and the nucleotide binding folds regulate chloride conductance through the channel. Over 600 mutations of the CFTR gene, that cause cystic fibrosis, have now been reported, but by far the commonest is a codon deletion that results in the loss of a phenylalanine residue at position 508 in the first nucleotide binding fold, the ΔF508 mutation.

The ΔF508 mutation

Approximately 50% of individuals with cystic fibrosis are homozygous for the ΔF508 mutation and in many countries, including Britain, over 90% carry at least one allele with the mutation.\(^10\) The prevalence of the mutation varies geographically with a white bias\(^9\) and is less common in other ethnic populations.\(^1\) A number of DNA sequences, both within and adjacent to the CFTR gene, show remarkable
F508 mutation was identified, the initial hypothesis 
was that deletion of the phenylalanine residue would 
result in reduced chloride transport, whereas 
this has not been the case. Two groups have investigated 
whether aberrant binding with chaperones is the cause 
of decreased chloride transport, with one group 
suggesting that the F508 variant binds preferentially 
to chaperone molecules, resulting in a reduced 
secretion of chloride from the cell. This hypothesis 
was later rejected after a seminal paper describing 
the glycosylation of CFTR with and without the 
F508 mutation. It appears that the F508 mutation 
causes the arrested maturation of CFTR, resulting 
in a reduced amount of functional CFTR protein 
produced.

The inability of F508CFTR to mature properly 
results in a reduced chloride channel activity, 
which is associated with the symptoms of cystic 
fibrosis. The protein is transported to the surface 
of the cell and is glycosylated, a process that 
remains incomplete in F508CFTR. This 
glycosylation is necessary for the proper folding 
and transport of the protein to the cell surface.

Correction of the maturation defect in F508CFTR 
has been studied using transgenic mice and 
cell culture systems. Several strategies have been 
tried, including the use of chaperone proteins 
to stabilize and fold the abnormal CFTR protein. 
One such strategy involved the use of calnexin, a 
transmembrane protein located across the endoplasmic 
reticulum and hsp70 is restricted to the 
golgi apparatus. Calnexin is known to bind to 
to normal CFTR and stabilize it, allowing for 
the correct folding of the protein. This strategy 
has shown promise in correcting the maturation 
defect in F508CFTR.

Why does F508 cause cystic fibrosis? 
It seems unlikely that a disease of the 
lymph system could account for the 
heterozygous advantage in CF population. 
This theory was later rejected after a seminal paper 
showing that the F508 variant binds preferentially 
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defect in F508CFTR.
It is now well established that the ion transport defect of cystic fibrosis cells in culture can be corrected by gene transfer incorporating DNA for normal CFTR.31 32 These cystic fibrosis cells then show normal chloride conductance in response to cAMP. Surprisingly, however, when DNA coding for ΔF508CFTR was incorporated into non-human cells, cAMP mediated chloride conductance was also generated, albeit at a reduced level.33 34 This appeared contradictory, however it soon became apparent that these non-human cell lines may have different pathways for processing CFTR. Protein folding mechanisms are known to be temperature sensitive35 and the primitive cell lines, in which mechanisms are known to be temperature sensitive phenomena in tissues, as opposed to individual cells. Epithelial tissues, dissected from the distal colon were maintained at temperatures of 25–30°C for periods of up to 18 hours. Although the tissues maintained their ion transporting capabilities, no significant cAMP mediated chloride secretion was demonstrated (KW Southern, AW Cuthbert, unpublished data). This was disappointing. However, when tracheal epithelial cells from the mice were examined by a halide efflux fluororescence assay (a method for detecting evidence of CFTR function from individual cells) CFTR-type function was evident at 25°C but not at 37°C.41

Novel therapeutic strategies
Discovery of the CFTR gene has focused attention on gene replacement therapy and a number of early clinical trials have been initiated.42 43 An alternative approach, however, is to utilise the functional capability of the ΔF508CFTR by overcoming the trafficking defect. Such a strategy, if successful, would be applicable to over 90% of people with cystic fibrosis.

In cultured cells, a non-specific stimulus generating overexpression of ΔF508CFTR resulted in small amounts of functional ΔF508CFTR reaching the plasma membrane.44 The provisional results of a clinical trial of phenylbutyrate (a non-specific stimulator of gene expression) were reported at the 1996 North American Cystic Fibrosis Conference (PL Zeitlin). They suggested partial correction of the cystic fibrosis ion transport defect in patients with ΔF508. The final results of this trial are awaited with interest, however preclinical data suggest that a more specific and stronger promoter of gene expression will be needed if this is to be a feasible approach.

A number of chemicals, including glycerol,35 have been shown to facilitate presentation of ΔF508CFTR to the cell surface membrane. Two novel compounds may have therapeutic potential. The xanthine derivative, CPX (8-cyclopentyl-1,3-dipropylxanthine), selectively activates chloride efflux from cells expressing ΔF508CFTR.46 47 CPX is an A1 receptor antagonist, but is the only member, to date, of this class of drugs shown to exhibit this specific property. CPX has no affect on cells expressing normal CFTR. Its intrinsic lack of toxicity makes it a compound that deserves further investigation.

A group of compounds, termed heterotrimeric G proteins, have been linked with the binding fold with the ΔF508 mutation does not alter its ability to bind ATP.48 ‘Patch clamp’ experiments have shown that ΔF508CFTR in the endoplasmic reticulum membrane functions as a chloride channel39 and purified ΔF508CFTR protein, inserted into artificial membranes, has as much chloride channel capability as normal CFTR.40

TRANSGENIC ΔF508 MOUSE MODEL
The generation of a transgenic mouse model, homozygous for the ΔF508 mutation, has enabled the ‘ex vivo’ examination of this temperature sensitive phenomena in tissues, as opposed to individual cells.18 Epithelial tissues, dissected from the distal colon were maintained at temperatures of 25–30°C for periods of up to 18 hours. Although the tissues maintained their ion transporting capabilities, no significant cAMP mediated chloride secretion was demonstrated (KW Southern, AW Cuthbert, unpublished data). This was disappointing. However, when tracheal epithelial cells from the mice were examined by a halide efflux fluorescence assay (a method for detecting evidence of CFTR function from individual cells) CFTR-type function was evident at 25°C but not at 37°C.41

Figure 2 Transport of CFTR through the cell.
regulation of plasma membrane proteins through presentation of vesicles to the cell surface. Inhibition of one of this class, G(o), has been shown to stimulate exocytosis and the appearance of Campbell mediated secretion in AF058CFTR cells. These early findings again deserve further investigation and encourage the hope that a pharmacological approach may be able to overcome the AF058CFTR processing defect.

Summary

Over 90% of cystic fibrosis patients carry at least one AF058 allele and approximately 50% are homozygous for the mutation. An intracellular trafficking defect prevents presentation of this mutated protein at the cell membrane. Once in the correct position, AF058CFTR can function as an ion channel. The processes involved in post-translational protein modifications are being unravelled. Mutations that disrupt these processes may be responsible for a large number of inherited conditions. Pharmacological manoeuvres aimed at correcting trafficking defects may allow us to utilise the functional potential of these abnormal proteins. Transgenic animal models will have an important role in this research.

Gene replacement therapy is not the sole therapeutic end point of molecular medicine. As knowledge of the AF058 mutation expands, further strategies will develop to overcome the molecular defect. These will have clinical significance to most patients with cystic fibrosis.

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21 Lukacs GL, Mohamed A, Kartner N, Chang X, Riordan JR, Grinstein S. Conformational maturation of CFTR but not its mutant counterpart (AF058) occurs in the endoplasmic reticulum and requires ATP. EMBO J 1995;14:6707-16.


