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The cystic fibrosis transmembrane conductance regulator: state of the art

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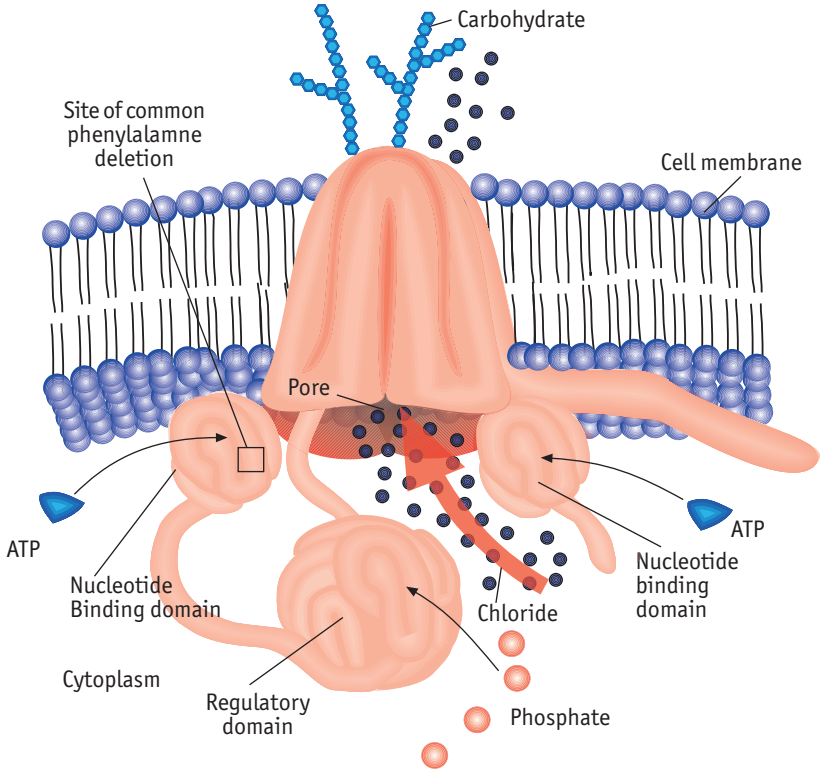
Provenance
Adapted from an ERS School course



Educational aims

- To provide an overview of current knowledge on the structure and function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein and its role in CF disease.
- To explore the different ways in which mutations in the CFTR gene can alter or abolish the function of the protein *in vivo*.

Summary

In the 20 years since the gene responsible for cystic fibrosis (CF) was cloned, more than 1,300 mutations have been documented, which interfere with the function of the CF transmembrane conductance regulator (CFTR) protein at every stage from gene transcription to the working of the expressed protein.

Investigation of the pathogenesis of CF has led to the development of the 'low volume' hypothesis, whereby CFTR dysfunction leads to a depletion in airway surface liquid (ASL) and a resultant reduction in mucociliary clearance (MCC). Infection begins early in life and elicits an exaggerated and prolonged inflammatory response, which is thought to be key in the irreversible tissue destruction characteristic of CF.

Hermes syllabus link: modules B15.1, A1.10

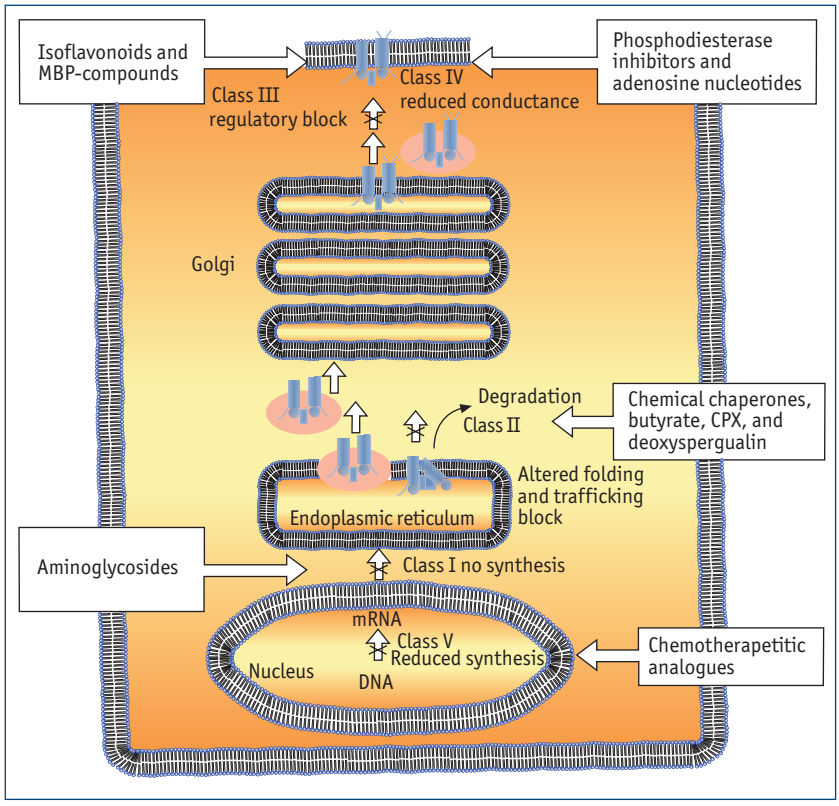


Figure 1
The effects of the five classes of CFTR mutation.

CF is an autosomal, recessively inherited disease caused by mutations in the CFTR gene. It is common in most Caucasian populations; in the UK, for instance, approximately one in 22 people is a healthy carrier of a single mutation, giving rise to an incidence of CF of ~1:2000 in the newborn population. In recent years, our understanding of the basics of the disease has expanded enormously. This article will review the nature of disease-causing mutations in the CFTR gene, the functions of the CFTR protein and current hypotheses relating to disease pathogenesis.

The CFTR gene

The CFTR gene was cloned in 1989 [1] through positional cloning approaches. It is located on chromosome 7q31.2, spans 250 kb of genomic DNA and contains 27 exons. The mRNA is 6.2 kb and encodes a polypeptide of 1,480 amino acids with a molecular mass of ~170,000 kDa.

Classes of disease-causing CFTR mutations

More than 1,300 mutations have been detected in the CFTR gene. Mutations have been found in every exon as well as in many of the flanking intron sequences. They include mis-sense, nonsense, frameshift and splice site mutations as well as amino acid deletions and substitutions. CFTR mutations can be divided into five classes (figure 1).

Class I: defective protein synthesis

Mutations in this class disrupt synthesis of the CFTR protein. They include nonsense and frameshift mutations, which lead to the creation of premature termination codons (PTCs). In some populations, such as Ashkenazi Jewish descendants, these mutations are those most commonly seen. In addition to leading to truncated proteins, they may also significantly reduce the half-life of mutant mRNA through the nonsense-mediated mRNA decay pathway. Therefore, such mutations are expected to produce little or no protein; this contention is supported by genotype-phenotype studies, which usually show an association with severe disease. Novel forms of treatment, directed at overcoming these PTCs, are currently in clinical trial [2].

Class II: defective protein processing

Normal CFTR protein undergoes a series of processes in the endoplasmic reticulum (ER) and the Golgi, including glycosylation and folding; these are required to enable the protein to traffic to the apical cell membrane. Class II mutations impair this process, and lead to degradation of the protein. The commonest mutation worldwide, ΔF508, leads to a protein that is unable to fold correctly. Consequently, it is retained in the ER and degraded. If the protein does succeed in reaching the apical cell membrane, it retains some ion channel activity, although its rate of turnover is also increased compared with wild-type protein. These observations have led to the exploration of several novel therapies aimed at correcting protein folding and trafficking, including the use of 4-phenylbutyrate [3]. Other compounds are being explored with the aid of high-throughput technology, which aims to screen large numbers of chemicals for the ability to restore chloride ion efflux.

Class III: defective protein regulation

Class III includes mutations leading to the production of proteins that successfully reach the plasma membrane, but that cannot be activated and therefore have a low open probability. Novel pharmacological agents directed at this class of mutation include VX770, which has recently

shown promise in clinical trials, leading to changes in sweat electrolytes, nasal potential difference and lung function [3].

Class IV: altered conductance

Class IV mutations lead to protein with altered conductance and thus reduced chloride transport. Mutations in this class (and in class V) are often associated with milder phenotypes and pancreatic sufficiency.

Class V: reduced CFTR level

Class V mutations lead to the production of normal proteins, but at reduced levels, and include splicing mutations. These can lead to variable levels of correctly spliced transcripts among different patients and even within organs of the same patient.

CFTR polymorphisms

In addition to the large number of mutations, about 130 genetic polymorphisms have been identified in the CFTR gene. Although these polymorphisms are variations in the gene sequence, they differ from mutations in that they are also frequently (>1%) found in the general population. However, it has been established that certain polymorphisms alter the amount of functional CF gene product. The best-studied example is the thymidine polymorphism in intron 8 of the CF gene [4]. This polymorphism exists as a 5-, 7-, or 9-thymidine (T) variant. The 5T variant significantly reduces the amount of normal CFTR transcript, because intron 8 is incorrectly spliced, which leads to mRNA lacking exon 9.

CFTR protein structure

The amino acid sequence of CFTR suggests a tandem repeat structure with two identical halves, each consisting of six putative transmembrane α-helices and an intracellular nucleotide-binding domain, which is capable of ATP hydrolysis (main image and figure 2). The two halves are linked through a highly charged intracellular regulatory domain (R domain), which contains numerous phosphorylation sites. This protein structure indicates that CFTR is part of the ATP-binding cassette transporter protein superfamily. This is a complex and rapidly evolving field and several review articles have been published on the subject [5].

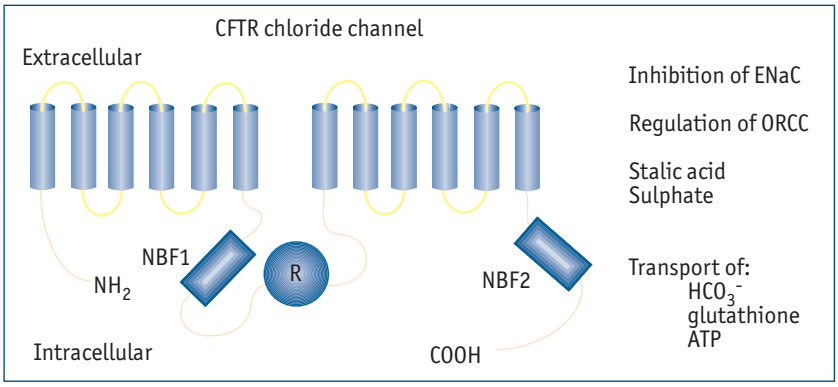


Figure 2
Schematic of the CFTR protein. ENaC: epithelial sodium channel; ORCC: outwardly rectifying chloride channel

CFTR function and the basic defect in cystic fibrosis

CFTR is expressed in the apical membrane of epithelial cells, including airway and intestinal epithelium, where it functions as a cyclic AMP-regulated chloride channel. CFTR has been shown to have additional functions, some of which also play a defined role in disease pathogenesis, including regulation of a variety of other channels, most importantly the epithelial sodium channel (ENaC). In CF, loss of the normal inhibition of ENaC leads to sodium hyperabsorption from the airway surface. Water follows down its osmotic gradient, dehydrating the airway surface. This forms the basis of the most favoured hypothesis of CF disease pathogenesis, termed the "low volume" hypothesis (figure 3). Exquisite regulation of ASL volume is required for normal function of a primary innate defence system, MCC. In normal health, cilia, bathed in periciliary liquid (PCL), beat in a coordinated fashion to transport the overlying mucus layer. In CF, both the PCL and the overlying mucus layer are depleted of volume due to sodium and water hyperabsorption, leading to failure of this mechanism [6].

Evidence in support of the low volume hypothesis

Evidence in support of this hypothesis comes both from cell culture and *in vivo* experiments. Primary airway epithelial cultures were shown to regulate the volume of ASL, maintaining the height of the PCL at ~7 μm, which corresponds to the height of an outstretched cilium. In

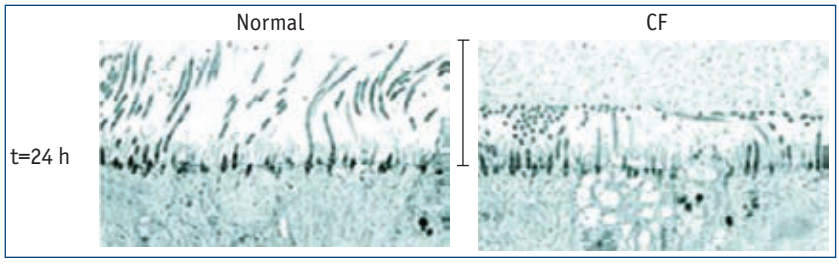


Figure 3
The loss of CFTR function results in increased sodium aborption and loss of water from the airway surface. Scale bar = 7 µm. Adapted from [4], with permission from the publisher.

contrast, over a period of 24 h, a similar volume of fluid placed on the surface of CF cultures became depleted; the cilia could no longer remain upright but collapsed onto the cell surface, which resulted in significantly reduced mucus transport rates (figure 3) [7]. Measurement of ASL height *in vivo* is much more difficult. Animal models of CF are of limited value in that they do not mimic well the CF lung phenotype. In contrast, the murine nasal epithelium is much more similar, with regard to ion transport abnormalities, to the human CF respiratory epithelium. In this organ, ASL height has been shown to be significantly reduced in mice deficient in CFTR [8].

MALL *et al*. [9] have focused on sodium hyperabsorption, developing a transgenic mouse overexpresses ENaC. In contrast to CFTR mutant mice, this model demonstrated reduced ASL height and impaired mucus clearance. In addition, there was a susceptibility to bacterial infection and neutrophilic inflammation very similar to that observed in human CF.

Other functions of CFTR

CFTR has also been shown to regulate the activity of the outwardly rectifying chloride channel, possibly through ATP transport, and probably helps to regulate basolateral potassium channels and aquaporins. It is involved in the transport of bicarbonate ions, which are likely to be important in the composition of mucus. In addition, intracellular enzymatic processes are impaired in the absence of normal CFTR; these include sulphation of mucins and the sialylation of surface glycoconjugates. The latter, in their nonsialylated form, have been identified as receptors for bacteria including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Although there is no evidence that direct adherence of whole pathogens to these receptors is a pathogenic mechanism *in vivo*, it is likely that shed bacterial products stimulate inflammation in this fashion.

Infection in the CF airway

It is hypothesised that impaired MCC allows inhaled infective organisms to gain a foothold in the CF airway. What is not clear from the low volume hypothesis is why such a fundamental defect in innate defence should selectively encourage infection with such a narrow range of organisms, such as *S. aureus*, *Haemophilus influenzae*, *P. aeruginosa*, *etc.* Patients with a complete absence of MCC due to genetic defects in the ciliary microstructure itself (various forms of primary ciliary dyskinesia) appear to be less severely affected by such infections, although this may be because cough clearance, which is well preserved, compensates for impaired MCC in a way that it does not in CF. Alternative hypotheses to explain the susceptibility of CF subjects to bacterial infections include: the suggestion that wild-type epithelial cells ingest such bacteria; the presence of increased numbers of receptors on the CF cell surface; and the decreased production of antimicrobial molecules such as nitric oxide and glutathione. *P. aeruginosa* seems to be particularly well adapted to survival within the CF airway. It secretes a complex variety of exoproducts that incapacitate various arms of the host's immune defence and is capable of rapid genetic mutations, many of which render it resistant to microbial agents. It also has an affinity for the hypoxic environment found inside thick mucus plugs, which is one of the triggers for biofilm formation, which further protects the organism from host recognition. The chronic presence of bacteria within the airway leads to a profound neutrophilic inflammation, although whether such inflammation is also seen in the absence of current or previous infection, is controversial.

Conclusion

Since cloning of the CFTR gene in the late 1980s, much has been learned about the effect of the many described mutations on protein expression, much of which has led to novel drug targets. Disease pathogenesis is better understood, although there are clearly areas where further research will offer new insights.

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Further reading

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