How do CFTR mutations cause cystic fibrosis?

The key defect in cystic fibrosis is loss of chloride conductance, but mutations of the cystic fibrosis gene product, the CFTR, have multiple effects on cell physiology; new results help to reconcile these facts.

Two major discoveries have transformed our understanding of cystic fibrosis, a genetic disease in which thick secretions accumulate in airways, digestive organs and sperm duct. The first was that cystic fibrosis involves a basic defect in epithelial ion transport [1], which is manifested primarily as the loss of chloride conductance [2]. The connection between the loss of epithelial chloride conductance and many of the symptoms of cystic fibrosis is explained by a well-established model in which fluid secretion is driven by chloride secretion, the last step of which occurs through a chloride channel [3]. Loss of conductance through the chloride channel reduces fluid secretion, leading to airway obstruction by condensed macromolecules; salt absorption is also compromised in some tissues. The other great advance was the cloning of the cystic fibrosis gene [4], which brought the enormous power of molecular biology to cystic fibrosis researchers. This led to the demonstration that the cystic fibrosis gene product is a chloride channel [5], validating earlier electrophysiological analyses and leading to the hypothesis that the fundamental physiological defect in cystic fibrosis is loss of the chloride conductance mediated by the cystic fibrosis gene product (Fig. 1).

Two major challenges to the chloride channel hypothesis remain. One arises from the structure of the cystic fibrosis gene product, named 'CFTR' for cystic fibrosis transmembrane conductance regulator. The CFTR contains two nucleotide-binding domains and is a member of the family of ATP-hydrolyzing transporter proteins that includes P-glycoprotein, which confers multidrug resistance on cells in which it is expressed, and STE6, which transports a polypeptide pheromone out of yeast cells. These similarities in structure suggested that CFTR might transport something involved in regulating chloride conductance. Indeed, CFTR was named in such a way as to include this possibility. When CFTR was shown to have intrinsic channel activity, this line of reasoning abated briefly, but it re-emerged with the claim that P-glycoprotein is both a transporter and a swelling-activated chloride channel [6]. The subsequent rejection of that hypothesis [7] has not dimmed enthusiasm for the basic idea, which can be restated for CFTR as follows: if CFTR is just a channel, why does it have such a complex structure?

A second challenge to the view that CFTR is simply a chloride channel arises from the pleiotropic consequences of CFTR mutations. Researchers — myself included — who would like to derive the pathophysiology of cystic fibrosis from decreased chloride conductance have been hard put to account for the pleiotropic effects of CFTR mutations. These include alterations in sodium conductance, in chloride conductance mediated by non-CFTR channels, in pH regulation and in properties of cell-surface glycoproteins. How does CFTR influence these varied cellular properties and, more importantly, what contributions do these alterations make to the pathology of cystic fibrosis? Several recent papers bring new prominence to these challenges to the chloride channel hypothesis.

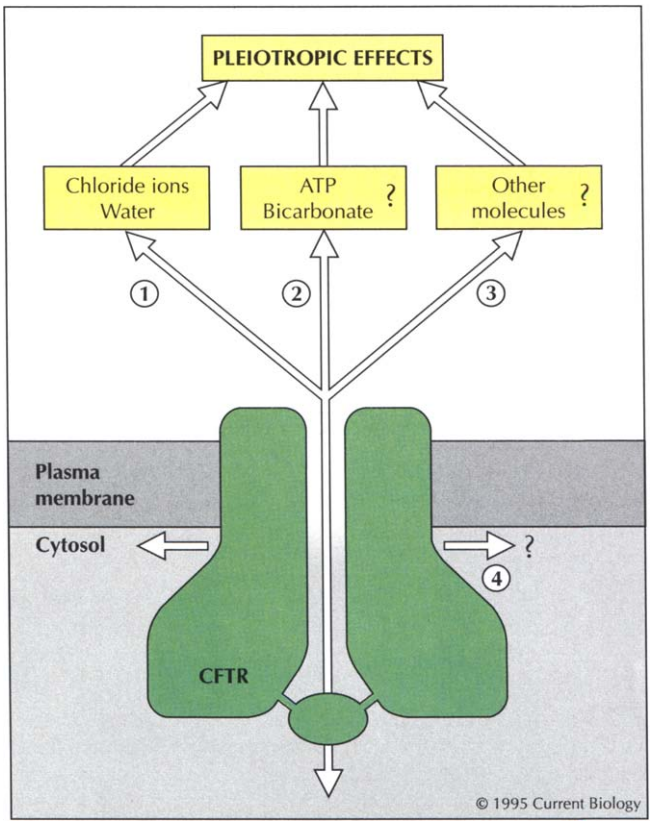

Fig. 1. How does loss of CFTR cause cystic fibrosis? The diagram shows known and suggested activities of CFTR. The only unchallenged function of the CFTR is as a chloride channel (**1**), which in some epithelia is essential for fluid secretion or salt absorption. The CFTR may transport ATP and bicarbonate ions (**2**), but this not been observed in all laboratories. The transport of other molecules (**3**) is speculative at present, as is the suggestion that CFTR can affect other membrane molecules, perhaps by direct protein–protein interactions (**4**). Each of these pathways could trigger complex downstream events; for example, evidence discussed in the text suggests that ATP is exported from the cell by the CFTR and activates external receptors which in turn activate other chloride channels.

CFTR and epithelial sodium channels

When an increased voltage across the nasal epithelium of cystic fibrosis subjects was discovered, it was attributed to increased sodium transport [1]. Quinton [2] subsequently proposed the alternative hypothesis that the increased voltage is the result of decreased chloride conductance. After numerous studies, most researchers were convinced that decreased chloride conductance is the fundamental problem in cystic fibrosis, but that both decreased chloride conductance and increased sodium conductance occur, at least in cystic fibrosis airway tissues.

Any remaining skepticism about the latter conclusion should be dispelled by a new study [8] in which the recently cloned epithelial sodium channel was expressed in epithelial cells and fibroblasts, either alone or with the CFTR. In each cell type, co-expression of CFTR caused a small, but significant, reduction in sodium transport. Moreover, sodium currents were reduced when the CFTR was stimulated by cyclic AMP (CAMP) in the absence of chloride ions. Because previous studies found that sodium channels in cystic fibrosis tissues spent more time in an open state, the combined results show that the activated CFTR inhibits sodium channels by a chloride-independent mechanism.

CFTR and outwardly rectifying chloride channels

A related issue is the role of an infamous chloride channel that allows chloride ions to enter the cell more easily than they exit (a property called outward rectification). Because this channel is commonly observed when patches are excised and depolarized, it is referred to as an 'ORDIC' (outwardly rectifying depolarization-induced chloride) channel, to distinguish it from other outwardly rectifying chloride channels. The ORDIC channel is defectively regulated by cAMP-regulated protein kinase (PKA) in cystic fibrosis cells, and was considered by many to be the 'cystic fibrosis channel', although this was controversial even before the cystic fibrosis gene was cloned.

The controversy arose because the ORDIC channels occur in most cell types examined, unlike the chloride conductance that is defective in cystic fibrosis, which has a restricted distribution. Also, the properties of the ORDIC channels — notably stilbene inhibition and a preference for iodide over chloride — are inconsistent with their mediating the chloride conductance that is missing in cystic fibrosis [9]. In patch-clamp studies of single-channel activity, the ORDIC channel is rarely observed in cell-attached patches, so most studies of this channel have employed excised patches. In such excised patches, however, activation by phosphorylation is erratic and often takes many minutes to work. During this long delay, the channel can activate spontaneously and irreversibly. When whole-cell recordings were used to avoid the problems of excised patches, elevation of cAMP levels induced non-rectifying currents! As a result of these confusing findings, most researchers dropped their studies of ORDIC channels with relief when it was demonstrated that CFTR is an intrinsic chloride channel with properties that fit both cAMP-mediated whole-cell currents and the chloride conductance that is missing in cystic fibrosis cells.

To his credit, William Guggino and his colleagues persisted in trying to reconcile the new data on CFTR channels and the older experiments with ORDIC channels, and in paper [10] they provide fascinating evidence to support a model of indirect activation of ORDIC channels. According to the model, when PKA and ATP are applied to excised patches, they activate CFTR channels. CFTR then conducts ATP across the cell membrane, where it activates purinergic receptors. The purinergic receptors then activate ORDIC channels, either directly or indirectly [10]. The model certainly explains why experiments with ORDIC channels are not easy to reproduce. If this model is correct, to observe ORDIC channels in a patch-clamp study, a patch must have a functional CFTR channel, an ORDIC channel, a purinergic receptor and perhaps associated G proteins (GTP-binding proteins that transduce signals from certain types of receptor).

Several lines of evidence support this model [10]. In normal cells, but not in cystic fibrosis cells, whole-cell currents activated by cAMP develop an outwardly rectifying component when the ATP in the pipet is increased above 2.5 mM, and in excised patches outwardly rectifying channels can be seen. Strikingly, the whole-cell and single-channel currents are blocked by extracellular scavengers of ATP. Also, with 500 nM ATP in bath and pipet, active ORDIC channels were observed right after patches were made, in either cystic fibrosis or normal cells (the channel activity decreased by 90 % within three minutes; the reason for this lability is not known, but it may also contribute to the variable results). Finally, ATP currents can reportedly be measured through CFTR [10,11], although this is also disputed. These results go a long way toward resolving old controversies, and should generate research to test the model further and to determine if a similar mechanism is responsible for the negative regulation of sodium channels by CFTR.

What is the clinical significance of these results?

Is the regulation of other channels by CFTR more or less important than its intrinsic chloride conductance? This question is of vital interest to the cystic fibrosis community, for if loss of the purinergic pathway is an important component of disease, then supplying purines exogenously should ameliorate the disease. And if enhanced sodium absorption is important, then blocking sodium channels should ameliorate the disease. Both of these predictions are now being tested clinically [12], but because clinical trials are notoriously slow and rarely unequivocal in their outcomes, we certainly need to look to other kinds of evidence to provide guidance on these questions.

Studies of cystic fibrosis 'knockout' mice, generated by gene targeting, together with genotype/phenotype

analysis of human cystic fibrosis subjects, have provided some of the best evidence that it is the loss of CFTR-mediated epithelial chloride conductance that is primarily responsible for cystic fibrosis disease. Four kinds of evidence are now available. First, the knockout mice, which lack CFTR entirely, have profound intestinal disease that is fatal without intervention, but, unlike their human counterparts, their lungs and pancreas are minimally affected, and male mice are fertile [13]. The sparing of mouse organs from cystic fibrosis disease correlates with the expression of alternative chloride channels in the mouse pancreas, lungs and sperm duct [14], suggesting that chloride conductance *per se* can protect against cystic fibrosis disease, presumably by allowing relatively normal fluid and electrolyte transport in these organs. Second, measurements of ion transport in intestinal biopsies from cystic fibrosis subjects having identical CFTR mutations but varying clinical status shows a positive correlation between the magnitude of an alternative chloride conductance and mildness of the disease [15]. Third, CFTR mutations that partially reduce chloride conductance through CFTR result in milder disease, especially in the pancreas [16]. Finally, in three cell types that are profoundly affected in cystic fibrosis, namely sweat duct cells, intestinal crypt cells and lung serous cells, CFTR channels appear to be the exclusive form of apical chloride channel. Taken together, these results indicate that chloride conductance is what matters, and the organ does not care if it is through CFTR or a different chloride channel. It is organs that rely exclusively on intrinsic CFTR-mediated chloride conductance that are most affected in cystic fibrosis.

Conclusion

A dozen years have passed since Paul Quinton [2] first proposed that lost epithelial chloride conductance might be the key defect in cystic fibrosis, and support for his hypothesis has never been stronger. However, the evidence that expression of CFTR has multiple consequences for cell physiology is now unequivocal. How CFTR influences other cell properties is not known, but the suggestion that CFTR exports ATP that binds to extracellular purinergic receptors is a precise, testable hypothesis that has received considerable initial support. This hypothesis deserves to be tested further, especially in tissues and conditions that are most relevant to cystic fibrosis disease.

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