to the prevention of many of these deaths in the future. At present, prenatal diagnosis contributes to the avoidance of new cases of cystic fibrosis in families with the disease; the detection of carriers would make it possible to avoid the conception or birth of many currently unavoidable cases. I disagree with the sentiments of Kerem *et al.*³, who suggest waiting until all the mutations have been identified — carrier testing is worthwhile now.

Finally, there is the hope that knowledge of the structure and function of CFTR will lead to the design of rational approaches for treating cystic fibrosis. That hope, however, should be tempered with the lesson of sickle cell anaemia: molecular advances do not always lead to molecular cures¹². □

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Protein joins transport family

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OBTAINING a gene sequence is, in itself, not always especially informative. *CF* is clearly an exception¹, its sequence giving a strong indication of the biochemical nature of the cystic fibrosis defect. The CFTR protein that *CF* encodes shares considerable organizational and sequence similarities with a family of ATP-dependent transport systems that have been extensively studied in bacterial cells and which have more recently been shown to have eukaryotic counterparts (see ref. 13). The best characterized members of this family are the bacterial transporters referred to as the periplasmic or binding-protein-dependent transport systems. Over 20 such systems have been identified, each specific for a different substrate (such as an amino acid, sugar, peptide or inorganic ion)¹⁴. Transport requires the function of a 'periplasmic binding protein' (hence the family name) that delivers substrates to a complex of membrane proteins that mediate substrate translocation across the membrane.

The 'basic' membrane complex consists of four protein subunits (although there are variations on this theme), two of which are highly hydrophobic and span the membrane five or six times, and two of which are hydrophilic and associated with the cytoplasmic face of the membrane. The hydrophilic subunits share considerable amino-acid sequence similarity over much of their length. The conserved sequences include a potential ATP-binding site, and several of the proteins, indeed, bind ATP^{15,16}. Although none of the purified proteins has yet been shown to hydrolyse ATP, there seems little doubt that they do and stoichiometric ATP

Schematic model of CFTR, modified from ref. 2. The two membrane-spanning subunits are joined by a nucleotide(ATP)-binding fold (NBF) and the large R subunit. The other NBF is at the carboxy(C)-terminus.

hydrolysis concomitant with transport has been demonstrated¹⁷.

Several eukaryotic counterparts to these bacterial transporters have been identified. All differ from the bacterial transporters in that the individual protein subunits are combined into a single multidomain polypeptide, but the overall organization and amino-acid sequences are strongly conserved. The first eukaryotic example to be found was the MDR protein, or P-glycoprotein, which is responsible for multidrug resistance in tumours. The protein functions as a transporter, pumping drugs out of cells. The CQR protein responsible for chloroquine resistance in the malarial parasite turns out to be closely related to MDR¹⁸, as is the product of the *STE6* locus of yeast, which mediates export of α -factor mating pheromone from the cell^{19,20}.

It is to this widespread group of eukaryotic transporters that CFTR, the product of the *CF* gene, belongs. In its overall structure, CFTR is very similar to the other transport systems, having two hydrophobic domains, which comprise potential membrane-spanning helices, and two ATP-binding domains (see figure). Furthermore, CFTR includes an additional, large cytoplasmic domain (R in the figure) which, based on consensus phosphorylation sites for protein kinase C and cAMP-dependent protein kinase, probably serves a regulatory role. Thus there seems little doubt that CFTR is a transport system, but for what?

It has become clear that cystic fibrosis patients show altered chloride transport across epithelia and that this is the root cause of the most severe clinical symptom — decreased fluid secretion and accumulation of dehydrated mucus in the bronchial tubes. Furthermore, this chloride channel is subject to complex regulation, only now being unravelled. Channel activity is increased by cAMP-dependent protein kinase in normal subjects but not in patients; activation by protein kinase C also seems to be defective in patients²¹⁻²⁴. Biochemically it is unclear whether the primary defect is in the chloride channel

or in another component of the complex regulatory network. The similarity of CFTR to other membrane transporters strongly suggests that it is part of the chloride transporter, and hence it is this pump that is altered in cystic fibrosis patients. But this has yet to be demonstrated. It may be significant that the predicted molecular mass of CFTR is rather different from that of functional chloride channels recently isolated from epithelia²⁵.

Over 60 per cent of cystic fibrosis patients seem to have the same mutation, a deletion of three nucleotides, removing a single phenylalanine residue from CFTR². This amino acid is in one of the highly conserved ATP-binding domains of the protein but is not one of the supposedly crucial amino acids in the 'core' site¹⁶. Does the mutation completely destroy the function of the CFTR or is it a specific missense mutation that alters the protein's activity? There is support for the latter view as patients' regulation of chloride flux is altered rather than absent.

If the cystic fibrosis phenotype involves protein misfunction, not loss of function, there is a better chance of devising a corrective treatment for the disease; it would be hard to replace an absent transport protein, but it may be possible to modulate the activity of one that is perturbed. In what way might such a missense mutation affect the activity of the CFTR protein? One possibility is a subtle effect on the coupling of ATP hydrolysis to the transport process. An understanding of this will probably require determination of the three-dimensional structure of these sites, not impossible given recent progress on bacterial transport proteins. Their ATP-binding domains are so similar to CFTR that there is no doubt that they provide an important structural model. □

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