CYSTIC FIBROSIS

A welcome animal model

Francis S. Collins and James M. Wilson

THE creation of mouse models for human disease by homologous recombination in embryonic stem cells has emerged as a major tool for studying molecular pathogenesis, but unfortunately these mouse mutants do not always resemble their human counterparts as closely as one would like. Two recent examples are the *hprt*$^-$ mouse[1,2], which lacks the enzyme hypoxanthine phosphoribosyl transferase and was constructed as a model for Lesch–Nyhan syndrome, but which has no detectable phenotype, and the p53 mutant mouse[3], whose phenotype is much less dramatic than would be expected from the homozygous obliteration of this tumour-suppressor gene. It was therefore with considerable anxiety and anticipation that cystic fibrosis researchers awaited the construction of a mouse model of this common and potentially lethal autosomal recessive disease. The results, reported in a pair of papers in the latest issue of *Science*[4,5], were well worth the wait.

Since its original clinical characterization, the unifying pathological feature of cystic fibrosis has been the obstruction of the respiratory, gastrointestinal and reproductive tracts, particularly their serous and mucous glands, by thick sticky secretions[6]. The identification of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by a positional cloning strategy[7–9] has done much to clarify the basic cellular defect, which involves a loss of the normal cyclic AMP-mediated apical chloride conductance of a wide variety of epithelial cells (for a review, see ref. 10). Presumably the resulting imbalance of chloride and water flux results in the dehydration of secretions characteristic of cystic fibrosis (CF), and is subsequently worsened by obstructive damage to involved organs, and by bacterial superinfection in the respiratory tree. But the absence of an animal model has prevented a better understanding of the pathophysiology of CF at the organ and whole animal level, and created significant obstacles to developing and testing new therapies.

Successful targeting of the murine CFTR locus turned out to be a formidable task, and has occupied the intense attention of several experienced groups over the past three years. On the way to achieving this goal, the University of North Carolina group, led by Bev Koller and Oliver Smithies[4], had to develop a series of elegant and rigorous controls, and discovered that the frequency of homologous versus non-homologous recombinant events was capable of tenfold variations from day to day; indeed, the identification of the factors responsible for this variability would be of enormous benefit to such experiments in the future. Eventually two cell lines harbouring an interruption of exon 10 of the CFTR gene and capable of germ-line incorporation were constructed, heterozygotes were generated, and these were bred to produce homozygous (–/–) animals.

In the light of the physiological differences between humans and mice, and in the face of previous failure to identify any spontaneous mouse model of CF, there was concern that the (–/–) animals might have an embryonic lethal phenotype. Gratifyingly, the (–/–) animals appeared in the expected mendelian ratio at birth, indicating no apparent intrauterine loss. Shortly after birth, however, these animals began dying, and few survived beyond 40 days. The cause of death appeared in all cases to be intestinal obstruction, perforation and fatal peritonitis. This part of the mouse CF phenotype bears striking similarity to the human syndrome of meconium ileus, which occurs in 5–10 per cent of CF newborns and can be fatal. Histological examination of the (–/–) mice revealed distention of the crypts of Lieberkuhn by dense concretions throughout the intestinal tract.

Physiological studies of the (–/–) mice[5] also confirmed similarity to the human phenotype at the cellular level. In both intestinal and respiratory epithelia, Ric Boucher and colleagues demonstrated the absence of the normal cyclic AMP-stimulated apical chloride channel, which is the physiological hallmark of CF in man. As in man, murine respiratory epithelium contains an alternative non-CFTR chloride channel which responds to externally applied ATP or UTP, and which provides an attractive pharmacological alternative for the treatment of CF.

Similarity between the pathology of the mouse model and the human disease, however, did not extend as well to the reproductive, pancreatic, hepatobiliary and respiratory systems. In the reproductive tract there was no evidence for abnormality in the mouse, and one long-surviving (–/–) male successfully reproduced. Similarly, there was no conclusive evidence for pancreatic or hepatobiliary disease except for inflammation of the gall bladder. In the lungs, which are responsible for 95 per cent of the morbidity and mortality of CF in man, no evidence for obstructive disease was found in the (–/–) mice, although there was pathology in the nasal sinuses and an increased frequency of patches of goblet cells in the proximal airway. This general lack of a pulmonary phenotype may reflect anatomical differences: rodents essentially lack the components of the human airway primarily responsible for mucous production, including submucosal glands, which are the predominant site of CFTR expression in human bronchi (J. Englehardt and J. M. W., unpublished results). The absence of clear pathological changes in the lungs, liver and pancreas of the (–/–) mice might be viewed as a disappointment. Given that such changes are quite limited in CF newborns, however, the possibility remains that these murine manifestations might have appeared had the animals survived longer.

The consequences of the availability of such a mouse model are likely to be many. The affected mice provide a golden opportunity to study the intestinal pathophysiology of CF in much greater detail than before. It will be of interest, for example, to determine whether heterozygotes are selectively resistant to intestinal exposure to cholera toxin, an advantage that might explain the high frequency of CF in the Caucasian population[11]. To address this and other questions about CF, it may also be necessary to produce a mouse homozygous for the ΔF508 mutation, which is a deletion of a phenylalanine residue at position 508 of the CFTR and is responsible for 70 per cent of CF cases in northern Europeans. This mouse might have a subtly different phenotype in both heterozygotes and homozygotes from a CFTR 'knockout' mouse. The recent development of 'hit-and-run' targeting protocols[12,13] should now make such a precise alteration of the murine CFTR gene possible.

The CF mouse will immediately find a role in the testing of new therapies. Although the absence of pulmonary manifestations and the (potentially related) discovery of a murine airway amiloride-sensitive sodium transport system with no human homologue[5] will

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significantly complicate the use of this model for evaluation of pharmacological approaches to CF lung disease, there are likely to be correlations between approaches that ameliorate the intestinal phenotype in the CF mouse and those that would be beneficial in the lungs in humans. For gene therapy protocols, issues of efficiency and safety of CFTR gene transfer can already be addressed in non-CF animal models. But a genetically deficient animal that models the clinical manifestations of CF, especially in the lungs, will be necessary to answer critical questions of therapeutic efficacy; this information is fundamental to the design of early clinical protocols. It will therefore be important to transfer the existing mutant onto a wide variety of mouse strain backgrounds, and also to breed long survivors from such crosses selectively. The identification of (–/–) animals with milder intestinal manifestations permitting long survival may allow emergence of a pulmonary phenotype, especially if these animals are exposed to pathogens. Such breeding experiments would also provide the opportunity to map modifier genes that determine the severity of the disease, which may well have human homologues.

Geneticists are fond of quoting the old (and perhaps somewhat self-serving) maxim: "whatever it is you're studying, you're better off if you have a mutant"[14]. The successful development of a mouse model for CF will undoubtedly have significant repercussions in the rigorous search for a better understanding and improved treatments for this debilitating disease, and stands as a significant milestone in the midst of a flurry of recent stunning advances in CF research. □

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CYSTIC FIBROSIS

Another protein out in the cold

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A SINGLE amino-acid deletion in the cystic fibrosis transmembrane conductance regulator (CFTR) is the mutation most frequently responsible for cystic fibrosis. How does this change affect the normal function of the protein? The mouse lacking functional CFTR which is described above by Collins and Wilson is already on track to provide some of the answers. And a report by Denning *et al.* on page 761 of this issue[1] rounds off a series of studies indicating that the problem lies not so much with what the mutant protein cannot do as with where it ends up in the cell. Like many of the altered membrane proteins constructed by cell biologists, the mutant CFTR is unable to leave the endoplasmic reticulum, but in the best traditions of classical genetics it is temperature-sensitive: at lower temperatures the protein travels onwards to the cell surface where it can then function, more or less.

Cystic fibrosis is one of the most common human genetic diseases. The story of the identification of the gene responsible has been widely reported as a triumph of the new genetics. Just as impressive has been the progress made in understanding what this piece of DNA encodes: CFTR appears to function as a regulated channel for chloride ions[2]. The most frequently occurring mutation, ΔF508, which causes disease in homozygotes, lacks a phenylalanine residue in the first nucleotide-binding domain of the protein. The simplest result of changing the structure of a protein is to damage its biochemical activity, but for a membrane protein there are other possibilities. CFTR must reach the plasma membrane to function. It does this in the usual way, by first assembling in the endoplasmic reticulum, then travelling via a series of vesicles through the Golgi complex to the cell surface. Failure to accomplish any of these steps will have consequences just as drastic as any loss of biochemical function.

When the faulty CFTR gene is transfected into cultured cells, much of the ΔF508 protein is trapped in the endoplasmic reticulum[3]. In contrast, when the defective protein is expressed in an alternative system, the *Xenopus* oocyte, it appears to travel normally to the cell surface, but is not correctly regulated by cyclic AMP[4]. This raised the possibility that the apparent accumulation of mutant CFTR in cultured cells is an artefact of the expression system. Two complementary papers that argue against this explanation both rely on reagents sufficiently sensitive to detect the protein at its normal expression level. In primary cultures of normal airway epithelial cells, CFTR was found largely at the apical surface, whereas in cells derived from ΔF508 patients it was inside the cell[5]. Likewise, direct antibody labelling of tissue samples from patients showed a lack of apical membrane protein in some tissues[6].

The latest work[1] neatly resolves this apparent discrepancy. It transpires that mutant CFTR is not absolutely blocked in the endoplasmic reticulum: when transfected cells are incubated at temperatures lower than 37 °C, the protein starts to appear at the surface. At the temperature used to incubate *Xenopus* oocytes, a significant proportion of the protein is transported correctly. Once at the surface, the behaviour of the mutant protein as a chloride channel is not completely normal, but nevertheless it retains enough of its function to imply that the main problem at body temperature is a failure of delivery to the plasma membrane.

Such behaviour is by no means unprecedented among membrane proteins. A temperature-sensitive mutant of vesicular stomatitis virus, tsO45, fails to bud at high temperature because its membrane protein, the G protein, is trapped in the endoplasmic reticulum[7]. The reversibility of this trapping has made tsO45 a favourite work-horse in studies of membrane traffic, because G protein can be accumulated in large amounts in the endoplasmic reticulum, then, by lowering the temperature, released as a 'pulse' through the secretory pathway to the surface. Some artificially mutated membrane proteins have been shown to behave similarly[8]; many more are trapped in the endoplasmic reticulum and never leave. Perhaps most remarkably, HLA class I proteins normally require binding of a peptide to leave the endoplasmic reticulum, but at lower temperatures they can escape and arrive empty-handed at the surface[9].

The mechanism responsible for retaining such proteins in the endoplasmic reticulum is less than clear. Some but not all of them are associated with BiP, a 'chaperone' protein whose normal function is probably to regulate folding and assembly[10]. Simple aggregation in the membrane could be enough to prevent these molecules from entering the regions of the membrane where transport vesicles bud, a 'quality control' process that may prevent misfolded proteins from reaching the cell surface[11]. Thermal instability, leading to unfolding and precipitation, is a frequent characteristic of mutant proteins. However, mutant CFTR can function as a channel at 35 °C, arguing that it cannot be grossly denatured[1]. In addition, it is not obvious that denaturation of a membrane protein, which can move only within the plane of the lipid bilayer, would cause it to aggregate. A satisfactory description of this two-dimensional process may not come readily from biochemists trained exclusively in three dimensions.

Thus cystic fibrosis appears to have joined the list of inherited human 'protein targeting' diseases, which between them illustrate several basic concepts. A