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The CF mouse: an important tool for studying cystic fibrosis

Donald J. Davidson and Julia R. Dorin

Cystic fibrosis (CF) is a common and fatal recessive disease, which is caused by dysfunction of a chloride ion channel, termed the CF transmembrane conductance regulator (CFTR). The CF gene was cloned in 1989; subsequently, several mouse models have been created using gene targeting within embryonic stem cells. This report describes how such animal models provide the opportunity to elucidate disease pathogenesis, correlate genotype with phenotype and develop novel therapies. The current models encompass mice with a complete knockout of CFTR function, with residual CFTR function, and with precise mutations corresponding to those in humans that precipitate CF. All the CF mice demonstrate the characteristic CF ion-transport defect and show some evidence of intestinal disease, but they have a variable level of survival. Genetic background has also been shown to affect the intestinal phenotype of CF mice and this has allowed identification of a genetic modifier locus of CF in humans. Lung disease in human CF is the major cause of death in early adulthood. This is not entirely reproduced in CF mice, but repeat exposure of the lung to clinical pathogens does reveal a significantly abnormal pathogen-related response in the residual-function mice. CF mice have been successfully used to investigate the safety and efficacy of various pharmacological and gene-therapy protocols. As new cloning techniques become available, the models can be refined to ensure that in vivo models continue to be an essential tool for studying CF.

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Research into the pathogenesis of cystic fibrosis (CF) has aimed to develop a hypothesis that unites the underlying genetic defect responsible for this disease with the well-documented clinical sequelae. Since the gene responsible for CF, the CF transmembrane conductance regulator (*CFTR*) gene, was isolated in 1989, understanding of this common genetic disorder has progressed rapidly. Details of over 950 disease-causing mutations have been recorded, extensive research has established the expression pattern of the gene, the predicted structure of the protein and its function as an ion channel, and studies have begun to reveal the cellular consequences of CFTR dysfunction. This progress has enabled the development of reliable screening programmes, the creation of animal models and the design of novel therapeutic approaches. Although the biological basis of many aspects of the disease phenotype are becoming clearer, the study of CF has also served to demonstrate the mechanistic complexity of this 'simple' single-gene defect. This review begins with a summary of the characteristics of CF, and then focuses on mouse models of CF, which provide a valuable resource with which to dissect the complexities of CFTR function and dysfunction in vivo and to develop novel therapies.

Human CF disease

CF is a common, lethal, autosomal recessive disorder with a carrier frequency estimated at 1 in 29 in Caucasian populations. It affects about 1 in 3300 live births and results in a median life expectancy of 30 years (Ref. 1). CF is caused by mutations in the *CFTR* gene, located on human chromosome 7, with the most common mutation ΔF508 occurring on ~70% of CF chromosomes (Refs 2, 3, 4). One important function of the CFTR protein is its activity as a chloride ion (Cl^-) channel in the apical membrane of epithelial cells (reviewed in Ref. 5; see Fig. 1 for model of protein structure). The ΔF508 mutation is a trinucleotide deletion in exon 10 that results in the absence of a phenylalanine residue from nucleotide-binding domain 1 (NBD-1). The mutant protein is still produced but, because it lacks this amino acid, it is non-functional, is not properly processed in the endoplasmic reticulum and does not reach the apical cell membrane. Many other mutations also cause CF; these include mutations that lead to dysfunctional or non-functional proteins, as well as complete 'null' mutations.

The characteristic manifestations of CF are salty sweat, pancreatic insufficiency, intestinal obstruction, male infertility and severe pulmonary disease (reviewed in Ref. 6). The intestinal obstruction is characterised by meconium ileus (i.e. failure to produce meconium stool) in 10–20% of patients in the first few days of life, and distal intestinal obstructive syndrome in 20% of patients in later life. The affected tissues in CF show characteristic abnormalities in electrolyte transport – in particular, the movement of Cl^- and sodium ions (Na^+) across epithelia (reviewed in Ref. 7). The clinical picture shows considerable variation between individuals. This is a consequence of environmental factors, independently segregating disease-modifying genes, and differences between specific *CFTR* mutations. Disease phenotype has been categorised as 'severe' or 'mild' on the basis of the degree of pancreatic disease. This pancreatic phenotype has been found to correlate with certain *CFTR* mutations (Ref. 8). No strong genotype–phenotype correlation has been demonstrated for CF pulmonary pathology.

Lung disease in CF is characterised by chronic microbial colonisation and repeated acute exacerbations of pulmonary infection, with a distinctive spectrum of pathogens (reviewed in Ref. 9). These episodes precipitate progressive, irreversible, inflammatory lung damage. Despite the recent advancement of various hypotheses, the precise aetiology of CF lung disease is still relatively poorly understood. Current theories place particular emphasis on the role of CFTR in regulating the volume and/or composition of the airway surface liquid (ASL).

As our understanding of the molecular and biological basis of CF becomes more comprehensive, the goal of successful treatments becomes more accessible. New insights into the disease pathogenesis create opportunities for novel therapeutic interventions while also revealing the limitations of current treatments. The major goals of traditional therapies have been to alleviate the pulmonary, gastrointestinal and pancreatic manifestations of the disease (reviewed in Ref. 10). The mainstays of current therapy are chest physiotherapy with postural drainage, antibiotic therapy and pancreatic enzyme supplementation. The use of an aggressive regimen incorporating these treatments has been responsible for the improvements in survival achieved thus far. However, novel approaches are

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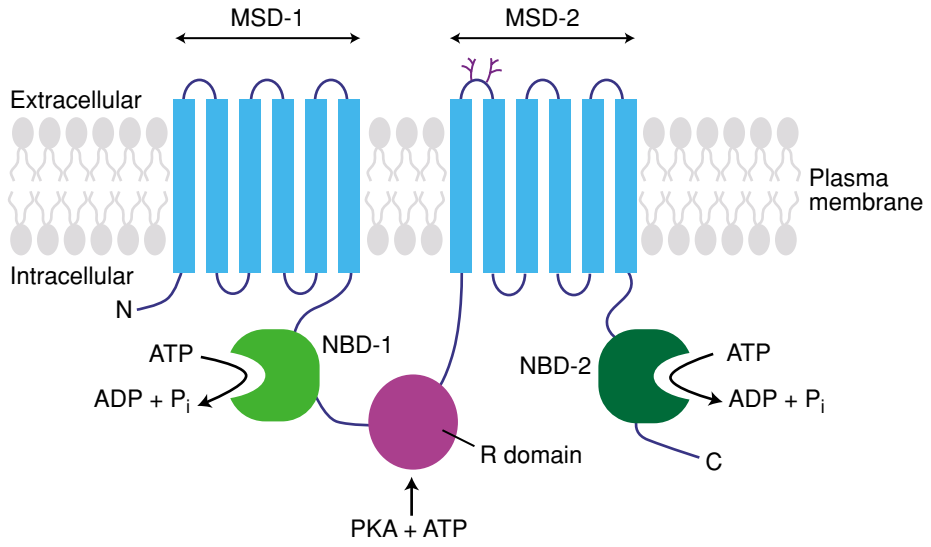

A model of the proposed structure of the cystic fibrosis transmembrane conductance regulator (CFTR)

Figure 1. A model of the proposed structure of the cystic fibrosis transmembrane conductance regulator (CFTR). The CFTR consists of two repeated motifs made up of six membrane-spanning domains (MSDs) and a nucleotide-binding domain (NBD), separated by a regulatory domain (R domain). Site-directed mutagenesis targeting the MSDs alters the conduction and permeation properties of the CFTR, suggesting that these domains form a pore in the cell. Studies suggest that this pore is kept 'closed' by the R domain in its unphosphorylated state. Phosphorylation by protein kinases (PKs) such as PKA and PKC appears to have two effects: (1) a permissive effect, perhaps releasing steric inhibition; and (2) a stimulatory effect, facilitating interaction between the NBDs and adenosine triphosphate (ATP). This phosphorylation is a requirement for pore opening and can be reversed by intracellular phosphatases. The 'opening' of the pore requires the hydrolysis of nucleoside triphosphates by the NBDs after the phosphorylation of the R domain **(fig001jde)**.

required to achieve further increases in life expectancy and to improve quality of life. Novel solutions are being sought utilising new-found genetic knowledge (reviewed in Ref. 11), and mouse models of CF provide an important resource for the development and testing of these new therapies.

The murine CF gene

The murine homologue of the human *CFTR* gene (*Cftr*) on mouse chromosome 6 was isolated in 1991 (Ref. 12). The genomic region has recently been sequenced and comparative analysis performed with human *CFTR* (Ref. 13). The *Cftr* gene spans ~152 kb, with all 27 exons being highly similar to the human homologue. The murine CFTR protein is very similar to the human (78% overall sequence identity at the amino acid level) and the majority of known CF mutations occur in well-conserved regions, suggesting conservation of function across species. The predicted mouse protein has a phenylalanine residue corresponding to the phenylalanine deleted in the common ΔF508 mutation, flanked by a 28 amino acid region that is identical to human CFTR. Although human and murine CFTR have many properties in common, some important differences in function (including channel-gating behaviour; Ref. 14) have been described. The degree of similarity between human *CFTR* and mouse *Cftr*, and the existence of the necessary molecular techniques, made the development of mouse models of CF a viable proposition.

The first mouse models of CF were created within three years of the isolation of the human *CFTR* gene, using gene targeting in embryonic stem cells to disrupt the murine homologue

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(Refs 15, 16; see Fig. 2). A further 12 mouse models of CF have been reported since, with varying degrees of characterisation (Refs 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). These models fall, broadly, into two categories: (1) those designed to disrupt *Cftr*; and (2) those that aim to model clinical mutations, such as ΔF508 (loss of a phenylalanine) and G551D (substitution of a glycine with an aspartic acid at amino acid position 551 in the protein) (Table 1). The first category can be further subdivided into those that used a replacement gene-targeting strategy to disrupt the *Cftr* gene and create absolute nulls, with no normal CFTR production, and those that used insertion into the target gene without loss of genomic sequence (Table 1). A comparison of the phenotype of mouse models of CF with the phenotype of human CF is given in Table 2.

The insertional mutants retain the potential for reversion to wild type and the production of normal *Cftr* mRNA (messenger RNA) by various mechanisms. This is particularly evident in the $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mouse, in which there is ~10% of normal levels of wild-type mRNA expression (Ref. 27). This has resulted in the description of this mutation as 'residual function'. The low level of normal CFTR is most likely responsible for the significant phenotype differences observed between the $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mouse and the absolute nulls. It should be noted that human compound heterozygotes, such as R347P/ΔF508 or R117H/ΔF508, are also predicted to retain some level of normal CFTR function (Ref. 28). In the case of R347P/ΔF508, this is estimated to be 15%. These individuals are still diagnosed as having CF and, although pancreatic sufficient, can develop severe lung disease. Thus, despite their residual function, the insertional mutant mice still mimic CF and are important models.

Table 1. Mouse models of cystic fibrosis (tab001jde)

Mouse	Mutation	*Cftr* mRNA	Original strain	Ref.
$Cftr^{tm1Unc}/Cftr^{tm1Unc}$	Exon 10 replacement	No wild-type mRNA detectable	C57Bl/6/129 BALBc/129	15
$Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$	Exon 10 insertional	10% of normal levels of wild-type mRNA	MF1/129	16
$Cftr^{tm1Cam}/Cftr^{tm1Cam}$	Exon 10 replacement	No wild-type mRNA detectable	MF1/129 C57Bl/6/129	17
$Cftr^{tm1Hsc}/Cftr^{tm1Hsc}$	Exon 1 replacement	No wild-type mRNA detectable	CD1/129	24
$Cftr^{tm1Bay}/Cftr^{tm1Bay}$	Exon 3 insertional duplication	<2% of normal levels of wild-type mRNA	C57Bl/6/129	18
$Cftr^{tm3Bay}/Cftr^{tm3Bay}$	Exon 2 replacement	No wild-type mRNA detectable	C57Bl/6/129	20
$Cftr^{tm2Cam}/Cftr^{tm2Cam}$	ΔF508 by exon 10 replacement	Mutant mRNA with 15% of normal expression levels	C57Bl/6/129	19
$Cftr^{tm1Kth}/Cftr^{tm1Kth}$	ΔF508 by exon 10 replacement	Decrease in mutant mRNA levels in intestinal tract	C57Bl/6/129	22
$Cftr^{tm1Eur}/Cftr^{tm1Eur}$	ΔF508 by exon 10 'hit and run'	Mutant mRNA expression at normal levels	FVB/129	21
$Cftr^{tm1G551D}/Cftr^{tm1G551D}$	G551D by exon 11 replacement	Mutant mRNA with 53% of normal expression levels	CD1/129	23
$Cftr^{tm2Hgu}/Cftr^{tm2Hgu}$	G480C by exon 10 'hit and run'	Mutant mRNA expression at normal levels	C57Bl/6/129	25

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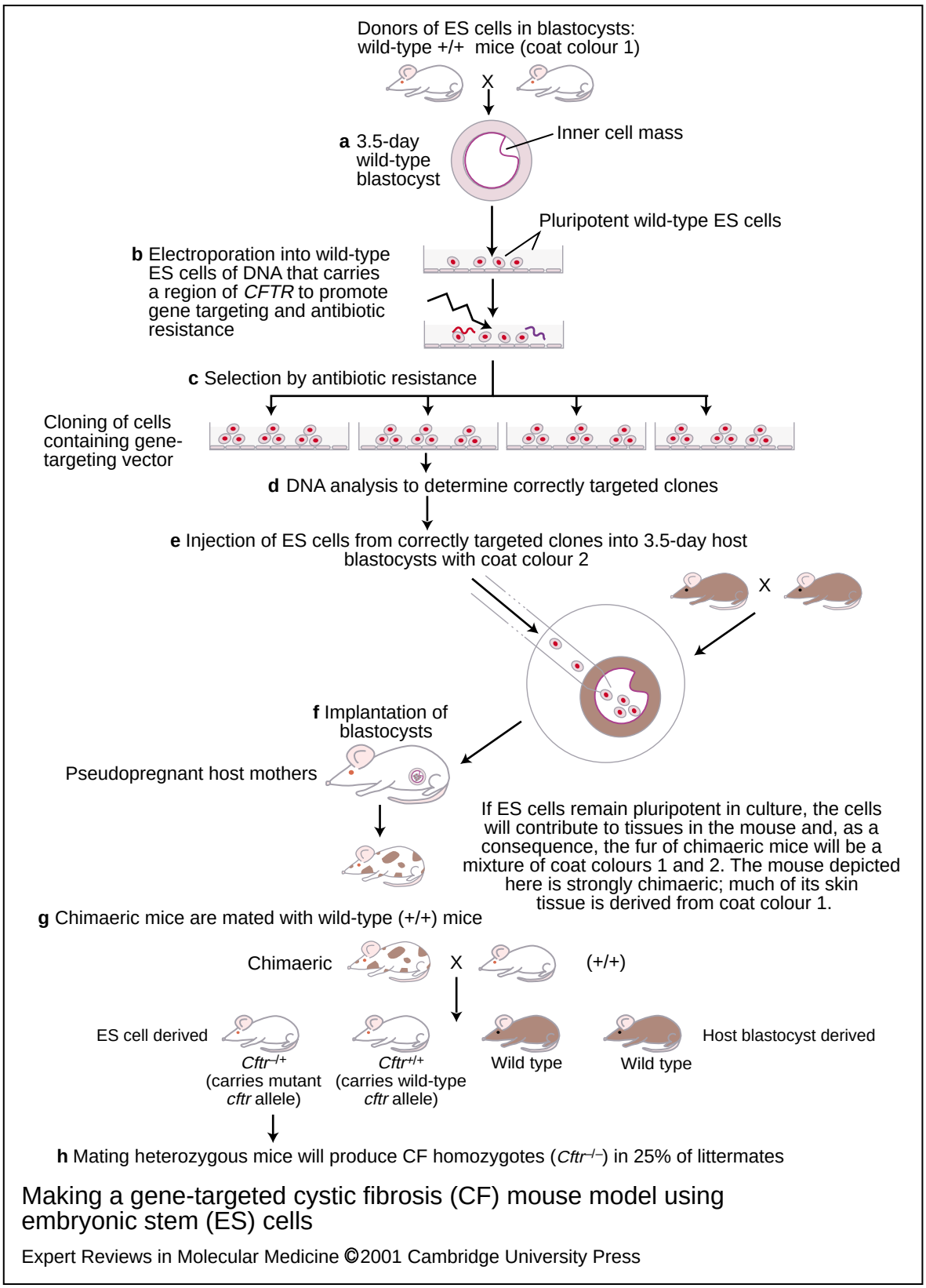

Figure 2. Making a gene-targeted cystic fibrosis (CF) mouse model using embryonic stem (ES) cells *(see next page for legend)* **(fig002jde)**

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Figure 2. Making a gene-targeted cystic fibrosis (CF) mouse model using embryonic stem (ES) cells. (a) ES cells derived from the inner cell mass of 3.5-day blastocysts (from mice with coat colour 1) can remain pluripotent when cultured under certain conditions [such as on a primary embryonic fibroblast feeder layer or in the presence of differentiation inhibitory activity (DIA)/leukaemia inhibitory factor (LIF)]. (b) DNA vectors for gene targeting can be introduced into the cultured ES cells by electroporation. (c) Cells that take up DNA are selected by their antibiotic resistance, which is conferred on them by a gene carried by the vector. The design of the targeting construct determines the type of mutation that will be introduced. (d) Clones that are correctly targeted are identified by DNA analysis and, (e), are injected into 3.5-day host blastocysts derived from mice with coat colour 2. (f) These are then implanted into pseudopregnant mothers and, providing the ES cells have been cultured correctly, the mice that are born will have some tissue derived from ES cells and some from the host blastocyst (i.e. are chimaeras). This can be visualised because the ES cells and host blastocysts are derived from mice with different coat colours. (g) The germline is the most difficult tissue for ES cells to colonise, but chimaeric mice mated with a wild-type (+/+) mouse with a suitable coat colour will produce mice of different colours depending on whether the gamete was derived from ES-cell tissue or the host blastocyst. The chromosome with the introduced mutation will be carried by 50% of mice derived from ES cells, and these mice will be *Cftr*$^{-/+}$ heterozygotes. (h) Mating *Cftr*$^{-/+}$ heterozygotes will produce *Cftr*$^{-/-}$ homozygote mice in 25% of the litter **(fig002jde)**.

The mouse models of CF designed to reproduce known clinical mutations were created using either a replacement gene-targeting strategy or a double homologous recombination ('hit and run') procedure (Ref. 29) (Table 1). The latter technique produces a mutated exon without selection marker genes or plasmid sequences in the intronic structure of the gene. The presence of such intronic 'debris', which arises during replacement gene targeting, could be responsible for the observed transcriptional interference. Thus, it is crucial that the possible consequences of the techniques used to generate the different mouse models are taken into account in any interpretation of the phenotypes observed.

Table 2. Phenotype of cystic fibrosis in humans and mouse models (tab002jde)

Characteristic	Human	Mouse[a]
Survival	Reduced survival due to lung disease	Varying degree of survival due to intestinal blockage
Intestinal disease	Meconium ileus and distal intestinal obstruction syndrome in 15% of patients; decrease or no change in baseline PD in the intestine; decreased cAMP-meditated Cl^- secretion; decreased Ca^{2+}-related Cl^- response	Intestinal obstruction fatal in majority at birth or at weaning; significant decrease in baseline PD in the intestine; decreased cAMP-meditated Cl^- secretion; decreased Ca^{2+}-related Cl^- response
Pancreatic disease	Pancreatic insufficiency	No severe pancreatic disease
Reproductive tissue abnormalities	Male infertility	Reduced fertility predominantly in females
Lung disease	Mucus build-up and chronic infection in lungs; hyperabsorption of Na^+ in nasal epithelium; decreased cAMP-stimulated Cl^- conductance in nasal epithelium	Lung disease only after chronic exposure to pathogens; hyperabsorption of Na^+ in nasal epithelium; decreased cAMP-stimulated Cl^- conductance in nasal epithelium
Other abnormalities	Salty sweat	Reduced enamel on teeth

[a] Variation between mouse models exists and the phenotype characteristics shown above may be found with variable severity between models.
Abbreviations: cAMP, cyclic adenosine monophosphate; PD, potential difference.

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Phenotype of mouse models of CF

Animal models of human diseases must be carefully characterised and demonstrate appropriate pathology in order to justify their use in the dissection of disease pathogenesis and in the design and testing of novel therapies. One possible source of concern is variations in the phenotypic consequences of identical genotypic abnormalities between different species. However, after detailed scrutiny, such differences, on a background of essentially similar pathology, also have the potential to prove as illuminating as the similarities. In addition, the existence of numerous different mouse models of CF provides the opportunity to study the contribution of a variety of factors within the same species.

Although the phenotypes of the different mouse models of CF bear most of the same hallmarks as each other, important differences have been observed. These phenotypic variations have been shown to relate to the specific mutation generated, to environmental influences and to independently segregating modifier genes. The level of significance now attached to such phenotype modifiers was not evident during the initial characterisation of the many mouse models of CF. As a result, direct comparisons between the different models must be made with considerable care and with attention to the environmental conditions and background strain (see Table 1). The models in which the majority of significant studies have been performed are *Cftr*^{tm1Hgu}, *Cftr*^{tm1Unc} and *Cftr*^{tm1Hsc}; thus, special attention will be paid to their characterisation in the following sections, which discuss the phenotype of mouse models of CF.

Survival

Intestinal pathology and the resultant mortality appear to be the predominant hallmark of *Cftr* mutation in the mouse. The survival rates reported in the initial characterisation of the different mouse models of CF vary from <5% survival to maturity in *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} nulls and *Cftr*^{tm1Cam}/*Cftr*^{tm1Cam} mice, to ~90% in *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice, and normal survival in *Cftr*^{tm2Hgu}/*Cftr*^{tm2Hgu} and *Cftr*^{tm1Eur}/*Cftr*^{tm1Eur} mice (Table 3).

Table 3. Survival and body weight in mouse models of cystic fibrosis (tab003jde)

Species	Mutation	Survival	Body weight	Ref.
Human	ΔF508	10% MI, 20% DIOS	Failure to thrive	6
Mouse	*Cftr*^{tm1Unc} (null)	<5% survival to maturity	10–50% reduction	15
Mouse	*Cftr*^{tm1Hgu} (residual function)	95% survival to maturity	No reduction	16
Mouse	*Cftr*^{tm1Cam} (null)	<5% survival to maturity	50% reduction	17
Mouse	*Cftr*^{tm1Hsc} (null)	25% survival to maturity	Delayed	24
Mouse	*Cftr*^{tm1Bay} (null)	40% survival by day 7	70% reduction	18
Mouse	*Cftr*^{tm3Bay} (null)	40% survival at 1 month	Reduced	20
Mouse	*Cftr*^{tm2Cam} (ΔF508)	<5% survival to maturity	NR	19
Mouse	*Cftr*^{tm1Kth} (ΔF508)	40% survival to maturity	50% reduction	22
Mouse	*Cftr*^{tm1Eur} (ΔF508)	Normal	20% reduction	21
Mouse	*Cftr*^{tm1G551D} (G551D)	27–67% survival by day 35[a]	30–50% reduction	23
Mouse	*Cftr*^{tm2Hgu} (G480C)	Normal	No reduction	25

[a] 27% survival at day 35 in standard animal facility, but 67% survival at day 35 in specific pathogen-free conditions.
Abbreviations: DIOS, distal intestinal obstruction syndrome; MI, meconium ileus; NR, not reported.

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The remaining 10% of *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} animals die around weaning as a result of intestinal obstruction, resembling meconium ileus. Interestingly, this is a similar proportion to that seen for meconium ileus in CF humans. The low-level production of normal CFTR has been proposed to be the explanation for the significantly greater survival rate in the *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice.

The mortality associated with intestinal disease has been reported to manifest itself at two distinct periods: (1) within a few days of birth; and (2) around the time of weaning to solid food (approximately 21 days). Significant differences in the survival rates at these time points in *Cftr*^{tm1Hsc}/*Cftr*^{tm1Hsc} mice, bred to congenicity on different inbred backgrounds, have revealed a modifier locus that partially determines the severity of the intestinal phenotype in mouse models of CF (Ref. 24). The power of mouse models of CF to identify such modifier loci, and the significance for novel therapy design, is discussed below.

The high mortality around the time of weaning appears to result from the consumption of solid food. Indeed, the use of a liquid diet has been found to prolong the lifespan of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice (Ref. 30). However, mice weaned on a liquid diet seem to suffer the effects of malnutrition (Ref. 31), which might impact upon phenotypic observations. Other, as yet undetermined, environmental factors have also been shown to influence survival of *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice maintained under specific pathogen-free (SPF) conditions as opposed to a standard animal facility (Ref. 23).

An alternative approach to address the poor survival rates of mouse models of CF has utilised the expression of human *CFTR* cDNA in the intestinal tract of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice, under the control of the rat intestinal fatty-acid-binding protein (*FABP*) gene promoter. This was demonstrated to correct the lethality of the intestinal defect in the mutant mice and to lead to longer-term survival, despite inappropriate cell-specific expression (Ref. 32). However, concern persists about the confounding effects of *CFTR* expression outside the gastrointestinal system.

Intestinal disease

The most pronounced pathology in mouse models of CF has been reported in the gastrointestinal system. Variation between models has been reported in the specific pathology observed and in the degree of severity. However, in most cases, characterisation of the mutant mice has revealed pathology similar to that initially reported for the *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} null mice (Ref. 15). These observations include runting and failure to thrive, goblet cell hyperplasia, mucin accumulation, crypt dilation and intestinal obstruction (bearing similarity to meconium ileus) with resultant perforation, peritonitis and death. Although rather more severe than in humans, this phenotype is sufficiently similar to suggest the same pathophysiological processes and validates the use of these animals as a model for human disease.

Characteristic intestinal pathology has been observed in *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice, but this model has been reported to display a lower mortality from intestinal complications compared with the null models (Ref. 23). It is interesting to note that compound heterozygote humans with G551D and DF508 mutations also have a reduced incidence of meconium ileus compared with ΔF508 homozygotes (Ref. 33). This finding suggests that the phenotype–genotype correlation observed in humans is well modelled in the *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice.

Although the *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice display a rather less-severe phenotype, without runting or failure to thrive, characteristic intestinal pathology is observed (Ref. 16). The intestinal phenotype reported for the *Cftr*^{tm1Eur}/*Cftr*^{tm1Eur} ΔF508 mouse is not severe, with only a small degree of runting and mild goblet cell hyperplasia observed (Ref. 21). This is surprising in light of the phenotypes of other ΔF508 mouse models and the 'severe' nature of the CF disease in individuals homozygous for ΔF508. The contrast could be due to the different background strains of the various mice, the difference in techniques by which they were generated or their housing conditions and pathogen status, or a combination of these factors (Table 1). Such observations highlight the caution that must be used when interpreting the phenotype of mouse models of CF and the importance of distinguishing clearly between the different models and background strains utilised.

Studies characterising the electrophysiological profile of the intestines in mouse models of CF have found broadly similar phenotypes in the different models (Refs 16, 17, 19, 20, 21, 22, 24,

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34, 35) (Table 4). On the basis of this form of analysis, mutant mice could be unequivocally distinguished from their wild-type littermates. Furthermore, these profiles closely model the electrophysiological phenotype in CF humans. All the mouse models of CF display a significant decrease in the baseline potential difference (PD) and short-circuit current (I_{SC}) in the intestine. This is likely to be the result of a decreased rate of unstimulated Cl^- secretion. In addition, a complete absence, or a significant decrease, in cyclic adenosine monophosphate (cAMP)-stimulated Cl^- secretion has also been reported in all of the models, which is indicative of the loss of CFTR function. The residual function nature of the *Cftr*^{tm1Hgu} mutation could provide the explanation for the mild intestinal phenotype of the *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice (Ref. 27). The reason for the improved survival rates of *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice and the mild nature of the intestinal disease in *Cftr*^{tm1Eur}/*Cftr*^{tm1Eur} ΔF508 mice is less clear, but probably relates to some residual function of the mutant protein produced from these alleles.

Table 4. Intestinal electrophysiology in mouse models of cystic fibrosis[a] (tab004jde)

Mutation	Tissue	Baseline PD	cAMP-mediated Cl^- response	Ca^{2+}-related Cl^- response	Ref.
CF human	GI tract	↔ or ↑	↓	↓	109
Cftr^{tm1Unc}	Jejunum	↓	↓100%	↓	110
	Caecum	↓	↓100%	↓	34
	Colon	↓	↓100%	↓	
Cftr^{tm1Hgu}	Jejunum	↓	↓65%	↓	35
	Caecum	↓	↓65%	↓	35
Cftr^{tm1Cam}	Caecum	↓	↓100%	NR	17
Cftr^{tm1Hsc}	Rectum	NR	↓100%	NR	24
	Ileum[b]	NR	↓100%	↑	24
Cftr^{tm1Bay}	Ileum	↔	↓80%	NR	18
Cftr^{tm3Bay}	Colon	NR	↓100%	↓	20
Cftr^{tm2Cam}	Colon	↓	↓100%	↓	19
Cftr^{tm1Kth}	Jejunum	↔	↓100%	NR	22
Cftr^{tm1Eur}	Ileum	↓	↓66%	↔	21
Cftr^{tm1G551D}	Jejunum	↓	↓99%	NR	23
	Caecum	↓	↓95%	NR	23
Cftr^{tm2Hgu}	Caecum	↓	↔	↓	25

[a] Comparison of the electrophysiological profiles of the intestinal epithelium in humans with cystic fibrosis (CF) and mouse models of CF (on the original background strain). Profiles (PD, potential difference) are shown as increased (↑), decreased (↓) or preserved (↔) in comparison with non-CF controls (NR, not reported).
[b] Patch-clamped, isolated ileal crypt cells.

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Pancreatic disease

In human CF, pancreatic insufficiency is a prominent manifestation of CFTR dysfunction. By contrast, pancreatic disease has not been convincingly demonstrated in most of the mouse models of CF. In one study in which *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice were weaned on a liquid diet, significant differences in pancreatic growth and specific enzyme activities were observed (Ref. 31). However, wild-type controls showed similar, although less-severe, abnormalities on this diet when compared with those on a solid diet, suggesting that the abnormalities were predominantly secondary to malnutrition. A further study, using a liquid elemental diet, reported luminal dilatation and the accumulation of zymogen granules at the apical pole of the ductal epithelial cells in *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice (Ref. 36). This phenotype has since been used, and corrected, in a study of the role of dietary fatty acids in CF (Ref. 37). The role of the original diet in this phenotype might yet prove to be significant.

These observations suggest that there might be important differences between the human and murine pancreas with regard to the role of CFTR. Whereas high-level expression of the gene encoding CFTR has been demonstrated in the human pancreas (Ref. 38), the level of expression of *Cftr* in the murine pancreas is low (Ref. 15). Furthermore, the existence of an alternative fluid secretory pathway, which is activated by increases in intracellular calcium ions (Ca^{2+}), has been demonstrated in murine pancreatic cells (Ref. 39). These Ca^{2+}-activated Cl^- currents (CACCs) can be observed in murine pancreatic duct cells that have no detectable CFTR. CACCs are of a similar magnitude in mouse models of CF and wild-type littermates, and are about 15-fold larger than CFTR currents. It appears likely that mouse models of CF do not develop pancreatic pathology of the same severity found in the human disease as a result of these physiological differences. Furthermore, these results indicate that other Cl^- channels could be capable of compensating for the loss of CFTR and suggest novel therapeutic approaches in humans, based on the identification and utilisation of such pathways.

Teeth abnormalities

The teeth of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice were reported to have significantly reduced enamel mineral content and an elevated level of magnesium ions (Mg^{2+}), compared with those of wild-type mice (Ref. 40). The *Cftr*^{tm1Eur}/*Cftr*^{tm1Eur}, *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} and *Cftr*^{tm2Hgu}/*Cftr*^{tm2Hgu} mice do not show this abnormality.

Reproductive tissue abnormalities

Male humans with CF produce functional sperm but are infertile as a result of agenesis (lack of growth) of the vas deferens. The male reproductive tract is particularly sensitive to CFTR dysfunction, in contrast to that of females. Many successful pregnancies have occurred in female CF subjects. High levels of expression of *Cftr* mRNA have been reported in the murine testes and epididymis (Ref. 15). Female mice homozygous for the *Cftr*^{tm1Unc}, *Cftr*^{tm1Cam} or *Cftr*^{tm1G551D} mutations are mostly infertile, while homozygote males have a reduced fertility. Both male and female mice homozygous for the *Cftr*^{tm1Hgu} mutation are fertile, but have a reduced fertility, which is more pronounced in the females. Although fertility is less severely affected in the *Cftr*^{tm1Eur}/*Cftr*^{tm1Eur} mice, the vas deferens has been reported to be obstructed with eosinophilic secretions, with sperm cells absent in the majority of animals examined, despite normal sperm production in the testes and epididymis (Ref. 41).

Lung disease

Lung disease is the primary concern for CF individuals, physicians and research scientists alike. Consequently, the value of mouse models of CF in dissecting the disease pathogenesis and in developing novel therapies is largely dependent upon the extent to which they mimic the lung disease seen in human CF patients.

Initial characterisation of the mouse models provided little indication of gross pulmonary abnormalities. These observations were greeted with some surprise. However, upon reflection, an expectation of mucus plugging, neutrophil accumulation and bronchiectasis in these mutant animals might be considered to be unrealistic. This is particularly true if bacterial interaction is required to initiate a cycle of infection and inflammation, given that most of these mutant animals were maintained in semi-sterile barrier facilities. Furthermore, the development of characteristic lung histopathology in CF individuals is a gradual process that occurs over years. The initial assessment of mouse models of CF was made after only a few weeks to months. Indeed, in the case of null mice, the vast

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majority died of gastrointestinal complications before a systematic assessment could be made. In this respect, the prolonged survival of the *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice proved to be particularly valuable.

Electrophysiology studies

Despite the apparent absence of gross pulmonary pathology, electrophysiological analyses of the mouse models of CF have been able to differentiate clearly between mutants and wild-type littermates. Studies have addressed the bioelectric profile of both the nasal epithelium (Table 5) and the conducting airways (Table 6).

The analysis of the nasal epithelium has demonstrated hyperabsorption of Na^+, manifest in a raised baseline PD and a significantly greater decrease in PD in response to amiloride (an inhibitor of the epithelial Na^+ channel) (Table 5). In addition, a decrease, or absence, of cAMP-stimulated Cl^- conductance was observed in all of the models tested, with the exception of the ΔF508 *Cftr*^{tm1Eur}/*Cftr*^{tm1Eur} mice. In these respects, the nasal epithelium in most of the murine models of CF accurately replicates the defects observed in the human airways. However, in contrast to human bioelectric profiles, the nasal mucosa of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice and long-lived *Cftr*^{tm1Hsc}/*Cftr*^{tm1Hsc} mice have been reported to display a raised Ca^{2+}-mediated Cl^- secretory pathway. This might be capable of partially alleviating the effects of CFTR dysfunction in these models.

The analysis of the conducting airways has proved to be more complex (Table 6). In contrast to the human airways, studies in the mouse models of CF report either no difference, or a reduction, in the amiloride-sensitive I_{SC}. This suggests that, in contrast to humans, the loss of CFTR does not result in the upregulation of the epithelial Na^+ channel in this tissue. The cAMP-stimulated Cl^- responses were mixed: whereas some showed no difference, others demonstrated a significant decrease. However, cAMP-stimulated Cl^- currents remained in this tissue in all the mouse models of CF.

It has been proposed that an alternative, Ca^{2+}-activated, Cl^- secretory pathway is dominant over CFTR in the murine trachea (Ref. 42). This channel can be stimulated by the cAMP-agonist forskolin, providing potentially misleading results and producing cAMP-stimulated Cl^- currents in the absence of CFTR. CACCs have been reported to be upregulated in *Cftr*^{tm2Cam}/*Cftr*^{tm2Cam} ΔF508 mice and *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice (Table 6). This might alleviate the effects

Table 5. Nasal electrophysiology in mouse models of cystic fibrosis[a] (tab005jde)

Mutation	Baseline PD	Amiloride response	cAMP-mediated or low Cl^- response	Ca^{2+}-related Cl^- response	Ref.
CF human	↑	↑	↓	←→	111
Cftr^{tm1Unc}	↑	↑	↓100%	↑	112
Cftr^{tm1Hgu}	↑	↑	↓70%	←→	35
Cftr^{tm1Cam}	↑	NR	NR	NR	100
Cftr^{tm1Hsc}	↑	↑	↓100%	↑	113
Cftr^{tm1Eur}	↑	↑	Response to Cl^- gradient ←→		21
Cftr^{tm1Kth}	↑	↑	↓100%	NR	22
Cftr^{tm1G551D}	↑	↑	↓100%	←→	23

[a] Comparison of the electrophysiological profiles of the nasal epithelium in humans with cystic fibrosis (CF) and mouse models of CF (on the original mixed genetic background strains). Profiles (PD, potential difference) are shown as increased (↑), decreased (↓) or preserved (←→) in comparison with non-CF controls (NR, not reported). Amiloride response reflects the activity of the epithelial Na^+ channel, as amiloride specifically blocks this channel.

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Table 6. Tracheal electrophysiology in mouse models of cystic fibrosis[a] (tab006jde)

Mutation	Baseline PD	Amiloride response	cAMP-mediated Cl^- response	Ca^{2+}-related Cl^- response	Refs
CF human	↑ or ↔	↑	↓	↔	111, 114, 115
Cftr^{tm1Unc}	↔	↔	↔	↔	42
Cftr^{tm1Hgu}	↓	↓	↓60%	↔	35
Cftr^{tm1Cam}	↓	↓	↓75%	↔	100
Cftr^{tm1Bay}	↔	NR	↓70%[b]	NR	18
Cftr^{tm2Cam}	↔	↔	↔ to ↓ 60%[c]	↑	19
Cftr^{tm1G551D}	↔	↔	↓60%	↑	23

[a] Comparison of the electrophysiological profiles of the tracheal epithelium in CF humans and mouse models of CF (on the original mixed genetic background strains). Profiles (PD, potential difference) are shown as increased (↑), decreased (↓) or preserved (↔) in comparison with non-CF controls (NR, not reported). Amiloride response reflects the activity of the epithelial Na^+ channel, as amiloride specifically blocks this channel.
[b] Greatest decrease observed in the youngest mice.
[c] Studied in cultured fetal tracheal cells.

of CFTR dysfunction in the murine trachea. However, recent studies have demonstrated that the ion-transport properties of the murine trachea are regulated by independently segregating modifier genes and show considerable variation between mouse strains (Refs 43, 44). These findings suggest that, although mice might be less severely affected by the loss of CFTR in the trachea, this could vary considerably between different models. Furthermore, these results raise the possibility of dissecting out the component parts of the electrophysiological response and establishing their relative contributions to disease pathogenesis. In addition, although differences might exist, the mouse models of CF nevertheless do display electrophysiological abnormalities as a result of *Cftr* mutation. Thus, any resultant pathology is of interest, interpreted in the context of knowledge of both the similarities and the differences between humans and mice. Furthermore, the extent to which these electrophysiological observations are replicated in the lower airways remains unknown.

Submucosal glands and airway epithelium

The role of airway submucosal glands (SMGs) in the pathogenesis of CF lung disease remains unclear. The serous cells of the SMGs are the site of the highest level of *CFTR* expression in the human airways (Ref. 45), and abnormalities of these glands are one of the earliest findings in CF lung pathology (Ref. 46). Because the glands are a source of ASL, mucus and airway antimicrobials, significant involvement of the SMGs in disease development appears probable.

Concerns have been raised about the distribution of SMGs and the cellular composition of the murine airways. Despite reports to the contrary, mice do have SMGs in the airways. However, unlike in the human, these glands are predominantly localised in the most proximal part of the trachea and do not extend into the bronchi (Ref. 47). Furthermore, the distribution pattern of these glands is affected both by strain background and by *Cftr* mutation (Refs 47, 48). The impact of these differences is as yet unclear, but, once again, the degree of variation observed provides an opportunity to investigate the contribution of SMGs to the development of disease in an in vivo model.

Whereas the proximal human airways are composed primarily of ciliated cells, the murine trachea and bronchi are predominantly composed of Clara cells (Ref. 49), which are non-ciliated

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columnar cells. As a result of this species difference, the murine airways might be a better model of human bronchioles, the site at which CF lung disease begins, than of the large airways of the human lung.

Lung pathology

Table 7 summarises the characteristic lung pathology observed in mouse models of CF. The first observations relating to abnormal lung pathology in a mouse model of CF were made in *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice. In initial studies of outbred MF1/129 *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice, no gross lung disease was observed at birth, or in animals born and raised in isolator conditions (Ref. 50). However, histopathological evidence of pulmonary pathology was noted in mice reared in normal animal-house conditions. Although no significant difference existed between the genotypes, there was a trend towards worse pathology in the CF mice.

An evaluation of the cytokine levels in bronchoalveolar lavage fluid from untreated MF1/129 *Cftr*^{tm1Hgu} mice revealed significantly increased levels of the pro-inflammatory cytokine tumour necrosis factor α (TNF-α) in the mutant mice in comparison with non-CF littermates (Ref. 51). Interestingly, this was demonstrated only in studies of animals maintained in standard animal facilities. In the animals maintained in a full-barrier SPF facility, the levels of TNF-α were significantly lower in both mutant and non-CF mice, with no significant difference between the genotypes observed. In addition, intravenous injections of lipopolysaccharide (LPS) into *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mutant mice induced S100A8 (an S-100 Ca^{2+}-binding protein with chemotactic activity) mRNA to a greater

Table 7. Lung pathology in mouse models of cystic fibrosis[a] (tab007jde)

Mutation	Observation	Ref.
Cftr^{tm1Hgu}	↑ TNF-α[b]	51
	↑ Inflammatory cells in tracheal lamina propria	53
	↓ Tracheal mucociliary transport	53
	↓ Pulmonary clearance of *Staphylococcus aureus* and *Burkholderia cepacia*	50
	↑ Pulmonary pathology after repeated exposure to *S. aureus and B. cepacia*	50
	←→ Pulmonary clearance of *Pseudomonas aeruginosa*	57
Cftr^{tm1G551D}	↑ S100A8 and TNF-α	52
Cftr^{tm1Unc}	↓ Mucociliary transport	54
	←→ Pulmonary clearance of *P. aeruginosa* and *S. aureus*	58
	↑ Mortality from *P. aeruginosa*-laden agar beads	61
	Cytokine abnormalities[b]	60
	↓ iNOS expression	66
Cftr^{tm1Kth}	←→ Pulmonary clearance of *P. aeruginosa*	59
	↓ iNOS expression	65

[a] Observations shown as increased (↑), decreased (↓) or no change (←→), in comparison with controls without cystic fibrosis.
[b] In bronchoalveolar lavage.
Abbreviations: iNOS, inducible nitric oxide synthase; S100A8, S-100 Ca^{2+}-binding protein; TNF-α; tumour necrosis factor α.

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extent than in control mice. Bone-marrow-derived macrophages from *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice were hypersensitive to LPS as measured by TNF-α induction (Ref. 52). These observations suggest that an abnormal lung phenotype might not be manifest without exposure to pathogens.

Further histological analysis of untreated MF1/129 *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice revealed a significant increase in the number of inflammatory cells (predominantly lymphocytes) present in the lamina propria, in comparison with non-CF littermates (Ref. 53). In addition, the mucociliary transport of inert particles in vivo was demonstrated to be significantly impaired in *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice in comparison with non-CF littermates (Ref. 53). A subsequent study, using embedded, cultured lung slices from mice homozygous for the *Cftr*^{tm1Unc} mutation, partially backcrossed onto the C57Bl/6 background, repeated these observations (Ref. 54).

Studies using the MF1/129 *Cftr*^{tm1Hgu} mice demonstrated an abnormal lung phenotype in response to aerosolised, CF-associated bacteria (Ref. 50). These studies showed significantly impaired airway clearance of aerosolised *Staphylococcus aureus* and *Burkholderia cepacia* in the mutant mice, in comparison with non-CF littermates. Furthermore, the MF1/129 *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice developed significantly more-severe, pathogen-specific, lung pathology after repeated exposure to these bacteria, in comparison with non-CF littermate controls. The spectrum of abnormal and variable pathology included lymphocytic aggregates, goblet cell hyperplasia and metaplasia, mucus retention, bronchiolitis, pneumonia and oedema. Subsequent studies using repeated exposure to *S. aureus* revealed similar findings in *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice bred onto a C57Bl/6N strain background and *Cftr*^{tm1Hgu}/*Cftr*^{tm1Unc} compound heterozygote mice (Ref. 55). The latter findings indicated that a decrease in the background level of normal CFTR in these 'residual-function' mutants did not impact upon this phenotype. Furthermore, they suggest that the level of normal CFTR in *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice could already lie below some critical threshold required for the development of abnormal lung pathology in response to bacterial exposure.

Although these early results indicated an abnormal response to bacterial burden, they did not reveal the prominent neutrophilia characteristic of CF lung disease in humans, nor address infection with *Pseudomonas aeruginosa*, the most serious and problematic lung pathogen for the majority of CF individuals (Ref. 56). The study of *P. aeruginosa* in mouse models of CF is complicated by a variety of factors. The most important of these is the phenotypic alteration that this organism undergoes over the course of chronic infection of the lungs of CF individuals. Although the initial infections in CF individuals are with planktonic strains, rapid deterioration of the CF lung usually occurs after transformation of *P. aeruginosa* into the mucoid form (Ref. 56). Thus, it is unclear whether the most revealing studies will arise from challenging mouse models of CF with non-mucoid *P. aeruginosa*, evaluating predisposition to infection, or modelling colonisation with mucoid strains in the absence of previous rounds of infection. The ideal study would of course demonstrate increased susceptibility to infection with non-mucoid *P. aeruginosa*, followed by the emergence of mucoid strains within the mouse lung and colonisation. In addition, the significance of previous infections with other organisms, and the consequent antibacterial chemotherapy received, is unclear with respect to priming the CF lung for *P. aeruginosa* infection.

A series of studies examined the response of an inbred strain of *Cftr*^{tm1Hgu} mice, derived from a mixed MF1/129 strain background, to repeated exposure with mucoid or non-mucoid clinical isolates of *P. aeruginosa* (Ref. 57). No significant difference was observed between the clearance profiles or the pulmonary histopathology of mutant mice in comparison with non-CF controls. The results of a further study, using *Cftr*^{tm1Unc} mice with induced allergic airways disease to increase mucus production, supported these findings (Ref. 58). Finally, a more recent report described no significant difference between the clearance of a clinical isolate of *P. aeruginosa* in *Cftr*^{tm1Kth}/*Cftr*^{tm1Kth} mice (carrying the ΔF508 mutation) and non-CF littermates, on a mixed C57Bl6/129 strain background (Ref. 59). These studies suggest that despite abnormal responses to other bacteria, mouse models of CF might not display increased susceptibility to pulmonary infection with *P. aeruginosa*. This is obviously a significant contrast to CF lung disease in humans.

The previous studies all attempted to model the process of initial lung infection with *P. aeruginosa* using relevant clinical strains. The intention of this approach was to elucidate the

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mechanisms underlying increased susceptibility to lung infection with this organism in CF individuals, as well as the transition to mucoidy and selection for mucoid variants in lung colonisation. In an alternative approach, agar beads laden with *P. aeruginosa* are directly instilled into the lungs of the experimental animals. This is purported to model the colonisation of the CF lung by mucoid *P. aeruginosa*. Using this technique, *Cftr*^{tm1Unc} mice were inoculated with laden agar beads, first on the original mixed C57Bl/6J/129 strain (Ref. 60) and later backcrossed onto an inbred C57Bl/6 strain (Ref. 61). In both cases, the mice were reared on a liquid diet. A significantly decreased survival rate of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice in response to bronchopulmonary instillation of sub-lethal doses of *P. aeruginosa*-laden agar beads, in comparison with non-CF mice, was reported in both studies. However, bacterial proliferation was observed regardless of genotype, and only one of the studies recovered significantly higher numbers of bacteria from the lungs of mutant mice. Analysis of the lung histopathology of these mice revealed severe, focal, neutrophil-dominated, endobronchial inflammation, as well as obstruction of the distal airways with agar beads and inflammatory cells, and excessive mucus production. However, no significant differences in lung histopathology were observed between *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice and non-CF littermates in either study.

In the earlier study, significantly higher levels of pro-inflammatory cytokines were reported in *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice, in comparison with non-CF controls, despite an absence of significant differences in the bacterial lung burden or pulmonary histopathology (Ref. 60). This study, and a subsequent report of decreased levels of the anti-inflammatory cytokine interleukin 10 (IL-10) in mutant mice (Ref. 62), support the theory that an inappropriate cytokine response to inflammatory stimuli might be important in the development of CF lung disease. However, a recent study examining the innate lung defences against *P. aeruginosa* in a malnourished mouse model reported some strikingly similar findings (Ref. 63). This suggests that malnourishment in the liquid-diet-fed mice might potentially contribute to the pulmonary phenotype observed in *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice in the previous studies, independent of any direct effects of CFTR dysfunction in the lung. Nevertheless, the agar-bead model reveals interesting phenotypic differences between mouse models of CF and control animals, and could prove effective in the study of the host response to established infection. However, this model might be less likely to provide information about the initiation and development of early-stage CF lung disease, or the increased susceptibility to initial infection secondary to CFTR dysfunction.

A further phenotype described in mouse models of CF is that of decreased levels of the inducible isoform of nitric oxide synthase (iNOS) in the lung. It has been suggested that iNOS-related antimicrobial activity might be compromised in CF, resulting in an increased susceptibility to lung infection. Nitric oxide (NO) appears to be involved in a wide range of processes: it acts as a signal molecule to induce vasodilatation and bronchodilation, as a powerful antimicrobial agent, and possibly as a regulator of the activity of epithelial Na^+ channels (reviewed in Ref. 64). The expression of iNOS is significantly reduced in mixed background strain *Cftr*^{tm1Kth}/*Cftr*^{tm1Kth} mice, homozygous for the ΔF508 mutation (Ref. 65), and *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice (Ref. 66). Furthermore, in *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice expressing human *CFTR* cDNA under the control of the *FABP* gene promoter in the intestinal tract but not the nose, iNOS expression is observed in the ileum but not in the nasal epithelium (Ref. 66). The exact mechanism by which CFTR affects the expression of iNOS remains to be determined.

In conclusion, despite some tantalising similarities between CF lung disease in humans and mouse models of CF, under the experimental conditions described, the suitability of these models remains controversial and significant differences are evident. Nevertheless, mouse models of CF clearly demonstrate a range of abnormal pulmonary phenotypes as a result of the *Cftr* mutation. Although these species differences could prevent mouse models of CF from accurately reproducing all aspects of CF lung disease in humans, they might prove as illuminating as the similarities. Rather than exclusively pursuing the development of all aspects of classical human CF lung disease in mouse models, studies should specifically address the effects of *Cftr* mutation upon the lung pathophysiology of mice. In this manner, the consequence of *Cftr* mutation in the mouse lung can be addressed and the underlying mechanisms evaluated. By recognising the key similarities and differences, mouse models of CF might provide

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useful in vivo systems for the analysis of specific aspects of CF lung disease and for testing the validity of specific hypotheses.

Pathogenesis of human CF lung disease

Although lung disease is the major clinical concern in CF, and has been extensively studied, the mechanisms by which mutation in *CFTR* results in the characteristic human pathology remain keenly debated. One current hypothesis emphasises the role of CFTR in determining the volume and the ionic concentration of the ASL lining in the lung epithelia (reviewed in Ref. 67). Thus, CF might be the result of an abnormally low ASL volume that compromises mucociliary clearance mechanisms, or a raised ASL salt content that inhibits salt-sensitive antimicrobial agents such as beta-defensins and lysozyme. Other theories include increased bacterial adherence to CF epithelial cells (Ref. 68) and decreased bacterial internalisation (Ref. 69). Despite important differences between lung pathophysiology in CF individuals and mouse models of CF, the presence of relevant abnormal pulmonary phenotypes in CF mice justifies their use in examining disease pathogenesis and addressing the conflicting hypotheses arising from predominantly cell-culture-based studies.

What do mouse models tell us?

The theories that consider the role of ASL to be central to the development of human CF lung disease are based largely on studies of primary culture systems (Refs 70, 71, 72). A series of contradictory results have been published reporting studies of the ionic composition of ASL in vivo and no conclusive results are yet available (reviewed in Refs 67, 73). Furthermore, studies using human subjects are limited by technical constraints, the requirement to minimise invasive procedures, the presence of infection in most of the available subjects and the lack of appropriate controls. However, three studies have circumvented some of these complicating factors by attempting to measure the ionic composition of ASL in murine airways.

A capillary electrophoresis fluid-collection technique has previously been used to show the hypotonic nature of ASL in rats (Ref. 74). This technique has also been performed on $Cftr^{tm1Unc}/Cftr^{tm1Unc}$ mice, backcrossed onto a C57Bl/6 strain background, and non-CF littermates (Ref. 75). A hypotonic ASL was also reported in mice; however, a limited analysis of $Cftr^{tm1Unc}/Cftr^{tm1Unc}$ mice showed no difference between these animals and their non-CF littermates. Interestingly, despite this observation, an elevated salt concentration was observed in these mice following the instillation of *P. aeruginosa*-laden agar beads.

A second study used a novel cryoprobe collection technique and X-ray microanalysis to analyse the elemental composition of tracheal ASL in $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mice, on a mixed outbred MF1/129 strain, and non-CF littermates (Ref. 76). This study also reported that ASL in mice was markedly hypotonic. ASL collected from $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mice was also not found to be significantly different in ion content in comparison with wild-type mice. However, the water content of samples was not determined and quantification of this in parallel would be of major interest.

A third study used a primary culture model of murine tracheal epithelium and assessed the ionic content of ASL on these cultured cells using a radiotracer technique (Ref. 59). This study also confirmed the hypotonic nature of ASL and found no difference between the Cl^- concentration of ASL from primary cultures from $Cftr^{tm1Kth}/Cftr^{tm1Kth}$ mice, with the ΔF508 mutation on a mixed C57Bl/6/129 strain background, and non-CF littermates.

The role of salt-sensitive antimicrobial agents, such as beta-defensins, in the development of CF lung disease remains contentious. However, studies have suggested that the dysfunction of these molecules in a high-salt environment could play a critical role in the increased susceptibility of CF individuals to pulmonary infection (Refs 70, 77). A homologous family of beta-defensins, displaying salt-sensitive antimicrobial activity, has recently been isolated in the mouse (Refs 78, 79, 80, 81, 82). Lines of transgenic mice with 'knockout' mutations in these genes have been established and are currently being characterised. The analysis of these mutant mice and the offspring intercrossed with mouse models of CF will help to determine the role of beta-defensins in the lung, particularly with reference to CF lung disease.

Mouse models of CF are also being used to examine the contentious hypothesis that CFTR is a receptor for bacterial internalisation into epithelial cells, as observed in vitro and in wild-type mice (Ref. 69). This mechanism is proposed to perform a protective role in the normal lung,

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via the internalisation of *P. aeruginosa*, and to be compromised in CF. However, a recent study using a range of transgenic mice expressing varying levels of human and murine CFTR found no direct correlation between the level of CFTR expression and the pulmonary clearance of *P. aeruginosa*, or the association of bacteria with epithelia cells in vivo (Ref. 83). The same region of CFTR that is reported to bind *P. aeruginosa* was found also to bind *Salmonella typhi* and mediate translocation of this enteric pathogen from the gastrointestinal lumen to the submucosa. In this case, the translocation is to the detriment of the host, being necessary for *S. typhi* infection of intestinal submucosa. *Cftr*^{tm2Cam}/*Cftr*^{tm2Cam} ΔF508 mice were used to study the translocation of *S. typhi* from the gut lumen to the submucosa (Ref. 84). In heterozygous ΔF508 *Cftr* mice, translocation of serovar Typhi cells was reduced by 86% from that in wild-type mice, and essentially no translocation occurred in homozygous ΔF508 *Cftr* mice. From this observation, it was proposed that the maintenance of the ΔF508 *CFTR* allele, and perhaps of other mutant *CFTR* alleles, at high levels (4–5% for ΔF508 *CFTR* in certain human populations) might be due to increased resistance of heterozygous carriers to typhoid fever.

The ability to pursue more-invasive techniques and to control for genetic and environmental influences in future studies makes mouse models of CF a powerful system. Appropriately controlled and defined studies, acknowledging the similarities and the differences between mice and humans, have the potential to answer questions about the basic pathophysiological processes regulating ASL in healthy lungs and in the presence of dysfunctional CFTR. In this manner, the use of mouse models of CF should help to dissect the pathogenesis of CF lung disease and define many of the critical components of disease development.

Phenotype modification

Initial characterisation of the different mouse models of CF did not place significant emphasis upon the importance of background strain and the role of independently segregating modifier genes, nor environmental influences.

Strains of laboratory mice have been specifically inbred over generations and, within such strains, all of the mice are practically 100% genetically identical. Repeated backcrossing of mouse models of CF with chosen inbred strains has been performed. The resultant offspring are considered to be congenic, and 99.9% inbred, after ten backcross generations. Congenic inbred mouse mutants of CF should have a more homogeneous phenotype than that observed in an outbred strain in which many genetic differences exist between littermates. Different alleles of *Cftr* have been established on the same genetic background. In these animals, the only genetic difference should be the nature of the mutation in *Cftr*. This allows the direct comparison of mutations and the elucidation of mutation-specific effects upon phenotype. In addition, the same *Cftr* mutations have been established on different inbred lines. This has introduced the possibility of comparing the effects of genetic differences between various inbred strains and has facilitated the search for independently segregating genetic modifiers of disease.

The first modifier locus was identified on the proximal region of mouse chromosome 7 (Ref. 24). The *Cftr*^{tm1Hsc} mouse was originally studied on a mixed outbred CD1/129 background and the mutant animals could be divided into three classes dependent upon when the mice died. The original founder chimaeric mice with sperm derived from the 129 strain ES cells were then bred with a selection of different inbred strains to produce F_1 heterozygotes. These were bred together to produce F_2 animals that were homozygous for the mutation but with different combinations of 129 and other strain alleles. Analysis of these mice demonstrated long-term survival (beyond weaning) in a proportion of the mutant mice on some backgrounds, but not others. A genome scan was then performed with polymorphic DNA markers to identify potential modifier loci. One major locus was identified and, although it has been suggested that a gene encoding a Ca^{2+}-activated Cl^- channel might be responsible (Ref. 85), the gene has not yet been reported. Nevertheless, as a direct consequence of this study, a genetic modifier locus for meconium ileus in humans has been identified on the conserved syntenic region of human chromosome 19 (Ref. 86). In other studies, the *Cftr*^{tm1Hgu} and *Cftr*^{tm1Unc} mutations were inbred to be congenic on the C57Bl/6 strain (Refs 87, 88). The inbred C57Bl/6J *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice demonstrate only 6% survival at day 20, despite weaning onto a liquid diet, whereas C57Bl/6 inbred mice with the *Cftr*^{tm1Hgu} mutation continue to show high rates of survival.

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The inbred *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice appeared to have a host of pulmonary abnormalities, and quantitative trait loci (QTL) analysis, based on alveolar thickening and collagen deposition in these mice relative to inbred BALB/c *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice, has identified one locus meeting the criteria for suggestive linkage (Ref. 89). Other abnormalities, including increased lung neutrophil counts and myeloperoxidase levels, are also being assessed in inbred *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice on different strain backgrounds.

Studies of the *Cftr*^{tm1Hgu} mice have shown that the distribution of SMGs in the murine trachea is determined by both the *Cftr* genotype and the genetic background (Ref. 48). In *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice, both inbred and outbred, the SMGs have been observed to extend more distally than in wild-type littermate controls. Using recombinant inbred strains, two putative loci associated with this phenotype have been identified; one of these, on mouse chromosome 9, has been confirmed by analysis of the F_2 generation (Ref. 48).

Analysis of inbred mouse strains has also revealed strain-specific variation in the electrophysiological properties of the murine airway epithelium (Refs 43, 44). These studies demonstrate the existence of genetic modifiers that determine the ion-transport profile of both the nasal and tracheal epithelium. Furthermore, these studies raise the possibility of independent genetic factors determining the expression and/or function of CFTR in these tissues.

Although genetic modifiers for lung disease in mouse models of CF are not necessarily relevant to the human disease process, the first example described above shows that, in some cases, they can reveal biologically relevant mechanisms for CF and might reveal possible targets for novel therapies.

Testing novel therapies in CF mice

Although CF mice do not spontaneously develop lung disease, the characteristic bioelectric abnormalities are observed in the nose of all CF mice and the trachea of some (Tables 5 and 6). Consequently, potential therapeutic approaches for CF can be assayed for efficacy by the correction of these electrophysiological parameters. This can be most easily and unequivocally measured in the nose. CF mice have been used successfully to monitor a variety of novel treatments. These can be divided into strategies that use drug therapy and those that use somatic gene therapy.

Drug therapies

Mutant CFTR protein treatment

One potential therapeutic approach for CF is to address the mutant CFTR proteins directly. These proteins are either mislocalised, as is the most common ΔF508 mutant, or reach the membrane but have altered conductance properties.

In those CF patients who have a mutant CFTR that is correctly localised in the cell membrane but is still dysfunctional, the use of CFTR agonists might induce a residual function by over-stimulation of the mutant channel. The potential of CFTR agonists has been investigated in *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice, which, as found in human CF, show defective cAMP-mediated stimulation of glycoprotein in their submandibular glands (Ref. 90). This defective response could be partially restored by elevating the level of cAMP using 3-isobutyl-1-methylxanthine (IBMX) or 8-(4-chlorophenylthio)-cAMP (CPT-cAMP). This suggests a rational pharmaceutical approach for treating CF patients.

Milrinone is a specific type III phosphodiesterase inhibitor that has been shown, in combination with forskolin, to induce Cl^- secretion from ΔF508 CF cells. *Cftr*^{tm1Kth}/*Cftr*^{tm1Kth} mice (carrying a ΔF508 mutation) have been used to show that this cocktail can induce a significant hyperpolarisation of the nasal epithelial PD of wild-type and ΔF508 mutants, but not null *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice, which completely lack the CFTR (Ref. 91). This implied that the ΔF508 mice produced some functional protein that could be stimulated by the cocktail. More recently, a combination of protein kinase A type II activators and phosphodiesterase class I and III inhibitors was used in the jejunum to show that ΔF508 CFTR in *Cftr*^{tm1Kth}/*Cftr*^{tm1Kth} mice can be activated to 4% of wild-type levels (Ref. 92). However, in contrast to these results, milrinone had no effect on the nasal PDs of *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} or *Cftr*^{tm2Cam}/*Cftr*^{tm2Cam} mice, whereas wild-type mice showed a small hyperpolarisation (Ref. 93). This lack of response was mimicked in a clinical trial with CF individuals with both the ΔF508 and G551D mutation, confirming the observations made in the latter murine study and indicating that milrinone was not appropriate for the induction of Cl^- secretion in CF airways (Ref. 93).

Thus, despite some conflicting results, mouse models of CF provide a resource for the testing of novel therapeutics. As mouse models of CF become more accurately characterised, and the

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most suitable background strains and mutations are identified, the reliability of this resource will be further increased.

Fatty acid metabolism defect and DHA treatment

Recently, a novel explanation for the increased inflammation and elevated arachidonic acid (AA) observed in CF patients was proposed (Ref. 37). AA is both an agonist of inflammatory pathways and a stimulant of mucus secretion, and is elevated in the phospholipid fraction of bronchoalveolar lavage fluid in CF patients. Docosahexaenoic acid (DHA) downregulates AA incorporation into membrane phospholipids and plays a major role in regulating membrane fluidity and membrane trafficking. AA is known to compete with DHA for elongation and desaturation enzymes and for the site of esterification of phospholipids.

A decreased level of phospholipid-bound DHA and increased AA has been demonstrated in pancreas, lung and ileum of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice compared with controls (Ref. 37). This imbalance of phospholipid-bound components was apparent only in CF-affected organs and not in plasma. Oral administration of DHA produced a dose-dependent increase in DHA and a reciprocal decrease in AA in pancreas and lung. This treatment also reversed the histological defect of massive luminal dilatation and zymogen granule accumulation at the apical pole of the acinar cells in the pancreas of *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice. In addition, a raised level of neutrophils in the *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice was described after a 3-day treatment with *P. aeruginosa* LPS, but this abnormal phenotype was no longer evident in mice pre-treated with DHA.

Although the phenotypes that are corrected by this therapy have not been previously well characterised in the literature, the observations in the *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice suggest that a primary defect in fatty acid metabolism might play a significant role in the pathogenesis of CF and indicate a novel therapeutic approach. However, it should be noted that, in order to keep the *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} mice alive past weaning, all mice were fed a liquid diet, which could itself impact upon phenotype in these animals (Ref. 63).

Somatic gene therapy

Even if CF disease pathogenesis is not fully understood, direct replacement of *CFTR* gene function should correct the disease status. However, this strategy is not expected to reverse pre-existing tissue damage. Therefore, gene-correction strategies must be demonstrated to be safe and effective so that intervention can be attempted early in life, before lung infection and inflammation precipitates extensive tissue damage. The feasibility of introducing purified CFTR protein via phospholipid liposomes to the apical membrane has been demonstrated in the nasal epithelia of null mice (Ref. 94). Although correction of the nasal PD defect could be achieved in about 30% of the treated animals, and CFTR could be visualised in the membrane, the effectiveness of the therapy appeared to be limited by the efficiency of the incorporation of CFTR into the membrane. Introduction of a recombinant *CFTR* gene into respiratory tissue might be an easier prospect.

Mouse models of CF have been extremely valuable for testing somatic gene therapy protocols. For effective somatic gene therapy, the major obstacle is in achieving efficient transfection of the correct cells to provide an appropriate level of gene expression. As CF is a recessive disease, we already know that gene correction of 50% of normal levels of CFTR will be sufficient. In fact the level of gene function required is likely to be much less than even 50%, as indicated by intercrossing the *Cftr*^{tm1Unc}/*Cftr*^{tm1Unc} null mice and residual-function *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mice (Ref. 95). The levels of intestinal *Cftr* mRNA in these mice correlated with their electrophysiological profiles and survival rates. The study indicated that quite small levels of gene activity (5%) could have an exaggerated effect on Cl^- channel activity and dramatic pathological consequences, as measured by survival. This suggested that even modest correction using gene therapy in humans might provide significant benefits.

To date, many of the somatic gene therapy approaches in humans have attempted to introduce vectors into any cells in the respiratory tract, with expression of the *CFTR* gene under the control of a ubiquitous promoter. Although this type of gene therapy could be beneficial and without deleterious consequence, more-precise temporal and spatial control will be of benefit. In another approach, a human *CFTR* cDNA transgene was introduced by homologous recombination into the mouse *Cftr* gene locus so that it was under the control of the mouse endogenous promoter (Ref. 96). However, mice

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homozygous for this transgene still displayed the fatal intestinal phenotype, indicating that tissue-specific expression and regulation of CFTR needs to be precisely understood.

The two types of somatic gene therapy vectors that have been used in the CF mouse models to date are adenovirus and DNA–liposome complexes. In both cases, the results observed in the mouse have been similar to those achieved in humans.

Adenoviral vectors

Adenovirus initially seemed to be a very promising vector for gene therapy. It has tropism for respiratory epithelia and can transfect quiescent cells. In vitro studies appeared to reveal that adenoviral vectors could introduce transgenes into cells at a high efficiency. Clinical trials were quickly initiated in the USA and evidence for successful correction of the CF ion-transport defect was reported (Ref. 97). However, subsequent studies could not repeat this initial success and it appeared that an unacceptably high multiplicity of infection was necessary to achieve efficient transfection in vivo. Studies carried out on the $Cftr^{tm1Unc}/Cftr^{tm1Unc}$ mouse showed that a single dose of adenovirus containing *CFTR* cDNA failed to correct the characteristic bioelectric defects (Ref. 98). However, treatment of the mutant mice with a high-dose vector on four successive days did demonstrate 50% correction with only an estimated 3% of the nasal epithelial cells expressing the gene. This provided further evidence that correction of only a small number of cells could have a large effect on the tissue but also that achieving even a low transfection level was difficult. Furthermore, as observed in the human studies (Ref. 97), this bioelectric correction was transient and had gone by day 10 post-transfection.

Although adenoviral transfection of the terminally differentiated cells in the nose is very inefficient, it could be enhanced in the presence of damage to the epithelium (Ref. 98). This is probably because the basal cells and/or basolateral membranes (now exposed to the vector) express the required viral adhesion molecules. Pre-treatment of $Cftr^{tm1Unc}/Cftr^{tm1Unc}$ mice with the non-ionic detergent polidocanol was subsequently demonstrated to increase transfection efficiency (Ref. 99). Functional correction was observed for up to 5 days after treatment, whereas untreated mice did not show this correction. The use of surface-active adjuvants and/or membrane-perturbing agents might thus provide an approach to enhance the efficiency of adenoviral gene transfer in patients and allow well-tolerated viral doses to be used.

DNA–liposome complexes

The residual-function $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mouse and the $Cftr^{tm1Cam}/Cftr^{tm1Cam}$ null mouse have both been used to demonstrate the efficacy and safety of DNA–liposome complexes to introduce *CFTR* into the mouse (Refs 100, 101). One group used the human *CFTR* gene cDNA in a plasmid vector complexed with a cationic liposome, delivered by direct tracheal instillation (Ref. 100). The DNA–liposome complex normalised bioelectric abnormalities in the trachea of $Cftr^{tm1Cam}/Cftr^{tm1Cam}$ mice, and in the nasal epithelia of $Cftr^{tm1Cam}/Cftr^{tm1Cam}$ and $Cftr^{tm2Cam}/Cftr^{tm2Cam}$ ΔF508 mice (Ref. 102). This strategy resulted in correction of the raised basal I_{SC}, Na^+ hyperabsorption, the increased Ca^{2+}-dependent Cl^- secretion and the reduced cAMP-dependent Cl^- secretion in the nasal epithelium, and the reduced cAMP-dependent Cl^- transport and Na^+ absorption in the trachea. Another group also achieved partial correction of the cAMP-dependent Cl^--transport defect in $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mice with a cocktail of plasmids carrying *CFTR* cDNA under the control of three different promoters (Ref. 101). In this case, a nebuliser was used to aerosolise the DNA–liposome complexes. Correction of the cAMP-dependent Cl^- transport defect was observed in the nose and trachea, as well as a return to wild-type values for the tracheal hypoabsorption of Na^+. Both groups reported no evidence that the DNA–liposome complexes at these concentrations had a toxic effect, and this work led directly to the first Phase I DNA–liposome trial in the UK for CF (Ref. 103). This clinical trial also demonstrated partial correction and no evidence for toxicity, using the DC-CHoL lipid complexed with *CFTR* under SV40 promoter control (Ref. 103). In addition, $Cftr^{tm1Hgu}/Cftr^{tm1Hgu}$ mice have also been used to show efficacy for an alternative lipid (DOTAP) and a *CFTR* cDNA under the cytomegalovirus (CMV) promoter (Ref. 104). As before, the results were very similar to the previous mouse studies, and the subsequent clinical trial again showed evidence for partial correction of the Cl^--transport defect in patients, without toxicity.

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Studies in *Cftr*^{tm1Cam}/*Cftr*^{tm1Cam} mice have shown that a second dose of DNA–liposome complex was as effective as the first dose in achieving cAMP-stimulatable Cl^- transport and was well tolerated with no discernible inflammation of lung tissue (Ref. 105). Further experiments to identify factors that affect the efficiency of cationic-lipid-mediated transfection to epithelium have resulted in both novel lipid formulations and naked DNA being tested in CF mice. The lipid GL-67 complexed with *CFTR* cDNA resulted in only marginal correction of the nasal Cl^--transport defect of null *Cftr* mutant mice after perfusion of the nasal epithelium (Ref. 106). Nasal transfection appeared to be less effective when using naked DNA, whereas the reverse was true in the lungs. The transfection was apparently affected by polarisation, differentiation and the proliferative state of the cells, with highly polarised and differentiated, non-mitotic cells being extremely refractive to transfection. Both *Cftr*^{tm1Cam}/*Cftr*^{tm1Cam} and *Cftr*^{tm2Cam}/*Cftr*^{tm2Cam} ΔF508 mice have been used to demonstrate that bicarbonate secretion in the mouse gallbladder can be restored by administration of a *CFTR*-cDNA–liposome complex to the airways of the mice by direct intratracheal administration (Ref. 107). Surprisingly, the route of administration appeared to be important, with intratracheal delivery being successful when oral, intravenous, intramuscular, subcutaneous or intraperitoneal routes were not.

Thus, mouse models of CF are proving to be an important resource in the continuing development and optimisation of gene therapy vectors and delivery protocols. Currently, a more efficient method of delivery to the respiratory tract is necessary. Some new vectors show great promise in this regard (see for example Ref. 108), and the CF animals will bridge the gap between cell-culture-based studies and human trials by providing both an in vivo system and relevant clinical endpoints to assess the efficacy and safety of such therapies.

Future directions

The challenges for future use of mouse models of CF lie, first, in refining existing models to replicate human disease as accurately as possible and, second, in understanding the mechanisms that underlie the development of the mutant phenotypes observed only in mice. The phenotypic variability observed in mouse models of CF is a result of different strain backgrounds, specific mutations in *Cftr* and environmental influences. This variability provides great potential, both for the definition of genetic modifiers and for the refinement of mouse models of CF to select the phenotypes that most closely resemble human disease patterns. These processes should define many of the key components involved in disease pathogenesis, establish the most suitable models for therapy testing, with clear, relevant clinical endpoints, and suggest novel therapeutic approaches for the treatment of human disease. The future availability of comprehensive gene expression arrays could prove particularly illuminating in studying the mechanisms underlying the phenotypic differences observed between different models, and between mouse models of CF and non-CF littermates, in response to different environmental stimuli, such as pulmonary infection. It might be the case that such studies will demonstrate the greatest value of inbred mouse models of CF.

Despite this powerful variability in phenotype, it is likely that key species differences could prevent mouse models of CF from accurately reproducing all aspects of CF lung disease in humans. However, it is possible that these differences might prove as illuminating as the similarities. Rather than exclusively pursuing the development of all aspects of classical human CF lung disease in mouse models, studies should specifically address the effects of *Cftr* mutation upon the lung pathophysiology of mice. In this manner, the consequence of *Cftr* mutation in the mouse lung can be addressed and the underlying mechanisms evaluated. Through our recognition of the key similarities and differences, mouse models of CF might provide ideal systems for the analysis of specific aspects of CF lung disease. In addition, the validity of specific hypotheses can be examined in an in vivo model. In this way, mouse models of CF can provide valuable contributions to our understanding of this disease process and support efforts towards organ-based treatment for CF patients.

In addition, the establishment of other transgenic mice in which specific genes implicated in the pathogenesis of CF disease have been mutated to prevent or increase expression has the potential to contribute greatly to our understanding of disease development. Targeting genes such as beta-defensins, cytokines and anti-proteases will help to define their relative roles in CF pathogenesis. The characterisation of

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these mutant mice will provide valuable data, both in isolation and when crossed with mouse models of CF.

Finally, the recent development of cloning technologies has paved the way for specific gene targeting in species other than mouse. The use of other animals to model disease affords new opportunities and complementary resources, with sheep and pigs or even ferrets offering the potential advantages of increased size and pulmonary anatomy that more closely resembles humans. In this manner, the future use of animal models of CF offers the potential to dissect the mechanisms underlying disease development in CF, reveal genetic and environmental modifiers of phenotype, and provide clinically relevant models for therapy testing and development.

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Further reading, resources and contacts

For an earlier review of cystic fibrosis mouse models see:

Dorin, J.R., Alton, E.W.F.W. and Porteous, D.J. (1994) Mouse models for cystic fibrosis. In Cystic Fibrosis Current Topics (Dodge, J.A. et al., eds), pp. 3–31, John Wiley & Sons

Comprehensive information on cystic fibrosis, including research and clinical trials, is provided at the North American Cystic Fibrosis Foundation website.
http://www.cff.org/

The Cystic Fibrosis Mutation Database, compiled by the Cystic Fibrosis Genetic Analysis Consortium, gives the full *CFTR* gene sequence, *CFTR* sequence polymorphisms and a table of *CFTR* mutations.
http://www.genet.sickkids.on.ca/cftr/

The UK's Cystic Fibrosis Trust website provides patient support.
http://www.cftrust.org.uk/

Dorin lab website
http://www.hgu.mrc.ac.uk/Research/Devgen/Cysfib/julia.htm

Features associated with this article

Figures

Figure 1. A model of the proposed structure of the cystic fibrosis transmembrane conductance regulator (CFTR) (fig001jde).
Figure 2. Making a gene-targeted cystic fibrosis (CF) mouse model using embryonic stem (ES) cells (fig002jde).

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