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GFP-Tagged CFTR Transgene is Functional in the G551D Cystic Fibrosis Mouse Colon

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Abstract. Trafficking of the cystic fibrosis transmembrane conductance regulator (CFTR) is central to its function, with the most common mutation, ΔF508, resulting in abnormal processing and trafficking. Therefore, there is a significant need to develop tools, which enable the trafficking of CFTR to be studied in vitro and in vivo. In previous studies it has been demonstrated that fusion of the green fluorescent protein (GFP) to the N-terminus of CFTR does lead to functional expression of CFTR chloride channels in epithelial cell lines. The aim of the present study was to examine whether it is possible to express GFP-tagged CFTR as a transgene in colonic and airway epithelial cells of cystic fibrosis (CF) mice and to correct the CF defect. Using the epithelial-specific human cytokeratin promoter K18, we generated bitransgenic mice *cftr*$^{G551D/G551D}$ K18-GFP-CFTR$^{+/-}$, designated GFP mice. Transcripts for GFP-CFTR could be detected in bitransgenic mice by use of RT-PCR techniques. Expression of GFP-CFTR protein was detected specifically in the colonic epithelium by both direct GFP fluorescence and the use of an anti-GFP antibody. Ussing chamber studies showed that the ion transport defect in colon and airways observed in *cftr*$^{G551D/G551D}$ mice was partially corrected in the bitransgenic animals. Thus, K18-GFP-CFTR is functionally expressed in transgenic mice, which will be a valuable tool in studies on CFTR synthesis, processing and ion transport in native epithelial tissues.

Key words: GFP-CFTR — Colon — Bitransgenic mouse — Cystic fibrosis — Airways — Epithelial transport

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Introduction

With the frequency of about 1 in 2500 newborns, cystic fibrosis (CF) is recognized as the most frequent genetic disease in the Caucasian population. CF is caused by abnormalities in electrolyte transport across epithelial cells caused by defective chloride channel function, enhanced amiloride-sensitive Na^+ conductance (ENaC) and other transport defects [21, 37]. The disease causing gene product cystic fibrosis transmembrane conductance regulator (CFTR) has been well characterized as a cAMP-mediated chloride channel and as a regulator of other ion channels [20, 33, 35, 36]. Some investigators have proposed that CFTR also plays an important role as receptor for binding, internalizing, and clearing of *Pseudomonas aeruginosa* infections in airway epithelial cells [31]. Thus, mutations in CFTR cause a hypersusceptibility towards chronic *P. aeruginosa* infections of the CF lung.

In CF cell lines, defective chloride channel function can be corrected by delivering CFTR cDNA into cells [6, 32] and bacterial killing activity is improved by CFTR gene transfer in CF respiratory cells [2]. Moreover, CFTR gene transfer in vivo in CF mouse airways was shown to correct the ion transport defect in airway epithelial cells [1, 17]. This, along with results from recent human gene therapy trials, indicates genetic complementation of the defective ion transport in CF in vitro and in vivo by delivering CFTR cDNA [41, 42]. An important aspect of gene transfer and gene therapy is the identification of markers indicating successful gene transfer, allowing for determination of efficiency of the transfer and selection of cells positive for the transgene. The green fluorescent protein (GFP) has been widely used as a marker in vitro and in vivo [40]. GFP and its recently developed derivatives offer the unique possibility to study maturation, trafficking and expression of proteins. They may even be used as fluorescent dyes for parallel measurements of intracellular ion concentrations such

as $[Cl^-]_i$ [7]. The yellow fluorescent protein YFP has been used for screening of combinatorial libraries in order to identify novel CFTR chloride channel activators [8]. A number of reports have shown that GFP-tagged proteins are targeted properly to their final destinations in the plasma membrane or mitochondrial membranes, where they form functional proteins [10]. A number of ion channels have been fused to GFP, such as voltage gated K^+ channels and CFTR [22, 23, 29]. However, it may occur that targeting of an ion channel with GFP interferes with its function or properties, as it has been described for the Ca^{2+}-dependent K^+ channel hSlo [27].

It is well-established that correct CFTR-dependent regulation of chloride ion transport in epithelial tissues is dependent upon interactions between CFTR and other ion channels and transporters, which may use PDZ domains containing proteins physically linking CFTR to other molecules. For example, the presence of functioning CFTR appears to regulate the epithelial sodium channel (ENaC), the outwardly rectifying chloride channel (ORCC) and an epithelial potassium channel (ROMK) [14]. The mechanisms by which CFTR achieves these pleiotropic effects are yet to be elucidated. However, it is clear that in order to understand these processes, there is a need to study the trafficking, subcellular localization and molecular interactions of CFTR. One powerful approach is to utilize GFP-tagged CFTR, which facilitates in vivo cell biology. The major concern with the use of any epitope-tagged cDNA is whether the tag and resultant fusion protein perturbs function. Previously, in vitro studies utilizing CFTR tagged at the N-terminus with GFP have demonstrated localization to the cell membrane and a normal Cl^- channel function, thus inducing cAMP-dependent chloride secretion in Madin Darby Canine Kidney (MDCK) cells [28, 29] and in cultured airway epithelial cells [19]. When MDCK cells expressing GFP-CFTR were exposed to *P. aeruginosa*, they demonstrated an enhanced ability to ingest bacteria compared to untransfected cells [9]. Taken together, these studies indicate that GFP-CFTR is functional under in vitro conditions.

It is, however, not clear, whether a GFP-CFTR fusion protein is also functional as a transgene when expressed in highly polarized native epithelial cells in vivo. In order to examine this question, we generated mice carrying GFP-CFTR as a transgene. The construct is driven by the human cytokeratin 18 (K18) promoter, which is an epithelium-specific promoter, known to direct transgene expression to all cell types in which CFTR is typically expressed, including airway and intestinal epithelial cells [3]. Previously, we have used this promoter to direct human CFTR expression in transgenic mice and this resulted in the correction of airway ion transport defects and improvements in defense against lung disease (29). In this study, we generated a line of transgenic mice expressing GFP-CFTR under the control of the K18 promoter. In order to assess the in vivo function of the GFP-CFTR bitransgenic mice *cftr*$^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ were obtained by breeding K18-GFP-CFTR mice to *cftr*$^{G551D/G551D}$ CF mice. Analysis of transcripts, protein and electrolyte transport in the colon and airways indicates functional expression of K18-GFP-CFTR and partial restoration of normal ion transport. Thus, despite the presence of a potentially disruptive foreign protein on the N-terminus, GFP-CFTR appears to be capable in vivo of supporting the complex interactions required to regulate epithelial chloride transport.

Materials and Methods

DNA Constructs

The GFP-CFTR construct, provided by Dr. Bruce Stanton (Dartmouth Medical School, Hanover, NH), consists of an enhanced GFP (EGFP) cDNA, a linker sequence of 23 amino acids and the wild-type human CFTR cDNA [29]. The human cytokeratin 18 (K18) promoter cassette in the pK18-CFTR construct was a generous gift from Dr. Jim Hu (University of Toronto, ON). The activity of this promoter has been characterized both in vitro and in vivo [3, 18]. The CFTR cDNA sequence in the pK18-CFTR was replaced by the GFP-CFTR, using the NcoI sites to generate the pK18-GFP-CFTR construct.

Generation of Transgenic Mice

The pK18-GFP-CFTR was cleaved with *Kpn*I and *Sal*I to remove the vector sequence. The insert was separated using sucrose-gradient centrifugation and injected into pronuclei of B6CB F1 fertilized ovum, and afterwards oocytes were transplanted into pseudopregnant females. The pups were screened for integration of the transgene by PCR using primers (forward) 5′-AGCAGAAGAACGGCATCAAGG-3′ and (reverse) 5′-TGTCAGCAGAATCAACAGAAGGG-3′, generating a 458 bp sequence spanning from nt 470 of the GFP gene to nt 141 of the CFTR cDNA. The transgenic founder was bred with CD1 mice and the transgenic offspring were crossed with G551D CF mice [5] to generate bitransgenic animals carrying the K18-GFP-CFTR transgene and the *cftr*^{G551D} allele. Four founder lines were obtained and included in this study. The four strains used in this study, *cftr*$^{+/+}$ (*wt*), *cftr*$^{G551D/G551D}$ (CF), *cftr*$^{G551D/G551D}$ *K18-CFTR*$^{+/-}$ (K18) [30], and *cftr*$^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ (GFP) have equivalent genetic backgrounds (a mixture of CD1, C57-B6 and 129). The *cftr*$^{G551D/G551D}$ allele was detected by PCR followed by restriction enzyme digestion [5].

Detection of GFP-CFTR Expression in the Colon of Transgenic Mice

Total RNA from excised colon was isolated using Trizol reagent (GIBCO). 1 μg of DNase I-treated total RNA was reverse-transcribed using MMLV-RT (GIBCO). Exogenous human CFTR transgene was PCR-amplified (791 bp) using primers (forward) 5′-GAGGACATCTCCAAGTTTGCAGAG-3′ and (reverse) 5′-TGTGGATGCTGTTGTCTTTCG-3′ with 93°C 1 min, 53°C 1 min, 72°C 1 min 30 sec for 35 cycles. Mouse hypoxanthine phosphoribosyltransferase (HPRT) message was amplified as a control, using oligos (forward) 5′-GTGGATACAGGCCAGACTTTG-

TTG-3′ and 5′-GAGGGTAGGCTGGCCTATGGCT-3′ (reverse), producing a 352 bp fragment. For EGFP detection, freshly excised colon was directly fixed for 2 h in 4% paraformaldehyde in PBS, followed by an overnight incubation in 15% sucrose at 4°C. On the next day, tissues were embedded in Tissue-Tek OCT compound (Sakura Finetechnical, Tokyo, Japan). Frozen sections were carried out using a LEICA cryostat model CM3050 (Leica, Germany), and mounted in DAKO fluorescent mounting medium (DAKO Corporation, CA). The fluorescence of the EGFP was visualized under an Olympus AX70 fluorescence microscope. To confirm EGFP expression, tissue sections were stained with rabbit anti-GFP antibody (Molecular Probes) using standard peroxidase methods.

Electrophysiological Studies

Distal colon and tracheas were removed from sacrificed animals. Connective tissues were removed from tracheas and the epithelial layer of the distal colon was stripped of the submucosal layer mechanically under a binocular microscope. Tissues were put into cold buffer solution of the following composition (mmol/l): NaCl 145, KH_2PO_4 0.4, K_2HPO_4 1.6, D-glucose 5, $MgCl_2$ 1, HEPES 5, Ca-gluconate 1.3, $MgCl_2$ 1, pH 7.4. The tissues were mounted into a micro-perfused Ussing chamber with a circular aperture of 0.95 mm^2. The luminal and basolateral sides of the epithelium were perfused continuously at a rate of 10 ml/min (chamber volume 2 ml). The bath solution had the following composition (mmol/l): NaCl 145, KH_2PO_4 0.4, K_2HPO_4 1.6, D-glucose 5, $MgCl_2$ 1, Ca-gluconate 1.3. pH was adjusted to 7.4. Bath solutions were heated by a water jacket to 37°C. Experiments were carried out under open-circuit conditions, which is more likely to reflect the in vivo situation. Values for transepithelial voltages (V_{te}) were referred to the serosal side of the epithelium. Transepithelial resistance (R_{te}) was determined by applying short (1 s) current pulses (ΔI = 0.5 μA). Voltage deflections obtained under conditions without the mucosa present in the chamber were subtracted from those obtained in the presence of the tissues. R_{te} was calculated according to Ohm's law ($R_{te} = \Delta V_{te}/\Delta I$). Tissue preparations were only accepted if transepithelial resistances exceeded that obtained for an empty chamber at least by a factor of 3. Distal colon and tracheas were obtained from wild-type (*wt*) mice, $cftr^{G551D/G551D}$ (CF) mice, $cftr^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ (GFP) mice and from CF mice expressing unlabeled wtCFTR $cftr^{G551D/G551D}$ *K18-CFTR* (K18). We previously demonstrated corrected bacterial clearance, inflammatory response and cAMP-mediated chloride transport in the airways of bitransgenic $cftr^{G551D/G551D}$ *K18-CFTR* mice [30].

Reagents and Data Analysis

Amiloride, 3-isobutyl-1-methylxanthine (IBMX) and carbachol were all obtained from Sigma (Australia). Forskolin was a gift from Dr. U. Gerlach, Aventis, Germany. All chemicals used were of highest grade of purity available. Data are shown as individual recordings or as mean ± SEM (n = number of tissue samples). Statistical analysis was performed using paired Student's t-test. P values < 0.05 were accepted to indicate statistical significance.

Results

Phenotypic Aspect of $cftr^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ Mice

Typically $cftr^{G551D/G551D}$ have a significant perinatal mortality primarily due to meconium ileus and survivors display an associated malabsorption leading to a low rate of post natal weight gain. Bitransgenic $cftr^{G551D/G551D}$ (CF) mice carrying the *K18-GFP-CFTR* transgene showed in general a good viability, which exceeded that of the $cftr^{G551D/G551D}$ mice. Typically, 30% to 40% of CF mice died before weaning, but no deaths were observed in the bitransgenic animals between birth and the age at experimentation. The bitransgenic CF mice were also significantly heavier than the $cftr^{G551D/G551D}$ mice and similar to non-transgenic $cftr^{+/+}$ mice. Mean weights (± standard deviation) of males at 10 weeks of age were 33.0 ± 2.5 g, 24.8 ± 3.0 g and 34.7 ± 2.9 g for bitransgenic $cftr^{G551D/G551D}$, $cftr^{G551D/G551D}$ and $cftr^{+/+}$ mice respectively ($n \geq 5$), indicating that the transgene was complementing the gastrointestinal defect at the broad phenotypic level.

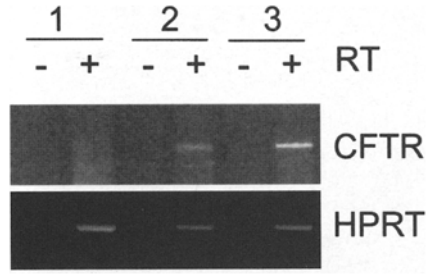

Fig. 1. RT-PCR analysis. Human CFTR was found to be expressed in the colon of bitransgenic $cftr^{G551D/G551D}$ animals carrying K18-GFP-CFTR (*2*) or K18-CFTR transgenes (*3*), but not in non-transgenic control mice (*1*). Transgene expression was detected by PCR amplification of reverse transcribed RNA (RT+), using primers specific for the CFTR transgene, but was not detected without reverse transcription (RT–). Additional control PCR of mouse HPRT was performed.

Expression of the GFP-CFTR Transgene in the Colon

Expression of the human CFTR transgene could be readily detected in the colon of bitransgenic $cftr^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ (GFP) and $cftr^{G551D/G551D}$ *K18-CFTR* (*K18*) animals by RT-PCR analysis (Fig. 1). However, no expression was detected in the wild-type littermates, indicating that the detection method was specific for human CFTR transgene and did not amplify endogenous mouse CFTR. These data are consistent with our previously published data utilizing the K18 promoter directing expression of wild-type CFTR. Since transcripts of GFP-CFTR were detected in the GFP mice, we aimed to detect GFP-CFTR protein as well, Thus, we monitored GFP fluorescence in colonic epithelial cells from GFP mice. Although we did not expect to find a strong signal due to limited expression of the transgene, we were able to detect GFP fluorescence in colonocytes of *GFP* mice but not in colonic epithelial

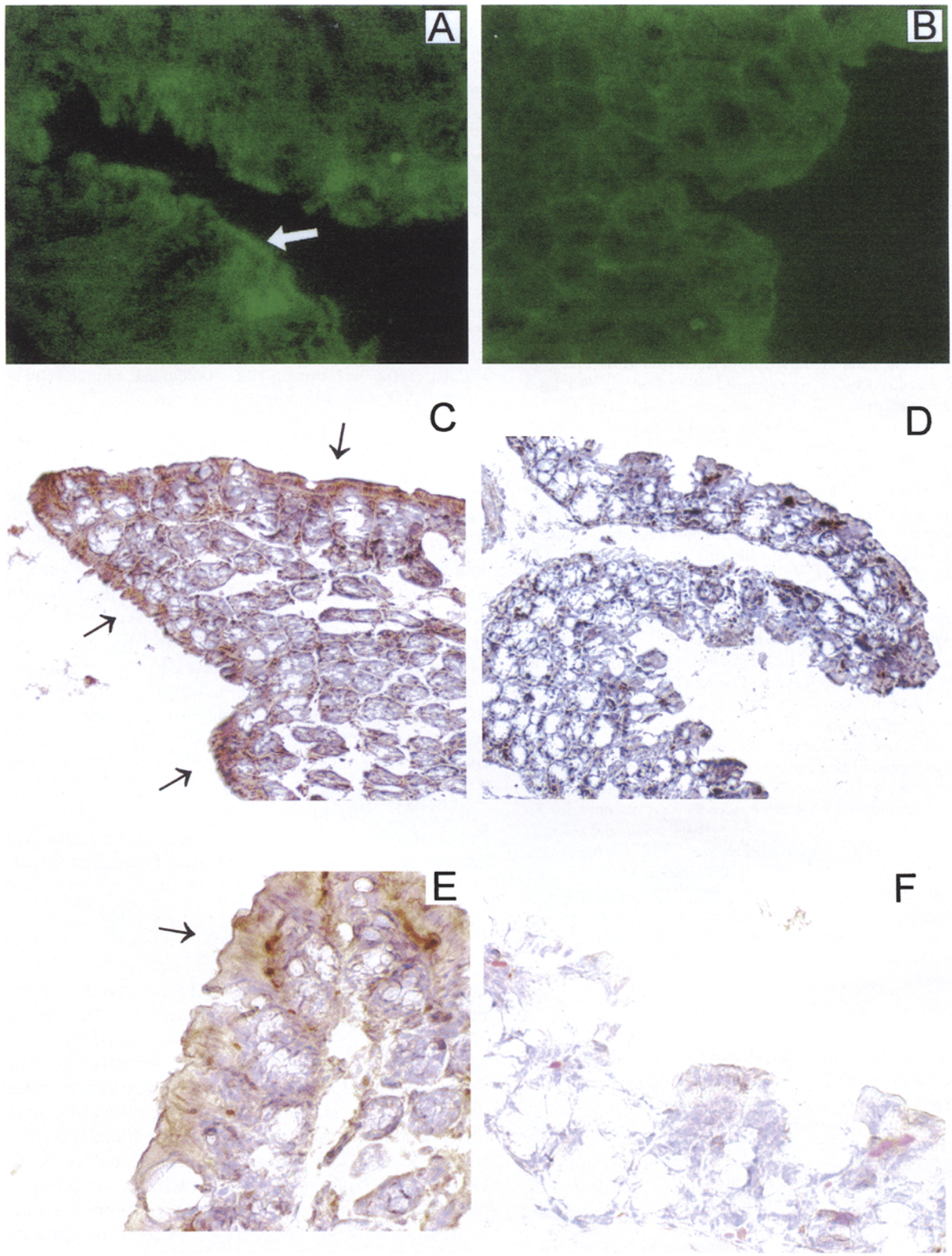

Fig. 2. Detection of GFP-CFTR protein in the colon of transgenic mice. GFP signals were detected in colon sections of K18-GFP-CFTR animals (*A*) (*arrows*) but not in the non-transgenic littermates (*B*). The presence of GFP-CFTR was further confirmed by immunostaining with anti-GFP antibody and utilizing peroxidase methods. Colon sections from a K18-GFP-CFTR animal (*C* and *E*) indicate the presence of GFP-CFTR in epithelial cells (*arrows*), whereas no signals were obtained from non-transgenic littermates (*D* and *F*). Original magnifications are 200× (*A* and *B*), 100× (*C* and *D*) and 400× (*E* and *F*).

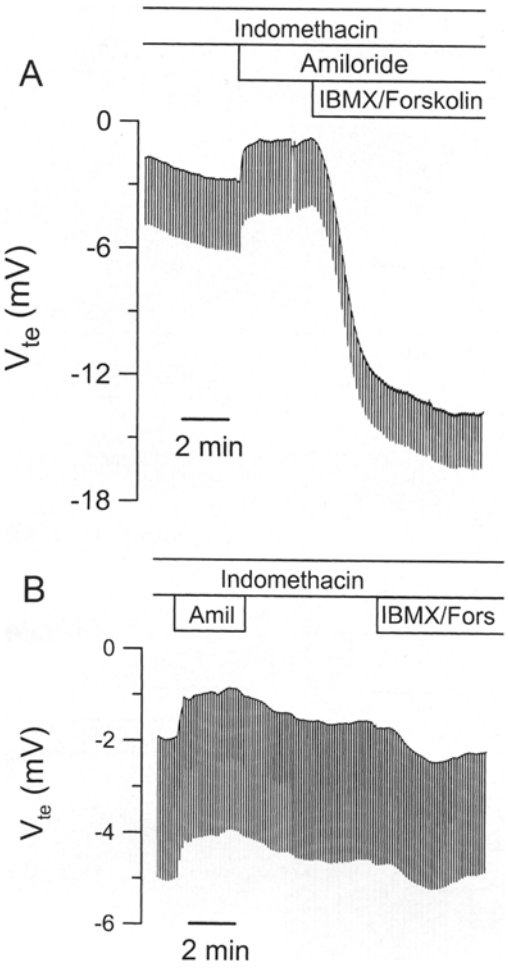

Fig. 3. Effects of amiloride (10 μM) and IBMX (100 μM)/forskolin 2 (μM)/amiloride on the transepithelial voltage V_{te} and transepithelial resistance R_{te} in mouse distal colonic mucosa. R_{te} was determined from the V_{te} downward deflections obtained by pulsed current injection. The original recordings were obtained from a normal wt mouse (*A*) and a bitransgenic $cftr^{G551D/G551D}$ CF mouse, expressing K18-GFP-CFTR (*B*).

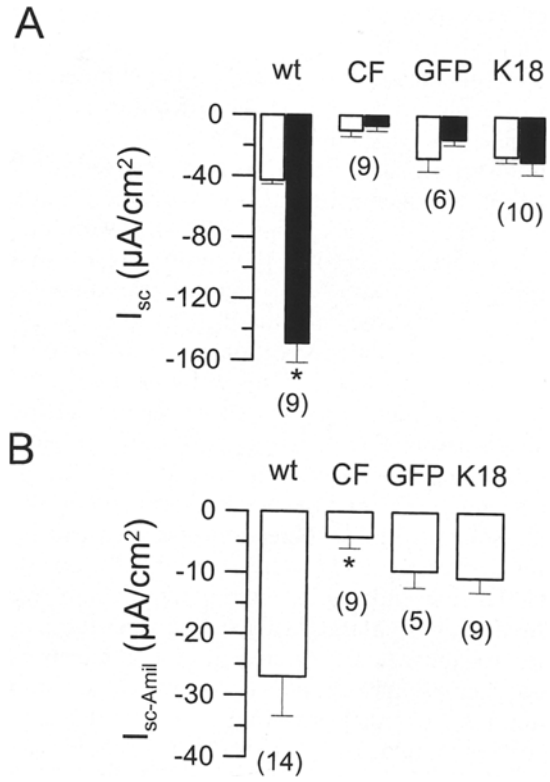

Fig. 4. Summary of the short-circuit currents (I_{sc}) calculated from transepithelial measurements as shown in Fig. 3. (*A*) A significant Cl^- secretion is activated by stimulation of the colonic epithelia from wild type (*wt*) mice with IBMX/forskolin (*black bars*). No increase of I_{sc} was observed in $cftr^{G551D/G551D}$ (*CF*) mice or the bitransgenic $cftr^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ (*GFP*) and $cftr^{G551D/G551D}$ *K18-CFTR*$^{+/-}$ (*K18*) mice. (*B*) Summary of amiloride-sensitive short-circuit currents ($I_{sc\text{-}Amil}$) measured in the four (*wt*, *CF*, *GFP*, *K18*) different mouse strains. $I_{sc\text{-}Amil}$ was significantly reduced in CF mice when compared to wt mice, but was not different in GFP or K18 mice. *Significant difference for the effect of IBMX/forskolin (paired *t*-test) and for $I_{sc\text{-}Amil}$ in CF cells (unpaired *t*-test). In parentheses, number of mice studied.

cells from control CF animals (Fig. 2*A*), indicating expression of GFP-CFTR protein in colonic epithelial cells in these bitransgenic mice. In order to further confirm these results, we performed an immunohistochemical analysis using an antibody that specifically recognizes GFP. The results show strong expression of GFP-CFTR in the surface epithelium of villi and weaker staining in the crypts of the colon in GFP mice (Fig. 2*C* and *E*).

Transport Properties of the Colonic Epithelium and in the Trachea of $cftr^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ Mice

We examined the ion transport in the colonic epithelium of GFP mice and compared the results with those obtained in the colon of $cftr^{G551D/G551D}$ mice, wt mice and bitransgenic $cftr^{G551D/G551D}$ *K18-CFTR* (K18) animals. Bitransgenic K18 animals have been demonstrated to have corrected ion transport properties in the airways when compared to $cftr^{G551D/G551D}$ [30]. Tissues were initially incubated with indomethacin to abolish endogenous prostaglandin-mediated stimulation of ion transport. In wt mice stimulation of the epithelium by forskolin (2 μM) and IBMX (100 μM) induced a large increase in the lumen-negative transepithelial voltage and short circuit current (I_{SC}) due to activation of Cl^- secretion in the presence of amiloride [4] (Figs. 3*A* and 4*A*). In contrast, the colonic epithelium of CF mice showed a significantly reduced baseline transport activity and no activation by IBMX/forskolin (Fig. 4*A*), which confirmed previous results [5]. In bitransgenic GFP mice, the baseline transport activity was not different from that of wt mice and a small IBMX/forskolin-induced Cl^- secretion could only be detected in some of these animals (Fig. 3*B*). However, the effects of

IBMX/forskolin on V_{te} and I_{SC} in GFP mice were not significant. Similar to the GFP mice, K18 mice also showed no significant changes in I_{sc} upon stimulation with IBMX and forskolin (Fig. 4*A*). From these data it was unclear if GFP-CFTR was functional.

Amiloride-sensitive transport was observed in wt mice and all transgenic animals but was significantly reduced in the CF mice (Figs. 3*B*, 4*B*). Although $I_{SC\text{-}Amil}$ appeared reduced in the colon of GFP and K18 animals, they were not significantly different from the $I_{SC\text{-}Amil}$ values measured in wt mice. These data suggest a partial correction of the transport defects observed in these CF mice, by expression of the K18-GFP-CFTR protein. In order to further determine whether there was actually a low level of cAMP-dependent Cl^- secretion present in the intestine of bitransgenic animals, we used a protocol that has been shown to allow detection of even weak intestinal Cl^- secretion, as in some human CF patients [26, 39]. To that end, mouse colonic epithelium was stimulated by basolateral carbachol (CCH, 100 μM) in order to activate Cl^- secretion. CCH has been shown to increase intracellular Ca^{2+}, which activates basolateral K^+ channels and thus increases the driving force for luminal Cl^- exit [24]. It is generally accepted that CFTR is the only luminal Cl^- channel in the mammalian colon [11, 21], thus both Ca^{2+}- and cAMP-mediated activation of Cl^- secretion rely on the activity of luminal CFTR Cl^- channels [26, 43]. As shown in Figs. 5 and 6*A*, after inhibition of prostaglandin synthesis by indomethacin and decrease of intracellular cAMP, which deactivates CFTR, stimulation by CCH induces a positive voltage deflection, which is due to K^+ secretion [24, 26]. After stimulation with IBMX and forskolin and activation of CFTR, a negative voltage deflection is induced in wt, GFP and K18 mice but not in G551D animals (Figs. 5 and 6). Moreover, CCH-induced secretions in wt, GFP and K18 mice were not significantly different from each other. In summary, these results indicate a partial correction of the defective Cl^- secretion in *cftr*$^{G551D/G551D}$ mice by expression of K18-GFP-CFTR.

Partial correction of defective Cl^- secretion in the colon may indicate limited activity of the K18 promotor in the intestinal tissue. We therefore also checked for functional expression of the *K18-GFP-CFTR* in tracheas of *cftr*$^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ mice by stimulating Cl^- secretion with a cocktail of IBMX (100 μM) and forskolin (2 μM). In fact, activation of Cl^- secretion was significantly reduced in CF airways and was restored in GFP and K18 mice to normal magnitudes as observed in wt animals (Fig. 6*B*). The cAMP response was not different between wt, GFP and K18 animals. This result suggests more efficient expression of K18-GFP-CFTR in tracheal epithelial cells when compared to the colonic mucosa.

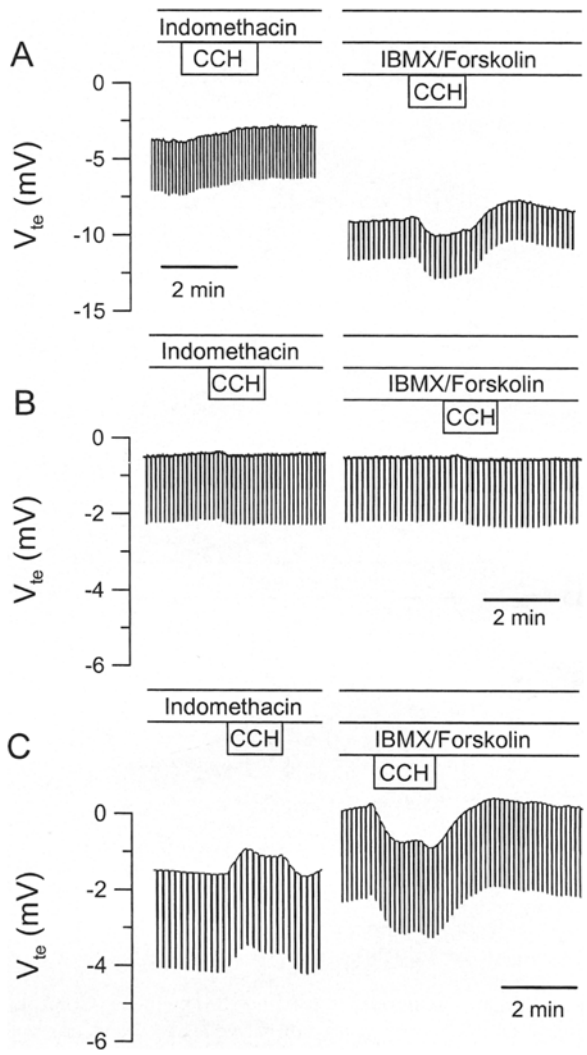

Fig. 5. Original recordings of the effects of carbachol (100 μM) on V_{te} in the absence or presence of IBMX/forskolin (100 μM/2 μM) and in the continuous presence of indomethacin/amiloride (both 10 μM). Recordings were obtained from wt mice (*A*), from *cftr*$^{G551D/G551D}$ CF mice (*B*) and from bitransgenic *cftr*$^{G551D/G551D}$ *K18-GFP-CFTR*$^{+/-}$ (*GFP*) mice (*C*).

Discussion

Transgenic Expression of GFP-CFTR

GFP is widely accepted as a marker in cell biology, gene transfer or gene therapy research. Although of relatively large size when compared with other immunocytochemical tags such as myc and HA, GFP can be fused to other proteins and numerous GFP-tagged proteins have been shown to be functional in vitro. GFP has been fused to cytoskeletal proteins, receptors, enzymes, nuclear proteins and ion channels [10, 40]. While many GFP-tagged ion channels function normally [16, 22, 23], abnormalities have been described in others [27]. However, little is known about transgenic

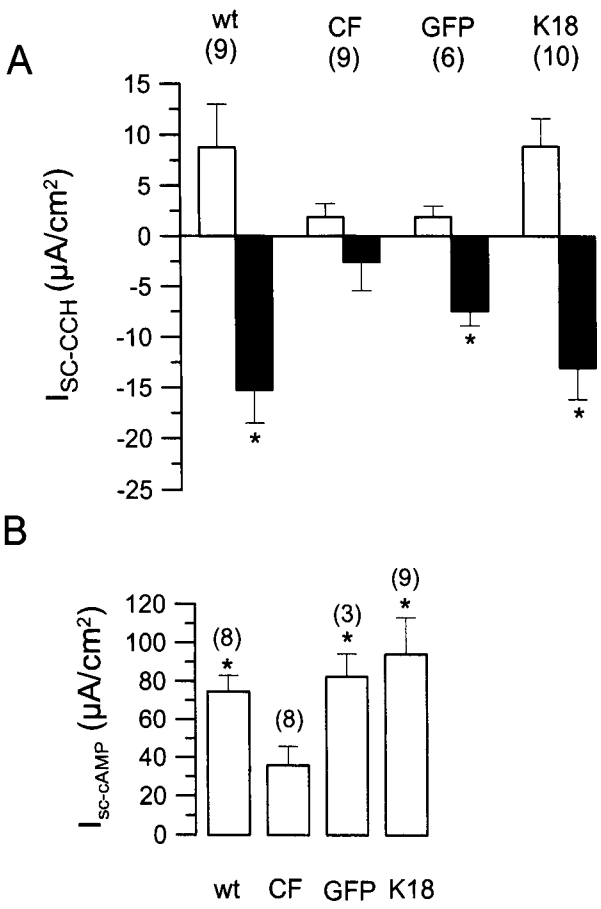

Fig. 6. Summary of carbachol-induced short-circuit currents ($I_{sc\text{-}CCH}$) in colonic epithelia of the four (*wt, CF, GFP, K18*) different mouse strains in the absence (*white bars*) or presence (*black bars*) of IBMX/forskolin, as calculated from the experiments shown in Fig. 5. A significant Cl^- secretion, i.e., activation of a negative I_{sc} by CCH, was only observed in wt, GFP and K-18 mice, but not in CF mice. *Significant difference for the effect of CCH (paired *t*-test) (*B*) Summary of cAMP-induced short-circuit currents (IBMX, forskolin) measured in tracheal epithelia of four (*wt, CF, GFP, K18*) different mouse strains. cAMP-induced transport was significantly larger in tracheas of wt, GFP and K18 mice, when compared to CF mice.

expression of GFP-tagged proteins in vivo. We were interested in studying expression of a GFP-CFTR transgene in $cftr^{G551D/G551D}$ mice. GFP tagging of CFTR did not affect chloride channel function when expressed in vitro [19, 29]. The present results indicate expression of transcripts and protein of GFP-CFTR in bitransgenic $cftr^{G551D/G551D}$ *K18-GFP-CFTR* mice. GFP fluorescence was not very strong but clearly detectable. A weak GFP fluorescence was not unexpected since the amount of endogenously expressed GFP-CFTR may be well below that detected in overexpression studies. Given the fact that the transgene can be detected by immunostaining and RNA as well as functional expression, we believe that this mouse model will be useful for further studies.

Generation of Cl^- Transport by GFP-CFTR

An obvious goal of the present study was to examine if the GFP-CFTR transgene is functional in the intact animal. We chose electrophysiological analysis predominantly of colonic ex vivo tissues rather than a detailed analysis of the airways, since airways have a dominating Ca^{2+}-mediated chloride transport in the absence of CFTR, probably compensating for the defect in cAMP-activated Cl^- conductance [13]. Moreover, expression of CFTR is generally very low in mouse trachea [34]. However, an increase of cAMP-activated Cl^- secretion could even be detected in tracheas of GFP as well as wt and K18 mice, suggesting functional expression of the CFTR transgene in the airways of these animals. In contrast to the limited expression in the airways, CFTR is clearly expressed in the colonic epithelium [21]. Both Ca^{2+}- and cAMP-activated Cl^- secretion are inhibited in the CF colon, since CFTR is the only luminal Cl^- conductance [4, 12, 24]. Using carbachol to induce Ca^{2+}-dependent Cl^- secretion, it is possible to detect even low levels of CFTR expression [26, 38]. We found in the present study that stimulation with carbachol activates a Cl^- secretion in GFP mice, which is not observed in the CF mice. However, Cl^- secretion could not be detected by stimulation with IBMX/forskolin only, suggesting low expression of the transgene and thus only partial correction of the colonic transport defect in GFP mice. Interestingly, a similar partial correction of colonic Cl^- secretion has been found in bitransgenic mice expressing wild type (without GFP) CFTR, which may indicate low activity of the K18 promoter in the intestine. Indeed, the results obtained in mouse trachea suggest more effective expression of the transcript in the airways. Along this line, corrected airway function and improved outcomes to lung disease were also found in these animals [30].

Apart from the partial correction of Cl^- secretion, we found a partial normalization of amiloride-sensitive Na^+ absorption. A reduced baseline transport activity has been described for $cftr^{G551D/G551D}$ CF mice in an earlier report [5]. We confirm this result by showing reduced amiloride-sensitive Na^+ absorption under control conditions along with abolished cAMP- and Ca^{2+}-dependent Cl^- secretion. Amiloride-sensitive Na^+ absorption can vary significantly when mice are kept on a normal diet. It has been shown to be increased in $cftr^{tm1Unc}$ mice when the animals were kept on Na^+-free diet [11]. However, $cftr^{G551D/G551D}$ mice are characterized by a lower amiloride-sensitive Na^+ absorption, which could be one of the reasons for the long-term survival of these animals and their reduced risk to develop a fatal intestinal blockage compared with 'null' mutants [5]. The reduced risk of intestinal blockage in these mice corresponds well to the threefold reduction in the

incidence of meconium ileus in CF patients, when compared to patients homozygous for the most common CFTR mutation, ΔF508 [15]. Thus, the present results, which show reduced amiloride-sensitive Na^+ transport in *cftr*$^{G551D/G551D}$ mice with lower risk for intestinal complications, confirm the importance of enhanced amiloride-sensitive Na^+ absorption for development of intestinal manifestations in CF [21, 25]. The present data indicate a partial recovery of the amiloride-sensitive Na^+ absorption along with partial correction of the Cl^- secretion in bitransgenic GFP mice. Because the four strains used in this study have equivalent genetic backgrounds (a mixture of CD1, C57-B6, and 129), the results most likely indicate functional expression of GFP-CFTR in vivo. Although multiple and also reciprocal interactions between CFTR and other ion channels such as ENaC have been described in several previous studies [reviewed in 20, 35], the reason and functional mechanism for the decreased Na^+ absorption in the colon of G551D animals, and the increase in Na^+ transport after expression of K18-GFP-CFTR remain obscure. However, it seems important to note that the colon of G551D (CF) mice is smaller than that of the other animals, that it demonstrates an altered quality and is difficult to prepare for Ussing chamber recordings. These circumstances may well contribute to the reduced amiloride-sensitive Na^+ transport found in these tissues. In summary, the data show that the K18 GFP-CFTR transgene complements the murine CF ion channel defect in airways and partially in the intestine. Thus K18-GFP-CFTR mice may be useful for further studies on CFTR maturation, trafficking and cellular function in native epithelial tissues.

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