

## Brief Report

# Spliceosome-Mediated RNA *Trans*-Splicing with Recombinant Adeno-Associated Virus Partially Restores Cystic Fibrosis Transmembrane Conductance Regulator Function to Polarized Human Cystic Fibrosis Airway Epithelial Cells

XIAOMING LIU,<sup>1,2</sup> MEIHUI LUO,<sup>1</sup> LIANG N. ZHANG,<sup>1</sup> ZIYING YAN,<sup>1,2</sup> ROMAN ZAK,<sup>1</sup> WEI DING,<sup>1</sup> S. GARY MANSFIELD,<sup>3</sup> LLOYD G. MITCHELL,<sup>3</sup> and JOHN F. ENGELHARDT<sup>1,2</sup>

### ABSTRACT

We previously reported that spliceosome-mediated RNA *trans*-splicing (SMaRT), using recombinant adeno-viral vectors expressing pre-*trans*-splicing molecules (PTMs), could partially restore cystic fibrosis trans-membrane conductance regulator (CFTR) chloride channel activity to polarized human  $\Delta$ F508 CF airway epithelia. Although these studies proved that SMaRT could correct CFTR mRNA defects, recombinant ade-noviral infection from the basolateral surface was required because of inefficient infection from the apical membrane. Hence, applications of SMaRT technology for CF gene therapy require further testing with al-ternative, more clinically viable, vector systems. Furthermore, because recombinant adeno-associated virus (rAAV) vectors have packing limitations with respect to the size of the CFTR transgene insert, SMaRT cor-rection of CFTR has the added attraction of a smaller transgene cassette. In the present study, we investi-gated whether rAAV vectors could effectively rescue CFTR chloride conductance in polarized human CF air-way epithelial cells, using a SMaRT approach. AAV vectors were generated to carry a PTM engineered to bind intron 9 of CFTR pre-mRNA and then *trans*-splice the normal sequence for human CFTR exons 10–24 into the endogenous pre-mRNA. Human CF polarized airway epithelia were infected from the apical mem-brane with rAAV2 or rAAV5 CFTR-PTM vectors in the presence of proteasome-modulating agents (dox-orubicin and *N*-acetyl-L-leuciny-L-leuciny-L-norleucinal) to enhance transduction. Epithelia were then eval-uated for cAMP-sensitive short-circuit currents 2 weeks postinfection. Levels of CFTR correction seen with rAAV2 ( $1.07 \pm 0.24 \mu\text{A}$ ) and rAAV5 ( $0.90 \pm 0.20 \mu\text{A}$ ) CFTR-PTM vectors were similar, representing con-ductance equivalent to 14.2 and 13.6% of that observed in non-CF human polarized epithelia, respectively. RT-PCR analysis demonstrated the existence of wild-type CFTR transcript in CFTR-PTM-corrected epithe-lia, whereas only  $\Delta$ F508 mRNA was detected in polarized cells infected with control rAAV LacZ-PTM vec-tors. These results provide evidence that rAAV vectors are capable of using SMaRT to correct CFTR func-tion after apical infection of human CF airway epithelia. The ability of CFTR-PTM-mediated correction to maintain endogenous CFTR regulation of the transgene product may further improve the efficacy of gene therapy for CF.

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<sup>1</sup>Department of Anatomy and Department of Cell Biology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242.

<sup>2</sup>Center for Gene Therapy of Cystic Fibrosis and Other Genetic Diseases, Carver College of Medicine, University of Iowa, Iowa City, IA 52242.

<sup>3</sup>Intronn Inc., Gaithersburg, MD 20878.

## INTRODUCTION

**S**UCCESSFUL GENE THERAPY for cystic fibrosis lung diseases relies on both an efficient and safe gene delivery system and the ability of such a system to physiologically reconstitute chloride channel and regulatory functions controlled by the cystic fibrosis transmembrane conductance regulator (CFTR) in airway epithelium. Gene therapy strategies have used adenoviral, adeno-associated viral, and retroviral vectors to deliver the wild-type CFTR cDNA into human airway epithelia (Engelhardt *et al.*, 1993; Flotte *et al.*, 1993; Crystal *et al.*, 1994; Goldman *et al.*, 1997; Zhang *et al.*, 1998; Wang *et al.*, 2000; Limberis *et al.*, 2002; Wagner *et al.*, 2002). However, on the basis of the cellular heterogeneity of CFTR expression and function in the lung, high levels of ectopic CFTR expression in all airway cell types may be toxic and/or may not properly correct the normal physiologic functions of CFTR in the airways (Schivi *et al.*, 1996; Jiang and Engelhardt, 1998). We previously reported that adenoviral vector-mediated spliceosome-mediated RNA *trans*-splicing technology (SMaRT; Intronn Inc., Gaithersburg, MD) was able to partially restore CFTR function in CF airway epithelia (Liu *et al.*, 2002). However, adenoviral vector-mediated gene transfer currently is unsuitable for clinical trials because it invokes a significant immune response, leads to transient gene expression, and poorly infects the apical surface of airway epithelial cells because of preferential localization of the coxsackie virus–adenovirus receptor (CAR) on the basolateral membrane (Walters *et al.*, 1999).

Recombinant adeno-associated virus (rAAV) has attracted considerable interest as a vector for gene therapy because of its ability to infect multiple tissue types and its low immunogenicity. The development of several new serotypes of rAAV, and the use of proteasome-modulating agents to augment transduction, have increased the effectiveness of rAAV vectors for gene delivery to human airway epithelia and mouse lung (Duan *et al.*, 1998a; Zabner *et al.*, 2000; Auricchio *et al.*, 2002; Sirminger *et al.*, 2004; Yan *et al.*, 2004). However, because of limitations in the packaging capacity of rAAV and the size of the CFTR cDNA, it has been difficult to include strong promoter regulatory elements without reducing the size of the CFTR cDNA. Approaches aimed at generating functional CFTR minigenes have been useful in overcoming this limitation (Zhang *et al.*, 1998; Ostedgaard *et al.*, 2002; Sirminger *et al.*, 2004); however, it is currently unclear whether these minigenes will completely complement CFTR function in humans. SMaRT technology, which targets endogenous pre-mRNA based on a mechanism of spliceosome-mediated RNA *trans*-splicing, is one approach that could solve potential limitations of rAAV delivery of CFTR. To this end, SMaRT technology requires that only a portion of the CFTR cDNA be delivered, reducing constraints on packing of the large, full-length cDNA. A second advantage is that CFTR correction occurs only in those cells that express CFTR pre-mRNA, allowing for a more endogenous pattern of transgene reconstitution.

In the present study, we demonstrate that functional correction of CFTR in polarized human CF airway epithelial cells by SMaRT can be achieved with rAAV. The level of CFTR functional correction reached ~14% of that of non-CF epithelia, and was similar for both rAAV2 and rAAV5 serotype vectors. These studies have now extended previous observations using

recombinant adenovirus to a more clinically suitable vector and apical delivery approach compatible with CF gene therapy.

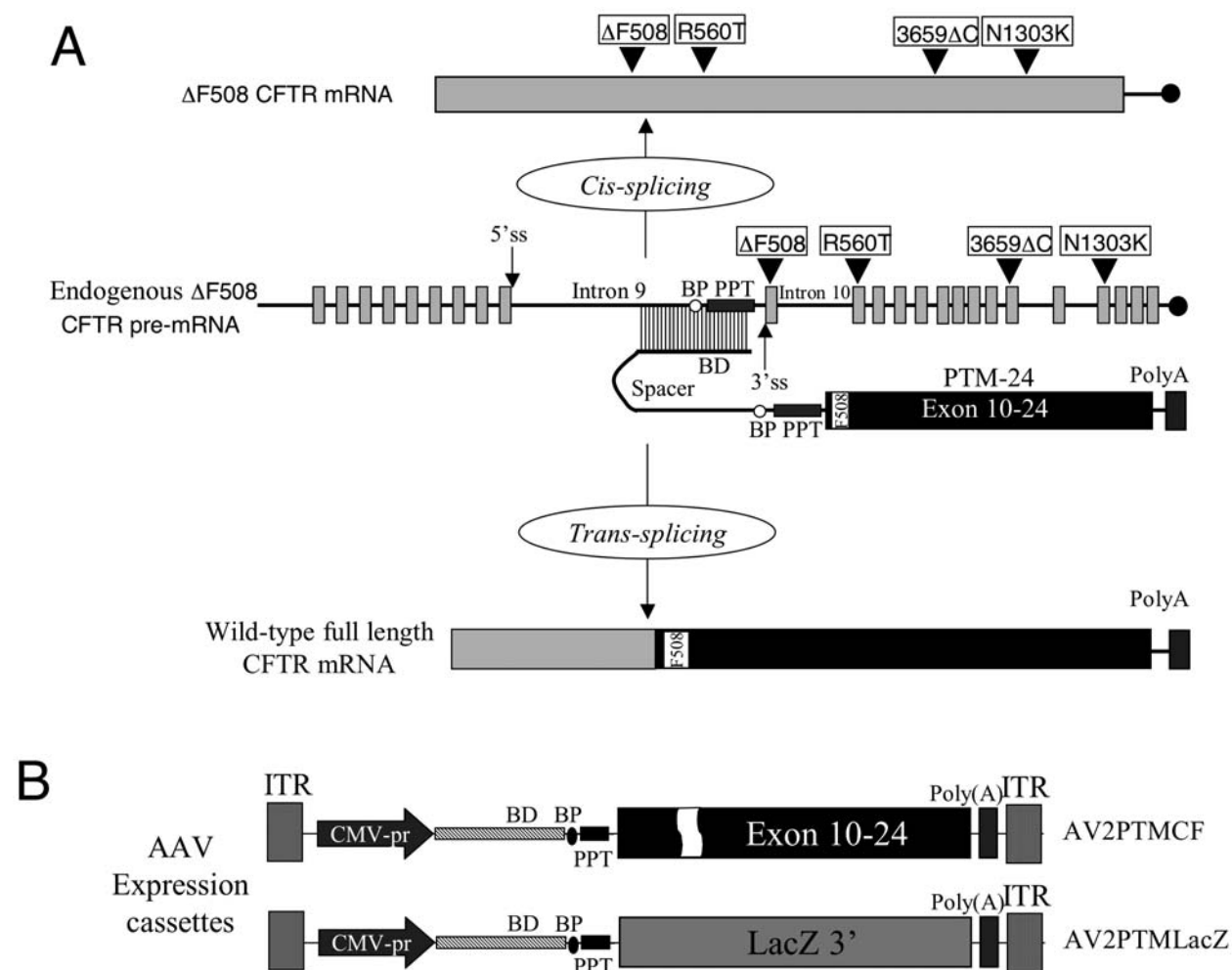
## MATERIALS AND METHODS

### *Generation of recombinant AAV vectors expressing pre-trans-splicing molecules*

The general design and structure of PTMs (pre-*trans*-splicing molecules) targeting intron 9 of CFTR have been previously described (Puttaraju *et al.*, 1999, 2001; Mansfield *et al.*, 2000; Liu *et al.*, 2002; Bhaumik *et al.*, 2004). The structure of the PTM used in this study, and the proposed mechanism of *trans*-splicing between the PTM and human CFTR pre-mRNA, are illustrated in Fig. 1A. These PTMs were incorporated into two rAAV vectors (AV2/2PTMCF and AV2/5PTMCF) and are designed to replace CFTR exons 10–24. Recombinant AAV vectors (Fig. 1B) were generated from proviral plasmid pAV2PTMCF as previously described, and the proviral vectors that harbored AAV2 inverted terminal repeats (ITRs) were packaged either into type 2 (AV2/2PTMCF) or type 5 (AV2/5PTMCF) capsids by triple-plasmid transfection in 293 cells (Duan *et al.*, 1998b; Yan *et al.*, 2004). Helper-free virus stocks were purified as previously described (Duan *et al.*, 1998b; Yan *et al.*, 2004) by high-performance liquid chromatography (HPLC) (for AAV2/2) or CsCl banding (for AAV2/5 pseudotyped vectors). Physical titers of rAAV were determined by slot-blot hybridization, and peak fractions were combined for analysis (Duan *et al.*, 1998b; Yan *et al.*, 2004). Similarly, two control rAAV vectors (AV2/2PTMLacZ and AV2/5PTMLacZ) were generated with a previously described PTM24LacZ fragment (Puttaraju *et al.*, 2001; Liu *et al.*, 2002), which incorporated the LacZ 3' exon downstream of the CFTR *trans*-splicing domain. The previously described CFTR *trans*-splicing adenoviral vector (Ad.CFTRPTM-24), and a full-length CFTR cDNA vector (Ad.CFTR), were used as positive control vectors (Liu *et al.*, 2002).

### *Generation of polarized human airway epithelia and rAAV infection*

Freshly isolated human airway epithelial cells from either non-CF bronchial airways, or CF bronchial airways with  $\Delta F508/\Delta F508$ ,  $\Delta F508/3659\Delta C$ ,  $\Delta F508/R560T$ , and  $\Delta F508/N1303K$  mutations, were seeded into collagen-coated 0.6-cm<sup>2</sup> Millicell culture inserts (Millipore, Bedford, MA) and grown at the air–liquid interface in 24-well tissue culture plates containing 0.5 ml of medium in the basolateral compartment as previously described (Karp *et al.*, 2002). Airway epithelial cells were infected with 50  $\mu$ l of rAAV-containing medium applied to the apical surface of the epithelia at a multiplicity of infection (MOI) of  $1.0 \times 10^4$  particles/cell for 16 hr in the presence of 40  $\mu$ M *N*-acetyl-L-leucyl-L-leucyl-L-norleucinal (LLnL) and 5  $\mu$ M doxorubicin (Yan *et al.*, 2004). This proteasome inhibitor cocktail significantly enhances nuclear transport of rAAV, and transduction after apical infection (Yan *et al.*, 2004). Infections with recombinant adenoviral vectors Ad.CFTR and Ad.CFTRPTM-24 were performed by applying 50  $\mu$ l of viral-containing medium to the basolateral side of the epithelia, using an MOI of 200–2000 particles/cell. Virally infected epi-



**FIG. 1.** Schematic approach for correction of CFTR RNA by SMaRT. **(A)** Structure of CFTR pre-RNA target and position of mutations evaluated in the current study are depicted in a schematic representation of the targeting mechanism. PTM-24 contains the *trans*-splicing transgene cassette, which is identical to a previously reported construct (Liu *et al.*, 2002). **(B)** The structure of rAAV2 *trans*-splicing cassettes for CFTRPTM-24 and LacZPTM-24. BD, binding domain; BP, branch point; CMV-pr, cytomegalovirus promoter; ITR, inverted terminal repeat; PPT, polypyrimidine tract; ss, splice site.

thelia were then incubated for 15 and 30 days after rAAV infection, or for 48 hr after adenoviral infection, before electrophysiological and molecular studies.

#### *Short-circuit current measurement in polarized airway epithelia*

A transepithelial resistance of  $>500 \Omega$  indicated the proper formation of tight junctions and the readiness of polarized airway epithelia for short-circuit current ( $I_{sc}$ ) measurement. Transepithelial short-circuit currents were measured with an epithelial voltage clamp (model EC-825) and a self-contained Ussing chamber system (both purchased from Warner Instruments, Hamden, CT) as previously described (Liu *et al.*, 2002). Throughout the experiment the chamber was kept at  $37^\circ\text{C}$ , and the chamber solution was aerated. The basolateral side of the chamber was filled with buffered Ringer's solution containing 135 mM NaCl, 1.2 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgCl}_2$ , 2.4 mM  $\text{KH}_2\text{PO}_4$ , 0.2 mM  $\text{K}_2\text{HPO}_4$ , and 5 mM HEPES, pH 7.4. The

apical side of the chamber was filled with low-chloride Ringer's solution containing 135 mM sodium gluconate, 1.2 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgCl}_2$ , 2.4 mM  $\text{KH}_2\text{PO}_4$ , 0.2 mM  $\text{K}_2\text{HPO}_4$ , and 5 mM HEPES, pH 7.4. Transepithelial voltage was clamped at zero, and the resulting  $I_{sc}$  was measured and recorded with a Quick DataAcq DT9800 series real-time data acquisition USB board (Data Translation, Marlboro, MA) and analyzed in Excel files.

#### *Detection of repaired CFTR mRNA by reverse transcription-polymerase chain reaction and Southern blotting*

After electrophysiological analysis, polarized airway epithelial cultures were harvested for molecular studies by reverse transcription-polymerase chain reaction (RT-PCR) and Southern blotting. The cells were lysed within the Millicell apparatus by the addition of lysis buffer (provided with the total RNA isolation kit [RNeasy micro kit]; Qiagen, Valencia, CA), and cell lysates from the same donor were collected and pooled for

each experimental group (at least four identical cultures were harvested for each group). Total RNA was isolated with the Qiagen total RNA isolation kit according to the manufacturer's instructions. Synthesis of complementary DNA was performed with a Qiagen Omniscript RT kit. Briefly, 2  $\mu\text{g}$  of total RNA from each sample was mixed with reaction reagents according to the manufacturer's instructions. Five microliters of a 10-fold dilution of the RT product was aliquoted and used for PCR analysis. To amplify the CFTR mRNA generated by either *cis*- or *trans*-splicing, nested PCR and Southern blotting were performed as previously described (Liu *et al.*, 2002). The outer primers (primers A and B) were designed to amplify a 500-bp region harboring the F508 region. The inner primers were composed of a common forward primer (primer C) for both the *cis*- and *trans*-spliced products ( $\sim 200$  bp), but a different reverse primer based on either the wild-type F508 sequence (primer D) or  $\Delta\text{F508}$  sequence (primer E). The primer sequences used are as follows: primer A, 5'-AGAATGTAACAGCCTTCTGGAGG-3'; primer B, 5'-AGAAATCTTGCTGGTTGACCTCC-3'; primer C, 5'-CTTCTGGTACTCCTGCTCTG-3'; primer D, ATCATAGGAAACACCAAA-GATGA-3'; and primer E, 5'-TCATAGGAAACACCAATGATAT-3'. The second-round PCR products were analyzed on a 2% agarose gel before being transferred onto nitrocellulose membranes. The membranes were then hybridized with  $^{32}\text{P}$ -labeled allele-specific oligonucleotides for the wild-type F508 CFTR products (5'-CATCTTTGGTGTTCCT-3').

## RESULTS

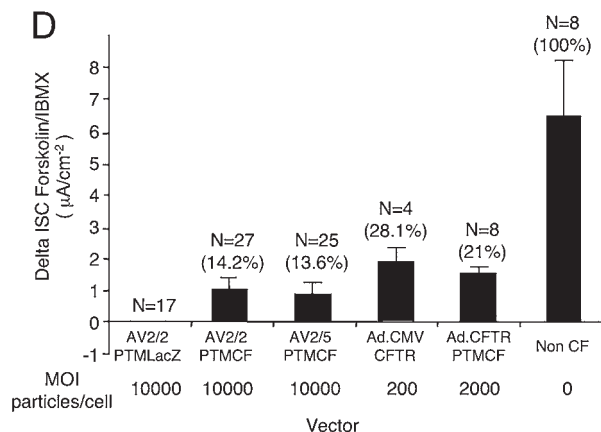
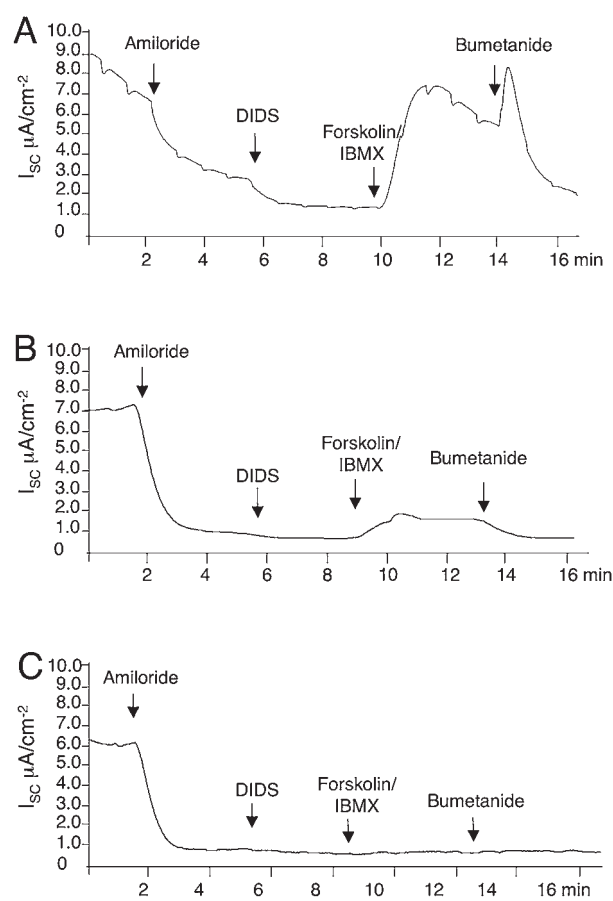
We have previously demonstrated that PTMs carrying exons 10–24 of the CFTR gene can repair RNA derived from a  $\Delta\text{F508}$ -CFTR minigene construct by spliceosome-mediated *trans*-splicing (Mansfield *et al.*, 2000, 2003; Liu *et al.*, 2002). In the present study, we investigate whether rAAV vectors are capable of mediating efficient SMaRT correction of endogenous CFTR pre-mRNA transcripts in polarized human CF airway epithelial cells containing the  $\Delta\text{F508}$  mutation (Scotet *et al.*, 2003). The polarized human CF airway epithelial cells used in this study included several genotypes, all of which contained at least one  $\Delta\text{F508}$  allele ( $\Delta\text{F508}/\Delta\text{F508}$ ,  $\Delta\text{F508}/3659\Delta\text{C}$ ,  $\Delta\text{F508}/\text{R560T}$ , and  $\Delta\text{F508}/\text{N1303K}$ ). All of these mutations resided distal to intron 9, and hence should be corrected by the SMaRT approach (the locations of mutations are summarized in Fig. 1A). rAAV2 genomes encoding CFTR- and LacZ- directed PTMs were packaged (Fig. 1B) into both serotype 2 and 5 capsids to generate the following recombinant viruses: AV2/2PTMCF, AV2/2PTMLacZ, AV2/5PTMCF, and AV2/5PTMLacZ.

Previous studies have demonstrated that proteasome-modulating agents can significantly augment rAAV2 and rAAV5 gene transduction from the apical surfaces of airway epithelia (Duan *et al.*, 1998b; Yan *et al.*, 2004). As seen in these previous studies, proteasome inhibitor cocktail enhanced transduction by  $\sim 1000$ -fold after apical infection when using either AV2/2CMVeGFP or AV2/5CMVeEGFP vectors (data not shown). At 15 days postinfection with AV2/2PTMCF or AV2/5PTMCF virus, short-circuit currents ( $I_{\text{sc}}$ ) were measured in epithelia after the sequential addition of (1) 100  $\mu\text{M}$  amiloride

to inhibit the epithelial sodium channel (ENaC), (2) 100  $\mu\text{M}$  4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid (DIDS) to inhibit non-CFTR chloride channels, (3) 10  $\mu\text{M}$  forskolin/100  $\mu\text{M}$  3-isobutyl-1-methylxanthine (IBMX) to activate CFTR channels, and (4) 100  $\mu\text{M}$  bumetanide to inhibit all transepithelial chloride current. As shown in Fig. 2, a significant increase in forskolin/IBMX-inducible  $I_{\text{sc}}$  was observed in CF epithelia apically infected with either AV2/2PTMCF ( $1.07 \pm 0.24 \mu\text{A}$ ) or AV2/5PTMCF ( $0.90 \pm 0.20 \mu\text{A}$ ) vectors, representing a conductance equivalent to 14.2 and 13.6% of that observed in non-CF human polarized epithelia, respectively. Forskolin/IBMX-stimulated  $I_{\text{sc}}$  was inhibited by the addition of bumetanide at the end of the experiment, indicating that the changes in  $I_{\text{sc}}$  were due to active CFTR  $\text{Cl}^-$  transport and not passive movement of  $\text{Cl}^-$  across damaged epithelia (Fig. 2A and B). No significant change in amiloride- or DIDS-sensitive current was seen with either rAAV-PTM vector (data not shown). The efficiency of CFTR functional correction was sustained for at least 30 days with no diminution of IBMX/forskolin-inducible  $I_{\text{sc}}$  (data not shown). In contrast, no IBMX/forskolin-stimulated  $I_{\text{sc}}$  was observed in the CF airway epithelia infected with negative control AV2/2PTMLacZ or AV2/5PTMLacZ vectors harboring the CFTR PTM fused to a 3' segment of LacZ (Puttaraju *et al.*, 1999, 2001; Liu *et al.*, 2002) (Fig. 2C).

The efficiencies of functional correction after apical infection with rAAV-PTM vector were compared with previous levels of correction observed using basolateral infection with full-length CFTR and CFTR-PTM adenoviral vectors (Liu *et al.*, 2002). CFTR correction produced with a recombinant adenovirus harboring a wild-type CFTR cDNA (Ad.CMVCFTR; MOI, 200 particles/cell) or the CFTR-PTM vector (Ad.CFTRPTM-24; MOI, 2000 particles/cell) were 28 and 21%, respectively. Results from the comparison of all vector groups are shown in Fig. 2D and demonstrate a significant increase ( $p < 0.01$ ) in CFTR-mediated  $I_{\text{sc}}$  for all CFTR-PTM vector types as compared with epithelia infected with the control LacZ-PTM virus.

To confirm that infection with the AAV-PTM vectors indeed repaired  $\Delta\text{F508}$  CFTR mRNA we confirmed *trans*-splicing in  $\Delta\text{F508}/\Delta\text{F508}$  homozygous samples, using nested RT-PCR followed by allele-specific oligonucleotide (ASO) hybridization. The strategy of this nested RT-PCR approach is illustrated in Fig. 3A. RT-PCR employed allele-specific primers and evaluated the PCR products in ethidium bromide-stained agarose gels followed by allele-specific hybridization for the wild-type F508 sequence as previously described (Liu *et al.*, 2002). PCR analysis of plasmid controls carrying either the  $\Delta\text{F508}$  or wild-type CFTR cDNA demonstrated the specificity of this assay (Fig. 3B, lanes 2 and 3). As shown in lanes 4–9 of Fig. 3B,  $\Delta\text{F508}$ -specific RT-PCR products were seen in all CF primary airway cultures tested and confirmed the expression of  $\Delta\text{F508}$  CFTR mRNA in these samples. No PCR products were detected in samples from which reverse transcriptase was omitted (see, e.g., lane 10 in Fig. 3B), demonstrating the specificity of amplification for mRNA. Wild-type F508-specific RT-PCR products were not detected in any of the  $\Delta\text{F508}$  human airway samples infected with a control AV2/2PTMLacZ vector (Fig. 3B, lane 6), but were seen in non-CF airway samples (Fig. 3B, lane 1),  $\Delta\text{F508}$  airway cultures infected with AVPTMCF vectors (Fig. 3B, lanes 4 and 5), Ad.CFTRPTM (Fig. 3B, lane 7), and the full-length Ad.CMVCFTR vector (Fig. 3B, lane 8). These re-



**FIG. 2.** Functional correction of CFTR-mediated chloride currents in polarized human CF epithelia. Polarized human CF airway epithelia containing at least once  $\Delta F508$  allele were infected with rAAV vectors (10,000 particles/cell) in the presence of proteasome-modulating agents from the apical surface. Adenoviral vector controls were used to infect epithelia from the basolateral surface at 200 or 2000 particles/cell as indicated. Functional correction of CFTR-mediated chloride current was evaluated by measuring the transepithelial short-circuit current 15 days after rAAV infection or 48 hours after adenovirus infection. (A–C) Typical short-circuit current ( $I_{sc}$ ) profiles under various sequential experimental conditions: (1) addition of amiloride to inhibit epithelial sodium conductance, (2) addition of DIDS to inhibit non-CFTR chloride channels, (3) addition of IBMX/forskolin to stimulate CFTR-mediated transepithelial  $Cl^-$  current, and (4) addition of bumetanide to block all  $Cl^-$  movement into cells from the basolateral membrane, which serves as a control for active transepithelial  $Cl^-$  transport under secretory

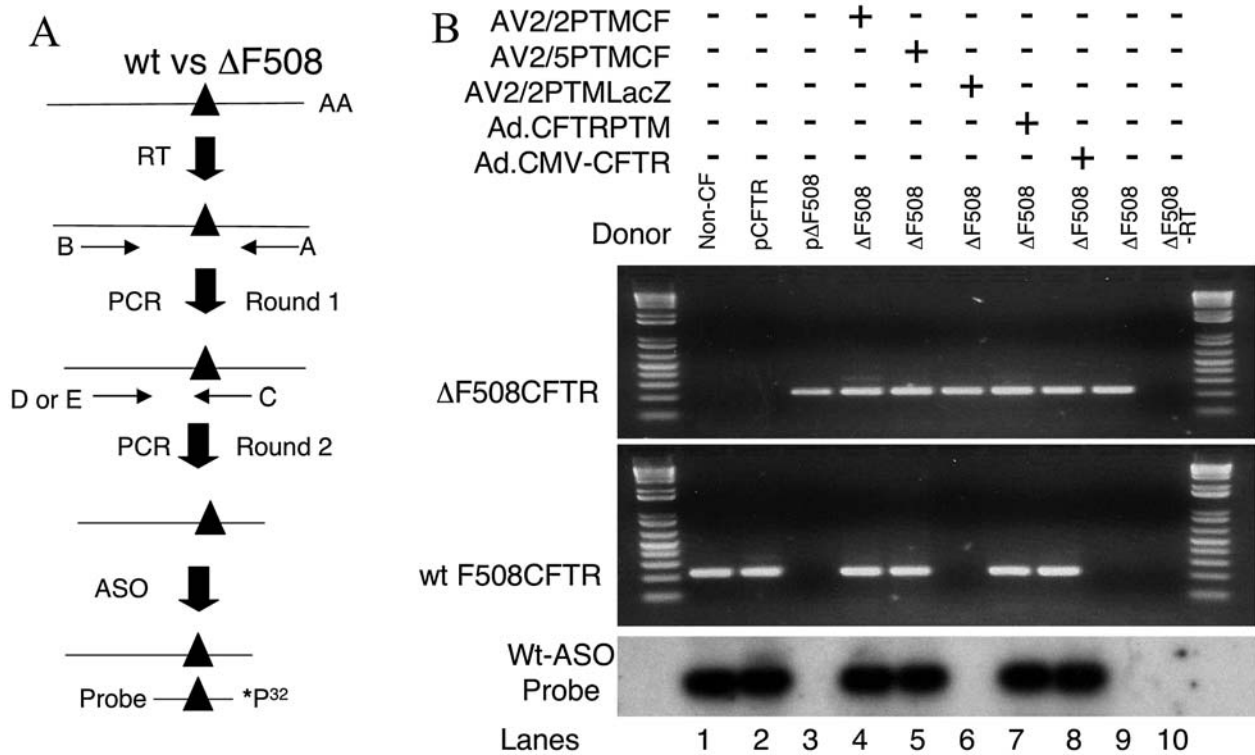
conditions. The amplitude ( $\mu A$ ) of the IBMX/forskolin response correlates with the magnitude of CFTR-mediated  $Cl^-$  transport across the epithelia, and was used to calculate  $I_{sc}$ . Shown are  $I_{sc}$  profiles of (A) non-CF airway epithelia, (B) AV2/2PTMCF-infected  $\Delta F508/\Delta F508$  CF epithelia, and (C) AV2/2PTMLacZ-infected  $\Delta F508/\Delta F508$  CF epithelia. (D) Cumulative summary of  $\Delta I_{sc}$  response to IBMX/forskolin in CF airway epithelia from all genotypes tested (see Materials and Methods) for the indicated vector infections. Results indicate the mean ( $\pm$ SEM) change in forskolin/IBMX-inducible  $I_{sc}$  for  $n$  experiments. The response from non-CF controls is given as a reference for fully functional CFTR. The percent correction is given in reference to non-CF for each CFTR vector-treated group. Significant differences between all CFTR vector-treated groups and the control LacZ vector-treated group ( $p < 0.01$ ) were observed, based on the Student  $t$  test.

sults confirmed that molecular correction of  $\Delta F508$  CFTR mRNA transcripts occurred in our primary culture model. Southern blotting analysis of the RT-PCR products, using allele-specific oligonucleotide (ASO) hybridization, was performed to confirm the presence of the F508 codon in wild-type CFTR amplified products (Fig. 3B, bottom). These Southern blots confirmed that the wild-type F508 CFTR codon was seen only in samples infected with AVPTMCF, Ad.CFTRPTM, or Ad.CMVCFTR. These results demonstrate that wild-type CFTR transcripts were produced by rAAV SMaRT repair of  $\Delta F508$  CFTR pre-mRNA.

## DISCUSSION

Gene therapy for CF lung disease, using rAAV-mediated CFTR cDNA gene transfer, presently has at least two technical barriers. First, because of the limited packaging capacity of

rAAV vectors ( $\sim 5.0$  kb), incorporation of the intact CFTR-coding sequence ( $\sim 4.5$  kb) leaves little room for promoter/enhancer elements. To this end, current clinical strategies have used the ITR as a weak promoter to deliver a functional CFTR cDNA to the maxillary sinus and/or lungs of CF patients (Aitken *et al.*, 2001; Wagner *et al.*, 2002; Flotte *et al.*, 2003). This strategy has failed to yield detectable transgene-derived CFTR mRNA in clinical trials. Although progress has been made in reducing the CFTR cDNA size by generating CFTR minigenes and by developing shorter but reasonably efficient promoters (Zhang *et al.*, 1998; Wang *et al.*, 1999; Ostedgaard *et al.*, 2002; Siminger *et al.*, 2004; Yan *et al.*, 2004), it remains to be determined whether such advances will be sufficient to correct CF lung pathology *in vivo*. Second, barriers to apical transduction appear to exist for at least two serotypes including rAAV2 and rAAV5 (Duan *et al.*, 1998b, 2000; Bals *et al.*, 1999; Yan *et al.*, 2004). However, studies suggest that this block in apical transduction can be overcome when proteasome-modulating agents,



**FIG. 3.** Evaluation of CFTR mRNA correction using allele-specific RT-PCR and allele-specific oligonucleotide (ASO) hybridization. (A) Schematic diagram of the nested RT-PCR and ASO Southern blotting strategies used to determine CFTR mRNA correction. (B) Allele-specific primers were used to specifically amplify mutant  $\Delta F508$  (*top*) or wild-type (wt) F508 (*middle*) mRNA sequences from polarized epithelial cultures infected with the indicated rAAV or adenoviral vectors. PCR products were evaluated on an ethidium-stained agarose gel using mutant  $\Delta F508$ -specific primers (*top*) and wild-type F508-specific primers (*middle*). The omission of reverse transcriptase (lane 10) was used as a control for mRNA-specific amplification. Allele-specific plasmid controls carrying either a wild-type F508 CFTR cDNA (lane 2) or a  $\Delta F508$  CFTR cDNA (lane 3), and RNA from non-CF polarized human epithelia (lane 1), were used to confirm the specificity of the PCR. PCR products from the wild-type CFTR PCR panel (*middle*) were further evaluated by Southern blotting (*bottom*) for the presence of the wild-type F508 codon, using ASO hybridization against a  $^{32}P$ -labeled wild-type CFTR-specific antisense oligonucleotide probe as previously described (Liu *et al.*, 2002).

such as doxorubicin and LLnL, are added at the time of infection or shortly after infection (Yan *et al.*, 2004).

Potential advantages of using SMaRT technology for CF gene therapy over conventional gene replacement strategies include specificity of gene correction and reduced size of the CFTR transgene cassette. Because SMaRT is dependent on the presence of a pre-mRNA target, repaired mRNA will be generated only in those cells that are expressing CFTR mRNA. Although it is presently unclear whether reconstituting the normal pattern of CFTR expression in the airway is beneficial for CF gene therapy, the fact that CFTR is a regulator of other ion channels and demonstrates a heterogeneous pattern of expression (Jiang and Engelhardt, 1998) suggests there may be potential merits of this approach.

We have previously demonstrated that adenoviral vector-mediated RNA *trans*-splicing was able to partially restore endogenous  $\Delta F508$  CFTR in human cystic fibrosis airway epithelia (Liu *et al.*, 2002). However, recombinant adenovirus most likely will not be the vector of choice for gene therapy of CF lung disease because it is relatively ineffective at transduc-

ing airway epithelia from the apical surface (Walters *et al.*, 1999). In the present report, we have demonstrated that apical infection of human CF airway epithelia with both rAAV type 2 and type 5 vectors was able to functionally correct endogenous mutated CFTR mRNA, using SMaRT. The maximal level of CFTR channel activity seen after AVPTMCF infection was similar to that seen after basolateral infection with recombinant adenovirus delivering the identical CFTR-PTM. Given that only 8% of normal CFTR mRNA transcripts are required to maintain a normal clinical phenotype (Chu *et al.*, 1992), these studies suggest that the level of correction seen with SMaRT may be clinically useful.

Previous studies using rAAV vectors in mice have demonstrated that rAAV5 is much more efficient at transducing airway epithelia than is rAAV2 (Zabner *et al.*, 2000; Auricchio *et al.*, 2002). However, using reporter genes, we have shown that rAAV2 and rAAV5 transduce polarized human airway epithelia from the apical surface with only marginally different efficiencies in the presence or absence of proteasome inhibitors (Yan *et al.*, 2002, 2004). Findings from this present study sup-

port this conclusion, using a functional readout for CFTR complementation. Hence, the optimal choice of serotype for human CF clinical trials is unclear.

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Address reprint requests to:

*Dr. John F. Engelhardt*

*Departments of Anatomy and Cell Biology*

*University of Iowa*

*School of Medicine*

*51 Newton Road, Room 1-111 BSB*

*Iowa City, IA 52242*

*E-mail: john-engelhardt@uiowa.edu*

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