Transcriptional Targeting in the Airway Using Novel Gene Regulatory Elements

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The delivery of cystic fibrosis transmembrane conductance regulator (CFTR) to airway epithelia is a goal of many gene therapy strategies to treat cystic fibrosis. Because the native regulatory elements of the *CFTR* are not well characterized, the development of vectors with heterologous promoters of varying strengths and specificity would aid in our selection of optimal reagents for the appropriate expression of the vector-delivered *CFTR* gene. Here we contrasted the performance of several novel gene-regulatory elements. Based on airway expression analysis, we selected putative regulatory elements from *BPIFA1* and *WDR65* to investigate. In addition, we selected a human *CFTR* promoter region (~ 2 kb upstream of the human *CFTR* transcription start site) to study. Using feline immunodeficiency virus vectors containing the candidate elements driving firefly luciferase, we transduced murine nasal epithelia *in vivo*. Luciferase expression persisted for 30 weeks, which was the duration of the experiment. Furthermore, when the nasal epithelium was ablated using the detergent polidocanol, the mice showed a transient loss of luciferase expression that returned 2 weeks after administration, suggesting that our vectors transduced a progenitor cell population. Importantly, the *hWDR65* element drove sufficient *CFTR* expression to correct the anion transport defect in *CFTR*-null epithelia. These results will guide the development of optimal vectors for sufficient, sustained *CFTR* expression in airway epithelia.

Keywords: gene transfer; lung; cystic fibrosis

Mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene cause cystic fibrosis, a multiorgan disease that affects the sinuses, lung, sweat glands, intestines, liver, pancreas, and reproductive tracts (1–3). However, most of the morbidity and mortality associated with the disease results from chronic bacterial infections and inflammation in the lung. Developing gene transfer vectors for efficient, safe, and effective therapeutic expression in the appropriate cell types of the airways is an important consideration in the treatment of cystic fibrosis.

CLINICAL RELEVANCE

Our results indicate that the novel endogenous genetic element from the human *WDR65* gene may provide a suitable alternative to the stronger viral promoters used in current gene-transfer vectors. Furthermore, our findings suggest that this promoter is active in progenitor cell populations in the airway. This promoter has the potential to mediate long-term cystic fibrosis transmembrane conductance regulator expression in the airway, an important step toward treating cystic fibrosis.

CFTR mRNA is present in low copy numbers (1–2 copies per cell) in nasal, tracheal, and bronchial epithelia (4, 5). Previous work in *Cftr*-null murine models showed that achieving as little as 5% of normal CFTR concentrations resulted in a much larger correction of the chloride transport defect (50% of normal), suggesting that modest levels of transgene expression may confer a significant therapeutic benefit (6). Conversely, CFTR overexpression in airway epithelia may cause protein mislocalization to the basal surface and an overall net decrease in chloride current (7). The entire suite of regulatory elements for the *CFTR* locus is unknown, but several regions have been identified that span several hundred kilobases (8–12), a size too large to package efficiently in standard viral vectors. Thus, a need exists for vectors with appropriate transgene expression levels in the airway.

To that end, we sought to develop vectors carrying transgenes driven by novel promoters from endogenous genes expressed in the airway. Transcript profiling in human airway epithelia indicated that a member of the bactericidal/permeability-inducing fold containing family A (*BPIFA1*, also known as *PLUNC*) is highly expressed in human airway epithelia (13). The *in situ* hybridization of murine nasal sections revealed that the expression of tryptophan, aspartic acid repeat protein 65 (*Wdr65*) is restricted to the respiratory epithelium (14). Based on the expression profile of *BPIFA1* and *Wdr65*, we selected putative gene-regulatory elements from these genes to investigate. In addition, we selected an approximately 2-kb human *CFTR* promoter region to study, which presumably encompasses the basic *cis*-regulatory elements.

In addition to achieving appropriate levels of transgene expression in the airway, expression in progenitor cell types is important for long-term therapeutic potential. Previously, we reported stable luciferase expression in human airway epithelia *in vitro* and in murine nasal airways *in vivo* for over a year after the administration of feline immunodeficiency virus (FIV) vector (15–17). This result may indicate that progenitor cells were transduced. Expression in these studies was driven by the ubiquitous and relatively strong Rous sarcoma virus (RSV) promoter. Here we show that firefly luciferase reporter expression from the candidate gene-regulatory elements human (*h*)

(*Received in original form December 21, 2011 and in final form March 13, 2012*)

This work was supported by National Institutes of Health grants P01 HL-51670 (P.B.M. and P.L.S.), P01 HL-091842 (P.B.M.), and HL-007638 (E.R.B.), by the Children's Miracle Network (P.L.S.), and by the Roy J. Carver Charitable Trust. The authors also acknowledge the support of the *In Vitro* Models and Cell Culture Core, the Gene Transfer Vector Core, and the Cell Morphology Core, partly supported by the Center for Gene Therapy for Cystic Fibrosis (through National Institutes of Health grant P30 DK-54759) and the Cystic Fibrosis Foundation.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 47, Iss. 2, pp 227–233, Aug 2012

Originally Published in Press as DOI: 10.1165/rcmb.2011-0444OC on March 23, 2012
Internet address: www.atsjournals.org

BPIFA1 and *hWDR65* also persists long term. In subsequent experiments, we used a murine model carrying a floxed termination codon directly upstream of the initiation codon (AUG) of luciferase expressed from the endogenous *Rosa26* promoter (18). We delivered FIV-expressing Cre-recombinase under the control of our candidate promoter elements to these mice. After intranasal administration of the detergent polidocanol, expression was ablated in the nasal airways. By 2 weeks after polidocanol administration, luciferase expression in the airways of these animals returned to pretreatment levels, consistent with the activity of our candidate elements in airway progenitor populations. Moreover, an *hWDR65*-driven *CFTR* transgene corrected the chloride current defect in *CFTR*-null airway epithelia.

MATERIALS AND METHODS

Primary Epithelial Cultures and Electrophysiology Studies

Tracheal epithelial cells from *CFTR*-null pigs were isolated by enzymatic digestion, seeded onto permeable filters, and grown at an air–liquid interface, as previously described (19). Adenoviral vectors expressing porcine *CFTR* from either the RSV or *hWDR65* promoter elements were administered basolaterally to CFTR-null porcine tracheal epithelia cultures. Three days after transduction, cultures were studied in modified Ussing chambers, as previously described (20). Briefly, epithelia were bathed on both surfaces with solution containing 135 mM NaCl, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM dextrose, and 5 mM Hepes (pH 7.4) at 37°C, and gassed with compressed air. Baseline transepithelial currents were measured. After an apical addition of 100 μM amiloride (Amil) and 100 μM 4,4′-diisothiocyanoto-stilbene-2,2′-disulfonic acid, currents were allowed to stabilize, and the apical solution was replaced with a 4.8-mM Cl^- solution containing 135 mM D-gluconic Acid, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM dextrose, and 5 mM Hepes (pH, 7.4) at 37°C, and gassed with compressed air. Cyclic adenosine monophosphate (cAMP)–dependent chloride current was stimulated by the apical addition of 10 μM forskolin and 100 μM 3-isobutyl-1-methylxanthine (IBMX), and CFTR-specific chloride transport was inhibited with 100 μM GlyH-101 (a generous gift from Cystic Fibrosis Foundation Therapeutics and R. Bridges) was measured. Transepithelial voltage (Vt) was maintained at 0 mV to measure the short-circuit current (I). Transepithelial electrical conductance was measured by intermittently clamping Vt to +5 and/or −5 mV. Spontaneous values of Vt were measured by transiently removing the voltage clamp.

Murine Studies

All mice for this study were housed at the Animal Care Facilities of the University of Iowa. All animal procedures were previously approved by the Institutional Animal Care and Use Committee of the University of Iowa, in accordance with National Institutes of Health guidelines. The FVB.129S6(B6)-*Gt(ROSA)26Sor*$^{tm1(Luc)Kael}$/J strain (stock number 005125; Jackson Laboratory, Bar Harbor ME) carries a firefly luciferase cDNA, preceded by a LoxP-stop-LoxP cassette, at the ubiquitously expressed ROSA26 locus (18). This strain received FIV vectors expressing our selected promoter-driven Cre. Balb/c mice (strain code 01B05; Charles River Laboratories, Wilmington, MA) received FIV expressing firefly luciferase. Six to 8-week-old mice were transduced intranasally with FIV or adenovirus (Ad) vector formulated 1:1 with methylcellulose (50 μl total volume), as previously described (21). Animals were imaged at various time points, using the In Vivo Imaging Systems (IVIS) system (Caliper Life Sciences, Hopkinton, MA). Two hundred microliters of luciferin substrate (15 mg/ml; Caliper Life Sciences) were administered intraperitoneally, and the mice were imaged for 5 minutes. Data were analyzed using Living Image software (Caliper Life Sciences). The negative controls in all *in vivo* bioluminescent assays were naive Balb/c mice. The typical background level of bioluminescence in a naive mouse is approximately 10^4 photons/second/cm^2. We included negative controls in every assay, and report only the background-subtracted levels of experimental groups.

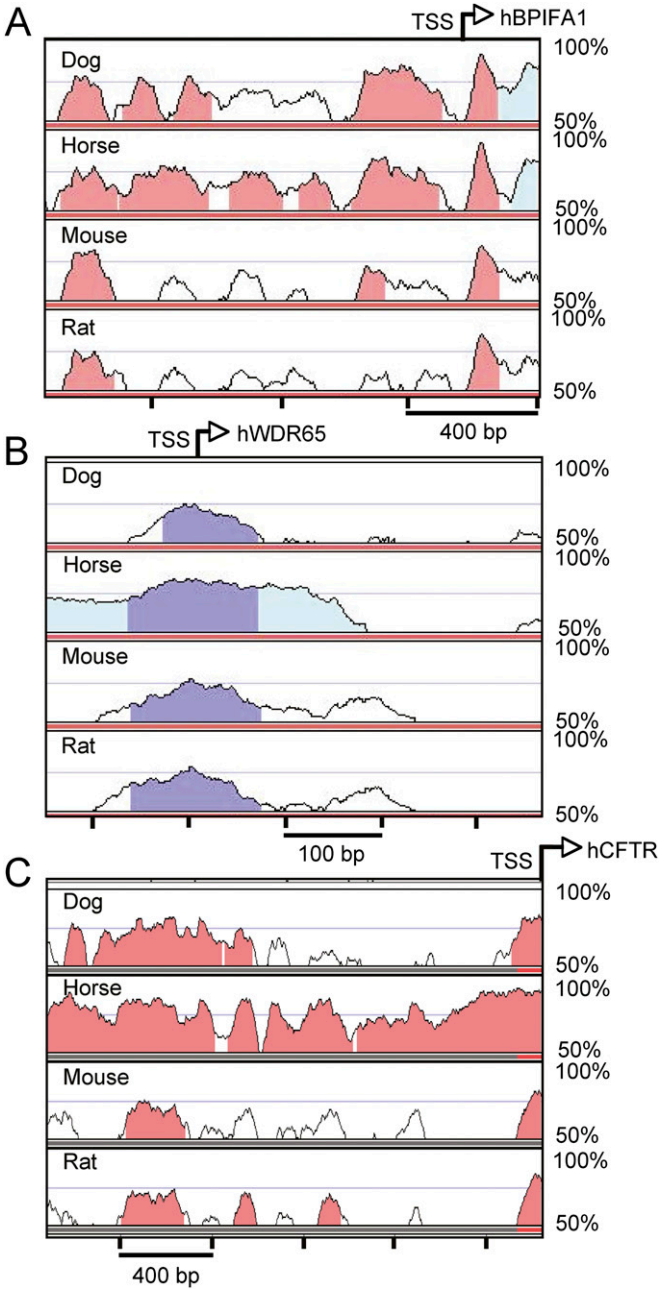

Figure 1. Visualizing global DNA sequence alignments of arbitrary length (VISTA) plot shows the genomic loci of novel genetic elements. Genomic loci from the human bacterialcidal/permeability-inducing protein family A 1 (BPIFA1) (*A*), tryptophan and aspartic acid (WD) repeat protein 65 (WDR65) (*B*), and cystic fibrosis transmembrane conductance regulator (*CFTR*) (*C*) genes were aligned to the homologous loci in dogs, horses, mice, and rats, using the VISTA Genome Browser (www.ucsc.org). The human locus is shown at the *top* of each plot, and the other vertebrate genomes are shown with their relative conservation (70% identity over 100 bp) to the human locus *below*, in separate tracks. The lower threshold of each track represents 50% conservation. *Dark blue* represents exons, *pink* represents the conserved nucleotide sequence, and *light blue* represents untranslated regions. The displayed region represents the portion of the sequence cloned for analysis in the airway (*BPIFA1*, chromosome 20:31286263–7563; *WDR65*, chromosome 1:43410454–962; *CFTR*, chromosome 7:116905142–7292 on the University of California Santa Cruz human genome 19 assembly). TSS, transcription start site.

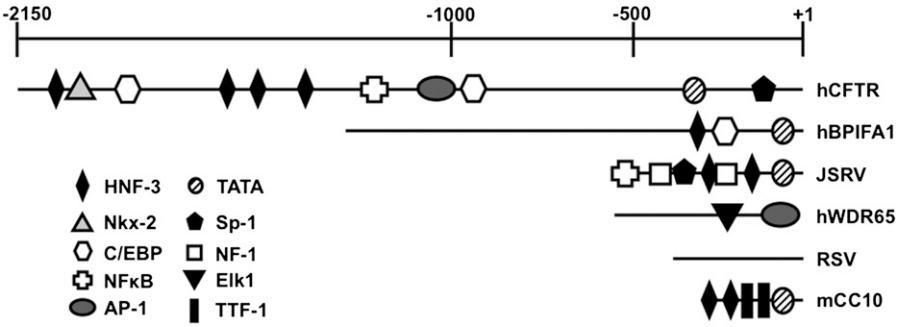

Figure 2. Transcription factor binding sites in novel genetic elements. Selected genetic element sequences were analyzed for predicted transcription factor binding sites (TFBs), using TFSearch (http://www.cbrc.jp/research/db/TFSEARCH.html). TFBs were selected using the default threshold score of 85.0. Only TFBs sharing 85% identity with the TFMATRIX transcription factor binding site database were predicted (31). TFBs corresponding to transcription factors relevant in the airway are indicated. C/EBP, CAAT/enhancer binding protein; mCC10, Clara cell secretory protein; h, human; RSV, Rous sarcoma virus; HNF-3, hepatocyte nuclear factor 3; Nkx-2, NK2 transcription factor–related locus 2; AP-1, activator protein 1; TATA, TATA box; Sp-1, specificity protein 1; NF-1, neurofibromatosis type 1; Elk1, E twenty-six (ETS)-like transcription factor 1; TTf, thyroid transcription factor 1; JSRV, Jaagsietke sheep retrovirus.

RESULTS

Promoters and Putative Upstream Regulatory Regions

To identify novel gene-regulatory regions for airway gene transfer applications, we selected upstream regions of genes expressed in epithelia as well as from viral vectors with airway tropism. The 3′ long terminal repeat (LTR) of the Jaagsietke sheep retrovirus (JSRV) was chosen because the virus bears a tropism for alveolar Type II pneumocytes and Clara cells (22). The minimal promoter region of the murine Clara cell secretory protein (*mCC10*) drives expression in Clara cells of the airway epithelia (23). We chose the upstream sequences of two novel endogenous genes (human *BPIFA1* and human *WDR65*), based on expression analysis. *BPIFA1* mRNA is highly expressed in human airway epithelia, as shown by transcript profiling using a microarray (13). *In situ* hybridization showed that *Wdr65* expression was restricted to the respiratory epithelia in murine nasal passages (14). However, the upstream regions of these genes are not characterized.

To identify elements to clone and test, we aligned upstream sequences from humans, mice, rats, dogs, and horses, using the University of California at Santa Cruz (UCSC) program for visualizing global DNA sequence alignments of arbitrary length (VISTA) browser (www.ucsc.org). We selected approximately 1 kb and 500 base pairs (bp) of the sequence upstream from *BPIFA1* and *WDR65*, respectively, based on multispecies homology (Figures 1A and 1B, respectively). We also tested approximately 2,150 bp of the sequence upstream from the human *CFTR* gene (Figure 1C). As we previously demonstrated (24), the 5′ LTR from the RSV drives strong, persistent expression from viral vectors in the airway epithelia, and thus serves as a control for comparisons. The analysis of predicted

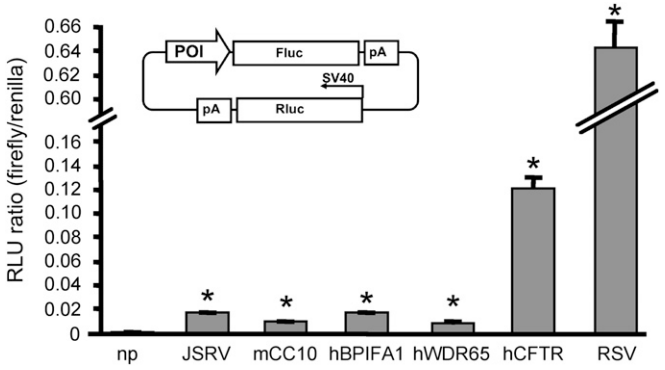

Figure 3. *In vitro* activity in an airway cell line. A549 cells were transfected with the dual luciferase plasmid (*inset*). Candidate promoter activity (firefly luciferase activity) was normalized to the internal renilla luciferase control activity. POI, promoter of interest; Fluc, firefly luciferase; Rluc, Renilla luciferase; SV40, spleen necrosis virus 40; RLU, relative light units; np, no promoter; pA, polyadenylation signal. *Bars* represent mean RLU ratios. *Error bars* represent standard errors ($n = 3$). *$P < 0.05$, as determined by Student *t* test.

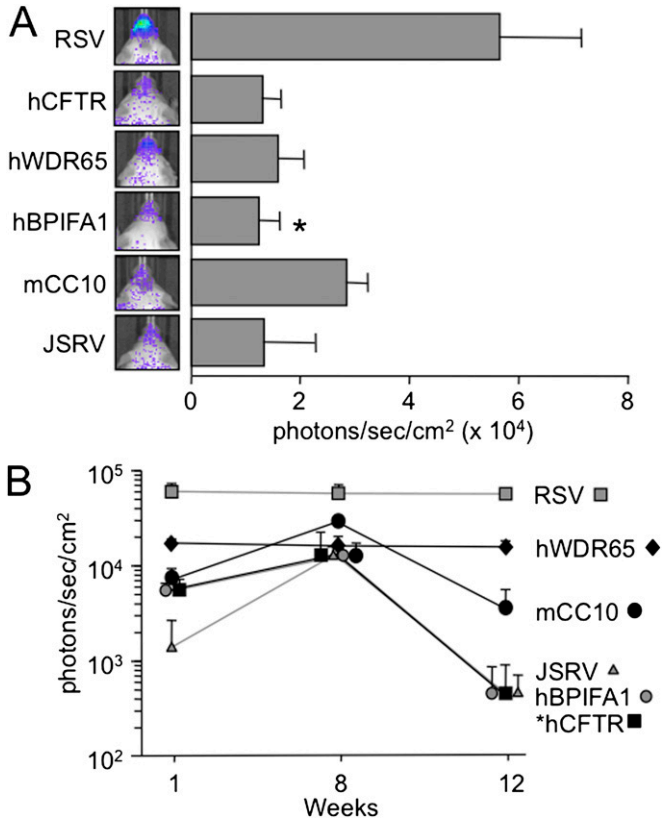

Figure 4. *In vivo* expression from novel genetic elements persists for 3 months. GP64-pseudotyped feline immunodeficiency virus (FIV) vector expressing firefly luciferase under the control of the candidate promoters and formulated with 1% methylcellulose was delivered to mice intranasally. Mice were imaged at indicated time points, and we measured photons/second/cm^2 as described in Materials and Methods. (*A*) *Bars* represent expression levels at 8 weeks. The RSV promoter drove significantly higher expression than the *hBPIFA1* genetic element ($P = 0.04$, according to Student *t* test). (*B*) Expression was monitored over time. Expression from the *hCFTR* genetic element was significantly lower at the conclusion of the experiment, compared with expression at 1 week ($P = 0.04$, according to Student *t* test). *Error bars* represent standard errors ($n = 3$–4 animals per group).

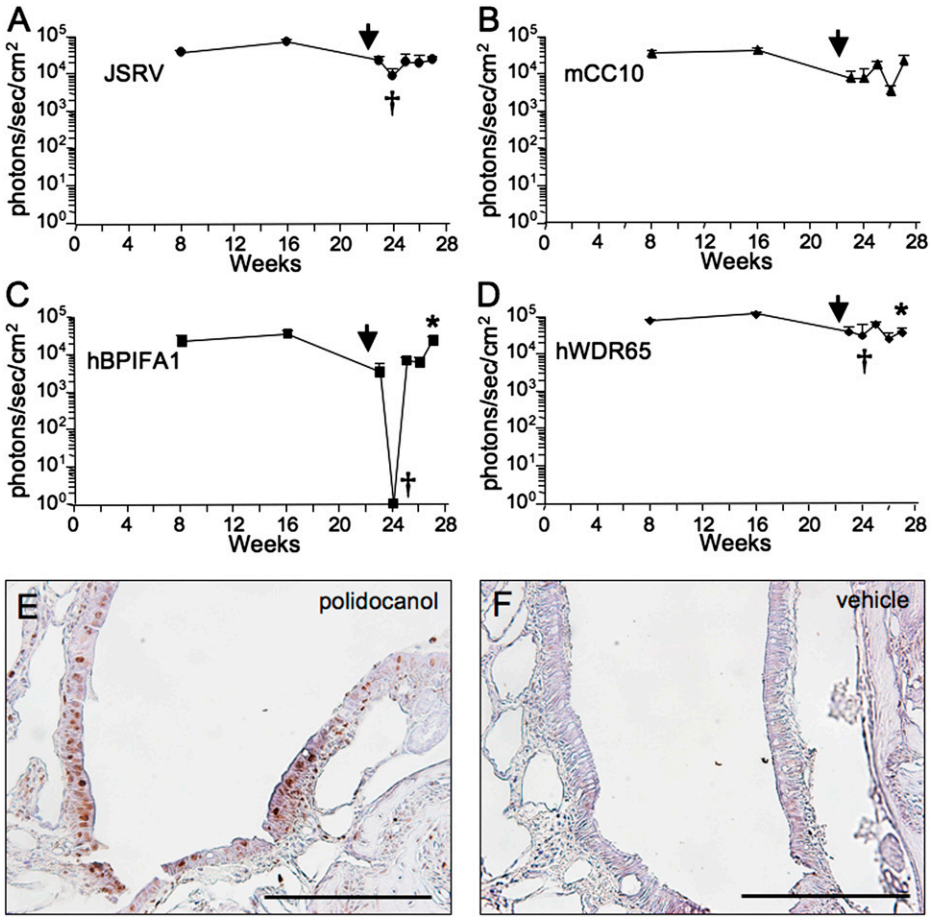

Figure 5. Progenitor cell populations were transduced in murine nasal epithelia. GP64-pseudotyped FIV vector expressing Cre recombinase under the control of the JSRV (*A*), *mCC10* (*B*), *hBPIFA1* (*C*), and *hWDR65* (*D*) candidate promoter elements and formulated with 1% methylcellulose was delivered to FVB.129S6(B6)-*Gt(ROSA)26Sor*$^{tm1(Luc)Kael}$/J mice intranasally. Animals were imaged, and luciferase activity was measured at various time points over 30 weeks. Luciferase activity reflects naive-subtracted values at each time point. At 24 weeks after administration, 2% polidocanol was delivered intranasally (*arrow*), and animals were imaged at various time points. *Error bars* represent standard errors ($n = 3$ animals per group). $^{\dagger}P < 0.05$, relative to values before polidocanol treatment. $^{*}P < 0.05$, relative to values 1 day after polidocanol treatment, as determined by one-tailed *t* test. Bromodeoxyuridine (BrdU) staining of nasal respiratory epithelia of mice that received polidocanol (*E*) or vehicle (*F*) was undertaken, as described in the online supplement's MATERIALS AND METHODS. Sections were counterstained with hematoxylin and eosin. *Scale bar* = 100 μm.

transcription factor–binding sites in the candidate promoter element sequences, using TFSearch (www.cbrc.jp/research/db/TFSEARCH.html), revealed several predicted motifs important in gene regulation in the airways, such as hepatocyte nuclear factor–3 (HNF-3) (22), CAAT/enhancer binding protein (25), and thyroid transcription factor–1 (22, 25, 26) (Figure 2).

In Vitro Candidate Gene-Regulatory Element Activity in Human Airway Cells

To verify the activity of the novel elements used in this study, we cloned promoter and/or upstream sequences of RSV, Jaagsietke sheep retrovirus (JSRV), *mCC10*, *hPLUNC*, *hWDR65*, and *hCFTR* upstream from a firefly luciferase reporter gene (please refer to the online supplement's MATERIALS AND METHODS). Renilla luciferase expression under the control of the SV40 promoter served as an internal control in each plasmid (Figure 3, *inset*). We transiently transfected the adenocarcinoma cell line A549 with the indicated reporter constructs, and collected cell lysates 48 hours later. Luciferase assays determined the relative activity from each element. Each gene-regulatory element drove significantly higher reporter expression in airway cells than did he control plasmid lacking a promoter (Figure 3). Functional promoter activity was also replicated in the airway epithelial lung adenocarcinoma cell line, Calu-3. However, the transfection efficiency and resulting luciferase expression were substantially lower than in A549 cells (data not shown).

In Vivo Activity and Persistence in Murine Airways

To determine the expression levels and persistence of each of the promoters *in vivo*, we cloned the promoter/upstream sequences upstream of the reporter firefly luciferase in a GP64-pseudotyped FIV lentiviral vector (Figure 4). We formulated 2.5×10^5 transducing units (TU) of each vector with methylcellulose (1:1) (21), and delivered it intranasally once a day for 5 consecutive days. Expression was monitored noninvasively, using the Xenogen IVIS200 bioluminescent imaging system (Caliper Life Sciences, Hopkinton, MA). Expression directed by the RSV promoter remained at a relatively high level ($\sim 6 \times 10^4$ photons/second/cm^2) for 3 months (i.e., the duration of the study). Expression from the *hWDR65* genetic element persisted for 3 months at a lower level ($\sim 2 \times 10^4$ photons/second/cm^2) than the RSV. Expression from the other genetic element vectors was reduced to background levels ($\leqslant 10^4$ photons/second/cm^2) by the end of the study (Figure 4B). We furthered these studies by cloning each candidate promoter element upstream from an alternate reporter gene, β-galactosidase, in a lentiviral FIV vector. We were unable to detect β-galactosidase activity in murine nasal sections after intranasal delivery, as previously described in our experiments using FIV vectors expressing luciferase (data not shown). The luciferase data suggest that RSV and *hWDR65* can direct persistent expression in the nasal airways. However, because of the variable gene activity driven by each of the candidate promoters, it is unclear whether the lack of persistence resulted from the limits of our ability to detect reporter activity.

Progenitor Populations Transduced

To address concerns regarding our limits of detection, we used a murine model in which luciferase is expressed from the endogenous *Rosa26* promoter. This mouse carries a floxed termination codon directly upstream from the AUG initiation codon (18). The JSRV,

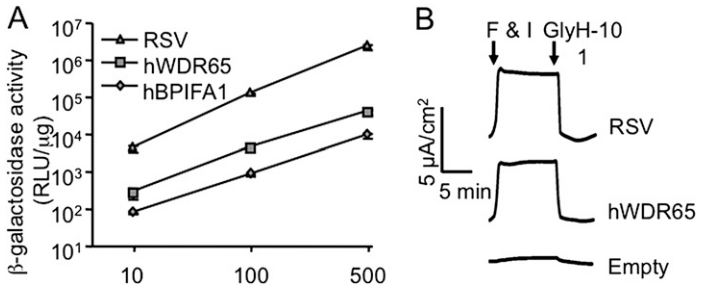

Figure 6. hWDR65-driven CFTR corrects chloride current defect in CFTR-null tracheal epithelia. (*A*) A549 cells were transduced with adenovirus vectors expressing β-galactosidase from each of RSV, *hBPIFA1*, and *hWDR65* candidate promoter elements at multiplicities of infection [MOIs] of 10, 100, and 500. Two days after transduction, β-galactosidase activity was measured via GalactoLight assay (Applied Biosystems, Bedford, MA; *see* MATERIALS AND METHODS). (*B*) Representative chloride current tracings from each of RSV, *hWDR65*, and empty control measurements. (*C*) Well-differentiated tracheal epithelia cultures from CFTR-null piglets were transduced basolaterally with adenovirus carrying porcine CFTR (pCFTR) driven by the *hWDR65* genetic element (MOI = 500) or the RSV promoter (MOI = 50). Adenovirus vector without pCFTR was used as a control (empty; MOI = 50). Three days after transduction, transepithelial Cl^- currents were measured in Ussing chambers (*see* MATERIALS AND METHODS). *Bars* represent means of change in chloride current upon the addition of forskolin (10 μM) and IBMX (100 μM) (*black*; F & I) or GlyH-101 (*gray*). *Error bars* represent SEMs. $n = 3$. *$P < 0.05$, according to *t* test. The *asterisk* represents significant change ($P < 0.05$ according to *t* test) relative to empty control cultures.

mCC10, *hBPIFA1*, *hCFTR*, or *hWDR65* candidate promoter elements driving Cre recombinase were packaged in a GP64-pseudotyped FIV vector. About 2.5×10^5 TU of each vector (maximum dose) were formulated with 1% methylcellulose (21) and delivered intranasally once a day for 3 days. Cre recombinase delivered to murine nasal epithelia excises the termination codon, which results in luciferase expression. This model allowed us to normalize candidate promoter strength. In addition, this model facilitated our ability to determine whether our candidate promoter elements were active in progenitor cell populations. To this end, we transiently ablated the surface epithelium with detergent. Polidocanol (26) was administered intranasally at 24 weeks after transduction. Bioluminescent expression was monitored over a 3-week period after polidocanol administration.

Luciferase expression in mice receiving the JSRV–Cre vector was stable for 23 weeks. After polidocanol administration, expression decreased to 47% of pretreatment levels (Figure 5A). Expression returned to pretreatment levels by 2 weeks in these mice. Expression at 1 day after polidocanol administration was significantly decreased in these mice, and did not significantly increase by Day 21 after treatment. Expression in the mice treated with the *mCC10*–Cre vector was reduced to 14% of pretreatment levels by 2 weeks after polidocanol administration, and returned to pretreatment levels by 21 days after treatment (Figure 5B). Expression in the mice receiving *CFTR*–Cre was not significantly different from that in naive control mice (data not shown). Expression in mice receiving the *hBPIFA1*–Cre vector was significantly reduced to 0.04% after 1 day. However, expression recovered to pretreatment levels after 14 days (Figure 5C). The increase in expression to pretreatment levels was significant ($P < 0.05$). Luciferase expression in mice receiving the *hWDR65*–Cre vector decreased to 30% of pretreatment levels ($P < 0.05$), and returned to approximately 59% of pretreatment levels ($P < 0.05$) by 3 weeks (Figure 5D). Although the expression in these animals did not completely disappear after polidocanol treatment, the decrease in luciferase expression was significant for each promoter except *mCC10*. These data suggest that the candidate promoter elements driving Cre are integrated in progenitor cell populations.

To examine proliferation after epithelial ablation, sections of nasal airways from animals receiving an intranasal delivery of polidocanol were stained for bromodeoxyuridine (BrdU) to indicate dividing cells (please see the online supplement's MATERIALS AND METHODS). In contrast to animals receiving vehicle only (Figure 5F), abundant BrdU signal was detected in the nasal epithelia of animals receiving the detergent (Figure 5E). In addition, epithelia were less well differentiated in polidocanol-treated mice, as evidenced by an absence of ciliated cells. The rapid drop and recovery of expression after detergent delivery was consistent with the rapid stripping and regeneration of surface epithelia.

CFTR Expression from the *hWDR65* Genetic Element Corrects the Chloride Current Defect in *CFTR*-Null Airway Epithelia

Based on our *in vivo* luciferase expression experiments, we selected the *hBPIFA1* and *hWDR65* candidate promoters as candidates for *CFTR* gene addition studies. To demonstrate the expression of an alternative transgene in human cells, we transduced A549 cells with Ad5-based vectors expressing β-galactosidase from each of the *hBPIFA1* and *hWDR65* candidate gene-regulatory elements. These vectors achieved β-galactosidase expression in a dose-dependent manner (Figure 6A). These results indicate that the candidate elements tested can direct expression in an airway cell line from multiple vector platforms.

Murine models of cystic fibrosis (CF) do not spontaneously develop lung disease, as seen in human patients, possibly because of the presence of alternative chloride channels in the airways. Thus, we chose a porcine model system to investigate corrections of the anion transport defect. *CFTR*-null pigs recapitulate the disease progression characteristic of CF in humans (27). To determine if the anion secretion defect in *CFTR*-null airway epithelial cells could be rescued with wild-type (WT) *CFTR* driven by the novel genetic elements, well-differentiated primary tracheal epithelia from *CFTR*-null pigs, cultured at an air–liquid interface (19), were transduced with an adenoviral vector expressing porcine *CFTR* (*pCFTR*) under the control of the *hWDR65* or *hBPIFA-1* genetic elements (multiplicity of infection [MOI] = 500). An Ad vector expressing *pCFTR* driven by the RSV promoter served as a positive control. As a negative control, an Ad vector without a transgene (empty) (MOI = 50 each) was delivered to cultured airway epithelia. Three days after transduction, chloride secretion was measured in modified Ussing chambers, as previously reported (20). Upon the addition of forskolin and IBMX, cAMP-stimulated chloride secretion was observed in cultures treated with *hWDR65-pCFTR* (Figure 6B). No chloride secretion was evident in cultures treated with *hBPIFA1-pCFTR* (data not shown). The chloride current (I) increased approximately 4 $\mu A/cm^2$ in these cultures. This increase in cAMP-stimulated chloride secretion was significant, compared with the empty control–treated cultures (Figure 6C; $P < 0.05$, according to Student *t* test). This observation indicates that the *hWDR65* genetic element can

drive sufficient expression to achieve functional correction in airway epithelia.

DISCUSSION

Here we demonstrate that several novel, putative promoters have activity in the airway. We show that the 5′ genetic regions from the human *BPIFA1* and *WDR65* genes direct persistent expression in the airway. The expression from these promoters was approximately 30% that of the control RSV promoter (2×10^4 photons/second/cm^2 versus 6×10^4 photons/second/cm^2, respectively). Expression from the upstream region of *CFTR* was also similar to that of the *hBPIFA1* and *hWDR65* promoters (2×10^4 photons/second/cm^2). The relative *in vivo* expression levels from the novel promoters correlate with our *in vitro* experiments. We note that the values of Renilla expression varied among dual promoter constructs from 2–40-fold. This range likely resulted from variability in the transfection efficiency, plasmid quality, and potential promoter read-through that could occur when both expression cassettes are delivered on a single plasmid. In the absence of Renilla normalization, the relative firefly luciferase activities were similar. Our results using 2,150 bases upstream of *CFTR* are supported by previous work using a luciferase reporter construct including 2,244 bases upstream from the transcription start site. Yoshimura and colleagues reported that this sequence supported expression in airway cells at less than 5% of that from the RSV promoter (5). Lower expression of the transgene may be desirable when attempting to correct the anion transport defect in *CFTR*-deficient cells, because the overexpression of CFTR in airway epithelia leads to mislocalization to the basolateral surface, thereby decreasing the net chloride conductance (7).

One of the goals of gene therapy for cystic fibrosis involves the long-term, efficient correction of the anion transport defect. Thus, developing vectors with persistent transgene expression is an important step toward achieving this goal. When we analyzed luciferase expression in animals receiving vectors carrying luciferase driven by the novel promoters *hBPIFA1* and *hWDR65*, expression remained stable over the course of the experiment (3 months), indicating that the promoter activity persists. However, expression from the *hCFTR* gene element significantly decreased at the conclusion of the study (5.5×10^3 photons/second/cm^2 versus 4.8×10^2 photons/second/cm^2, respectively). The transcriptional regulation of *CFTR* remains under investigation, and studies indicate that the regulatory elements are spread over several kilobases (10–12). The 2,150 base fragment we used in these studies may not have contained the necessary regulatory elements to confer sustained expression, or else human-specific regulatory mechanisms were missing in our murine models. The novel *hWDR65* promoter may offer a suitable alternative because it drives expression at a level similar to that of *CFTR*, and it also persists in airway epithelia.

When the detergent polidocanol was administered intranasally, bioluminescent imaging indicated that expression transiently decreased in the nasal passages of mice transduced with vectors carrying JSRV-driven, *hBPIFA1*-driven, and *hWDR65*-driven transgenes. Two weeks after detergent administration, significant expression was again detected only in the animals receiving vectors carrying *hBPIFA1*-driven and *hWDR65*-driven transgenes. The polidocanol treatment of nasal airways causes epithelial cell disruption and ablation, whereas the basal cell layer and basement membrane remain intact (28). This is followed by epithelial regeneration within 7 days, mediated by progenitor cells (29). Luciferase expression decreased after polidocanol administration. We can only speculate about the reasons why polidocanol treatment exerted a much more dramatic effect in *hBPIFA1*–Cre–treated mice, compared with *hWDR65*–Cre–treated mice. Based on previous evidence using FIV-expressing RSV–β-galactosidase (15), *hBPIFA1*–Cre has likely integrated into a broad range of airway epithelial cells, including ciliated and nonciliated surface cells and basal cells. Human *BPIFA-1* may only be active in terminally differentiated surface epithelial cells, which are more susceptible to removal by polidocanol. Perhaps *hWDR65*–Cre is active in a broader range of epithelial cells, including surface and basal epithelia. In this case, polidocanol would not ablate all luciferase-expressing cells. The return of luciferase expression in mice treated with the detergent supports the conclusion that a progenitor cell population was transduced. The transduced cell population may include basal cells, because they are a source of multipotent stem cells (30) in the airways of both humans and mice. Stable expression from a progenitor cell population would lessen the need for repeated administration. However, the overexpression of CFTR in progenitor cells may be detrimental to progenitor cell function. This is an important question to consider for future studies.

When we delivered an adenoviral vector carrying porcine *CFTR* under the control of the novel genetic element from the *hWDR65* gene to *CFTR*-null airway epithelia, we observed a rescue of the anion transport defect. The cAMP-stimulated increase was specific to *CFTR* expression, because the addition of the CFTR inhibitor GlyH-100 decreased the current to levels measured before forskolin/IBMX treatment. This level of chloride current is approximately 10% of that measured in WT epithelia (20). However, our results are similar to those observed by Zabner and colleagues (30), using the lower-expressing Elongation Factor 1 alpha (EF1a) promoter to drive CFTR expression in airway epithelia. EF1α-driven CFTR sufficiently and persistently corrected the chloride secretion defect in human CF airway epithelia (31). Because CFTR overexpression in the airway results in a net decrease in anion transport (7), lower therapeutic transgene expression from endogenous gene internal promoters may be desirable when investigating strategies to treat CF.

Taken together, our results indicate that the novel endogenous genetic element from the *hWDR65* gene may provide a suitable alternative to the stronger viral promoters used in current gene transfer vectors. Furthermore, our findings suggest this novel promoter is active in progenitor cell populations in the airway. This promoter has the potential to mediate long-term CFTR expression in the airway, an important step toward treating CF.

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgments: The authors thank Dr. Barry Stripp for providing the mCC10 promoter sequence. The authors also thank John Fisher, Sarah Ernst, and Phil Karp for technical assistance. The authors acknowledge the assistance of the DNA Sequencing, Central Microscopy, and Gene Transfer Vector Core Facilities at the University of Iowa.

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