Differential Regulation of Rat Aquaporin-5 Promoter/Enhancer Activities in Lung and Salivary Epithelial Cells*

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Aquaporin-5 (AQP5) is a water channel protein that is selectively expressed in respiratory, salivary, and lacrimal tissues. In order to establish the tissue-specific transcriptional programs that underlie its lung- and salivary-specific expression, a 4.5-kilobase pair DNA fragment encompassing the 5′-flanking region of the rat AQP5 gene has been characterized in detail. A major transcription start site utilized in lung and salivary glands has been localized downstream of a TATAA-like motif. Transient transfection assays of –4.3- and –1.7-AQP5-luciferase constructs in AQP5-expressing lung (MLE-15) and salivary (Pa-4) cells and nonexpressing fibroblast (NIH3T3) and epithelial (HeLa) cells demonstrate preferential transcriptional enhancement of reporter activities in MLE-15 and Pa-4 cells. Transient transfection assays of a series of 5′ → 3′ deletion constructs of –4.3-AQP5-luciferase suggest that a common salivary and lung enhancer is located between nucleotides –274 and –139, and a lung-specific enhancer is located between nucleotides –894 and –710. There is one putative lung-specific repressor located in the region of nucleotides –1003/–894 and a common lung and salivary repressor located at nucleotides –503/–385. Moreover, 3′ → 5′ deletions up to –171 and –127 base pairs almost abolish transcriptional activation in salivary and lung cells, respectively. Together, our findings indicate that the combination of enhancer/repressor elements within the proximal 5′-flanking region of rat AQP5 gene dictates its restricted expression in both lung and salivary cells.

The aquaporins constitute a family of homologous intrinsic membrane proteins that function as highly selective water channels and are strongly expressed in tissues the function of which involves rapid water movement across the cell membrane (1–3). Expression of many of the aquaporins is regulated in a highly tissue- and cell-specific manner (4–6). Aquaporin-5 (AQP5)1 is an aquaporin family member that in the adult is selectively expressed in distal lung, salivary glands, lacrimal glands, and corneal epithelium (4, 7–10). Within the distal lung, AQP5 is restricted to the apical surface of alveolar type I (AT1) cells and is not expressed in alveolar type II (AT2) cells, vascular endothelium, or interstitial cell types (11–13). AQP5 expression in rat lung is developmentally regulated, first appearing just before birth, coincident with the appearance of morphologically recognizable AT1 cells, and continuing to increase through adulthood (4, 13). Its restricted spatial and temporal expression patterns have led to the suggestion by us and others that AQP5 transcription is coordinately regulated with AT1 cell differentiation (11–14). However, the cis-active elements that are required for AQP5 cell- and tissue-specific expression and its mode of transcriptional control have not yet been characterized.

The alveolar epithelium of the mammalian lung is composed of two highly specialized epithelial cell types, AT1 and AT2 cells, that differ markedly in their morphological appearances and presumed functional properties (15–17). AT2 cells have been extensively investigated with regard to their role in surfactant production (18, 19). Despite the fact that AT1 cells cover the majority of the alveolar surface of the lung and their presumptive importance for normal gas exchange, little is known of their biochemical or functional properties. The restricted expression of AQP5 in type I pneumocytes in distal lung (11), together with its ability to confer mercury-sensitive osmotic water permeability when expressed in Xenopus oocytes (7), implies a role for AQP5 in transepithelial water movement across the alveolar epithelium. A role for AQP5 in water transport in AT1 cells of the alveolar epithelium is further supported by the recent demonstration that immunoconcentrated populations of AT1 cells exhibit significantly higher mercury-sensitive osmotic permeability than AT2 cells (20).

AT2 cells are believed to serve as the progenitors of AT1 cells for normal maintenance of the alveolar epithelium and during repair following injury (21, 22). In vitro studies also indicate that isolated AT2 cells in primary culture undergo a process of transdifferentiation toward an AT1 cell-like phenotype over time (23–26). The process of transdifferentiation appears to be reversible under certain culture conditions, with expression of AT2 and AT1 cell characteristics being reciprocally regulated (14, 27–29). However, the transcriptional programs responsible for activation or repression of differentiation-related genes in AT2 and AT1 cells are little understood. In particular, the mechanisms underlying the molecular phenotype of AT1 cells and the regulatory processes that lead to distinct patterns of gene expression in AT1 cells in the adult have not been char-

1 The abbreviations used are: AQP5, aquaporin-5; AT1, alveolar type I; AT2, type II; TIS, transcription initiation site; kb, kilobase(s); bp, base pair(s); kbp, kilobase pair; PCR, polymerase chain reaction; TK, thymidine kinase.
acetylated. In the context of the role of AQ5p as a differentiation marker for AT1 cells and in conjunction with its putative role in water transport in AT1 cells, establishment of AQ5p transcriptional control mechanisms and characterization of its cis-elements that underlie its cell- and tissue-specific expression was undertaken.

MATERIALS AND METHODS

Immunofluorescence Microscopy of Whole Lung—Frozen sections were prepared from unfixed, inflated rat lung, snap-frozen in isopentane at –136 °C, and embedded in optimum cutting temperature compound. 2–4-μm sections were briefly fixed in methanol at –20 °C and incubated with a polyclonal rabbit anti-AQ5p (Chemicon International, Temecula, CA) antibody. Sections were then incubated with affinity-purified secondary antibodies conjugated to fluorescein isothiocyanate. Labeled specimens were viewed with an Olympus BX-60 microscope equipped with epifluorescence optics. Controls include substitution of normal rabbit serum.

Isolation and Culture of Alveolar Epithelial Cells—AT2 cells were isolated from adult male Harlan Sprague-Dawley rats by disaggregation with elastase (2.0–2.5 units/ml) (Worthington Biochemical, Freehold, NJ) followed by panning on IgG-coated bacteriologic plates (30, 31). The enriched AT2 cells were resuspended in a defined serum-free medium supplemented with 2% fetal bovine serum (NLogelcorp dI) and incubated at 37 °C. AT2 cell purity (>90%) was measured by trypan blue dye exclusion.

Expression of AQ5p in Alveolar Epithelial Cells in Primary Culture—Steady-state levels of AQ5p mRNA increase markedly as a function of time in cultured alveolar epithelial cells (14). To evaluate the mechanisms underlying the increase in AQ5p that accompanies the transition toward an AT1-like phenotype in vitro, mRNA stability was compared at early (day 3) and at later (day 6) times in culture. Actinomycin D (1 μg/ml) was added to alveolar epithelial cells (AEC) on day 3 or day 6 in culture, and total RNA was harvested at intervals up to 18 h (see below). At a concentration of 1 μg/ml, actinomycin D has been demonstrated to inhibit >90% of transcription (33). Levels of AQ5p mRNA as a function of time were analyzed by Northern analysis.

RNA Isolation and Northern Analysis—RNA was isolated by the acid phenol-guanidine-chloroform method of Chomczynski and Sacchi (34). Equal amounts of RNA (5 or 10 μg) were denatured with formaldehyde, size-fractionated by agarose gel electrophoresis under denaturing conditions, and transferred to nylon membranes (Hybond N +, Amersham Pharmacia Biotech). Blots were probed with an AQ5p-specific cDNA probe (P. Agre, Baltimore, MD) or with PCR-generated probes that hybridize to discrete regions of the AQ5p 5′-flanking region described above. Probes were labeled with [α-32P]dCTP (Amersham Pharmacia Biotech) by the random-primer method using a commercially available kit (Roche Molecular Biochemicals). Blots were washed at high stringency (0.5× SSC (75 mM NaCl, 7.5 mM sodium citrate, pH 7.0) with 0.1% SDS at 55 °C) and visualized by autoradiography. Differences in RNA loading were normalized using a 24-mer oligonucleotide probe for 18S rRNA end-labeled with [32P]ATP.

Cloning and Sequencing of the 5′-Flanking Region of Rat AQ5p Gene—A P1 plasmid rat genomic library (Genome Systems, St. Louis, MO) was screened by PCR using oligonucleotide primers (sense primer nucleotides –2 to +21 and antisense primer nucleotides +198 to +222 relative to the ATG start codon) in order to isolate genomic clones that overlap the 5′-end of the rat AQ5p cDNA (7). Three genomic clones containing the expected 224-bp fragment were identified. Digestion of these clones and of rat genomic DNA with EcoRI, HindIII, PstI, and BamHI, followed by Southern blotting, yielded fragments of identical size that hybridized to a PCR-generated probe that encompasses the 5′-end of the cDNA. An ~5-kb HindIII fragment from one clone was gel-purified, further digested with KpnI, and subcloned into the multiple cloning site of pBluescript (Stratagene, La Jolla, CA) for further characterization. A proximal 1.5-kbp fragment (which spans 164 bp of protein coding sequence and ~1.4 kbp of 5′-flanking DNA) was manually sequenced by the dideoxynucleotide termination method (Amersham Pharmacia Biotech), using a combination of restriction digestion and nested deletions. Sequences were aligned and analyzed using MacAlign and MacVector 6.0 software (Oxford Molecular Group, Campbell, CA).

Analysis of the Transcription Initiation Site—A series of probes specific for contiguous and/or overlapping discrete regions of the 5′-end of the AQ5p cDNA and the adjacent proximal 5′-flanking region was amplified by PCR using rat genomic DNA. The location of the oligonucleotide primer pairs used for amplification of each probe is included in Fig. 3A. Probe labeling and hybridization were performed using the random-primer method. The 5′-end of the ATG start codon was characterized. In the context of the role of AQ5p as a differentiation progenitor determining to AT2 cells located at the corners of the alveoli, whereas the AT2 cell marker SP-A was clearly detected in these AQ5p-negative cells (data not shown).

Transcriptional Regulation of AQ5p Expression in Cultured Alveolar Epithelial Cells—As previously shown, freshly iso-
that on day 6 (t\(_P\) 6) days in culture (acti-
mycin D for 18 h) were approximately 60% and were not signif-
ically different from each other on day 3 and day 6 (p > 0.05). One represent-
ative Northern blot out of three separate experiments is shown. B. alveolar epithelial cells were exposed to actinomycin D (1 \(\mu\)g/ml) on day 3 or day 6 (0 h), and RNA was harvested at intervals up to 18 h. The reductions in AQP5 mRNA levels by 18 h following addition of actinomycin D at t = 0 were not significantly different from each other on day 3 and day 6 (p > 0.05). One representative Northern blot out of three separate experiments is shown.

The reductions in AQP5 mRNA steady-state levels following exposure to actinomycin D for 18 h were approximately 60% and were not significantly different from each other on early (day 3) and later (day 6) days in culture (p > 0.05) (Fig. 2B). Because the rate of degradation on day 3 (t\(_d\) = 15.8 ± 2.4 h) was no greater than that on day 6 (t\(_d\) = 15.4 ± 1.8 h), the increment in AQP5 mRNA on later days likely reflects an increase in the rate of transcription on later days in culture.

**Cloning of the 5‘-Flanking Region of the Rat AQP5 Gene**—A rat P1 plasmid library was screened by PCR using oligonucleotide primer pairs designed according to the published sequence of the most 5‘-end of the rat AQP5 cDNA (7). Three positive genomic clones were identified and analyzed by restriction digestion and Southern blotting. Based on the results of our Southern analyses, we concluded that rat AQP5 exists as a single copy gene and ruled out the possibility that an AQP5 pseudogene or a closely related AQP5 gene was cloned by our approach. Because all three positive clones harbored the same 5-kbp HindIII fragment, one clone was selected for further characterization.

**Structure of the 5‘-Flanking Region of the Rat AQP5 Gene**—The transcription initiation site (TIS) of rat AQP5 gene was first mapped in AQP5-expressing lung cells by Northern analysis with a set of probes generated by PCR using combinations of primer pairs that span overlapping and/or contiguous regions of the 5‘-end of the published rat cDNA sequence and the cloned proximal 5‘-flanking region. Total lung RNA was hybridized with each of the four individually radiolabeled probes as shown in Fig. 3A. Both probes a and b yielded a comparable distinct signal corresponding to the published size of 1.6-kb for AQP5 mRNA, whereas probe c was weakly positive, and probe d was negative. The low signal obtained with probe c implies that only a small region of lung AQP5 mRNA was detected by this probe, indicating the putative TIS is located in the vicinity of the 3‘-end of this probe. Primer extension localized a major TIS to −128 relative to the ATG within the sequence CCAAGGT (Fig. 3B, designated * on the antisense strand) in both lung and salivary gland. In salivary gland, another extended product was reproducibly identified at approximately 150 bases further upstream from the above-identified TIS, suggesting the usage of an additional tissue-specific transcription initiation site. The intensity of the signal for this upstream TIS is relatively less than the product at −128 bp, indicating that the more proximal TIS is the major transcription initiation site in salivary gland in vivo.

Through DNA sequence analyses and comparison with the published rat AQP5 sequence, the 3‘-end of the HindIII-KpnI genomic fragment was determined to be identical to the first 164 bp of the rat AQP5 protein coding sequence and to extend an additional ~4.3 kbp 5‘-upstream of the previously identified AQP5 translator initiator, methionine. The nucleotide sequence of the proximal 1.4 kbp of AQP5 5‘-flanking region is shown in Fig. 4A. A TATAA-like motif (TATAT) is located at –25 bp relative to the TIS identified at –128 bp in lung and
salivary glands, consistent with the notion that transcriptional regulation of AQP5 mRNA is initiated via a TATA-dependent mechanism. The region upstream of this TATAA-like motif is extremely GC-rich, and the exact nature of this GC-rich region in regulating AQP5 expression remains to be determined. Interestingly, no potential TATA box or CCAAT consensus sequence is located immediately upstream of the distal TIS identified in salivary gland. Computer analysis using MatInspector (35) and the TRANSFAC data base (36) of the sequence of the proximal 5'-flanking region reveals putative binding sites for a number of ubiquitous and tissue-enriched transcription factors.

**Fig. 3. Analysis of the transcription initiation site(s) of rat AQP5 gene.** A, Northern analysis. A diagram for a series of probes that span overlapping and/or contiguous regions of the 5'-end of the published rat cDNA sequence and the adjacent proximal 5'-flanking region is shown. This representative Northern blot demonstrates that probes a and b yield a comparable ~1.6-kb signal corresponding to the size of rat AQP5 mRNA. The low intensity signal obtained with probe c, together with the absence of signal with probe d, localizes the putative transcription initiation site in lung to the vicinity of the 3'-end of probe c. B, primer extension. Primer extension was performed on total rat lung or submandibular gland RNA as described under "Materials and Methods." Extended products were analyzed on an 8% denaturing polyacrylamide gel. A major transcription initiation site at –128 bp (*) was identified in RNA prepared from both lung and salivary glands. An additional upstream extended product was identified at ~276 bp (** in salivary gland RNA exclusively. An adjacent sequencing ladder generated using the same primer and the original HindIII-KpnI genomic fragment indicates the nucleotide sequence on the antisense strand surrounding the transcription initiation sites. • represents the possible adjacent nucleotide from which transcription begins.

**Fig. 4. Structure of 5'-flanking region of rat AQP5.** A, nucleotide sequence of the proximal 1453 bp of rat AQP5 5'-flanking region. Putative transcription factor binding sites and a TATAA-like motif identified by computer analysis are underlined. Proximal and distal transcription initiation sites are indicated by bent arrows at –128 and –276 bp, respectively, upstream of the translation initiation site (+1). The primer used for primer extension shown in Fig. 3 is underlined. B, schematic representation of rat AQP5 5'-flanking region shows the restriction map and arrangement of the transcription initiation sites (bent arrows) and TATAA-motif relative to the translation initiation site.
elements to direct expression in a cell- and/or tissue-specific fashion was further evaluated in several different epithelial and nonepithelial cell lines. Among five different lung epithelial cell lines (H441, H358, SV40T11, A549, and MLE-15) investigated for endogenous AQP5 mRNA expression, only MLE-15 cells were found to express measurable levels of AQP5 mRNA by Northern analysis (data not shown). MLE-15 cells were therefore used in transient transfection assays to delineate the location of the promoter/enhancer mediating lung-specific AQP5 expression. Rat Pa-4 cells, an immortalized parotid epithelial cell line that expresses abundant amounts of AQP5 mRNA (data not shown), were also used to elucidate transcriptional activation by transient transfection of AQP5 promoter-reporter constructs in salivary cells.

As shown in Fig. 5, robust luciferase activities, 220- and 670-fold above that of promoterless pGL2-Basic, were detected in transiently transfected AQP5-expressing lung and salivary cells, respectively, using an AQP5-luciferase construct that harbors AQP5 DNA spanning from –4300 to –6. In contrast, only modest activity, 7- and 18-fold above background, was detected in AQP5-nonexpressing NIH3T3 and HeLa cells, respectively. Moreover, the observed preferential transcriptional activation in Pa-4 and MLE-15 cells compared with HeLa and NIH3T3 cells is retained by an AQP5-luciferase construct deleted from –1716 to –1003 bp (data not shown). Together, these results demonstrate that the 5′-flanking region encompasses regulatory elements that can confer preferential lung- and salivary-specific promoter activity.

To delineate regions important for regulating AQP5 gene expression, a series of 5′ → 3′ deletion constructs starting at –1716 bp (Fig. 6, construct A) was analyzed by transient transfection assays in Pa-4 and MLE-15 cells. In MLE-15 cells, there was a drastic 90% decrease in normalized luciferase activity with 5′-deletion to –1003 (Fig. 6, construct A versus construct D), indicating the presence of a distal enhancer between –1716 and –1003 bp that may be involved in modulating AQP5 expression in lung cells. An increase in expression with 5′-deletion from –1003 to –894 (Fig. 6, construct D versus construct E) and from –503 to –385 (Fig. 6, construct G versus construct H) suggests the presence of putative repressor elements within these deleted fragments. A second major decrease in luciferase activity in MLE-15 cells was observed when the DNA fragment of –894/–710 is deleted (Fig. 6, construct E versus construct F), implicating the existence of a putative lung-specific enhancer. By contrast, the variations of luciferase activity in transfected Pa-4 cells by constructs A to I were more moderate with the exception of the deletion of –503/–385 and –1716/–1638. However, an approximate 8- to 10-fold decline in transcriptional activity with further deletion to –139 was noted in both lung MLE-15 and salivary Pa-4 cells, indicating the presence of a putative proximal enhancer element, located between –274 and –139, which appears to be essential for AQP5 expression in both lung and salivary cells. Moreover, it suggests the aforementioned proximal TATAA-like element is necessary for directing AQP5-reporter expression in lung and salivary AQP5-expressing cells.

Transient transfection of a series of progressive 3′ → 5′ deletions of –4300-AQP5-luciferase demonstrated a dramatic reduction in transcriptional activation in MLE-15 cells with deletion of the region between –6 and –127 bp (Fig. 7, construct K versus construct L), indicating the importance of the proximal TIS and its surrounding sequences for directing lung-specific expression. Further deletion to –171 (Fig. 7, construct M) leads to further decreases in transcriptional activity, confirming the importance of the TATA region in directing AQP5 expression in salivary cells and possibly lung cells. The modest decrease in luciferase activities when DNA fragment –385/–274 was deleted (Fig. 6, construct H versus construct D) cannot unequivocally differentiate the contribution of both TISs in salivary Pa-4 cells. However, the remaining activity in construct M transfected Pa-4 cells was not negligible, suggesting that the second distal TIS is functional in salivary cells.
Fig. 7. Effect of AQP5 3′-deletions on transcriptional activity in lung and salivary cells. A series of progressive 3′ → 5′ deletions of −4300/-6-AQP5-luciferase was analyzed by transient transfections in Pa-4 and MLE-15 cells. Cells were co-transfected with a total of 2 μg of the indicated AQP5-luciferase reporter construct and pRL-TK, a Renilla luciferase internal control plasmid. Luciferase activity, assessed 48 h after transfection relative to the promoterless construct pGL2-Basic, is shown as a percentage relative to the −4300/-6-bp AQP5-luciferase construct (construct K). Results represent the mean ± S.E. for three of more transfections assayed in duplicate.

**DISCUSSION**

We have reported herein that AQP5 is expressed exclusively in AT1, but not in AT2 or other cells, by immunofluorescence microscopy in the distal lung. We also demonstrate that the increase in AQP5 expression that occurs during transdifferentiation of primary cultured AT2 cells toward the AT1 cell phenotype is likely regulated, at least in part, at the transcriptional level. As a first step toward elucidating the transcriptional mechanisms that underlie its tissue- and cell-specific expression, we cloned and functionally characterized the 5′-flanking region of the rat AQP5 gene. We have identified a common TIS, 128 bp upstream of the translation initiation site, in both lung and salivary gland mRNA and an additional upstream start site in salivary gland. Furthermore, the region upstream of the TIS mapped to the vicinity of −128 bp constitutes a functional promoter that, in transient transfection assays, is able to direct both lung- and salivary-specific gene expression.

Using primer extension, the major TIS in rat lung and salivary cells has been mapped to a region −30 bp downstream of a TATAA-like motif and 128 bp upstream of the translation initiation site, methionine. The sequence upstream of the TATA-box is GC-rich, similar to promoters of constitutively expressed or “housekeeping” genes. However, unlike constitutively expressed genes (which usually lack a TATA-box and in which transcription is frequently initiated at multiple start sites distributed over a 15–20-bp region), we observed a single major extension product downstream of the TATA motif (37, 38). The importance of the region immediately surrounding this TATAA-like motif for transcriptional activation of the rat AQP5 gene in both lung and salivary cells is confirmed by our functional analyses of 5′- and 3′-deletion constructs of the AQP5 5′-flanking region in transient transfections. 5′-deletion of the DNA region between −274 and −139 bp, encompassing the TATA motif located at −153 to −158 bp, markedly reduces luciferase activity of reporter constructs in both lung and salivary cells, whereas 3′-deletion to −171 virtually abolishes activity in MLE-15 cells and dramatically reduces activity in Pa-4 cells. The close correlation of these functional data with the results of primer extension confirm the importance of the proximal TIS in both tissues and strongly suggests that, similar to other aquaporin family members characterized to date, transcription of the rat AQP5 gene is mediated via a TATA-dependent mechanism (39–42).

Recent analyses of the 5′-flanking regions of the human and mouse AQP5 genes mapped the primary TISs to 518 and 494 bp, respectively, upstream of the translation start site. Despite the comparable patterns of developmental and tissue-specific expression of human, mouse, and rat AQP5 (7, 10, 43), no defined TATA element was identified within the 5′-flanking region immediately upstream of either mouse or human TIS. However, functional characterization of human and mouse AQP5 promoters was not undertaken. It is possible that despite close sequence homology and the expectation that transcriptional activation might be conserved through evolution, the adaptation of a TATA-dependent transcription mechanism in the rat AQP5 gene may represent a genuine species variation. As discussed further below, the TISs identified in previous studies may also represent additional upstream start sites that could function in a tissue- and/or stimulus-specific fashion. Clarification of this issue will await studies to define the functional promoter of human and mouse AQP5 genes.

Interestingly, primer extension using salivary gland RNA yields an additional upstream extension product suggesting additional promoter usage in salivary glands. Transient transfection in MLE-15 cells of an AQP5-luciferase construct deleted to −127 bp from the 3′-end demonstrates an ~90% decrease in transcriptional activity relative to a construct extending to −6 bp. In contrast, deletion of the −127/−6 fragment has negligible effects on transcriptional activity in Pa-4 cells, supporting the notion that the identified upstream promoter is functional and contributes significantly to transcriptional activity in salivary cells. The region upstream of the distal TIS identified in salivary gland is also GC-rich but, unlike the proximal TIS, contains no TATA motif. The use of additional promoters is a common mechanism for regulating differential gene expression in different tissues as well as responsiveness to extracellular stimuli in a tissue-specific fashion (44). For example, the α-amylase gene alternate promoter usage determines different levels of gene expression in salivary gland and liver. In contrast to other aquaporin family members, the expression of which has been shown to be regulated at the transcriptional level in response to exogenous stimuli, such as vasopressin and corticosteroids, few agents have been identified to date that induce AQP5 mRNA expression (45). However, the recent demonstration that interferon-α is able to up-regulate AQP5 expression in human salivary tissue suggests that there may be stimuli that function in a cell-specific fashion to up-regulate AQP5 at the transcriptional level (46).

AQP5 is selectively expressed in lung, salivary glands, lacrimal glands, and corneal epithelium, and within these tissues, it is restricted to specific subsets of epithelial cells (4, 7, 8, 10). The distinctive patterns of developmental and tissue-specific expression of AQP5 suggest that its expression is actively regulated. We demonstrate that the −4300 bp genomic fragment of AQP5 is able to direct preferential expression in lung and salivary epithelial cells, indicating that the 5′-flanking region encompasses the necessary cis-active elements to determine cell specificity. Analysis of the sequence of the 5′-flanking region reveals putative binding sites for both tissue-specific and ubiquitous transcription factors, including thyroid transcription factor 1, hepatocyte nuclear factor-3β, activator protein 1, activator protein 2, nuclear factor I, and SP1. Several of these transcription factors have been shown to trans-activate the promoters of several lung-epithelium-specific genes, including SP-A, SP-B, SP-C, CC16, and T1a, indicating that none of these factors alone determines the characteristic pattern of gene expression.
expression that defines the phenotype of specific subsets of lung epithelial cells (47–51).

Transient transfections of a series of AQP5-luciferase deletion constructs identify distinct regions within the 5′-flanking DNA that differentially regulate transcriptional activation in lung and salivary cells. The region between −894 and −710 appears to be essential for high levels of expression in lung cells. Deletion of this region leads to a marked reduction in luciferase activity in MLE-15 cells, whereas the effects on activity in Pa-4 cells are minimal, suggesting that the deleted fragment encompasses elements that may function as a lung-specific enhancer. The observed increase in activity following deletion of the fragment −1003/−894 is also much more dramatic in lung cells, suggesting the presence of a lung-specific repressor that may potentially be involved in suppressing AQP5 expression in other cell types within the lung other than AT1 cells. Several regions are also identified that appear to function similarly in both lung and salivary cells, with deletion of these regions having somewhat similar effects on transcriptional activation in both cell types. In this regard, deletion of the regions between bp −1716 and −1638 and bp −274 and −139 markedly reduces transcriptional activation in both lung and salivary cells, consistent with the presence of distal and proximal enhancers common to both cell types. In a similar fashion, deletion of −503/ −385 results in enhanced expression in both lung and salivary cells, suggesting the presence of a common repressor in this region that may be important for inhibiting expression in AQP5-nonexpressing tissues. Differential interactions of these distinct cis-regions of the AQP5 promoter/enhancer with transcription factors that are expressed in a cell-specific fashion will be characterized in future studies in order to determine how the balance between these enhancers and repressors leads to cell- and tissue-specific gene expression within lung and salivary gland.

Characterization of the molecular phenotype of AT1 cells and the processes that lead to distinct patterns of gene expression in AT2 and AT1 cells has been limited until recently by the availability of reliable AT1 cell differentiation markers with which to dissect the pathways that underlie cell-specific gene expression. T1a/RTI40, to which as yet no function has been ascribed, is the other known AT1 cell gene the transcriptional regulation of which has been studied to date (51, 52). Initial studies identified a proximal region of the T1a/RTI40 gene that was able to confer preferential expression in rat lung epithelial cells relative to a human fibroblast cell line (51). However, subsequent analyses have been unable to delineate promoter elements in the T1a/RTI40 gene that restrict expression of reporter genes specifically to epithelial cells in vitro or to AT1 cells in the adult lung in vivo (53, 54).

In this study, we confirm localization of AQP5 to AT1 cells of the distal alveolar epithelium in rat lung. Consistent with its exclusive localization to AT1 cells in situ (Fig. 1), we have also previously demonstrated that isolated AT2 cells do not express AQP5 mRNA or protein (14). Furthermore, expression of AQP5 increased in alveolar epithelial cells after several days in primary culture (Fig. 2A), indicating that AQP5 is up-regulated during the transition toward the AT1 cell phenotype that occurs in vitro (14). The close correlation of AQP5 expression with the AT1 cell phenotype, both in vitro and in situ, suggests that analysis of its transcriptional regulation will provide useful insights into the molecular mechanisms that determine the differentiated AT1 cell phenotype.

We have previously demonstrated that under appropriate culture conditions, transdifferentiation of AT2 cells toward the AT1 cell phenotype and the accompanying increase in AQP5 in alveolar epithelial cells with increasing time in culture are reversible. Addition of soluble factors (e.g., keratinocyte growth factor or rat serum) or changes in cell shape induced by retraction of collagen gels after the AT1 cell phenotype has been acquired lead to a reduction in AQP5 levels and reexpression of surfactant apoprotein genes, indicating reacquisition of an AT2 cell phenotype. These findings suggest a model in which expression of either the AT2 or AT1 cell-differentiated phenotype is the result of reciprocal activation and repression of specific sets of differentiation-related genes in each cell type. The absence of changes in mRNA stability demonstrated here makes it likely that the progressive increase in mRNA levels observed in cultured cells over time is associated with an increase in AQP5 gene transcription. These findings suggest that expression of AQP5 in AT1 cells is regulated by interactions with cell-specific trans-activating factors that function to induce or repress gene expression in AT1 or AT2 cells, respectively. The variation in AQP5 levels that occurs as a function of phenotype in alveolar epithelial cells in primary culture offers a unique system for following the interactions between the AQP5 promoter and its cognate trans-activating factors that are up- or down-regulated in a cell-specific fashion during the transition from one phenotype to another.

In summary, we have characterized the 5′-flanking region of rat AQP5, a functional gene with a unique tissue and cellular distribution that in lung and salivary gland has been implicated in water movement across AT1 cells and saliva secretion, respectively. We have identified a functional promoter and delineated the limits of a promoter/ enhancer(s) that drives preferential expression in lung and salivary epithelial cells. The tissue-specific distribution of AQP5 and its differential expression in AT2 and AT1 cells provide a unique means to compare the molecular mechanisms that mediate its expression in lung and salivary gland or that lead to distinct patterns of gene expression in AT2 and AT1 cells. Functional characterization of the AQP5 promoter will form the basis for further analyses of transcriptional regulation of AQP5 in vitro and in vivo in order to understand the molecular mechanisms that determine cell-specific gene expression in AT1 cells.

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