Aqp1 expression in erythroleukemia cells: genetic regulation of glucocorticoid and chemical induction

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Moon, Chulso, Landon S. King, and Peter Agre. Aqp1 expression in erythroleukemia cells: genetic regulation of glucocorticoid and chemical induction. Am. J. Physiol. 273 (Cell Physiol. 42): C1562–C1570, 1997.—The aquaporin-1 (AQP1) water channel protein is expressed in multiple mammalian tissues by several different developmental programs; however, the genetic regulation is undefined. The proximal promoter of mouse Aqp1 contains multiple putative cis-acting regulatory elements, and mouse erythroleukemia (MEL) cells are a well-characterized model for erythroid differentiation. Corticosteroid or dimethyl sulfoxide (DMSO) exposure induces AQP1 protein expression in MEL cells, and transcriptional regulation was investigated by transient transfections with Aqp1 promoter-reporter constructs. Dexamethasone induction is abrogated by deletion of two glucocorticoid response elements −0.5 kilobases (kb) from the transcription initiation site. Mutation of the GATA element at −0.62 kb has no effect, whereas mutation of the CACCC site at −37 bp significantly reduces DMSO-induced promoter activity. Hydroxyurea induces expression of AQP1 protein without acting through the proximal promoter. The MEL cell line is a reproducible erythroid model system for studying transcriptional regulation of the Aqp1 gene while determining the consequences on AQP1 protein biosynthesis.

transcriptional regulation; water channels; erythroid tissue; promoter; protein biosynthesis; aquaporin-1

THE PLASMA MEMBRANES of certain mammalian and plant tissues are highly permeable to water due to the presence of aquaporin water transport proteins (for review, see Ref. 5). Rapid flow of water across cell membranes was first demonstrated by expression of human aquaporin-1 (AQP1) cRNA in Xenopus laevis oocytes (21) and subsequently by reconstitution of highly purified red cell AQP1 protein into proteoliposomes (30). Although freely permeated by water, the AQP1 protein does not conduct protons, cations, anions, or other small molecules (21, 31). On the basis of studies of AQP1, homologous proteins have been sought in other tissues and a family of mammalian aquaporins is now known (for review, see Ref. 11).

Originally isolated from red cell membranes and renal proximal tubules (7, 26), AQP1 is expressed in several secretory and absorptive epithelia, as well as capillary endothelia in many organs (18). Complex patterns of developmental expression of AQP1 have been defined in rat (3, 10, 27): 1) prenatal expression of mRNA with downregulation after birth (cornea, peritoneum), 2) perinatal and postnatal expression (red blood cells, kidney, lung), and 3) expression throughout fetal development and maturity (choroid plexus). Although less pronounced, human developmental expression patterns appear to be similar (2).

Aqp1 RNA was also identified among delayed early response genes expressed in growth factor-stimulated mouse fibroblasts (13) and was noted in freshly dispersed vascular smooth muscle but not after proliferation in culture (25). AQP1 protein expression is induced by corticosteroids in perinatal rat lung, but it is not known whether other hormonal controls exist (10). Genetic understanding of the developmental and tissue expression patterns is limited, and no detailed examination of the AQP1 promoter has been reported.

The structure, organization, and chromosomal localization have been established for human AQP1 (16), and the organization of the gene encoding the mouse homologue Aqp1 was found to be similar (17). Because none of the genetic mechanisms responsible for complex developmental and tissue expression patterns of human AQP1 are known, analysis of the regulation of the mouse gene Aqp1 may provide molecular insight relevant to both species. AQP1 protein is highly abundant in red blood cells (7, 26), and erythroid gene regulation has reached an advanced level of understanding (for review, see Ref. 19). Thus we employed the mouse erythroleukemia (MEL) cell line, a well-established model for inducible erythroid differentiation and globin biogenesis, to delineate the genetic mechanisms behind Aqp1 expression.

EXPERIMENTAL PROCEDURES

Materials. Electrophoresis reagents were from Bio-Rad (Melville, NY). DNA sequencing kits were from United States Biochemical (Cleveland, OH). Enhanced chemiluminescence reagents, [α-32P]dCTP (3,000 Ci/mmol), Rediprime DNA-labeling kit, and d-threo[(chloroacetyl)-1-14C]chloramphenicol were from Amersham (Arlington Heights, IL). RNAzol B solution was from Tel-Test, (Friendswood, TX). Bicinchoninic acid protein assay was from Pierce (Rockford, IL). Promoterless chloramphenicol O-acetyltransferase (CAT) plasmid vector (pBLCAT3) was provided by Dr. M. Daniel Lane, Johns Hopkins University School of Medicine. pSV-bgalactosidase control vector, CAT assay system, and β-galactosidase assay system were from Promega (Madison, WI). pBluescript II (pBSII) was from Stratagene (La Jolla, CA). The Muta-Gen phagemid in vitro mutagenesis kit was from Bio-Rad. Cell culture reagents and molecular biology enzymes were from Gibco-BRL (Gaithersburg, MD). Other reagents and supplies were from Sigma Immunochemicals (St. Louis, MO) or Eastman Kodak (Rochester, NY).

Antibodies. The polyclonal, affinity-purified rabbit antibody to AQP1 reacts with the 4-kDa COOH-terminal domain of the protein (26). Affinity-purified rabbit antibodies to red cell spectrin, band 3, and ankyrin were provided by Dr. Vann Bennett, Howard Hughes Medical Institute, Duke University Medical Center (Durham, NC) (1).
General methods. Except as indicated, standard methods were employed (23). cDNA probes were gel purified and labeled with [α-32P]dCTP (3,000 Ci/mmol) using a Rediprime DNA-labeling kit. DNA sequencing was performed by the dyeoxyribonucleotide chain termination method (24).

Cell culture and membrane isolations. MEL cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 1× antibiotic-antimyocotic mixture (100 units of penicillin, 100 µg of streptomycin, and 0.25 µg of amphotericin B/ml of medium). Cells were grown at 37°C in a humidified atmosphere containing 5% CO2. Cell density was maintained no higher than 106 cells/ml by subculturing every 2 or 3 days. MEL cells were incubated with dimethyl sulfoxide (DMSO; 2%), dexamethasone (1 µM), or hydroxyurea (50 or 100 µM) for a varying number of days. Cells were harvested by centrifugation at 500 g for 5 min and frozen at −85°C for subsequent isolation of membranes or RNA. Cells were homogenized by vigorous pipetting in ice-cold lysis buffer containing 7.5 mM sodium phosphate (pH 7.4), 0.25 M sucrose, and the protease inhibitors phenylmethylsulfonyl fluoride (1 mM) and leupeptin (4 µg/ml) in ice. Homogenates were centrifuged at 800 g for 5 min at 4°C to remove the nuclear fraction. Supernatants were collected, and protein concentrations were measured spectrophotometrically and the bicinchoninic acid protein assay, using bovine serum albumin as the standard. Red blood cell membranes were prepared by five cycles of hypotonic lysis in 5 mM sodium phosphate with the above-mentioned protease inhibitors, followed by centrifugation at 39,000 g. Membranes were solubilized in 1.5% (wt/vol) sodium dodecyl sulfate (SDS).

Immunoblot analysis. SDS-polycrylamide gel electrophoresis (PAGE) was performed using the buffer system of Laemmli (12) and 0.1× 9 × 6-cm 12% acrylamide slabs. Total membrane protein was loaded at 10 µg/sample; SDS-PAGE gels were stained with Coomassie brilliant blue to confirm equivalent loading. Immunoblotting was performed as described (6), using enhanced chemiluminescence and autoradiography to visualize immunoblots. Expression of AQP1 protein was assessed by scanning the films with an UltroScan XL laser densitometer (Pharmacia LKB, Piscataway, NJ). Although the relative units should be regarded as semiquantitative, the films contained signals shown to be with the linear range of dilutional immunoblots (2). Contact prints of autoradiographs were made for presentation of data.

Northern blot analysis. Total RNA was isolated from MEL cells using RNAzol B solution. RNA concentration was measured spectrophotometrically and confirmed with formaldehyde agarose gel electrophoresis. Total RNA (10 µg/sample) was electrophoresed into 1% formaldehyde agarose gels, transferred to nitrocellulose, and probed with 1.6-kilobase (kb) mouse Aqp1 cDNA (800 base pairs (bp) of coding sequence and ~800 bp of 3′ untranslated sequence) previously labeled with [α-32P]dCTP (7 × 106 counts·min−1·ml−1). After overnight incubation, the blot was washed at high stringency (0.1× SSC, 0.1% SDS, 65°C; 1× SSC is 0.15 M NaCl and 15 mM sodium citrate) and exposed to film. After the initial exposure, the blot was stripped by immersion for 5 min in a boiling solution containing 0.5× SSC and 0.1 M EDTA (pH 8.0). The stripped blot was reprobed with 1.6-kb mouse globin cDNA. Mouse globin cDNA plasmid (provided by Dr. Terry Bishop, Johns Hopkins University School of Medicine) was digested with Hha I and labeled with [α-32P]dCTP (7 × 105 counts·min−1·ml−1). After overnight incubation, the blot was washed at high stringency and exposed to radiographic film.

Sequencing and analysis of Aqp1 promoter. The mouse Aqp1 genomic clone was recently isolated and characterized (17). The 8.0-kb Sac I-Sac I subclone contains the 4.4-kb proximal promoter, the first exon and part of the first intron. Multiple overlapping DNA fragments corresponding to the 4.4-kb Aqp1 promoter were subcloned into pBSII to generate templates for DNA sequencing. Selected subclones were further deleted using exodeoxyribonuclease III (23) to make nested deletions of each subclone. Bidirectional DNA sequencing was performed by using T3 and T7 primers, and oligonucleotides were used to resolve sequencing discrepancies. The sequenced 4.4 kb of the Aqp1 proximal promoter was analyzed by comparison with available databases (8).

Plasmids for transient transfections. pBLCAT3 (14) was used to make all of the promoter-CAT constructs in this study. The 2-kb Bgl II-BamHI I Aqp1 proximal fragment was subcloned into the Bgl II site of pBLCAT3 vector to generate a 2-kb CAT construct. Orientation and accuracy of ligation were confirmed by sequencing. This 2-kb CAT construct contains the Aqp1 proximal promoter, the transcription start site (17), and ~50 bp of the 5′ untranslated sequence of Aqp1 ligated into the plasmid 5′ to the initiating ATG in the CAT coding sequence. From this 2-kb CAT construct, exodeoxyribonuclease III deletions and restriction digestions with Hind III and Xba I were used to generate smaller promoter-CAT constructs.

Transient transfections and reporter assays. The 2-kb and smaller promoter-CAT constructs were transfected into MEL cells with pSV-β-galactosidase by electroporation. Immediately after transfection, the cells were divided into groups: control cells, test cells treated with DMSO, or test cells treated with dexamethasone. In the DMSO induction experiments, cells were grown in RPMI 1640 medium and 10% fetal bovine serum with or without 2% (vol/vol) DMSO. In the DMSO induction experiments, cells were incubated in RPMI 1640 medium (without serum) with or without 1 µM dexamethasone. After 24 h, the cells were harvested using reporter lysis buffer (Promega), and CAT assays using...
D-threo[dichloroacetyl-1-14C]chloramphenicol and the β-galactosidase assay were performed as described by the supplier (Promega). The pSV-β-galactosidase vector (Sigma) was used to evaluate transfection efficiency in each experiment; promoter-CAT activities are expressed in relative units (CAT activity normalized to β-galactosidase activity). All promoter activities are representative of two or more independent experiments.

Generation of mutant promoter-CAT constructs. The 2-kb, 0.85-kb, and 68-bp Aqp1 promoter fragments from the corresponding CAT constructs were used as templates for site-directed mutagenesis after subcloning into pBSII. Site-directed mutagenesis was performed with the Muta-Gene phagemid in vitro mutagenesis kit using antisense oligonucleotides: CACCC to CCGGG mutation, 5'-TTTATAGGGCTGCCC-3'; GATA to TATA mutation, 5'-GGAGCCACCTATAATTCTTTAGACAAT-3'. The GATA element (-2619) on the promoter of 0.85-kb CAT construct was mutated to TATA, and CACCC element (-37) on the promoter of 2-kb, 0.60-kb, and 68-bp CAT constructs was changed to

Fig. 2. Inducible Aqp1 mRNA expression in mouse erythroleukemia (MEL) cells. A: MEL cells were incubated with 2% (vol/vol) DMSO or 1 µM dexamethasone, and RNA was harvested on 4 consecutive days. Total RNA (10 µg) from DMSO-treated or dexamethasone-treated (Dex) MEL cells was electrophoresed into a formaldehyde agarose gel, transferred to nitrocellulose, and probed with an [α-32P]dCTP-labeled 1.6-kb mouse Aqp1 cDNA (top). Subsequently, nitrocellulose blot was stripped and reprobed with [α-32P]dCTP-labeled globin cDNA (middle). 18S ribosomal RNA was visualized on blot by ultraviolet light (bottom). B: autoradiographic films corresponding to Northern blots from A were analyzed by densitometry. Amount of Aqp1 mRNA (left) and globin mRNA (right) are expressed in relative units calculated from arbitrary signal intensities. C: after 4 days of incubation, control, DMSO-treated, or dexamethasone-treated MEL cells were spun by cytocentrifuge onto glass slides and stained with Wright's reagent.
RESULTS

Analysis of 4.4-kb Aqp1 proximal promoter. Genomic cloning of the mouse Aqp1 gene was recently reported (17). An 8-kb Sac I-Sac I subclone contains the 4.4-kb promoter, the first exon, and part of the first intron. Multiple overlapping subclones containing the 4.4-kb Aqp1 proximal promoter were generated and used as templates for complete nucleotide sequencing. Computer analysis of available databases (8) identified >20 putative cis-acting regulatory elements of potential relevance to the transcriptional regulation of the Aqp1 gene as well as known regulators of erythroid-specific genes (for review, see Ref. 19). Likewise, multiple CACCC elements reside very close to the transcription start site, and this element is also known to be important in globin gene regulation (15). EKLF (erythroid Kruppel-like factor) is a transcription factor that binds to CACCC, and disruption of the EKLF gene produced β-thalassemia in a knockout mouse model (20).

Induction of Aqp1 mRNA in MEL cells. MEL cells were incubated with 2% (vol/vol) DMSO or 1 µM dexamethasone for 4 consecutive days. Northern analysis clearly demonstrates that DMSO increases mRNA levels for both Aqp1 and mouse β-globin (Fig. 2A). In contrast, dexamethasone increases the Aqp1 message level without increasing mouse globin mRNA. Densitometric analyses of the autoradiography films demonstrated increasing levels of Aqp1 mRNA that reached a maximum after 2 days in DMSO or after 3 days in dexamethasone (Fig. 2B). After 4 days of incubation, control cells and DMSO- or dexamethasone-treated cells were examined for morphological alterations (Fig. 2C). As reported (29), the DMSO-treated cells show a distinct morphological pattern of erythroid differentiation with markedly reduced cell size and synthesis of hemoglobin. In contrast, dexamethasone-treated MEL cells are modestly reduced in size but lack striking morphological alterations.

Induction of AQP1 protein in MEL cells. MEL cells were incubated with 2% (vol/vol) DMSO or 1 µM dexamethasone, and total membranes were isolated over 4 days. Immunoblotting reveals a lack of AQP1 protein expression in the control cells incubated without inducing agent, whereas the nonglycosylated 28-kDa AQP1 polypeptide (AQP1) and the higher-molecular-weight glycosylated AQP1 polypeptide (gly-AQP1) are induced by both DMSO and dexamethasone (Fig. 3A). The 28-kDa band of the autoradiography film was quantitated by densitometry and shows a gradual increase of AQP1 protein, reaching a maximum level after 3 days of DMSO incubation but appearing to

1 The nucleotide sequences reported in this paper have been submitted to the GenBank database with accession no. U67925.

Fig. 3. Inducible AQP1 protein expression in MEL cells. A: MEL cells were incubated in culture medium with 2% (vol/vol) DMSO (in 10% bovine fetal serum) or 1 µM dexamethasone (in serum-free medium), and total cellular membranes were isolated on 4 consecutive days. Total protein (10 µg) was electrophoresed into 12% SDS-polyacrylamide gel electrophoresis (PAGE) slabs and stained with Coomassie blue or analyzed by immunoblot with anti-AQP1 antibody. Rat red cell membranes (RBC) were used as positive control. Immunoblot reveals 28-kDa monomer (AQP1) and glycosylated AQP1 (gly-AQP1). B: 28-kDa band of immunoblot from A was quantitated by densitometry. Duplicate immunoblots were incubated with affinity-purified antibody to band 3 or spectrin (not shown) and analyzed by densitometry. Amount of AQP1 protein (top), band 3 (middle), and spectrin (bottom) are expressed as relative units calculated from arbitrary signal intensities. Although not precisely quantitative, lower signal intensities for band 3 and spectrin reflect substantially lower protein levels.
reach a plateau after 2–4 days in dexamethasone (Fig. 3B). Duplicate immunoblots were incubated with affinity-purified polyclonal antibodies to other red cell membrane proteins, band 3, or spectrin. Densitometric analyses show that DMSO induces a minimal increase of band 3 protein above the low baseline level, whereas dexamethasone induces a somewhat higher level of expression. Spectrin protein biosynthesis is slightly induced by either DMSO or dexamethasone (Fig. 3B).

Chemically inducible transcriptional activation of Aqp1 promoter-CAT constructs. The 2-kb Bgl II-BamH I DNA fragment containing 2 kb of proximal promoter, the transcription start site, and ~50 bp of the 5’ untranslated region of Aqp1 gene was ligated into the pBLCAT3 vector, generating a 2-kb CAT construct from which promoter activity may be determined by the CAT reporter. Smaller promoter-CAT constructs were generated by digestion by exodeoxyribonuclease III or restriction enzymes (Fig. 4A). Each of these constructs was transfected into MEL cells by electroporation along with pSV-β-galactosidase as a transfection efficiency control. Cells were then incubated for 24 h in culture medium containing 10% bovine fetal serum with or without 2% DMSO (vol/vol). The promoter-CAT activities were each normalized to the β-galactosidase activity and are expressed as relative units (Fig. 4B). All constructs exhibit promoter-CAT activities that are severalfold above the level of cells transfected with the promoterless pBLCAT3 vector. The highest basal activity is produced by transfection with the construct containing 0.12 kb of promoter, suggesting the presence of possible repressor elements in the 5’ DNA or inducer elements in the 3’ DNA. Notably, incubation in DMSO induces promoter-CAT activities that are still severalfold above the control cells but vary in magnitude (Fig. 4B, left). For example, the 2-kb and 0.60-kb CAT
constructs are induced sixfold above the control, whereas the 0.85-kb construct is induced to a 12-fold higher level. Even the smallest 68-bp CAT construct shows a 4.5-fold induction.

Dexamethasone-induced expression was evaluated similarly. The 0.85-kb and smaller CAT constructs were transfected into MEL cells along with pSV-β-galactosidase. Cells were cultured for 24 h with or without 1 µM dexamethasone (Fig. 4B, right). After this incubation, the promoter-CAT activities of the 0.85-kb and 0.60-kb CAT constructs are induced five- to sevenfold above the control cells. The highest basal activity is exhibited by the 0.45-kb construct, but this is not further stimulated by dexamethasone, and the smaller promoter-CAT constructs exhibit very small activities that are not enhanced by dexamethasone. Two glucocorticoid response elements (GREs) are located between the 0.60-kb and 0.45-kb DNA sequences, providing a genetic explanation for the dexamethasone induction.

Functional analysis of GATA and CACCC sites. Two potentially important erythroid cell-specific regulatory consensus sequences were identified that could explain the DMSO-induced promoter activity. A GATA consensus sequence resides in the promoter at position −619 from the transcription initiation site. This GATA element is present in the 0.85-kb CAT construct but not the 0.60-kb CAT construct. A CACCC consensus sequence resides in the promoter at position −37 and is present in the 68-bp CAT construct. It was considered that the CACCC element may be critical for DMSO induction and the GATA element may augment the induction.

To test the significance of the GATA element for DMSO induction, the sequence was changed from GATA to TATA in the 0.85-kb CAT construct (Fig. 5). Promoter-CAT assays were performed on cells transfected with the wild-type or mutant constructs. Basal promoter-CAT activities of the wild-type and mutant 0.85-kb CAT constructs are equivalent, and both are induced ~12-fold by DMSO. The 0.60-kb CAT construct that lacks the GATA element has a higher basal level and is induced approximately sixfold by DMSO to a...
comparable level. These data suggest that the GATA element plays no major role for Aqp1 induction by DMSO.

To test the potential significance of the CACCC element in DMSO or dexamethasone induction, a mutation was introduced into the sequence. By using site-directed mutagenesis, the CACCC element located at position −37 with respect to the transcription initiation site was altered to CCGGG in each of the 2-kb, 0.60-kb, and 68-bp CAT constructs (Fig. 6). These wild-type and mutant constructs were tested for DMSO induction. The wild-type 2-kb CAT construct exhibits a sixfold induction above the control level. In contrast, the mutant 2-kb construct shows less than a twofold induction. The wild-type 68-bp CAT construct exhibits a 4.5-fold induction above the control level, but the mutant 68-bp CAT construct shows only a 1.4-fold induction. Similar studies were undertaken after dexamethasone incubation; wild-type and mutant 0.60-kb CAT constructs exhibit equivalent levels of induction. These data implicate the CACCC element as a regulator of Aqp1 promoter activity induced by DMSO but not by glucocorticoids.

Hydroxyurea-inducible expression of AQP1 protein. Certain cancer chemotherapeutic agents including hydroxyurea have been found to ameliorate some of the pathological complications of sickle cell anemia (4). The major pharmacological mechanism of action of hydroxyurea is inhibition of ribonucleotide reductase. Although some clinical benefits of hydroxyurea are derived from induction of fetal globin synthesis that dilutes the concentration of sickle globin, other changes, including reduced red cell hydration, are unexplained and may reflect alterations in AQP1 expression. To explore this possibility, MEL cells were incubated with 50 or 100 µM hydroxyurea for 1 or 3 days, conditions that did not reduce cell viability. Immunoblotting reveals no AQP1 protein expression in untreated cells, however, hydroxyurea treatment induces biosynthesis of the 28-kDa AQP1 polypeptide and the higher molecular weight gly-AQP1 polypeptide (Fig. 7). The 28-kDa band of the immunoblot was quantitated by densitometry, revealing a dose- and time-dependent increase in AQP1 protein expression by hydroxyurea incubation. When promoter–CAT constructs were assayed in hydroxyurea- incubated cells, no significant increase in activity was measured (data not shown), suggesting that hydroxyurea induction of AQP1 protein synthesis is not a result of specific promoter-induced transcriptional control.

DISCUSSION

Our studies were undertaken to define molecular genetic controls behind the complex developmental patterns of AQP1 protein expression. Although dissection of Aqp1 promoters may be analyzed by transfection into representative cell lines, interpretation of such studies is difficult if undertaken in cell lines that fail to express the native protein. The studies reported here represent initial efforts to correlate the regulation of Aqp1 gene transcription with expression of the AQP1 water channel protein in MEL cells. The mouse origin...
of MEL cells should be fully compatible with the mouse origin of the 4.4-kb proximal promoter DNA. In addition, the 80% sequence identity shared by the mouse and human proximal promoters includes complete conservation of the cis-acting regulatory elements analyzed in this study, indicating that the findings are not likely to be species-limited.

MEL cells are a well-studied model for erythroid differentiation and globin biogenesis, and several features of this system underscore the potential usefulness. The induced differentiation pattern of MEL cells in culture closely reproduces erythroid differentiation in vivo, and MEL cells were found to express the AQP1 protein in an inducible manner. Thus the relevance of promoter-CAT assays is directly supported by corresponding determinations of AQP1 protein expression under identical conditions. By this approach, it was found that multiple different promoter elements are active and that the Aqp1 promoter is functionally distinct from the promoters of genes encoding globin, spectrin, and band 3 (the anion exchanger AE1).

DMSO is believed to mimic the actions of hormone and is known to be a potent and reproducible inducer of MEL cell differentiation and globin expression (29). Although GATA1 sites are known to be important in globin gene expression, the GATA1 consensus sequence closest to the transcription start site of the Aqp1 gene was not found to be functional (Fig. 5). In contrast, the CACCC site at position −37 appears to be a major determinant of Aqp1 promoter activity in response to DMSO. It should be noted that the overall biological significance of AQP1 expression in red blood cells is not yet understood. The human blood group antigen Co was identified as an amino acid polymorphism in the AQP1 protein (28), and humans with the Co null phenotype are known to have beneficial actions for treatment of pulmonary dysfunction in premature infancy, asthma, and other clinical disorders of lung and kidney (10). Analyses of induced AQP1 expression in cultured lung or kidney epithelia may prove useful for development of therapeutic agents for clinical disorders.

Induction of AQP1 was also observed in MEL cells treated with low concentrations of hydroxyurea, a drug known to stimulate fetal globin biosynthesis and increase hydration of red blood cells in patients with sickle cell disease (4). The importance of hydroxyurea-induced expression of AQP1 is not yet clear, since the amount of AQP1 protein is small in comparison to DMSO induction and the observation was not found to result from the Aqp1 promoter. Moreover, sickle cell patients treated with hydroxyurea do not appear to have increased levels of AQP1 protein (J. C. Mathai and P. Agre, unpublished observations). Thus, although our studies provide molecular explanations for AQP1 expression in erythroid tissue in response to selected chemical agents and glucocorticoids, the system may be further used to screen for other hormones or naturally active agents that may induce AQP1. Perhaps more importantly, the studies indicate that cell lines representative of other tissues may be similarly analyzed for induction of the gene encoding AQP1 as well as genes encoding other aquaporins.

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