The Human Aquaporin-5 Gene
MOLECULAR CHARACTERIZATION AND CHROMOSOMAL LOCALIZATION*

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The CDNA for the fifth mammalian aquaporin (AQP5) was isolated from rat, and expression was demonstrated in rat salivary and lacrimal glands, cornea, and lung (Raina, S., Preston, G. M., Guggino, W. B., and Agre, P. (1995) J. Biol. Chem. 270, 1908–1912). Here we report the isolation and characterization of the human AQP5 cDNA and gene. The AQP5 CDNA from a human submaxillary gland library contains a 795-base pair open reading frame encoding a 265-amino acid protein. The deduced amino acid sequences of human and rat AQP5 are 91% identical with 6 substitutions in the 22-amino acid COOH-terminal domain. Expression of human AQP5 in Xenopus oocytes conferred mercurial-sensitive osmotic water permeability (Pf) equivalent to other aquaporins. The human AQP5 structural gene resides within a 7.4-kilobase Sall-EcoRI fragment with four exons corresponding to amino acids 1–121, 122–176, 177–204, and 205–265 separated by introns of 1.2, 0.5, and 0.9 kilobases. A transcription initiation site was identified 518 base pairs upstream of the initiating methionine. Genomic Southern analysis indicated that AQP5 is a single copy gene which localized to human chromosome 12q13; this coincides with the chromosomal locations of the homologous human genes MIP and AQP2, thus confirming 12q13 as the site of an aquaporin gene cluster. The mouse gene localized to distal chromosome 15. This information may permit molecular characterization of AQP5 expression during normal development and in clinical disorders.

Discovery of the aquaporin family of water transporters provided a molecular explanation for osmotically driven water transport (Pf) across cell membranes of mammalian and plant tissues (reviewed by Chrispeels and Agre (1994)). When expressed in Xenopus oocytes, aquaporins confer large increases in Pf without conducting small molecules or ions. AQP1 is present in red cells, renal proximal tubules, lung, and other tissues (reviewed by Agre et al. (1993)). The major intrinsic protein of lens (MIP, AQP0) was recently confirmed as a weak water channel (Mulders et al., 1995a). CDNAs encoding AQP2 through AQP5 were isolated by homology cloning (reviewed by Knepper (1994)). In response to vasopressin, AQP2 (Fushimi et al., 1993) is targeted to the apical surface of renal collecting duct principal cells (Nielsen et al., 1993). AQP3 is located at the basolateral membranes of renal collecting ducts (Ishibashi et al., 1994, Ma et al., 1994; Echevarria et al., 1994). AQP4 is the major water channel in brain (Jung et al., 1994; Hasegawa et al., 1994). AQP5 is expressed in rat salivary and lacrimal glands, corneal epithelium, and lung (Raina et al., 1995).

The human genes for several aquaporins have recently been characterized (Moon et al., 1993; Uchida et al., 1994; Pisano and Chepelninsky, 1991; I nase et al., 1995; Mulders et al., 1995b; Yang et al., 1995). Identification of the Colton blood group antigens on the first exofacial loop of AQP1 led to the identification of three Colton-null individuals who had different mutations in AQP1, but total lack of AQP1 is not associated with an apparent phenotype (Smith et al., 1994; Preston et al., 1994). In contrast, a subset of patients with nephrogenic diabetes insipidus have mutations of the AQP2 gene resulting in a lack of clinical response to vasopressin (Deen et al., 1994; van Lieburg et al., 1994). Mice with mutations in MIP (AQP0) suffer from congenital cataracts (Shiels and Bassnett, 1996). Disease relevance of AQP3 and AQP4 remains to be determined. These studies reported here were undertaken to elucidate the gene structure and chromosomal localization of AQP5 as initial steps needed to understand regulation of the gene and to identify possible linkages to human disease.

MATERIALS AND METHODS
Isolation of Salivary cDNA Clones—The 1018-bp rat AQP5 cDNA containing the coding region plus 109-bp 5'-UTR and 114-bp 3'-UTR (Raina et al., 1995) was labeled with [α-32P]dCTP (Amersham) by random DNA labeling (Boehringer Mannheim). This probe was used to screen 3 × 106 plaques of an adult human submaxillary gland xgt11 CDNA library (Clontech) under conditions of moderate stringency (0.5 × SSC, 0.1% SDS, 55°C). Two positive plaques were purified. DNA inserts were released with EcoRI, gel-purified, and ligated into pBS II KS(−) (Stratagene). Both strands were sequenced by the dideoxy nucleotide termination method (U. S. Biochemical Corp.). A 1348-bp cDNA containing the entire coding region was utilized for all future studies. 

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¶§ The abbreviations used are: Pf, coefficient of osmotic water permeability; UTR, untranslated region; RFLP, restriction fragment length polymorphism; bp, base pair(s); kb, kilobase(s); PIPES, 1,4-piperazineethanesulfonic acid.
Isolation and Analysis of Human AQP5 cDNA—The rat AQP5 cDNA was used to isolate a recombinant from a human submaxillary gland cDNA library. The 1348-bp insert contained an open reading frame encoding a 265-amino acid protein. Further sequencing revealed the presence of 513 bp of 5'-UTR and only 40 bp of 3'-UTR. No other ATG translation initiation signals were identified within the 5'-UTR; the 3'-UTR did not contain a polyadenylation consensus sequence. Human and rat AQP5 cDNAs were compared by GAP analysis program (Fig. 1). The deduced amino acid sequences were 91% identical. Like all members of the aquaporin family, tandem repeats of the NPA motif (Asn-Pro-Ala) were present in rat and human cDNAs. The consensus for CAMP-protein kinase A phosphorylation (SRRTS) present in rat AQP5 was also in the human cDNA. Greatest divergence between the rat and human cDNAs was found within the 22-residue carboxyl-terminal domain where six substitutions were identified.

Transmembrane water flow by human AQP5 was evaluated by expression in Xenopus oocytes and measurement of osmotic induced swelling. Oocytes injected with 5 ng of AQP5 cRNA or 50 nl of water were incubated for 3 days at 18 °C. Oocytes were then transferred from 200 to 70 mosm modified Barth’s solution, and increases in volume were detected by videomicroscopy. The human AQP5 oocytes exhibited a 20-fold increase in P, when compared to the water-injected oocytes; this increase was reversed by incubation with β-mercaptoethanol (Fig. 2). Thus, the human AQP5 cDNA encodes an aquaporin which is functionally equivalent to the rat homolog.

Structure of the Human AQP5 Genomic Locus—Three differ-
gent genomic clones were isolated from a human genomic DNA library using the rat cDNA as a molecular probe. The insert from one isolated plaque was released with EcoRI and SalI and subcloned. The restriction map for AQP5 (Fig. 3) was confirmed by digestion with single restriction enzymes or combinations of enzymes followed by Southern analysis (data not shown).

Nucleotide sequencing with primers corresponding to the human AQP5 cDNA revealed the exon-intron boundaries (Fig. 4). Exons 1–4 corresponded to amino acids 1–121, 122–176, 177–204, and 205–265. Nucleotide sequences of these exons were identical to sequences obtained from the human submaxillary cDNA. Exon-intron class 0 boundaries were identified for all four exons. Using exon-specific sense and antisense oligonucleotide primers, introns 1–3 were determined by polymerase chain reaction to be 1.2, 0.5, and 0.9 kb (Fig. 3). Sequencing of 500 bp of the 3′-flanking sequence of the human AQP5 genomic clone revealed a polyadenylation consensus sequence 490 bp from the last amino acid of AQP5 (Fig. 4).

Analysis of the 5′-flanking region of the human AQP5 gene was undertaken to determine the site of transcription initiation and identify possible regulatory elements (Fig. 5A). Sequence obtained from the genomic clone was identical with that found in the 5′-UTR of the human AQP5 cDNA. Using human lung mRNA as a template, a single transcription start site was identified 518 bp upstream from the translation start site of the AQP5 gene (Fig. 5B). This site was 5 bp upstream of the 5′ terminus of the cDNA clone isolated. A major band of identical size was identified using human submaxillary RNA (data not shown). RNase protection using antisense RNA to the DNA region of interest confirmed the presence of a major band of the same approximate size as that seen with primer extension (data not shown). Several common response elements (Fig. 5A) were identified upstream of the transcription initiation site (Prestridge, 1991; Ghosh, 1990). A defined TATA consensus was not identified within the 406 bp of 5′-flanking sequence upstream of the transcription initiation site (Fig. 5A).

Genomic Southern analyses were performed to determine if AQP5 exists as a single copy gene. Human leukocyte DNA from two unrelated individuals was digested with restriction enzymes and hybridized at high stringency with the coding region of the human AQP5 cDNA (Fig. 6). Identical hybridization patterns were found in the DNA from the two individuals. Based on the restriction map for the AQP5 genomic clone (Fig. 3), the anticipated sizes of BamHI-digested DNA are 1.7 and 2.9 kb, which is consistent with the hybridization pattern observed.

**FIG. 2. Osmotic water permeability of oocytes expressing human and rat AQP5.** Oocytes were injected with 5 ng of indicated cRNAs or 50 nl of water. Oocyte swelling was determined as described under "Materials and Methods." Depicted are mean values ± S.D. of 4–6 oocytes (stippled bars), oocytes incubated for 5 min in 1 mM HgCl2 (black bars), or oocytes incubated for 5 min in 1 mM HgCl2 and subsequently incubated for 30 min in 5 mM β-mercaptoethanol (open bars).

**FIG. 3. Restriction map and exon-intron organization of human AQP5.** The 7.4-kb EcoRI-Sall fragment of genomic DNA was used to determine the sites of exons 1–4 using 32P-labeled probes prepared from the human AQP5 cDNA (see "Materials and Methods"). Black rectangles represent coding regions, and open rectangles represent untranslated regions established by cDNA and genomic sequencing (see text). S = Sall, B = BamHI, A = AccI, H = HindIII, K = Kpnl, X = Xhol, E = EcoRI.

**FIG. 4. Exon-intron boundaries of the human AQP5 gene.** Nucleotide sequences surrounding the coding regions of each exon (numbered black boxes) along with corresponding 5′ splice-donor and 3′ splice-acceptor regions were determined by dideoxynucleotide sequencing using oligonucleotide primers generated from the AQP5 cDNA. Partial 5′- and 3′-untranslated sequences with a polyadenylation consensus sequence (underlined) are represented.
served from the genomic Southern (Fig. 6). Moreover, digestion of the original phage clone with EcoRI yielded a fragment of approximately 7.7 kb (not shown) similar to that seen with human DNA (Fig. 6). Like the other aquaporins, AQP5 exists as a single copy gene (Moon et al., 1994; Pisano and Chepelinsky, 1991; Uchida et al., 1994; Yang et al., 1995).

Human Chromosomal Localization of AQP5 Gene—The 7.4-kb genomic clone of the human AQP5 gene was used as a molecular probe for in situ hybridization with chromosomal spreads from normal male lymphocytes (Fig. 7A). Analysis of 71 metaphase cells showed that 28 cells (40%) had at least one pair of signals involving both chromatids of a single chromosome. When these 28 metaphases were photographed on color slide film, 30 paired signals were seen. All signals were located on the long arm of a C-group chromosome (numbers 6–12). To determine the specific chromosome and band location of the signals, the hybridized slides were G-banded by fluorescence plus Giemsa, photographed, and aligned with the color slides to determine sub-band location (Fig. 7B). Twenty eight signals were analyzed after banding and were located on chromosome 12, band q13 (25 signals) and q12 (2 signals).

Mouse Chromosomal Localization of Aqp5 Gene—Mouse genetic studies may provide another approach for analysis of the patterns of aquaporin gene expression. Mouse chromosomal localization of Aqp5 was determined by interspecies backcross analysis using progeny derived from matings of ((C57BL/6J × M. spretus) F1 × C57BL/6J). This interspecies backcross mapping panel has been typed for over 2000 loci that are well distributed among all autosomes and the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and M. spretus DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative RFLPs using the rat AQP5 cDNA probe. The 9.4-kb SpH1 RFLP for M. spretus was used to follow segregation of the Aqp5 locus in backcross mice. The mapping results identified Aqp5 in the distal region of mouse chromosome 15, linked to Pdgfca, Wnt1, and Rarg. Although 104 mice were analyzed for every marker as shown in the segregation analysis (Fig. 8), up to 181 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. Ratios of the total numbers of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere-Pdgfca, Wnt1, and Rarg. Note that Wnt1 and Aqp5 were typed in common in 151 animals, suggesting that the two loci are within 2.0 centimorgans of each other (upper 95% confidence limit).

The interspecies map of mouse chromosome 15 was compared to a composite linkage map that reports map locations of many uncloned mouse mutations (Mouse Genome Data Base, a computerized data base maintained in The Jackson Laboratory, Bar Harbor, ME). Aqp5 mapped to a region of the composite map that did not include any known mouse mutations with a phenotype consistent with defects in the tissues of Aqp5 expression (not shown). Nevertheless, the distal region of
mouse chromosome 15 shares homology with human chromosomes 22q and 12q (Fig. 7). In particular, Wnt1 has been placed on human 12q13. The tight linkage between Wnt1 and Aqp5 suggests that Aqp5 resides on 12q, which was confirmed by our in situ hybridization (Fig. 7).

**DISCUSSION**

These studies have defined the genomic organization of human AQP5. The AQP5 gene is structurally similar to MIP (AQP0), AQP1, and AQP2 (Pisano and Chepelinsky, 1991; Moon et al., 1993; Uchida et al., 1994). The lack of a defined TATA consensus within the region of the transcription initiation site of AQP5 is the first example of a TATA-less promoter in the aquaporin gene family. Transcription begins approximately 30 bp downstream of the TATA box in most eukaryotic genes (reviewed by Sawadogo and Sentenac, 1990). A growing number of eukaryotic genes lack a typical TATA box (reviewed by Roeder, 1991 and Weis and Reinberg, 1992), but some of these genes contain an initiator element (Inr) that serves to organize the various transcription factors at the site of transcription initiation (Smale and Baltimore, 1989). Analysis of the AQP5 promoter failed to reveal any close sequence similarities with previously recognized Inr families (O'Shea-Greenfield and Smale, 1992), although there is currently no clear Inr consensus sequence (O'Shea-Greenfield and Smale, 1992). Studies directed at defining the active promoter regions of the AQP5 gene are currently underway to clarify this issue. The recognition of developmental expression patterns, identification of possible pharmacological modulation of expression, and definition of cellular and subcellular sites of expression may provide additional questions which may be answered by studies of AQP5 gene regulation.

Precise chromosomal localizations of candidate genes may permit linkage to mutant phenotypes. AQP1 has been mapped to human chromosome 7p14 (Moon et al., 1993). The genes for MIP (AQP0) and AQP2 have both been localized to human chromosome 12q13 (Saito et al., 1995). Although the chromosomal localization site for the AQP3 gene was reported to be...
7q36 (Inase et al., 1995), this has been corrected to 9p12-21 (Mulders et al., 1995b). Nevertheless, localization of AQP5 to human chromosome 12q13 is well supported: (i) fluorescence in situ hybridizations with large genomic DNA probes is highly reliable; (ii) AQP5 colocalized with Wnt1 to the distal arm of mouse chromosome 15 which corresponds to human chromosome 12q; (iii) colocalization of AQP5 to the same human chromosomal region as MIP (AQP0) and AQP2 identifies 12q13 as the site of an aquaporin gene cluster. To date, no obvious mutations in mice or humans suggesting AQP5 dysfunction have been mapped to the AQP5 gene locus. It is likely that AQP5 participates in the generation of pulmonary secretions, saliva, and tears, as well as prevention of corneal edema. Thus, definition of the AQP5 gene structure may aid in the identification of the role of AQP5 in normal physiology and may possibly reveal clinical disorders related to this protein.

The chromosomal clustering of MIP (AQP0), AQP2, and AQP5 may reflect similar modes of regulation of the proteins, since AQP5 is most closely related to AQP0 and AQP2 at the amino acid level, and preliminary evidence indicates that phosphorylation is involved in the regulation of both MIP and AQP2 (Nauntofte, 1992). The mechanism by which AQP2 is targeted to the cell surface in response to phosphorylation has been the subject of numerous investigations (reviewed by Agre et al. (1995)). Studies designed to determine the mechanism of AQP5 protein regulation as well as AQP5 gene expression are currently underway.

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