cDNA Cloning, Gene Organization, and Chromosomal Localization of a Human Mercurial Insensitive Water Channel

EVIDENCE FOR DISTINCT TRANSCRIPTIONAL UNITS*

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Two distinct cDNAs encoding a human mercurial insensitive water channel (hMIWC) were cloned from a fetal brain cDNA library. The longest open reading frame of cDNA clone hMIWC1 encoded 301 amino acids with 94% identity to rat MIWC (Hasegawa, H., Ma, T., Skach, W., Matthy, M. M., and Verkman, A. S. (1994) J. Biol. Chem. 269, 5497–5500). A second cDNA (hMIWC2) had a distinct 5′-sequence upstream from base pair (bp) −34 in clone hMIWC1 and contained two additional in-frame translation start codons. Expression of hMIWC cRNAs in Xenopus oocytes increased osmotic water permeability by 20–fold in a mercurial insensitive manner. Cell-free translation in a reticulocyte lysate/microsome system generated single protein bands at 30 kDa (hMIWC1) and 32–34 kDa (hMIWC2) without glycosylation. Northern blot and polymerase chain reaction/Southern blot analysis showed expression of mRNA encoding hMIWC in human brain ~ muscle ~ heart, kidney, lung, and trachea. Analysis of hMIWC genomic clones indicated two distinct but overlapping transcription units from which multiple hMIWC mRNAs are transcribed. The promoter region of hMIWC1 was identified and contained TATA, CAAT, AP-1, and other regulatory elements. Primer extension revealed hMIWC1 transcription initiation at 46 bp downstream from the TATA box. There were three introns (lengths 0.9, 0.2, and 6 kilobases) in the hMIWC1 coding sequence at bp 381, 546, and 627. A distinct 5′-sequence in clone hMIWC2 suggested an alternative upstream transcription initiation site. Two alternatively spliced, nonfunctional hMIWC transcripts with exon 3 deletion and partial exon 4 deletion were identified. A poly(A) + signal sequence was identified at 138 bp downstream of the translation stop codon. Genomic Southern blot analysis indicated the presence of a single copy hMIWC gene; chromosome-specific polymerase chain reaction and in situ hybridization localized hMIWC to human chromosome 18q22. The structural organization of the hMIWC gene represents a first step in definition of hMIWC differential expression, regulation, and possible role in human disease.

Several water-selective channels (aquaporins) have been identified and cloned in mammals, including channel-forming integral protein (CHIP28) (1, 2), water channel-collecting duct (WCH-CD or AQP-2) (3), mercurial insensitive water channel (MIWC)² (4), and possibly glycerol intrinsic protein (GLIP or AQP-3) (5, 6). CHIP28 is widely expressed in epithelial and endothelial cells in fluid-transporting tissues, including kidney proximal tubule and thin descending limb of Henle, choroid plexus, ciliary body, conjunctive tissue, and others (7–9). WCH-CD is expressed exclusively in the apical membrane and subapical vesicles in collecting duct principal cells, where it functions as a vasopressin-regulated water channel (10, 11). GLIP is expressed in the basolateral membrane of collecting duct principal cells and in several extrarenal tissues (5, 6, 12, 13).

MIWC is unique in that it encodes a water-selective channel that is not inhibited by high concentrations of mercurial compounds such as HgCl₂ (4). Rat MIWC is a 301-amino acid hydrophobic protein that spans the membrane six times with its NH₂ and COOH termini in the cytosol (14). An unusual feature of rat MIWC is the tissue-specific expression of both a full-length transcript encoding the functional protein and a short transcript, which had a 165-bp deletion and did not appear to be translated (4). Immunohistochemistry with MIWC peptide-derived antibodies localized rat MIWC protein to the basolateral membrane of kidney collecting duct, ependymal cells lining brain ventricles, astrocytes in brain and spinal cord, and epithelial cells in stomach, trachea, bronchi, ciliary body, conjunctive tissue, salivary gland, and lacrimal gland (12, 13). Immunoblot analysis detected a 30-kDa band in kidney, stomach, and lung and two bands (at 30 and 32 kDa) in brain. Interestingly, MIWC was also expressed strongly in the plasmalemma of skeletal muscle, where immunoblot showed a single band at 26 kDa (13). The functional analysis and tissue distribution of MIWC suggests an important physiological role in the urinary concentrating mechanism, cerebrospinal fluid reabsorption, airway hydration, and glandular secretion.

We report here the cDNA and genomic cloning of a human mercurial insensitive water channel (hMIWC) with greatest mRNA expression in human brain and skeletal muscle. Two distinct cDNAs with different 5′-sequences (hMIWC1 and hMIWC2) were identified that encoded functional water channels. Analysis of cDNA and genomic sequences defined the

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1 The abbreviations used are: MIWC, mercurial insensitive water channel; AQP, aquaporin; MIP, major intrinsic protein of lens fiber; bp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction; PIPES, 1,4-piperazinediethanesulfonic acid.
promoter/transcriptional unit corresponding to hMIWC1, including upstream genomic regulatory elements, transcription initiation sites, intron/exon gene structure, and a downstream polyadenylation signal sequence. A single copy hMIWC gene was localized to chromosome 18q22. Several interesting features of hMIWC expression included the expression of two protein isoforms corresponding to distinct transcriptional units and the presence of short, nonfunctional mRNAs with exon 3 or partial exon 4 deletions. The genomic analysis reported here should permit the examination of hMIWC regulatory mechanisms and the role of hMIWC in a subpopulation of cases of congenital nephrogenic diabetes insipidus, which are not associated with mutations in the V2 receptor or AQP-2 proteins.

**MATERIALS AND METHODS**

Cloning of hMIWC cDNA—A human fetal brain cDNA library (HL3001, Clontech) was screened with a 903-bp DNA probe corresponding to the coding region of rat M1WC (4) labeled with [α-32P]dCTP (random priming, Amersham). Five positive clones yielding two distinct sequences (hMIWC1 and hMIWC2) were isolated out of 2 x 10^6 plaques screened. Fragments released by EcoRI from each clone were subcloned into plasmid pGEM3Zf(+) (Promega) and sequenced by the dye-exchange chain termination method (U. S. Biochemical Corp., sequencing version 2.0, Life Technologies Inc.). hMIWC cDNA was amplified either from cloned hMIWC1 or human brain or kidney cDNA using primers with engineered BglI-5' and XbaI-3' restriction sites. The plasmids were linearized with XbaI, and cRNAs were transcribed/capped using SP6 RNA polymerase (Life Technologies, Inc.). Stage V and VI Xenopus laevis oocytes were defolliculated by collagenase and microinjected with 0.5–5 ng of cRNA in 50 nl of water as previously described (15). After 48 h in Barth's buffer (18°C), a 32P-labeled (random priming) in vitro translated product was separated from the time course of oocyte swelling in response to a 5-fold dilution of the extracellular Barth's buffer with distilled water. For cell-free translation, hMIWC cRNAs were added to rabbit reticulocyte lysate containing [35S]methionine for 1 h at 24°C (16). Microsomal membranes from dog pancreas were added to a final concentration of 8 A260 pmol of hMIWC cDNA coding region. Membranes were washed twice in 2 x SSC, 0.1% SDS at 65°C, and then twice in 0.2 x SSC, 0.1% SDS at 65°C for 15 min each. For PCR-Southern blot analysis, DNA samples were reverse transcribed from 1 μg of human brain, kidney, trachea, and skeletal muscle poly(A)+ RNA using dig(dT)70 random hexomer primers and superscript II reverse transcriptase (Life Technologies, Inc.). After RNase H treatment, the cDNAs were PCR amplified (94°C, 1 min; 55°C, 1 min; 72°C, 2 min; 30 cycles) using primers 5′-GAAGATCTATGGTGGCTTTCAAAGGGGT-3′ corresponding to hMIWC1 bp -7 to +22 (sense) and 5′-CCATGGTGACTCCCAG-3′ corresponding to hMIWC1 bp +80 to +359 (antisense). Positive bands were subcloned and sequenced. For chromosomal in situ hybridization, whole blood from a normal male human adult was cultured in RPMI 1640 medium containing 10% fetal calf serum and phytohemagglutinin (Sigma) for 72 h. Metaphase chromosomes were prepared by standard methods. A 7-kb XbaI fragment of genomic clone G4 was nick-translated with biotin-16 dUTP (Boehringer Mannheim). After denaturation and predenaturation with 1.0 mg of human brain tRNA (Life Technologies, Inc.) for 30 min at 37°C, the probe was hybridized to chromosome spreads for 16 h at 37°C in 50% formamide, 2 x SSC, 20% dextran sulfate, and 0.2 ng/μl human genomic DNA. Slides were washed three times in 50% formamide, 4 x SSC, 3 times in 50% formamide, 2 x SSC at 42°C, and twice in 0.1 x SSC at 23°C. Hybridization signals were detected with FITC-conjugated avidin (Boehringer Mannheim) followed by two rounds of amplification with biotin-conjugated anti-avidin antibody (Sigma) and fluorescein isothiocyanate-conjugated avidin.

**RESULTS**

Two hMIWC cDNA clones with different 5′-nucleotide sequences were isolated from a human fetal brain cDNA library probed by the coding sequence of rat MIWC. One clone (hMIWC1, 1.9 kb) had the most open reading frame encoding a 301-amino acid protein with 94% identity to rat MIWC (Fig. 1A). The amino acid sequence contained NPA and other amino acid motifs conserved in MIP family members (18, 19). There were three consensus sites for N-linked glycosylation and four sites for phosphorylation by protein kinases A and C. As in rat MIWC, residue 188 of hMIWC, corresponding to the mercaptoethanol sensitive residue C189 of CHIP28, was alanine rather than cysteine. Two short forms of the hMIWC transcript were identified (see below), corresponding to indicated deletions of bp 547–627 and bp 628–697. A polyadenylation signal sequence AATAAA was found 138 bp downstream of the translation stop codon.

A second cDNA clone (hMIWC2, 1.5 kb) had identical DNA sequence to hMIWC1 from position –34 onward but a different upstream sequence. The additional 5′-sequence encoded two in-frame methionines, designated M1 and M2 (Fig. 1B), which extended the amino terminus by 40 and 22 amino acids, respectively. The extended hydrophilic amino terminus contained two consensus sequences for phosphorylation by protein kinases C and three cysteine residues. The ATGs in hMIWC1 and hMIWC2 were favorable for Kazak’s sequences for translation.
The Genomic Structure of Water Channel MIWC

Fig. 1. cDNA and deduced amino acid sequence of hMIWC. A. Amino acids in parentheses are for rat MIWC (4) where they differ from hMIWC. Intron positions are indicated. Boxes correspond to deletions in exon 3 (solid) and a portion of exon 4 (dashed). An in-frame stop codon in the 5′-untranslated region and a downstream poly(A) signal sequence are underlined. B. Different 3′-sequence of clone hMIWC2. The sequence to the left bracket is identical with that of hMIWC1. An upstream in-frame stop codon is underlined, and two additional consensus sites for phosphorylation by protein kinase C are shown.

initiation, whereas hMIWC2M1 had a poor Kozak's sequence (T at position –3). cRNAs encoding three full-length forms of hMIWC (hMIWC1, hMIWC2M1, and hMIWC2M2) and two truncated forms (hMIWC1[33] and hMIWC1[44]) were in vitro transcribed and expressed in Xenopus oocytes. hMIWC2M1 and hMIWC2M2 refer to cDNA constructs in which M1 or M2 comprised the amino terminus (see "Materials and Methods"). hMIWC1[3] and hMIWC1[4] indicate cDNAs with exon 3 or partial exon 4 deletion. Fig. 2A shows that osmotic water permeability (Pf) was strongly increased in oocytes expressing hMIWC1, hMIWC2M1, and hMIWC2M2, and rat MIWC compared to water-injected oocytes, although expression of hMIWC2M1 conferred a lesser increase in Pf. Both short forms of MIWC did not increase oocyte water permeability. Oocyte Pf was not inhibited by HgCl2 in the three groups of hMIWC-expressing oocytes. Measurements of 14C-urea and glycerol uptake of control and hMIWC-expressing oocytes indicated that hMIWC functioned as a water-selective channel (data not shown). Cell-free translation of hMIWC1 (Fig. 2B) in rabbit reticulocyte lysate generated a single protein band of 30 kDa that did not become glycosylated when pancreatic endoplasmic reticulum-derived microsomes were present during translation. Interestingly, translation of hMIWC2M1 and hMIWC2M2 produced single bands of 34 and 32 kDa, respectively, without a 30-kDa band.

The tissue distribution of hMIWC was determined by Northern and PCR-Southern blot hybridization. Northern blot analysis showed strong expression of a 5.5-kb mRNA in brain and muscle, with two less intense bands at 3.2 and 1.4 kb (Fig. 2C). Prolonged film exposure revealed a similar pattern in heart, kidney, and lung (not shown). To identify spliced hMIWC transcripts, PCR-Southern analysis was carried out using as template cDNAs from human brain, kidney, trachea, and skeletal muscle and exon-derived primers corresponding to exon 1 (bp +325 to +345) and exon 4 (bp +753 to +733) (Fig. 2D). PCR products were blotted and hybridized with a DNA corresponding to the hMIWC1 coding sequence. A major band of ~430 bp and a band of smaller size were revealed. Sequence analysis of the subcloned bands revealed a full-length 428-bp hMIWC fragment, as well as two short forms of hMIWC with an (81 bp) exon 3 deletion or a partial (70 bp) exon 4 deletion (see Fig. 1A). The deleted segment in exon 3 corresponds to a hydrophilic segment between the 5th and 6th transmembrane domain; the partial deletion in exon 4 corresponds to the 6th transmembrane domain of MIWC and results in a shift in reading frame (14). Immunolocalization studies were performed in human tissues with a rabbit polyclonal antibody raised against a synthetic COOH terminus peptide as previously described (12, 13); hMIWC protein localized to basolateral membrane of principal cells in kidney-collecting duct and to skeletal muscle sarcolemma, similar to results reported in rat (data not shown).

Screening of a human genomic library using a DNA probe corresponding to the hMIWC coding sequence yielded two overlapping fragments of 20 and 18 kb, designated G4 and G7, respectively (Fig. 3). Initial maps of the coding regions were constructed by Southern hybridization after digestion with multiple restriction enzymes. Exon-intron boundaries were determined by sequence analysis of subcloned genomic fragments with exon-specific primers. All exons and introns 1 and 2 were fully sequenced from a subcloned 7-kb XbaI fragment from clone G7. The intron positions are summarized in Table I and intron-exon boundary sequences are given in Fig. 4A. The three introns in the hMIWC coding sequence were of class 0 and followed the gt-ag rule. Sequence analysis of 860 bp into the 5′-flanking region revealed a TATAAAA element (TATA box) at 385 bp upstream from the ATG translation initiation codon of hMIWC1. In addition, one CAAT box, two E-boxes, and SP1, AP-1, two AP-2, and APRRE elements were identified. A polyeuclytic signal sequence AATAAA was found at 138 bp downstream of the translation stop codon, identical to that
found in cDNA clone hMIWC1. Because cDNA clone hMIWC1 contained 604 bp downstream from the polyadenylation signal sequence, additional downstream polyadenylation sites must exist.

The 5′-untranslated sequence in the hMIWC gene upstream from the translation initiation codon was identical to that of cDNA clone hMIWC1. The transcription initiation site was determined by primer extension using human brain mRNA as template. A single transcription start site was identified at a T residue 332 bp upstream from the translation start of hMIWC1 (Fig. 4B). The different 5′-flanking sequence in cDNA clone hMIWC2 indicates the existence of a splice site at 34 bp upstream from the translation initiation codon (position 300 of the hMIWC gene in Fig. 4A, arrow), where an A-G splicing acceptor consensus sequence was found.

To determine the copy number of the hMIWC gene in the human genome, genomic Southern analysis was performed using human genomic DNA digested with EcoRI, XbaI, SacI, and Apal. The blot was hybridized with a probe corresponding to the hMIWC1 cDNA coding sequence. Fig. 5A shows two positive bands in the XbaI lane (7 and 3.6 kb), EcoRI lane (15 and 0.4 kb), and Apal lane (15 and 12 kb), consistent with the restriction map of the overlapping G4 and G7 genomic clones (Fig. 3). A single positive band of 10 kb was found in SacI lane, consistent with the absence of a SacI site in both the hMIWC cDNA and genomic introns analyzed. The genomic DNA hybridization pattern indicated that hMIWC gene is present as a single copy per haploid human genome.

Chromosomal assignment using the NIGMS human/rodent somatic cell hybrid mapping panel 2 indicated localization to chromosome 18 (Fig. 5B). The hMIWC sequence was confirmed in the positive band obtained with chromosome 18 DNA as template. The same positive band was observed with genomic clone G4 as template (lane G) and with total human genomic DNA (lane H). Chromosomal localization by fluorescence in situ hybridization using a 7.0-kb hMIWC genomic fragment as probe indicated localization to chromosome 18q22 (Fig. 5C).

DISCUSSION

This study reports the cDNA and genomic cloning of a human mercurial insensitive water channel (hMIWC) with 94% amino acid identity to a rat homolog (rMIWC) cloned previously from the laboratory (4). hMIWC and rMIWC transcript expression differed in several respects. Northern blot analysis revealed hMIWC transcripts with three different sizes (5.5, 3.2, and 1.4 kb) in brain and skeletal muscle, whereas a single
mRNA band was found for rMIWC. Two nonfunctional short forms of hMIWC were identified by reverse-transcriptase PCR analysis with exon 3 and partial exon 4 deletion, whereas only a single short form of rMIWC was found with an exon 2 deletion. Furthermore, two additional upstream translation start codons were identified in cDNA clone hMIWC2 from human brain. The different 5′-sequence extended the amino terminus of hMIWC1 protein by 22 and 40 amino acids and indicated a different but overlapping transcription unit. The hMIWC1 and hMIWC2 cDNAs probably explain the two distinct MIWC bands reported on immunoblots of brain tissue (13) and results of the cell-free translation of rat brain cRNA (20). Interestingly, cRNAs corresponding to each of the three ATG codons were in vitro translated as single protein bands and functioned as...

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**TABLE I**

Genomic organization and chromosomal localization of MIP family members.

|                      | MIP     | CHIP28  | WCH-CD  | MIWC    |
|----------------------|---------|---------|---------|---------|
| Exon span (amino acids) |         |         |         |         |
| Exon 1               | 1–120   | 1–128   | 1–120   | 1–127   |
| Exon 2               | 121–175 | 129–183 | 121–175 | 128–182 |
| Exon 3               | 176–202 | 184–210 | 176–202 | 183–209 |
| Exon 4               | 203–263 | 211–269 | 203–271 | 210–301 |
| Intron length (kb)   |         |         |         |         |
| Intron 1             | 0.5     | 9.6     | 3.0     | 0.9     |
| Intron 2             | 0.4     | 0.4     | 0.3     | 0.2     |
| Intron 3             | 1.6     | 0.8     | 0.7     | 6.0     |
| mRNA size (kb)       | 1.5     | 2.8     | 1.6     | 5.5, 3.2, 1.4 |
| Coding sequence (bp) | 789     | 807     | 813     | 903, 969 |
| Chromosome location   | 12q13   | 7p14    | 12q13   | 18q22   |
| Reference             | 23      | 21      | 22      |         |
water channels when expressed in Xenopus oocytes. The presence of multiple transcription units and translation initiation sites has not been found for other proteins in the water channel family.

Table I compares the genomic organization of human MIP and three human water channels examined to date. The hMIWC gene spans >9 kb and contains three introns at positions identical to those of hCHIP28 (21), hWCH-CD (22), and hMIP26 (23), suggesting origin from a common ancestor (19). A Southern blot and fluorescence in situ hybridization indicated that hMIWC is present as a single copy gene at chromosome location 18q22.

Two distinct hMIWC cDNA clones, hMIWC1 and hMIWC2, corresponded to distinct transcriptional units. Primer extension using an antisense oligonucleotide corresponding to 5′-cDNA flanking region of hMIWC1 revealed a single transcription initiation site at a T residue 332 bp upstream from the translation start codon. The sequence contained a TATA box 46 bp upstream from the transcription initiation site, as well as CAAT and E-boxes, and other regulatory elements. These findings, taken together with the identity in the upstream sequences of the hMIWC1 cDNA and hMIWC gene, suggested that this region constitutes the promoter for transcription of hMIWC1 mRNA. Primer extension using oligonucleotides corresponding to 5′-cDNA flanking region of hMIWC2 was not successful, possibly due to the long 5′-untranslated region of hMIWC2.

However, a splice site with an A-G consensus acceptor sequence was suggested by the identity of the hMIWC1 cDNA and hMIWC genomic sequences downstream from position −34 in hMIWC1. The transcription initiation site and promoter corresponding to the hMIWC2 transcription unit is thus located upstream of the hMIWC1 promoter.

Transcription initiation from alternative promoters has been previously reported. Two different size mRNAs of the myosin light chain of the myosin light chain were transcribed by two promoters located 10 kb apart. mRNAs made from distinct transcription initiation sites encoded proteins with different amino terminus sequences in different muscle cell types (24). Similarly, for the angiotensin-converting enzyme, different amino terminus sequences were transcribed from a single gene by a tissue-specific choice of alternate transcription initiation sites (25). Transcription of hMIWC gene from alternative initiation sites appears to produce proteins of different size with presumably different amino termini. The reason for synthesis of different hMIWC isoforms is unclear. The data here indicate that both isoforms are functional water channels. Because hMIWC2 contains additional consensus sites for protein kinase C phosphorylation, the existence of two hMIWC isoforms may be related to regulation of hMIWC protein targeting and/or expression.

Alternative choice of transcription termination and polyadenylation sites (26) appears to be another mechanism to produce multiple hMIWC mRNAs. A poly(A)′ signal sequence AATAAA was found at an identical positions (138 bp downstream of the translation stop codon) in hMIWC1 cDNA and genomic clones. The shortest hMIWC transcript on Northern blot of 1.4 kb probably corresponds to an mRNA initiated at the hMIWC1 transcription initiation site and terminated at this poly(A)′ sequence. The presence of an additional 604 bp downstream of this poly(A)′ signal in the hMIWC1 cDNA clone indicates additional downstream polyadenylation sites corresponding to the longer mRNAs.

The mRNA diversity of hMIWC related to alternative choices of transcription initiation and polyadenylation is increased further by alternative splicing. In contrast to the alternative splicing pattern of rat MIWC mRNA in which a 165-bp coding sequence corresponding to exon 2 is deleted, shorter forms of hMIWC were identified with an (81 bp) exon 3 and a (70 bp) partial exon 4 deletion. In the latter case, a splicing donor site was identified in exon 4. However, protein was not translated in vitro, and cRNA expression did not confer increased water permeability in Xenopus oocytes. The mRNAs containing specific deletions, which appear to be produced in a tissue-specific manner (4), may provide a mechanism at the post-transcriptional level to regulate the expression of functional hMIWC water channels.

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REFERENCES
1. Preston, G. M., and Agre, P. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 11110–11114
2. Zhang, R., Skach, W., Hasegawa, H., van Hoek, A. N., and Verkman, A. S. (1993) J. Cell Biol. 120, 359–369
3. Fushimi, K., Uchida, S., Hara, Y., Hirata, Y., Marumo, F., and Sasaki, S.
(1993) Nature 361, 549–552
4. Hasegawa, H., Ma, T., Skach, W., Matthey, M. A., and Verkman, A. S. (1994) J. Biol. Chem. 269, 5497–5500
5. Ma, T., Frigeri, A., Hasegawa, H., and Verkman, A. S. (1994) J. Biol. Chem. 269, 21845–21849
6. Ishibashi, K., Sasaki, S., Fushimi, K., Uchida, S., Kuwahara, M., Saito, H., Furukawa, T., Nakajima, K., Yamaguchi, Y., Gotoh, T., and Marumo, F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6269–6273
7. Hasegawa, H., Zhang, R., Dohrmann, A., and Verkman, A. S. (1993) Am. J. Physiol. 264, C237–C245
8. Nielsen, S., Smith, B. L., Christensen, E. I., and Agre, P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7275–7279
9. Hasegawa, H., Lian, S. C., Finkbeiner, W. E., and Verkman, A. S. (1994) Am. J. Physiol. 266, C893–C903
10. DiGiovanni, S. R., Nielsen, S., Christensen, E. I., and Knepper, M. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8984–8988
11. Deen, P. M., Verkijk, M. A., Knoers, N. V., Wieringa, B., Monnens, L. A., Van Os, C. H., and Van Oost, B. A. (1994) Science 264, 92–95
12. Frigeri, A., Gropper, M. A., Turck, C. W., and Verkman, A. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4328–4331
13. Frigeri, A., Gropper, M., Katsura, M., Brown, D., and Verkman, A. S. (1995) J. Cell Sci. 108, 2993–3002
14. Shi, L. B., Skach, W. R., Ma, T., and Verkman, A. S. (1995) Biochemistry 34, 8250–8256
15. Zhang, R., Logee, K., and Verkman, A. S. (1990) J. Biol. Chem. 265, 15375–15378
16. Skach, W., Shi, L.-B., Calayag, M. C., Frigeri, A., Lingappa, V. R., and Verkman, A. S. (1994) J. Cell Biol. 125, 803–816
17. Sambrook, J., Fritsch, E. F., Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, p. 2.60, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Verkman, A. S. (1993) Water Channels, R. G. Landes Co., Austin, TX
19. Reizer, J., Reizer, A., and Saier, M. H. (1993) Crit. Rev. Biochem. Mol. Biol. 28, 235–257
20. Jung, J. S., Bhat, R. V., Preston, G. M., Guggino, W. B., Baraban, J. M., and Agre, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 13262–13266
21. Moon, C., Preston, G. M., Griffin, C. A., Jabs, E. W., and Agre, P. (1993) J. Biol. Chem. 268, 15772–15778
22. Uchida, S., Sasaki, S., Fushimi, K., and Marumo, F. (1994) J. Biol. Chem. 269, 23451–23455
23. Pisano, M. M., and Chepelnisky, A. B. (1991) Genomics 11, 981–990
24. Leff, S. E., Rosenfeld, M. G., and Evans, R. M. (1986) Annu. Rev. Biochem. 55, 1091–1117
25. Kumar, R. S., Thekkumkara, T. J., and Sen, G. C. (1991) J. Biol. Chem. 266, 3854–3862
26. Smith, C. W., Patton, J. G., and Nadal-Ginard, B. (1989) Annu. Rev. Genet. 23, 527–577
Baoxue Yang, Tonghui Ma and A. S. Verkman

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