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Short sequence-paper

Characterization of the 3′ UTR sequence encoded by the AQP-1 gene in human retinal pigment epithelium [1]

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Abstract

The complete 3′ UTR sequence encoded by the human aquaporin-1 gene is reported. The sequence encompassed by two cDNA clones showed, 33 nucleotides of 5′ UTR sequence, a coding sequence of 807 nucleotides and 1886 nucleotides corresponding to the complete 3′ UTR sequence. High similarity with 3′ UTR sequences from rat and mouse counterparts was found. Northern blot analysis of several human tissues revealed a 2.8 kbp transcript. These data confirm the existence of water channels in the human retinal pigment epithelium.

Keywords: Water channel; Untranslated region; 3′-UTR sequence; Aquaporin; *AQP-1*; Retinal pigment epithelium; (Human RPE)

The transport of water across the plasma membrane of living cells may occur by simple diffusion through the lipid bilayer or by regulation of the membrane permeability. The description of a new family of proteins involved in the active movement of water through aqueous pores known as water channels (reviewed in Ref. [1]), and most recently referred to as aquaporins [2]; has given insight into the molecular mechanisms of water transport. At least five members of this family have been described currently. The channel-forming integral protein (CHIP) initially described in erythrocytes and renal tubules [3] and functionally defined as a water channel by expression in *Xenopus* oocytes [4] was the first established aquaporin (AQP-1). Subsequently, a water channel localized in the apical membrane of kidney collecting ducts (WCH-CD), whose activity is regulated by vasopressin and has only 42% amino-acid sequence identity with AQP-1, was defined as the second aquaporin (AQP-2) [5]. The third aquaporin (AQP-3) is mainly expressed in the basolateral membrane of kidney collecting ducts and is differentiated from the previous ones by its ability to also transport nonionic molecules such as glycerol and urea [6,7]. A mercurial-insensitive water channel (MIWC) initially described in lung [8], also highly expressed in brain and defined as the hypothalamic osmoreceptor and regulator of vasopressin secretion is recognized as the fourth aquaporin (AQP-4) [9]. Finally, a water channel implicated in the generation of saliva, tears and pulmonary secretions constitutes the fifth aquaporin member (AQP-5) [10]. Among all of them, AQP-1 seems to be the protein most frequently found in polarized epithelia with high water permeability. In the eye for example, AQP-1 has been immunolocalized among other tissues in ciliary body, lens epithelium and corneal endothelium [11,12]. As pointed out by Stamer et al. [12], these tissues are also known to express Na,K-ATPase, an integral membrane protein essential for the active fluid movement across transport epithelia in the eye.

Since this association is also observed in the choroid plexus epithelium, a brain tissue in which the AQP-1 and Na,K-ATPase are apically localized [11,13]. We decide to search for *AQP-1* in the retinal pigment epithelium (RPE), a highly polarized monolayer in the eye, where the Na,K-ATPase is also apically expressed and where all of the subunit sequences of this protein have been recently analyzed [14–16]. Because immunohistochemical studies have failed to demonstrate the presence of AQP-1 in RPE, we have undertaken this issue at the molecular level.

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[1] The sequence data reported in this paper have been deposited in the GenBank under accession numbers, U41517 for clone 1.7 and U41518 for clone 2.3, respectively.

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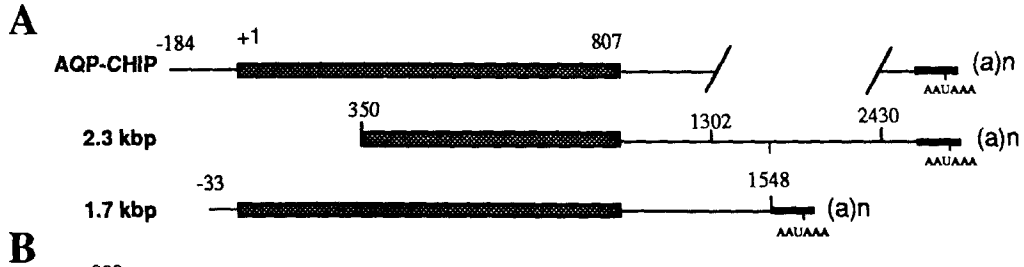
In this study, we describe the molecular characterization of *AQP-1* cDNA clones from human RPE (hRPE) and report the entire 3′ untranslated region (UTR) sequence encoded by this gene. To that end, we extracted poly(A)$^+$ RNA from hRPE cells and obtained an RT-PCR fragment of 680 bp corresponding to position 10–689 of the coding sequence of *AQP-1* [17]. The 680 bp fragment was used as a probe for screening a cDNA library which was primed with oligo(dT) and constructed in the UNI-ZAP XR vector (Stratagene) by using poly(A)$^+$ RNA from cultured fetal hRPE cells obtained from abortuses of 15–24 weeks gestation. Positive cDNA clones were further sequenced with the dideoxy chain termination method by using the Sequenase 2.0 system (USB). Seven positive clones out of $2.5 \cdot 10^5$ plaques were isolated. Four of these clones had a 1.7 kbp insert while in three the insert was 2.3 kbp. As illustrated in Panel A of Fig. 1, both 2.3 and 1.7 kbp cDNAs were overlapping with respect to the published *AQP-1* sequence [17,18]. The sequence encompassed by these two clones contained 33 nucleotides of 5′ UTR, 807 nucleotides of coding sequence encoding a polypeptide of 269 residues and 1886 nucleotides for the entire 3′ UTR sequence. The coding sequence and 5′ UTR of *AQP-1* from hRPE was identical to that previously described for human bone marrow and kidney [17,18]. The 1886 nucleotides of 3′ UTR contained in the 2.3 kbp clone is displayed in Fig. 1, Panel B. The sequence indicated in capital letters, represents a segment of 1129 bp which links the flanking sequences previously reported [17]. Except for an unpublished fragment of 413 bp from human brain (accession # R20024), no clones matching this sequence were found after searching the GenBank.



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gtctctttggagttggaatttcattatatgttaagaaaataaaggaaaatgacttgtaaggtc(a)n      2693
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Fig. 1. Analysis of the human *AQP-1* cDNAs. (A) Schematic representation of the AQP-CHIP clones from human bone marrow and kidney [17,18] and mapping of the 2.3 and 1.7 kbp cDNAs from hRPE. Hatched boxes represent coding sequence, whereas (a)n represents the poly(A) tail. (B) Complete nucleotide sequence of the 3′ UTR of *AQP-1* from hRPE. The new sequence (capital letters) links the flanking sequences previously reported [17]. The polyadenylation signal (underlined) and sequence indicated in boldface at the 3′ end, are represented as solid black boxes in panel A.

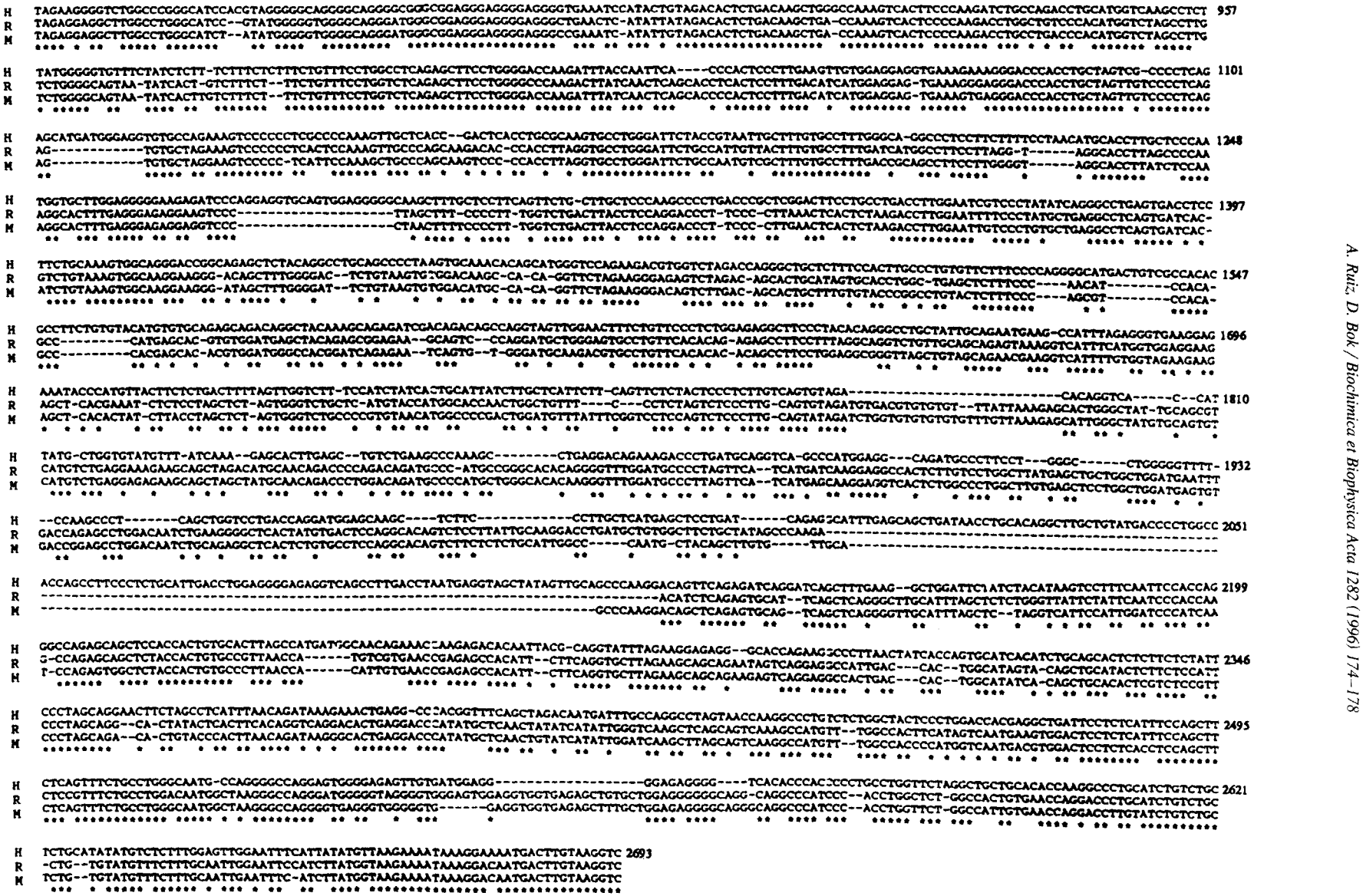

Fig. 2. Alignment of the 3′ UTR sequences encoded by the *AQP-1* gene from several species. Nucleotide sequences from hRPE (H), rat brain (R) [19] and mouse fibroblasts (M) [20] were analyzed using the Clustal multiple sequence alignment included in the PCGENE program (IntelliGenetics) with the following parameter settings, window size: 10, unit gap cost: 10, gap penalty: 5. Asterisks represent identity. Dashes were incorporated to maximize alignment. Nucleotide positions for the human sequence are indicated on the right.

A peculiarity in these two clones was the fact that clone 1.7 showed a shorter 3′ UTR, than the 2.3 clone. However, they conserved the same AAUAAA polyadenylation signal and 3′ end sequence (Panel B, sequence in boldface) as in the published *AQP-1* (Panel A, solid black boxes). In order to analyze this finding, we performed RT-PCR experiments using poly(A)$^+$ RNA from hRPE and a set of primers which spanned the region between 1302 and the 3′ end. The idea was to generate two possible fragments of different size, to account for messages with a long and a short 3′ UTR, respectively. Only the fragment corresponding to the large 3′ UTR was amplified (data not shown). Therefore, this observation could be the result of a complex secondary structure in this region of the *AQP-1* RNA messenger.

Alignment of 3′ UTR sequences of *AQP-1* from rat brain [19], mouse fibroblasts [20] and hRPE showed a high nucleotide similarity (Fig. 2). The conservation of the 3′ UTR from these species may have important implications for the mRNA distribution and expression of the active protein. Interestingly, a large region of 118 nucleotides (from 2004 to 2120) was unique to the human sequence. A region which could be used as a human specific probe in future in situ hybridization assays.

Northern blot analysis was performed to determine relative abundance and tissue distribution (Fig. 3). 1 μg of poly(A)$^+$ RNA from hRPE was electrophoresed in a 1.2% agarose-formaldehyde gel and blotted onto a nylon membrane. A filter membrane containing 2 μg of poly(A)$^+$ RNA from several human tissues (Clontech cat. # 7760-1) was also included in the analysis. These filters were hybridized with the 2.3 kbp cDNA labeled with [α-^{32}P]dCTP by Nick translation. A 2.8 kbp transcript was detected in hRPE mRNA in close agreement with the 2728 bp spanned by our cDNA clones (panel A). A transcript with the same size was also found in heart, lung, skeletal muscle, kidney and pancreas (panel B). After longer exposure of samples in panel B, weak bands were also detected in brain, placenta and liver mRNAs (panel C). The same results were obtained when the filters were probed with the 1.7 kbp cDNA clone. Interestingly, no transcripts other than 2.8 kbp were detected in skeletal muscle and lung mRNAs unlike the ones found in total RNA from the same tissues in rat [18].

Recently, using polyclonal anti-AQP-1 antibodies kindly provided by Dr. Peter Agre at Johns Hopkins University we have been able to detect a 27.5 kDa band by Western blot analysis of hRPE cells. This molecular mass is typical of the AQP-1 protein (data not shown). Our current experiments on protein localization will resolve the question whether this protein is apically localized like the Na,K-ATPase. If this is the case, it is tempting to hypothesize that other undiscovered Aquaporins could be responsible for the promotion of water transport across these cells.

Recently, the *AQP-1* gene has been suggested as a potential candidate for Dominant Cystoid Macular Dystrophy (DCMD), a disease characterized by dysfunction of the RPE [21]. Thus, it is reasonable to consider our results as a tentative starting point for comparative analysis with samples from patients carrying this disease.

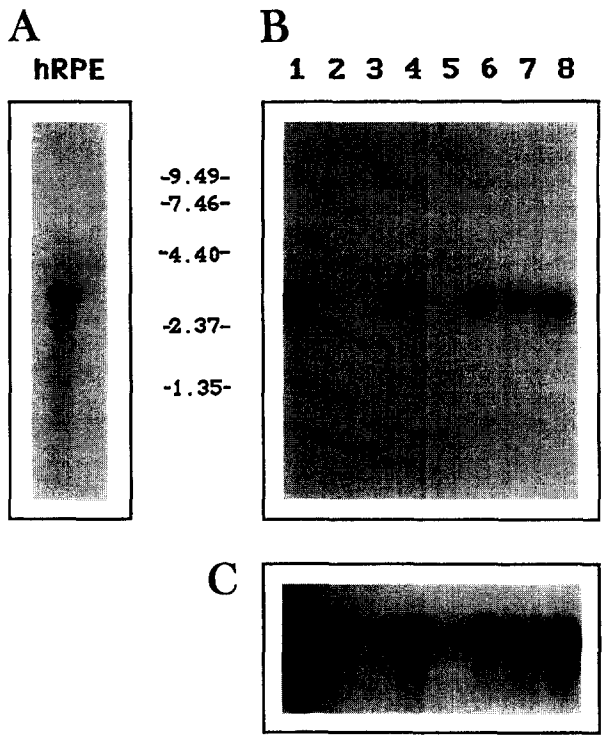

Fig. 3. Northern blot analysis of *AQP-1* mRNA in human tissues. (A) 1 μg of poly(A)$^+$ RNA from hRPE cells, and (B) 2 μg of poly(A)$^+$ RNA from heart (1), brain (2), placenta (3), lung (4), liver (5), skeletal muscle (6), kidney (7) and pancreas (8) were hybridized with a ^{32}P-labeled 2.3 kbp cDNA probe specific for *AQP-1* and exposed for 2 h (A and B). A 16 h exposure of panel B is also shown (C). Size of the RNA markers is indicated in kbp.

In summary, we have demonstrated the presence of *AQP-1* in hRPE. The data support the existence of water channels which provide a mechanism for movement of water in conjunction with the active ion transport performed by the Na,K-ATPase and other transporters in these cells.

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