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Identification of New M23A mRNA of Mouse Aquaporin-4 Expressed in Brain, Liver, and Kidney

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Abstract—Aquaporins (AQPs) belong to a transmembrane protein family of water channels that are permeable to water by the osmotic gradient. There are two isoforms of mouse AQP4 – M1 and M23. Their balance in the cell determines water permeability of the plasma membrane. These two isoforms are encoded by three mRNAs: M1 isoform is encoded by M1 mRNA and M23 isoform is encoded by M23 and M23X mRNAs. Here we found a new fourth mRNA of mouse AQP4 – M23A mRNA. The start of transcription is different for M23A mRNA from all the known AQP4 mRNAs. The 5′-untranslated region (5′-UTR) of M23A mRNA is encoded by four new exons (A, B, C, and D), which are located in the 5′ region from exon-0 of the *AQP4* gene. Alternative splicing between the exons-A, -B, -C, and -D leads to formation of multiple variants of M23A mRNA. We cloned six of these variants, all of which code full length M23 isoform of AQP4. Using RT-PCR we detected tissue-specific expression of the new M23A and already known M23, M23X, and M1 mRNAs. The M23A mRNA is expressed mostly in kidney, liver, and brain. Analysis of mRNA 5′-UTR structure showed low translation efficacy for M1 mRNA in comparison with high translation efficacy for M23A, M23X, and M23 mRNAs. We propose that AQP4 expression is controlled tissue-specifically by independent promoters. Thus multiple AQP4 mRNAs may allow long-term regulation of the balance between M1 and M23 AQP4 isoforms in the cell and thus water permeability of the plasma membrane.

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Key words: aquaporin-4, mRNA, gene, exon, alternative splicing, mouse

Aquaporin-4 (AQP4) is a member of a transmembrane protein (water channel) family that provides passive regulated transport of water molecules across the cell membrane by the osmotic gradient. It plays a pivotal role for tissue water homeostasis. AQP4 is expressed in astrocyte end-feet in the brain, epithelial cells of the kidney collecting duct, Muller cells of retina, lung epithelia, salivary gland, parietal cells of the stomach, liver cholangiocytes, colon and small intestine epithelia, skeletal muscle, and cardiocytes [1, 2]. It is suggested that AQP4 regulates water permeability of the blood–brain barrier and plays a key role in the development of brain edema [3]. *AQP4* deletion in mice reduces brain edema after acute water intoxication and ischemic stroke [4]. In humans, AQP4 was shown to be upregulated in brain inflammations associated with edema [5]. It is believed that inhibition of AQP4 could be the new therapeutic strategy for treatment of brain edema [3, 5].

In mammals there are two main AQP4 isoforms expressed – M1 (323 a.a.) and M23 (301 a.a.). They differ with regard to additional 22 a.a. at the N-terminus of the M1 isoform. In the plasma membrane, AQP4 forms tetramers from M1 and M23 isoforms. AQP4 is unique among aquaporins because its homo- and heterotetramers can assemble in oligomers in the plasma membrane, the so-called orthogonal array particles (OAPs) [6]. Homotetramers consisting only of M1 isoform show equal [6] or larger [7] water permeability than the M23 homotetramers. However, when M23 isoform is in the OAP it increases significantly single-channel osmotic water permeability of M23 isoform compared with the same of M1 isoform [8]. The proportion of M1/M23 depends on the tissue it is expressed in, for example in

Abbreviations: a.a., amino acid residue; AQP4, aquaporin-4; bp, base pair; OAPs, orthogonal array particles; ORF, open reading frame; UTR, untranslated region.

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astrocytes this ratio is 1 : 3 [6, 9]. The size of OAPs grows with increasing ratio of M23/M1 expressed in the cell [10]. It is believed that organization of AQP4 into OAPs is regulated by expression levels of M1/M23 in the cell, while AQP4 in the OAPs show single-channel osmotic water permeability increased by one order of magnitude and therefore increased water permeability of the plasma membrane [11]. In rat there is a third AQP4 isoform – Mz, which is 41 a.a. longer from N-terminus than M1 isoform [12]. Mz is not expressed in human or mouse, since in these species *AQP4* gene has in-frame stop codons at corresponding sites. Mz AQP4 is unable to form OAPs on its own, but it is able to associate with M23 AQP4 isoforms in heterotetramers [13].

Ratio of AQP4 protein isoforms in the cell may reflect expression levels of corresponding mRNAs and depend on their translation efficacy. M1 isoform in all mammals is encoded by one mRNA, while M23 isoform can be encoded by several mRNAs. They have different 5′-UTRs (untranslated regions), and their transcription starts are located at significant distance from each other on the *AQP4* gene. For mouse AQP4 there are two mRNAs – M23 [14, 15] and M23X – that we described previously [16, 17]. It was known that the mouse *AQP4* gene consists of six exons (0, X, 1, 2, 3, 4). M23 mRNA is encoded by exons-1, -2, -3, and -4. M23X mRNA is coded by exon-X, 5′-spliced exon-1, and exons-2, -3, and -4. M1 mRNA is coded by exon-0, 5′-spliced exon-1, and exons-2, -3, and -4. Two ATG starts of translation for M1 isoform (Met1) and for M23 isoform (Met23) are in the same open reading frame (ORF) and are located in exons-0 and -1, correspondingly. The stop codon is identical for all isoforms and is located in exon-4. In other mammals the following AQP4 mRNA homologs have been identified: in human M1 and M23 and its shorter variant M23sv [9, 18], in bull M1 and M23X [19], in sheep M1 and M23 [20], in kangaroo rat M1 and M23 [21]. However, in *Rattus norvegicus* rat six AQP4 mRNA forms have been identified. Three variants M1, M23X, and Mz code full length AQP4. Three other variants of M1, M23X, and Mz are lacking exon-2 and encode nonfunctional proteins [12].

By semiquantitative RT-PCR analysis of total RNA from brain, kidney, and lungs of 2-day-old and adult mice, we previously demonstrated that expression of M23 and M23X mRNAs that encode the same AQP4 M23 isoform is tissue- and age-specific [16, 17]. There are several factors known to affect *AQP4* gene expression: hypoxia, hyper osmolality, PKC activation, testosterone, estradiol, and erythropoietin [22-28]. However, it is not known the expression of which AQP4 mRNAs these factors can regulate. Water permeability can be regulated not only by total AQP4 expression levels, but also by ratio between AQP4 isoforms expressed. When using laboratory animals as disease models associated with AQP4 malfunctioning or for studies of AQP4 expression regulation, species-specific organization of the *AQP4* gene and mRNA forms should be taken into consideration. Here we present new data about a previously unknown M23A mRNA and four new exons of mouse *AQP4* gene (A, B, C, and D), together significantly changing our understanding of mouse *AQP4* gene structure and expression.

MATERIALS AND METHODS

DNA sequencing. RT-PCR fragments were separated using electrophoresis in agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen, Germany). Purified DNA fragments were sequenced with the Sanger method using BigDye Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA). For each 15-20 μl of the reaction 0.5-1.0 μl BigDye reagent, 0.3-0.5 pmol DNA, 5 pmol primer, and corresponding volume of 5× reaction buffer were used. Following temperature conditions were used for the reaction: 96°C for 1 min, then 33 cycles (98°C for 10 sec, 50°C for 5 sec, 60°C for 4 min). Purification of reaction from residual BigDye reagent was performed by isopropanol (60% v/v) precipitation.

Total RNA purification. Organs of adult mice (BALB and C57B strains) were obtained in the Laboratory of Gene Expression Regulation (Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences). Total RNA was purified using the RNeasy Total RNA Kit (Qiagen).

RT-PCR. Reverse transcription reaction was performed using M-MLV Reverse Transcriptase (Promega, USA), RNAsin, and oligo$(dT)_{15}$ primer in total volume of 25 μl with 2 μg total RNA in accordance with the manufacturer's protocol. The cDNA obtained was used as template for PCR with AmpliTaq Gold DNA Polymerase (Applied Biosystems). Temperature conditions for PCR were the following: 94°C (7 min), then 35-40 cycles (93°C (30 sec), 60°C (30 sec), 72°C (35 sec-1 min 35 sec)), 72°C for 7 min.

For crossover PCR (Fig. 1) common sense primer from exon-1 ExA69F (5′-gtgacccttgtcctggagacgcag-3′) was used in a couple with antisense primers located in other exons ExB/-132B (5′-gcttcgcttgctttcctcagtc-3′), Ex/B317B (5′-taatacgctggaaacaggca-3′), ExC358B (5′-gctcataacgctgttagttgtttgtat-3′), ExD500B (5′-aggga-gttgagcagagcgta-3′), AGBPE1 (5′-gaaagattctgcaccatgtg-gc-3′), CBN (5′-ccctctccagagacctaatc-3′), 045 (5′-ggc-gatggtttgctgaacac-3′), Ex/1-658B (5′-tgaaagccaccatgat-gctctctct-3′), Ex2-1078B (5′-aaagtgattattaactccaccagg-3′), Ex3-1250B (5′-atgataactgcgggtccaaa-3′); Ex4-985B (5′-tacggaagacaatacctctcccga-3′).

To localize by RT-PCR the transcription start of M23A mRNA, a number of sense primers placed in consecutive order, ExA35F (5′-tgtgctagccagagttgcattgtgc-3′), ExA69F (5′-gtgacccttgtcctggagacgcag-3′), ExA80F (5′-

cctggagacgcagcacaagaccttt-3′), ExA116F (5′-cctgtcacaacacacaacatcagca-3′) and common antisense primer A4SHTGA (5′-gagtccggaacaaatctgtttccttaatgggtgg-3′) were used.

For RT-PCR analysis of expression of AQP4 mRNA forms in different tissues following sense primers ExA116F for M23A (exon-A), 58F (5′-catcgccaaactgcaagac-3′) for M23 (exon-1), 77F (5′-agcaactgagtttcttgattagg-3′) for M23X (exon-X), 135F (5′-acagagctgcggcaagg-3′) for M1 (exon-0) and a common for all isoforms antisense primer from exon-2 1078B (5′-aaagtgattattaactccaccagg-3′) were used. Mouse GAPDH cDNA was amplified as a control using sense GAPDHFW (5′-gaaggtcggtgtgaacggatttg-3′) and antisense GAPDHBK (5′-tgtaggccatgaggtccaccac-3′) primers. Negative control RT reaction was performed with water instead of reverse transcriptase.

5′-RACE-assay. A dsDNA copy of the 5′-region of M23A mRNA with unknown nucleotide sequence was obtained using total RNA from mouse kidney and liver using SMART RACE cDNA Amplification Kit (Qiagen) and FirstChoice RLM-RACE Kit (Ambion, USA) in accordance with the manufacturer's protocol. Gene-specific primer 1 (GSP1) was Ex4-985B. GSP2 primers were the same as used for crossover PCR analysis from exons-A, -B, -C, and -D.

Cloning of AQP4 M23A mRNA alternatively spliced variants. The following PCR primers were used: sense ExA79F_KpnI ((5′-aacggtacctcctggagacgcagcacaagacct-3′) from exon-A with added *Kpn*I restriction site) and antisense A4SHTGA (from exon-4 with added *Kpn*2I restriction site). Total RNAs purified from mouse kidney, brain, and liver were used for RT. PCR fragments were separated on agarose gel. Marked gel zones were cut out and PCR fragments purified using QIAquick Gel Extraction Kit (Qiagen). PCR fragments were restricted with *Kpn*I and *Kpn*2I and ligated into pBlueScript II SK(+) preliminarily linearized with *Kpn*I and *Xma*I. Clones obtained were analyzed by sequencing. Six variants of M23A cDNA covering 5′-UTR and full-size protein encoding region were subcloned into ORF of pEGFP-N1 vector (Clontech, USA). The stop codon was deleted by use of Quick Change Mutagenesis Kit (Stratagene, USA). The structure of cDNA constructs was confirmed by sequencing. Control experiments of functionally active AQP4 protein expression were made as previously described using a short-term transfection with these cDNAs of CTX TNA2 astrocyte cell line, where endogenous AQP4 is not expressed [29, 30].

Computer analysis of nucleotide sequences. All nucleotide sequences were analyzed by Lasergene (DNASTAR, USA) and Vector NTI 10 (Invitrogen, USA) software. Efficacy of AQP4 mRNAs translation was estimated using Leader_RNA (http://wwwmgs.bionet.nsc.ru/mgs/gnw/leader/, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences).

RESULTS

Identification of new A, B, C, and D exons and a new M23A mRNA of mouse *AQP4*. Previously we cloned the mouse *AQP4* gene and sequenced its 5′-flanking fragment (13,006 bp, GenBank (GB) No. AF219992) overlapping with exons-0, -X and -1 as well as a 8629 bp sequence upstream of exon-0 [16, 17]. By comparing the *AQP4* gene sequence obtained with GB sequences, we found short fragments of the gene that were identical to "AQP4-like" mRNA from mouse kidney cDNA library (GB No. AI115947). Two homology fragments 39 bp (A) and 130 bp (B) were located at a considerable distance upstream of exon-0. Two other homologous fragments corresponded to the central part of exon-1 and 3′-end part of exon-4 of the mouse *AQP4* gene. Analysis of the "AQP4-like" mRNA ORF revealed that it can encode a protein resembling a partial AQP4, consisting of a single transmembrane domain with an intracellular N-terminus and an extracellular C-terminus. We aimed to determine whether these homologous fragments A and B transcribe within known AQP4 mRNAs or within some pre-mRNA that is common for M1, M23, and M23X mRNAs. Using RT-PCR with total RNA from mouse kidney, brain, and liver and a combination of primers from different exons of the gene, we obtained cDNA fragments connecting above-mentioned distant upstream homologous fragments A and B with known exons-1, -2, -3, and -4 of the mouse *AQP4* gene (Fig. 1). Sequencing of these cDNA fragments confirmed the presence of the A and B fragments in gene transcripts. Moreover, in some cDNA structures we found sequences of two more fragments C (105 bp) and D (184 bp) that were identical with the *AQP4* gene. By comparison of cDNA obtained and *AQP4* gene sequences, we found that the A, B, C, and D border sequences are in good agreement with consensus sequences for donor–acceptor sites between exons and introns (Fig. 2).

We concluded that the A, B, C, and D fragments are previously unknown exons of the mouse *AQP4* gene. Using crossover RT-PCR analysis with all possible combinations of sense and anti-sense primers from exons-A, -B, -C, -D, -/1 and sequencing of obtained transcripts, we found an alternative splicing point inside exon-B (Figs. 1 and 2). It is transcribed in two variants – full length exon-B (219 bp) and exon-B shortened from its 5′-end – B2 (130 bp). We have not found in the structure of the mouse *AQP4* gene an exon corresponding to rat Mz mRNA [12], nor any deletion in exon-2, nor other exons that differ from known ones, except for the new exons-A, -B, -C, and -D.

We did not obtain any RT-PCR fragments using sense primers from exons-A, -B, -C, and -D and antisense primers from exons-0, -X, and -1/, where starts of transcription for known AQP4 mRNAs (M1, M23X, and M23, correspondingly; Fig. 1) are located. Therefore exons-A, -B, -C, and -D do not transcribe within any known AQP4 mRNA, but form a fourth distinct AQP4

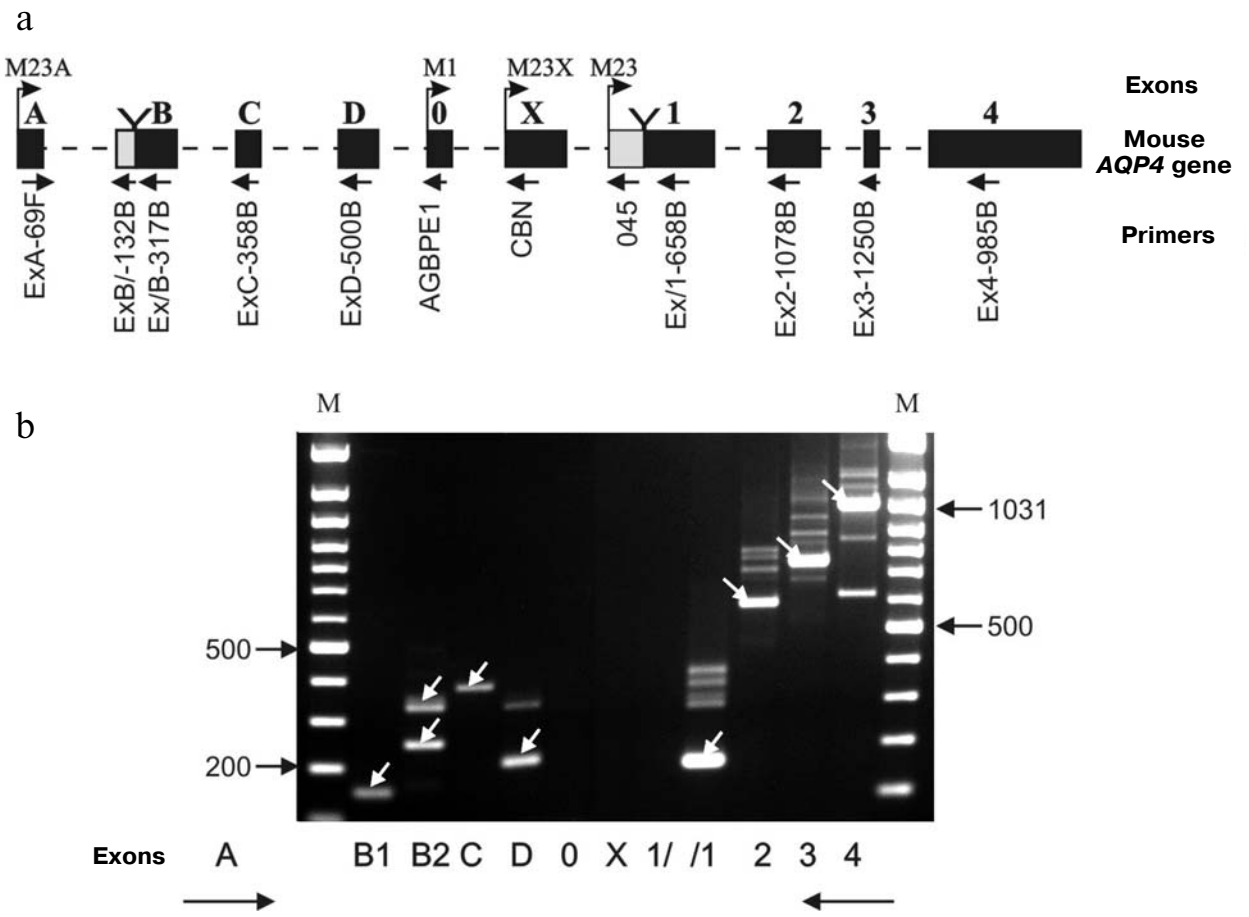

Fig. 1. Crossover RT-PCR between exon-A and other exons of mouse *AQP4* gene. a) Scheme of mouse *AQP4* gene. Primer location is marked by arrows. Exons-B and -1 have internal splicing sites marked with symbol "V", which divide them into B1 and B2 and into 1/ and /1, correspondingly. Starts of transcription for four mRNAs are marked with symbol ⌐→. b) Total RNA from mouse kidney was used as a template for RT-PCR. M line marks Gene Ruler 100 bp Plus DNA marker (Fermentas, Lithuania), length of fragments is in base pairs (bp). Sense primer ExA-69F (exon A) was used in all reactions to confirm the link between 5′-end of new M23A mRNA and all the other exons of the gene. Fragment indicated with arrows were purified from the agarose gel and sequenced, confirming their belonging to the M23A mRNA.

mRNA form. This mRNA also cannot be a common pre-mRNA for three known AQP4 mRNAs. ORF analysis revealed that this new mRNA encodes M23 isoform of AQP4, just like known previously M23 and M23X mRNAs. The new AQP4 mRNA was named as M23A in accordance with location of its transcription start in exon A and isoform M23 that it encodes.

Using crossover RT-PCR with sense primers from exons-A, -B, and -C, and antisense primers from exons-/1, -2, -3, and -4 we obtained several DNA fragments of different length in the same PCR reaction (Figs. 1b and 3). We purified the individual fragments from agarose gel, re-amplified, and sequenced them. These transcripts were also cloned into pBlueScript II SK(+) and analyzed by sequence. Comparative analysis of the nucleotide sequences and the *AQP4* gene sequence revealed that these transcripts correspond to alternative splicing variants of M23A mRNA in A, B, C, and D exon region. A set of alternative splicing variants of M23A mRNA seems to be dependent on tissue where *AQP4* is expressed (Fig. 3). Approximately 50% of clones represented the shortest M23A mRNA variant – A-/1-2-3-4. This mRNA variant is expressed in all three organs, just like A-D-/1-2-3-4. A-B2-C-/1-2-3-4 and A-B2-C-D-/1-2-3-4 variants were cloned only from the brain. Crossover RT-PCR and following sequencing of shorter cDNA fragments demonstrated existence of six other A, B, C, and D exons combinations between themselves (GB No. AY728046-AY728055) and exons-/1-2-3-4 (GB No. AY728043-AY728045). We propose that other alternative splicing variants of M23A mRNA may transcribe but that we were unable to clone them.

Thereby, we demonstrated that mouse AQP4 M23 isoform is encoded by at least three mRNAs – M23, M23X, and the new M23A.

Identification of transcription start of mouse AQP4 M23A mRNA using RT-PCR and 5′-RACE. We determined the approximate transcription start of AQP4

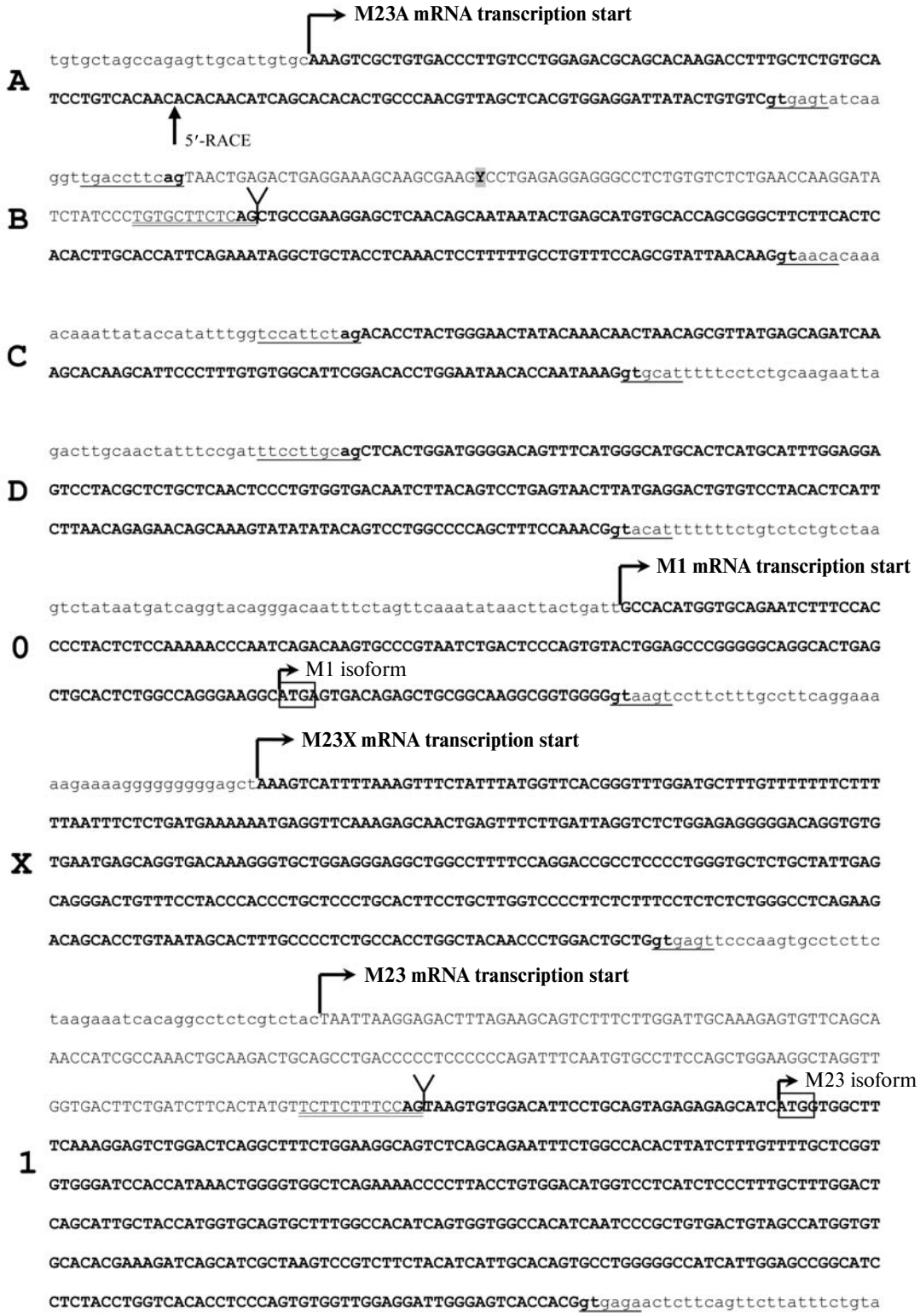

Fig. 2. Nucleotide sequence of mouse *AQP4* gene 5′-region. Exons names are indicated on the left. Exon nucleotide sequences are fully presented with upper-case letters in bold font. Intron nucleotide sequences are presented partially with lower-case letters. Intron borders are homologous with known consensus sequence of exon–intron splicing sites (intron - yyyyyyn**ag**↓N - exon - AG↓**gt**aagt - intron) and are underlined with one line. Consensus internal sites of splicing in exons B and -1 are underlined with two lines and splicing site is marked with symbol "Y". Starts of transcription for different mouse AQP4 mRNAs are marked with symbol ⌐→. Translation initiation codons of M1 and M23 isoforms are marked as ATG with bent arrow symbol. M23A mRNA start of transcription determined with RT-PCR is marked with text. Start of M23A mRNA transcription determined by 5′-RACE is marked with ↑.

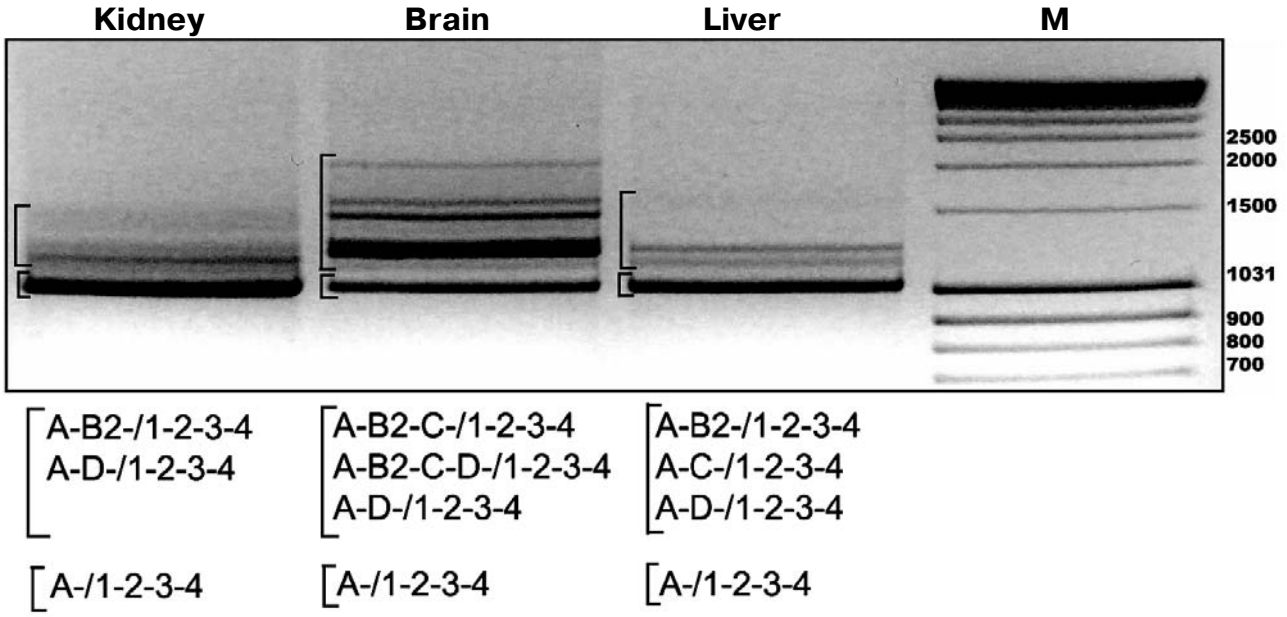

Fig. 3. Alternative splicing of mouse AQP4 M23A mRNA in kidney, brain, and liver. Total mouse RNA from kidney, brain, and liver was used as template for RT-PCR with sense primer from exon A and antisense primer from exon-4. The M line marks DNA Ladder Mass Ruler Mix (Fermentas, Lithuania); length of fragments is in bp. Two PCR band areas (marked with black brackets) were purified and cloned from each electrophoresis gel lane. The shortest fragment for all three organs (kidney, brain, and liver) corresponds to A-/1-2-3-4 M23A mRNA. Multiple longer PCR fragments correspond to alternative splicing variants of M23A mRNA between exons-B, -C, and -D.

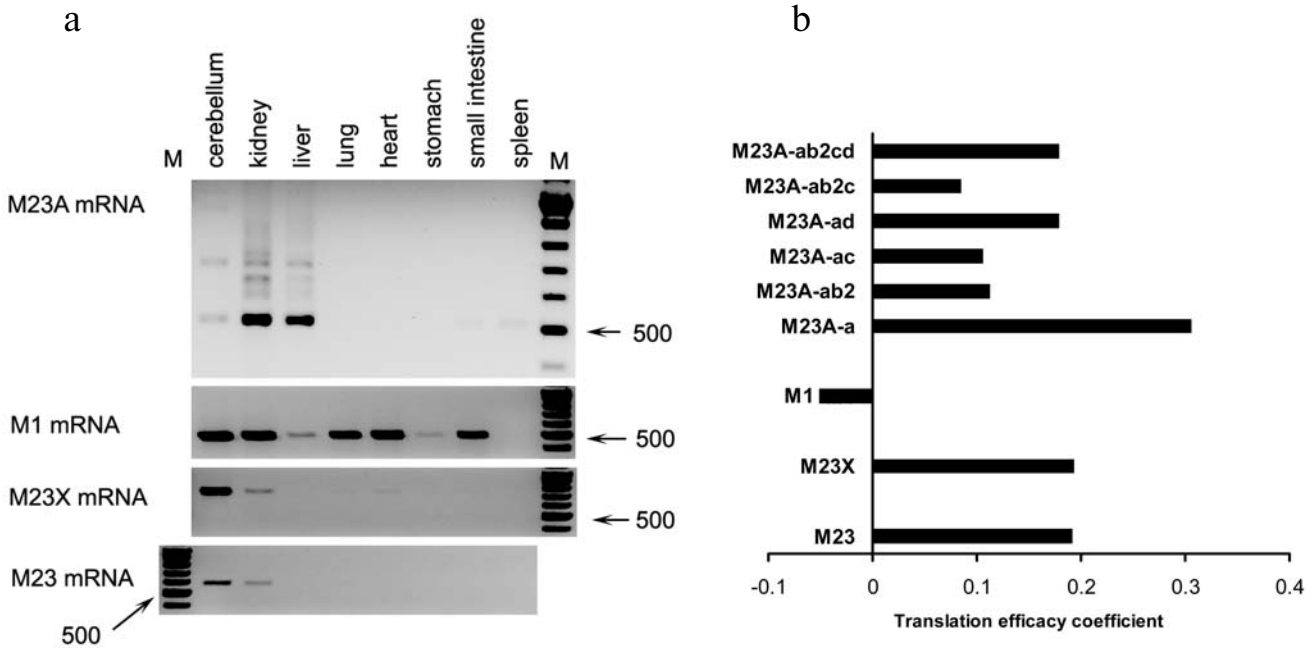

Fig. 4. RT-PCR detection of tissue-specific expression in mouse organs and translation efficacy of four AQP4 mRNA forms. a) For RT-PCR detection, total RNA from mouse organs was used. Sense primers specific for four AQP4 mRNAs and a common antisense primer were selected. GAPDH amplification was used as a positive control (data not shown). M line marks Gene Ruler 100 bp Plus DNA marker (Fermentas); length of fragments is in bp. b) Theoretical estimation of mouse AQP4 mRNA translation efficacy. Reduced name forms are used for M23A mRNA alternative splicing variants (M23A-a corresponds to M23A (A-/1-2-3-4)).

M23A mRNA using 5′-RACE and 5′-RLM-RACE (Fig. 2). For detection of the transcription start we also used another approach – RT-PCR and direct sequencing of PCR products. Fixing an antisense primer, we located sense primers sequentially (ExA35F, ExA69F, ExA80F, ExA116F) to cover an entire region of exon-A and upstream of it (in accordance with 5′-RACE results). We choose sense primers based on the comparative analysis of this genome region between mouse, rat, and human. To detect the functional transcripts, we used antisense primer A4SHTGA located in exon-4 in the 3′-region after the TGA stop translation codon. The longest PCR fragment between primer ExA35F (located at the 5′-end of high homology *AQP4* gene region between mouse and rat) and A4SHTGA primer was obtained only on freshly prepared sample of total mRNA. According to our data, approximate transcription start of new M23A mRNA should be located in the gene region corresponding to the ExA35F primer (Fig. 2).

Theoretical analysis of mouse AQP4 mRNA translation efficacy. We cloned six M23A mRNAs (Fig. 3), that are formed as a result of alternative splicing in the region of exons-B, -C, and -D: A-/1-2-3-4 (GB No. AY484964), A-B2-/1-2-3-4 (GB No. AY484966), A-C-/1-2-3-4 (GB No. AY803942), A-D-/1-2-3-4 (GB No. AY484965), A-B2-C-/1-2-3-4 (GB No. AY803943), A-B2-C-D-/1-2-3-4 (GB No. AY803944). All of them encode AQP4 M23 isoform. Using analysis of 5′-UTR structure features, we estimated theoretical translation efficacy for M1 mRNA as low and for M23, M23X, and M23A mRNAs as high (Fig. 4). Among the cloned six variants of M23A mRNA, the shortest variant A-/1-2-3-4 showed highest translation efficacy.

RT-PCR analysis of tissue-specific expression of mouse AQP4 mRNA forms. Qualitative analysis of all AQP4 mRNA forms (M1, M23, M23X, and the new M23A) expression in organs of adult mouse demonstrated significant differences in the tissue specificity for M23A and other forms (Fig. 4). M1 mRNA is detected in all tested organs (brain, kidney, liver, lung, heart, stomach, and small intestine) except for spleen. M23X mRNA is expressed in cerebellum, kidney, lung, and heart. M23 mRNA is detected in cerebellum and kidney. The new M23A mRNA is expressed predominantly in kidney, liver, and cerebellum. In these organs, M23A mRNA in contrast to other forms is represented by several transcripts corresponding to different variants of its alternative splicing (Fig. 4). The lowest cDNA fragment in kidney, liver, and cerebellum corresponds to the shortest splicing variant of AQP4 M23A mRNA (A-/1-2-3-4).

DISCUSSION

Identification of new exons-A, -B, -C, and -D and new fourth AQP4 mRNA form significantly changes our understanding of the mouse *AQP4* gene functional structure (Fig. 5). We found that the mouse *AQP4* gene has 10 exons and 9 introns. The new exons are located at a significant distance of several thousand base pairs upstream of exon-0, except for exon-D, which is located ~400 bp upstream of exon-0. The new exons transcribe within the fourth AQP4 mRNA form (M23A), determining its 5′-UTR structure and possibly affecting its translation efficacy. We identified six functional variants of M23A mRNA that are formed by alternative splicing in B, C, and D exon region. In exon-B we also found an alternative splicing site. The mouse *AQP4* gene is expressed as four mRNA forms (M1, M23X, M23, and M23A) having transcription starts located in four distinct exons (0, X, 1, and A, correspondingly). The 5′-end sequences of all AQP4 mRNAs are different. The splicing site inside exon-1 is identical for all mouse AQP4 mRNAs. Unlike the rat pool of AQP4 mRNAs, the mouse AQP4 mRNAs encode only two protein isoforms – M1 and M23 (Fig. 5). We did not find deleted (exon-2 for rat AQP4), extended (Mz isoform for rat AQP4 [12]), or shortened (M23sv isoform for human AQP4 [9]) variants of the mouse AQP4 mRNAs.

Among mammalian AQPs, aquaporin-4 has the most complex gene organization, scheme of mRNAs transcription, and protein isoform formation. In general a comparison of the *AQP4* genes and mRNAs for mouse, rat, sheep, bull, and human demonstrates similarities in their exon–intron structure (exons-0, -X, -1, -2, -3, -4 are characteristic for almost all these species). High interspecies homology of individual AQP4 mRNA sequences (for example M1 or M23X) and highly conservative protein isoforms reflect an important functional role of AQP4. In different species the set of known AQP4 mRNA forms is different. The most studied is the set of mouse mRNAs (four mRNA forms, including new M23A variants that encode two AQP4 isoforms) and rat mRNAs (three mRNA forms, including Mz that encode three AQP4 isoforms; and three spliced deletion variants of these mRNAs that encode nonfunctional AQP4 proteins). Currently there is no data about human homologs of mouse M23A and M23X mRNAs or rat Mz mRNA. Neither is there an analog of the new mouse M23A mRNA in the other mammals.

Multiple transcripts with different 5′-ends of the same gene reflect the presence of alternative promoters [31]. For many genes where multiple promoters have been described, no variation in the resulting proteins was reported. In these genes, although the mRNAs have alternative initial exons, they have the same ORF. The mRNA variants differ in their transcriptional patterns and translational efficiencies, as for example, for the human and the mouse genes of glucocorticoid receptor [32, 33] and for the gene of mouse estrogen receptor α [34]. These alternative promoters have different tissue-specific activity, developmental activity, and/or level of expression. We

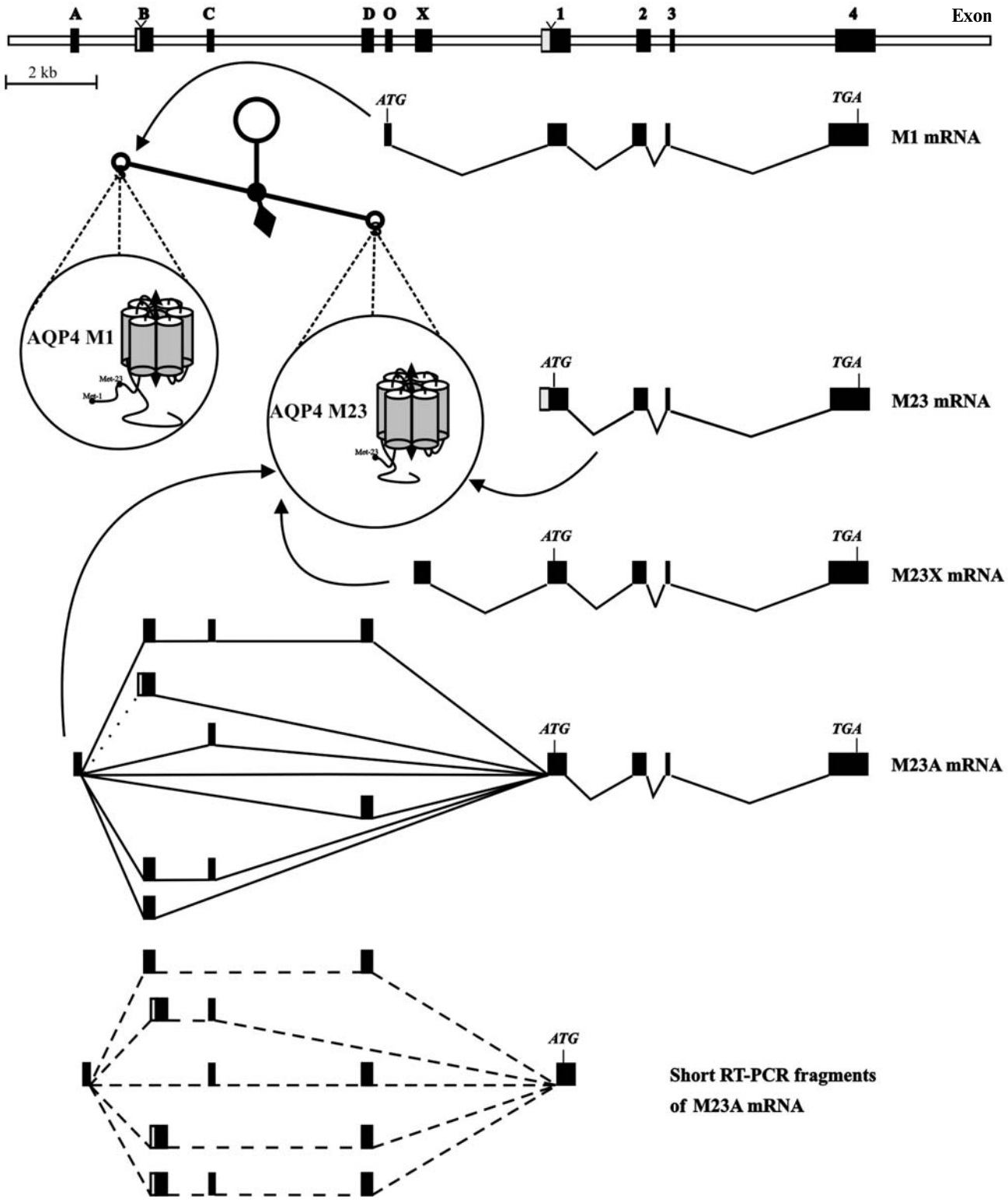

Fig. 5. Gene scheme, mRNA forms, and protein isoform formation of mouse AQP4. Above – cloned *AQP4* gene fragment. Exon-B and -1 internal splicing site is marked with symbol "Y". Below – a scheme of AQP4 mRNA formation coding the M1 and M23 isoforms. The mRNA sequence fragments corresponding to exons are shown with analogous bars. Solid lines connect cloned alternative splicing variants of M23A mRNA; dashed lines connect short RT-PCR fragments of M23A mRNA. The M23A mRNA (B-/1-2-3-4) variant was obtained using RT-PCR. A possible connection between exon-A and full exon-B is shown with a dotted line. A scheme of the balance indicates that the ratio between M1 and M23 isoforms in the cell determines water permeability of the plasma membrane.

revealed several M23A mRNA variants formed by alternative splicing of four 5′-untranslated exons. Three other mouse AQP4 mRNAs are transcribed from three different 5′-end exons (Fig. 5). Transcription starts of four mouse AQP4 mRNAs are separated from each other by several thousand base pairs, and possibly that transcription of these mRNAs is controlled by alternative promoters.

The proposed function of mRNA 5′-UTR is control over mRNA degradation [35] and regulation of translation [36]. The 5′-UTRs of mRNAs differ in their secondary structure, length, GC content, and presence of upstream ORFs, which can affect mRNA translation [31]. Recently a role of translation regulation on M1/M23 isoform expression was studied. It revealed that in primary culture of rat astrocytes alternative ORFs in 5′-UTR of the rat AQP4 M1 mRNA modulate M1/M23 ratio through the mechanism of re-initiation during translation of M1 mRNA [37]. An expert system of 5′-UTR structure feature analysis for mammals [38] estimates theoretical translation efficacy of mouse AQP4 M1 mRNA as the lowest, and all the other mouse AQP4 mRNAs as relatively high with the maximum translation efficacy for the identified here shortest M23A mRNA variant (A-/1-2-3-4). We propose that tissue-specific expression of different AQP4 mRNAs, which are controlled by alternative promoters, could be a mechanism for M1 and M23 expression levels regulation in the cell. The ratio of M1/M23 affects the size and organization of OAPs from AQP4 tetramers, and thus it affects water permeability of the plasma membrane. The M23 isoform has the largest single-channel water permeability when organized into OAP. Therefore, several mRNAs with different translation efficacy that encode the same M23 isoform may allow fine-tuned regulation of tissue-specific AQP4 expression.

In conclusion, we propose that existence of several AQP4 mRNAs may reflect so far unknown long-term tissue-specific regulation of M1 and M23 isoform expression levels; the M1/M23 ratio is believed to affect the water permeability of the plasma membrane.

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