

Nucleotide sequence of a cDNA for the $\beta 2$ subunit isoform of Na^+, K^+ -ATPase from human retina

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Using as probe the entire human liver cDNA clone coding for the the $\beta 2$ subunit isoform of the Na^+, K^+ -ATPase, which lacks the initiation codon ATG, and the entire 5'-untranslated region (Martin-Vasallo, P., Dackowski, W., Emanuel, J.R. and Levenson, R. (1989) *J. Biol. Chem.* 264, 4613–4618), we isolated a larger clone from a directional human adult retina cDNA library (Swaroop, A. and Xu, J. (1993) *Cytogenet. Cell Genet.* 64, 292–294). This clone, pNH $\beta 2$, shows 100% homology with the nucleotide sequence of the human liver cDNA clone and also contains additional 407 nucleotides in the 5'-untranslated region, the initiation codon and a poly(A) tail. Northern blot hybridization analysis reveals that the human mRNA (3.6 kb) is approx. 300 nucleotides larger than the major transcript size expressed in rat (3.3 kb). The larger human size mRNA for the human $\beta 2$ Na^+, K^+ -ATPase indicates species differences in gene processing.

The Na^+, K^+ -ATPase is an integral membrane enzyme that couples the hydrolysis of ATP to the active exchange of intracellular Na^+ out of the cell, for extracellular K^+ inside the cell. The enzyme is comprised of two subunits: the α or catalytic subunit; and the β subunit, a glycoprotein that appears to direct the proper insertion of the α subunit into the membrane. Three different α isoforms ($\alpha 1$, $\alpha 2$, $\alpha 3$) and three β isoforms ($\beta 1$, $\beta 2$, $\beta 3$) were isolated by recombinant DNA techniques [1]. These studies demonstrated that the Na^+, K^+ -ATPase α and β subunits are encoded by a multigene family that is expressed in a tissue-specific and developmentally regulated fashion [2,3].

$\beta 2$ isoform cDNAs have been isolated from rat and mouse brain and human liver cDNA libraries [4,5]. However, the human cDNA lacked the entire 5'-untranslated region and the initiation codon [4]. To delineate the structural and regulatory features within the 5'-untranslated region that may influence the transla-

tional efficiency, we isolated a larger human cDNA clone by screening a directional human retina cDNA library (AR2) [6], using a $\beta 2$ human liver cDNA as probe. A clone of 2.7 kb (pNH $\beta 2$) was obtained. The sequence was determined on both strands using specific oligonucleotides as primers. The resulting cDNA sequence of 2773 bp and deduced amino acid composition is shown in Fig. 1. Comparison of this nucleotide sequence with the previously reported sequence [4], revealed 100% homology in the open reading frame and the 3' region. In addition, pNH $\beta 2$ contains a stretch of 410 nucleotides of new sequence in the 5'-untranslated region including the initiation codon ATG.

Primer extension analysis was performed to determine how far the sequence of human retina $\beta 2$ mRNA extends 5' to that determined from the cDNA [7]. Since the transcription start site for the rat and mouse $\beta 2$ was reported [8,9], we compared the extension patterns of RNA from human retina with that of rat brain. Two antisense oligonucleotide primers, located 269 bp and 275 bp, respectively, upstream from the initiation codon, were designed based on the sequence of human $\beta 2$ cDNA (Fig. 1). A third antisense oligonucleotide primer (5'-GAGGAAGGGATGCCAAGG-

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The nucleotide sequence data of the pNH $\beta 2$ cDNA clone have been submitted to the EMBL/GenBank and assigned the accession number M81181.

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-407 CCACCTCTCTGCGCTTTTGTACCCGGCTTTTCTGCGTCTGCTCGGTTTTGTAG
-347 CGGTCGTTTTTGGACCCCATTCGTTTTGTTTCTAGACGGTTTGGGGGGTGAAGTC
-287 GCATTCATACCCCTTCCCTCTGTTATTCGCCCTGTCTGACAGACCCCTTTTCAATGG
-227 AGTGGGGGGCTAGGATCGGTGCATCTTCGCCGGCTGCCAGACCCCGCAGCCGGC
-167 GTCGTGACCCCGGAATCTGACAGAGTCGATATCTGAGGGGGTCTCTTTGCCCGCCG
-107 CGCCTTCTGCTCCCGTCTTTGGGTGTGGAGGGCTTCAGTGCAGCGCCCGCCGCTT
-47 CTCGCCAACCCCGCCCGCCCGGACTCGCCCGCCGCCACCAAGATGGTCTATCAGA
      H V I Q K
13  AAGAGAAGAGAGCTGCGGCAGGTGGTTGAGGATGGAAGGAGTTCGTGTGGAACCCGA
   E K R S C Q V V E G E W K E F V W N P R
73  GGACGCACCAAGTTTATGGGCCGCCAGCCAGCTGGGCCCTTATCTCTCTTCTTACC
   T H Q F M G R T G T S W A F I L L F Y L
133 TCCTTTTTATGGGTTCCCAACGCCATGTTCACTCACCATGSGGGTATGCTGAGAA
   V F Y G F P T A H F T L T H W V H L Q T
193 CTGTCTCCGACCATACCCCAAGTACCAGGACCGCTGGCCACACCGGGCTTATGATTC
   V S D H T P K Y Q D R L A T P G L M I R
253 GCCCAAGACTGAGAACCTTGATGTCATGTCAATGTCAGTGACACTGAAAGCTGGGACC
   P K T E N L D V I V N V S D T E S W D Q
313 AGCATGTTGAGAAGCTCAACAAGTCTTGGAGCCTTACAACGACTCTATGCAAGCCAAA
   H V Q K L N K F L E P Y N D S M Q A Q K
373 AGAATGATGCTGCGCCGCTGGCGCTTATACGAAACAGCAGATAATGGAGTCCCTCAACT
   N D V C R P G R Y Y E Q P D N G V L N Y
433 ACCCAAACTGGCCTGCCAATTCAACCGGACCCAGCTGGGCACTGCTCCGGCATTGGGG
   P K L A C Q F N R T Q L G N C S G I G D
493 ACTCCACCCACTATGGTTACAGCACTGGCGAGCCCTGTCTTCATCAAGATGAACCGGG
   S T H Y G Y S T G Q P C V F I K M N R V
553 TCATCAACTTCTATGAGGAGCAAAACAGAGCATGAATGTTACCTGTGCTGGGAGCGAG
   I N F Y A G A N Q S M N V T C A G K R D
613 ATGAAGATGCTGAGAATCTGGCAACTTCGTCATGTTCCCGCCCAACGGCAACATCGACC
   E D A E N L G N F V M F P A N G N I D L
673 TCATGACTTCCCTACTATGGCAAAAAGTCCAGCTGAACCTACACAGCCCTGGTGG
   M Y F P Y Y G K K F H V N Y T Q P L V A
773 CTGTGAAGTTCCTGAATGTGACCCCAACGTGGAGGTGAATGTAGAATGCTGCATCAACG
   V K F L N V T P N V E V N V E C R I N A
793 CGCCCAACATCGCCACAGAGATGAGCGAGACAAGTTCGCCCGCCGGTGGCCTTCAAAC
   A N I A T D D E R D K F A G R V A F K L
853 TCCGCATCAACAJAACCTGGAGCCCTTCTCCACCCCATCTCTCTCTGTGGATGCTC
   B I N K T *
913 CTGGAATGCTCCCTGACCCCTGCTGATCCCTCCCTCACCCACCCAAAGGATTTTTGATA
973 ACAGAGCTATGACTTGTCTGAGCCTCACATCCTTTCTCTGACTTCTCAACCCAGCCCTGA
1033 AGTCCATTGCGGTTCGCTCACTCGCCCTTCCCAACAACTTCTCCCAACTCAGATCACTC
1093 AGACAGGAGGCTGGGCTAAGATGGCCACAGAGGAGTTAGGAGCCCTTCTAGTCTCGGTT
1153 AGCTGTGAGAGCTATCCACTCTCCGCTGCATATCCCTGAGAGTATCGGAAGTGGCC
1213 ACTGACCCACCCACCCACTACACCCCGCCACACACACACAAAGCGGACAGCCGAG
1273 TCTCATTTGACCCCTTGGCTTCGAGAGTGAATGGGACTCCCTCTTCCATTCCTCAAG
1333 CTGTAGCCACCGCTCTGATCTCTCACTCTCTCTCTCTGACTGACAGTGGCCACTTTG
1393 GTGACTTTGAATTTAATGCTGCTCTGGCAGGCTCTCTCTCTCTCTCTCTCTCTCTCTCT
1453 TCTCTGAAATGCACCCCTTTGTAATGAGGACAAGTGGTTCGTGGCCCTTTTCCCTCT
1513 TTGCTGGCAGCTTCTGCTTCTCAACCCCTGCTGAGCTCTGTGAGCTGGGAAATGGGGACT
1573 GGAAGTGAGGCTGTGTTGACCCCTTCTGAAATCTCTAGCAGCCCGCACTTCAGGAG
1633 TTTCTTTCTTTGTTTTTGTAGATGGAGTTTCGCTCTGTTGCGCAGGCTGGAGTGCAAT
1693 GGTGCAATCTCAGCTCACTGCAACTTCCGCATCCAGGTTCAAGCGATCTCCCGCCCA
1753 GGTCCCGAGTAGTGGGACTACAGGCAATGTCACCAATGCCCGGCTAATTTCTTTCTTT
1813 CTTTTTTTTTTTTTTTTGCAATTTTTAGTAGAGATGGGGTTTCTCTTTGTGGTCAGG
1873 CTGGTCTCGAATCCCGACCTCAGGTGATCCACTGCCTCGGCCCTCCCAAGTGTGGGA
1933 TTACAGCGGTGAGCCACCGCGCCTGGCCTCAGTTTCTCTAGCCGCTTCTGTCAACCA
1993 AATAGCTGCTACAGAGGGCGGGTGAACCTAGCGGAATCTCACTTTGTTTATG
2053 GATGGCTCCCTCCCACTCGCCTTCCAGAAATCTCTCAAGTCCACTTCCAGGGA
2113 GCTCTGGGGAGGGGGCCATTCGGCTCCGCTCCAGTGCCACCTTGGAAACATCGG
2173 CTGGCTTTGGGACTATCCACCTCTCTCCCTGAGCCAGATCTGCCCCACCATCTTT
2233 CTCTGGCTTCTTTAGCAAGTATCAACTAATCACTAATCTCTCTTTCTCTCTGCAATG
2293 CCAGCTGAAAAATCCAAATCTAGCCTTGAATGCTTGGCTCATCTCTCAGACCCCT
2353 TTGCCTTAAAAA
    
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Fig. 1. Nucleotide and deduced amino acid sequence of the $\beta 2$ subunit isoform of the Na^+, K^+ -ATPase from human retina. The sequence was determined for both strands by sequencing, with specific oligonucleotides as primers. Nucleotide sequence was compared to the previously published human liver sequence [4] using the BESTFIT programs. Nucleotides are numbered on both sides of the sequence in the 5' \rightarrow 3' direction. Nucleotide 0 is the A of the ATG codon for the initiator Met. Negative numbers of the 5'-untranslated region are numbered in the 3' \rightarrow 5' direction. The deduced amino acid sequence (one letter code) is also numbered on both sides of the sequence. The nucleotide sequence complementary to the oligonucleotides used for primer extension analysis is underlined.

CACCTTC-3'), was also designed based on the rat $\beta 2$ cDNA (accession number D90048) and is located 259 bp from the initiation codon. The respective primers were end labeled and hybridized in separate reactions to total human retina and rat brain RNAs. After extension with reverse transcriptase, the products were resolved on a denaturing 6% polyacrylamide gel (Fig. 2). The primer extension with the two human primers gave similar results, yielding two bands in human retina

of about 230 and 340 nucleotides (nt), which are indicated by arrowheads in Fig. 2, lane 1. The extension pattern with the rat primer in rat brain was more complex. Only the 340-nt band in human retina was closer in size with the 330 nt found in rat brain (indicated by an arrowhead in Fig. 2, lane 2). The latter band correlates with the transcription start site assigned to the rat $\beta 2$ [8], found 595-nt upstream from the initiation codon or 330-nt upstream from the primer used in our extension experiment. This result also appears to agree well with nucleotide sequence derived

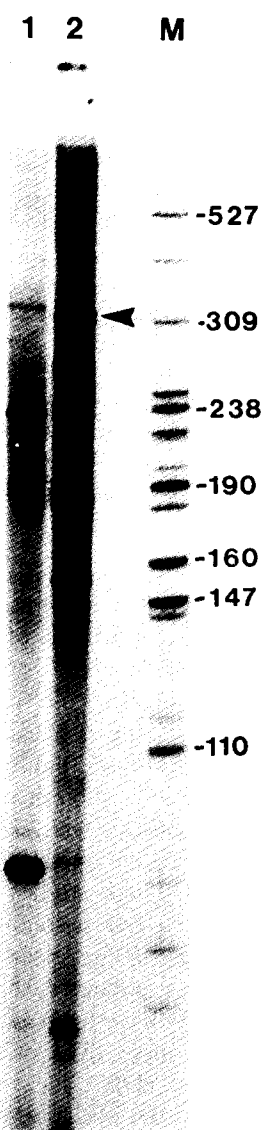


Fig. 2. A primer extension analysis was carried out with total human retina RNA and rat brain RNA as described (Ghosh et al. 1980). In lane 1, one oligonucleotide (from nt -269 to -293) complementary to sequence located at the 5'-untranslated region of the human $\beta 2$ cDNA (see Fig. 1) was used. In lane 2, one oligonucleotide (from nt 312 to 336) complementary to sequence located at the 5'-untranslated region of the rat $\beta 2$ cDNA [8] was used. Arrowheads indicate the positions at which termination occurred. ^{32}P -end labeled DNA size marker is shown at right (lane M).

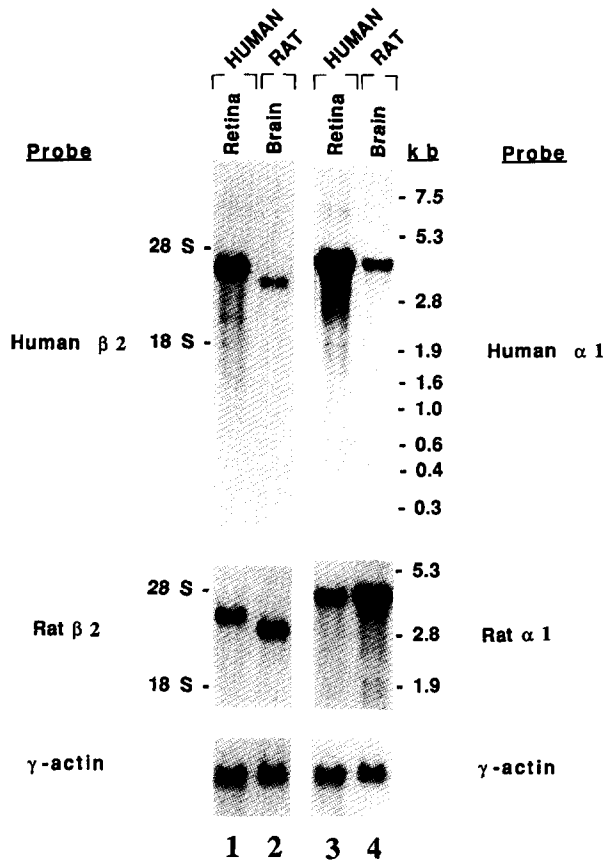


Fig. 3. $\beta 2$ Na^+, K^+ -ATPase mRNA expression in human retina and rat brain. Total RNA ($20 \mu\text{g}$)/lane was electrophoresed, transferred to a nitrocellulose filter and hybridized sequentially with: (1) human $\beta 2$ (pNH $\beta 2$) (left panel), (2) rat $\beta 2$ (left panel), (3) human Na^+, K^+ -ATPase $\alpha 1$ subunit isoform (right panel), (4) rat Na^+, K^+ -ATPase $\alpha 1$ subunit isoform (right panel), and (5) γ -actin (bottom panel). After each hybridization, filter was stripped and reprobated as previously described by Ghosh et al. [10]. Positions of the RNA markers, 7.5, 5.3, 2.8, 1.9, 1.6, 1.0, 0.6, 0.4, 0.3 kb, 28S and 18S rRNAs are indicated on the right.

recently from genomic human clones flanking the 5'-end of the human Na^+, K^+ -ATPase $\beta 2$ gene (accession number L23414). The additional bands in lanes 1 and 2 of Fig. 2, may represent alternately spliced messages of the $\beta 2$ primary transcript and/or copies of homologous but different mRNA expressed in retina and brain. To exclude the possibility of an artifact due to the degradation of RNA, we analyzed human retina

RNA and rat brain RNA. A Northern blot containing RNA from human retina and rat brain was sequentially hybridized with ^{32}P -labeled human and rat $\beta 2$ probes. The results (Fig. 3) revealed a major transcript of about 3.6 kb in human retina and a transcript of about 3.3 kb in rat brain. Transcripts of lower molecular weights of 2.4 kb and 1.7 kb were also detected after a 72-h exposure (data not shown). These results contrasted with the previous studies which failed to reveal $\beta 2$ RNA size differences between human and rat tissues [4]. To exclude the possibility that the difference in mRNA size was due to abnormal electrophoretic mobility, we sequentially hybridized the same Northern blot to Na^+, K^+ -ATPase $\alpha 1$ subunit and actin probes. The human and rat $\alpha 1$ probes recognized only one transcript with similar size in both human and rat tissues. Also, the actin probe hybridized to a transcript of the same size in human and rat tissues. Thus, these studies demonstrated that the main human $\beta 2$ mRNA transcript is approx. 0.3 kb bigger than the rat $\beta 2$ mRNA.

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