Volume 257, number 2, 439–442 FEB 07810 November 1989

Human Na^+,K^+-ATPase genes

β-Subunit gene family contains at least one gene and one pseudogene

Yu.A. Ushkaryov, G.S. Monastyrskaya, N.E. Broude, N.N. Nikiforova, D.A. Bessarab, M.Yu. Orlova, K.E. Petrukhin, N.N. Modyanov and E.D. Sverdlov

Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 117871 Moscow, USSR

Received 8 August 1989

The existence of a chromosome gene family containing at least one gene and one pseudogene was shown for the Na^+,K^+-ATPase β-subunit. A partial structure of the β1-gene was determined, the coding part of which was completely homologous to cDNA of the Na^+,K^+-ATPase βI-subunit from HeLa cells [8]. The region encoding the putative protein transmembrane domain was shown to be bordered by two introns. The structure of a pseudogene ($\beta\psi$) was determined. This pseudogene is processed and contains multiple stop codons. Its homology to the βI-subunit cDNA from HeLa cells is about 88%.

ATPase, Na^+,K^+-; β-Subunit; Family of gene; Exon/intron structure; Pseudogene sequence

1. INTRODUCTION

Na^+,K^+-ATPase (EC 3.6.1.3) of animal cell plasma membranes carries out the ATP hydrolysis-coupled transport of sodium and potassium ions across the plasmalemma against their electrochemical gradients. The enzyme consists of two polypeptide subunits. The α-subunit ($\approx$112 kDa) carries all known functional sites of the holoenzyme [1,2]. The function of the β-subunit, which is a sialoglycoprotein with a molecular mass of the protein component of about 35 kDa, has not yet been established. The β-subunit was hypothesized [3] to serve as a receptor for incorporation of the α-subunit into the membrane. (It cannot be ruled out that the β-subunit possesses another, yet unknown, activity required for holoenzyme functioning [4].) Both subunits are expressed coordinately and at approximately equal levels in most tissues except rat liver, but not human liver. The latter may indicate the presence in rat liver of another isoform of the β-subunit [5]. The possible existence of the family of β-subunit proteins may be confirmed by the localisation of the regions, homologous to the β-subunit cDNA on two different chromosomes (1 and 4) [6]. In a recently published work [7], the structures of rat brain and human liver mRNAs were established which encode two proteins having a weak homology to the earlier characterized β-subunit of Na^+,K^+-ATPase from rat brain (only 55.8% nucleotide homology). The authors designated the deduced proteins as the βII-isoforms. On the other hand, Kawakami and co-authors [8] postulated the uniqueness of the Na^+,K^+-ATPase β-subunit gene in the human genome. In this light, it is of interest to elucidate whether there are various isoforms of the β-subunit in animal cells and, if so, how many different genes encode these isoforms.

Earlier [9,10], we determined the complete cDNA nucleotide sequences corresponding to mRNA translated regions and deduced the protein primary structures of pig kidney Na^+,K^+-ATPase α- and β-subunits. The investigation of human chromosomal genes [11,12] evidenced the existence of a gene family for the Na^+,K^+-ATPase catalytic subunit, including no less than 5 genes and/or pseudogenes. The complete structure of one of the genes of this family (α3) has been determined [13].

Here, we present evidence for the existence of a family of related genes and pseudogenes of human Na^+,K^+-ATPase β-subunit and report a structure of a pseudogene ($\beta\psi$).

2. MATERIALS AND METHODS

To construct genomic libraries, human sperm DNA was partially digested with *Sau*3A I, and a fraction with fragments 10–20 kb in length was cloned into λEMBL3. The libraries obtained were screened as described [11] using the full length cDNA of the pig kidney Na^+,K^+-ATPase β-subunit cloned into plasmid pGEM2. Isolation of recombinant phages, DNA physical mapping, labelling of fragments

Correspondence address: Yu.A. Ushkaryov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 16/10 ul. Miklukho-Maklaya, Moscow GSP-7, 117871, USSR

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

and hybridization were carried out according to the methods described [11].

3. RESULTS AND DISCUSSION

The homology of structural genes for Na^+,K^+-ATPase β-subunits from phylogenetically distant species (which is 89.4% for pig kidney/HeLa cells cDNAs, and 66% for pig/torpedo cDNAs) made probing of human genomic libraries with the help of pig kidney cDNA possible.

Eight positive clones were isolated during several library screening procedures. By the restriction site maps, the clones were divided into two types: RH11 and RH4 (fig.1), the latter being represented by several overlapping clones.

In fig.2, the DNA sequences from both the phage λNKβRH11 and the phage λNKβRH4 are shown in parallel with cDNAs for Na^+,K^+-ATPase β-subunits from pig kidney and HeLa cells. It is evident that all 4 structures are highly homologous (see also table 1).

Analysis of the above data shows that, firstly, clone λNKβRH11 contains a genomic DNA which, in its exon part, is completely identical to the structural gene of the HeLa Na^+,K^+-ATPase β-subunit and, secondly, this DNA region has at least two introns. These introns, designated in figures as 1 and 2, have typical splice sites of exon-intron boundaries [15] and show no homology to the respective structural gene regions. We conclude that clone RH11 apparently contains a fragment of the gene for the enzyme's βI-subunit. It is also interesting to note that these introns border the exon which encodes the putative transmembrane domain of the protein molecule (amino acid residues 34–61, fig.3). This observation is in good agreement with the hypothesis proposed by Hogness [17].

Table 1

Homology of the nucleotide sequences of isolated clone fragments to the cDNA of Na^+,K^+-ATPase β-subunits from pig kidney and human HeLa cells (in %)

	cDNA of β-subunit from:		Human genomic DNA fragments from:	
	Pig kidney [10]	HeLa cells [8]	λNKβRH11[a]	λNKβRH4
Pig	–	89.2	97.6	82.3
HeLa		–	100.0	88.1
RH11			–	89.1
RH4				–

[a] For phage λNKβRH11 only the exon part of the insert was compared (residues 102–224 in coordinates of pig kidney cDNA which is the most conserved region)

As seen in the figures and table 1, the structure of the human genomic insert from clone λNKβRH4 is highly homologous but not identical to cDNA from HeLa cells. Its homology to the structural genes for Na^+,K^+-ATPase β-subunits from pig kidney and HeLa cells undoubtedly proves the close relationship of all these structures. Characteristically, the RH4-type gene lacks introns which are present in RH11 type gene. Besides, the reading frame includes the stop codons and nucleotide substitutions leading to multiple non-isofunctional amino acid substitutions; the insertion of 5 nucleotides in the region of the residue 215 causes a shift of the reading frame. These data may be an indication that the RH4-type gene is a pseudogene designated here as $\beta\psi$.

It is well known that the distribution of substitutions in the sequences of β-subunit cDNAs from different species is non-uniform (fig.4B). The amino-terminal sequence is highly conserved (underlined in fig.4 by one

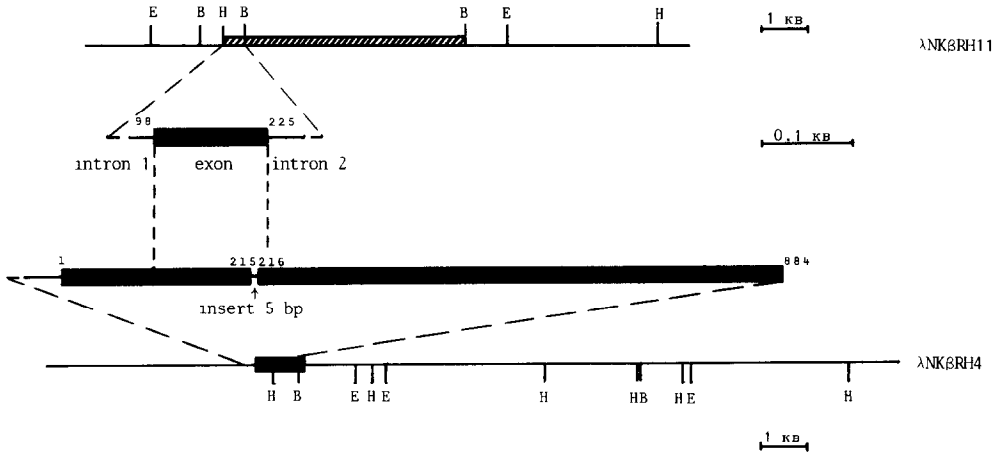

Fig.1. Comparison of structural organization of two types of genes related to the β-subunit of Na^+,K^+-ATPase. Top and bottom lines are restriction maps of phages λNKβRH11 and λNKβRH4, respectively. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; the scale is given as a bar. In the center, the sequenced and collinear portions of both genes are given enlarged. (Nucleotide numbers are marked according to pig kidney cDNA.) (■) Exons or areas homologous to coding regions; (–) introns or areas nonhomologous to coding regions; (///) fragments hybridizing to the probe used.

```
-70-1 a              gacgA-CGCa AGaGGCaCGC gGCAatCACC CAtCtTCaat cCCcGCaGCA GCTGCTGACC CGCCA---CC
      b              CCTCATCGCC AGGGGCGCGC CGCAGCCACC CACCCTCCGG ACC-GCGGCA GCTGCTGACC CGCCATCGCC
      c
      d                  cagaga Actacgag GC tGCcGgaAtC gcgaCcCttc cgactCGGCA -CTGCTGcCg atCtcTgcCg

 1-80 a  ATGGCCCGCG GaAAAGCCAA GGAGGAGGGC AGCTGGAAGA AATTCATCTG GAACTCcGAg AAGAAGGAGT TTtTGGGCAG
      b  ATGGCCCGCG GGAAAGCCAA GGAGGAGGGC AGCTGGAAGA AATTCATCTG GAACTCAGAG AAGAAGGAGT TTCTGGGCAG
      c        intron 1 -----t tcatccgtgg gaagattaaa gtttcattct gacggatatg acgtcatcac agctttttgt
      d  ATGaCCtcga GGAAAGCCAA tGAGGAGGGC AGtTGGAAGA AATTCtTCTG GAACTCAGAG AAGAAGGAGT TTtTaGGCAg

81-160 a  GACCGGTGGC AGTTGGTTTA AGATCCTTCT ATTCTACGTt ATATTTTATG GCTGCCTGGC TGGCATCTTC ATtGGAACCA
      b  GACCGGTGGC AGTTGGTTTA AGATCCTTCT ATTCTACGTA ATATTTTATG GCTGCCTGGC TGGCATCTTC ATCGGAACCA
      c  ttctgttctt cttgcagTTA AGATCCTTCT ATTCTACGTA ATATTTTATG GCTGCCTGGC TGGCATCTTC ATCGGAACCA
      d  GACCGtgGGC AGTTGGTTTA AGATCaTTCT ATTCTACaTA ATATTTTATG GCTGCCTGaC TGGCATCTTC ATCGGAAtgA

161-235 a  TCCAAGTGAT GCTGCTCACC ATCAGTGAAT TTAAGCCCAC ATATCAGGAC CGAGT----- GGCCCCaCCA GGATTAACAC
      b  TCCAAGTGAT GCTGCTCACC ATCAGTGAAT TTAAGCCCAC ATATCAGGAC CGAGT----- CGCCCCCCCA GGATTAACAC
      c  TCCAAGTGAT GCTGCTCACC ATCAGTGAAT TTAAGCCCAC ATATCAGGAC CGAGT----- GGCCCCGCCA gtaaatgcac
      d  TCCAAGTGgT GCTGCTCACC ATCAGTGAAT TTAAaCCCAC ATATCAGaAC CGcaTatcag GaCCCCaCCA GGATTAACtC

236-315 a  AGATTCCTCA GAgCCAaAAG ACTGAAATTT CtTTTCGTCC TAATGATCCC cAaAGCTATG AatCcTATGT ggTaAgCATA
      b  AGATTCCTCA GATCCAGAAG ACTGAAATTT CCTTTCGTCC TAATGATCCC AAGAGCTATG AGGCATATGT ACTGAACATA
      c  acatgatagc ttcatttcct tcagagatag cat----- intron 2
      d  AGATTaCTCA GATCCAGcAG ACTGAAATTg CCTTTCaTCC TAATGATCCC AAGcaCTgTG AGGCATATGT gCTGAACgTA

316-395 a  GTgAGGTTCC TGGAgAAGTA CAAAGATTtg GCgCAGAaGG ATGAtATGAT TTTTGAAGAT TGTGGCaATG TGCCCAGcGA
      b  GTTAGGTTCC TGGAAAAGTA CAAAGATTCA GCCCAGAGGG ATGACATGAT TTTTGAAGAT TGTGGCGATG TGCCCAGTGA
      c
      d  GTTAGGTTCC TGGAAAAGTA CAAAGATTCA GCCCAGAaGG ATGACATGAT TTTTGAAaAT TGTGaCaATG TGCCCAGTGA

396-475 a  ACtcAAAGAA CGAGGAGAaT aTAAcaAcGA ACGAGGAGAG CGAAAaGTgT GCAGgTcCAg GCTcGAATGG tTGGGAAAcT
      b  ACCGAAAGAA CGAGGAGACT TTAATCATGA ACGAGGAGAG CGAAAGGTCT GCAGATTCAA GCTTGAATGG CTGGGAAATT
      c
      d  ACCcAAAGAA CGAGGAGACT TTAATCAaGA AtGAGGtGAG CGAAAGGTCT GCAGATTCAA GCTTGAgTGG CTGGGAAATT

476-555 a  GCTCTGGATT AAATGATGAA ACcTATGGCT ACAAAGAtGG CAAACCcTGt gTcATTATAA AGCTCAACCG AGTTCTgGGC
      b  GCTCTGGATT AAATGATGAA ACTTATGGCT ACAAAGAGGG CAAACCGTGC ATTATTATAA AGCTCAACCG AGTTCTAGGC
      c
      d  GCTCTGGATT AAATGAcAAA ACTTAcaGCT ACgAAGAGGG CAAACCacat gTTATTATAA AGCTCAAgtG AGTTCTAGGC

556-635 a  TTCAAACCTA AGCCTCCCAA GAATGAGTCC TTGGAGACTT ACCCAGTGAT GAAGTATAAt CCAtATGTCC TgCCCGTTCA
      b  TTCAAACCTA AGCCTCCCAA GAATGAGTCC TTGGAGACTT ACCCAGTGAT GAAGTATAAC CCAAATGTCC TTCCCGTTCA
      c
      d  TTCAAACCTA AGtCTCCCgA GAATGAGTCC TTGGAGACTT ACCCAGTGAT GAAGTATAAC gCAtcTGTgt TTCCtGttCA

636-715 a  tTGCACTGGC AAGCGtGAcG AAGATAAGGA gAAAGTTGGA AccaTGGAGT ATTTTGGcCT GGGCggCTaC CCTGGTTTTC
      b  GTGCACTGGC AAGCGAGATG AAGATAAGGA TAAAGTTGGA AATGTGGGAT ATTTTGGACT GGGCAACTCC CCTGGTTTTG
      c
      d  GTGCACTGGC tAGCaAGATG AAGATAAGGA TAAAaaTaGA AATGTGGAGT ATTTTGGACT GGGCAACTaC CtTGGTTTTC

716-795 a  CTCTaCAGTA TTAcCCtTAC TAcGGCAAgC TCCTGCAGCC CAAgTACCTG CAGCCCCTGa TGGCtGTgCA GTTCACCAAc
      b  CTCTGCAGTA TTATCCGTAC TATGGCAAAC TCCTGCAGCC CAAATACCTG CAGCCCCTGC TGGCCGTACA GTTCACCAAT
      c
      d  CTCTGCAGaA TTATCCcTAC TATGGCAAAC TCCTGCAGCC CAAATACCTG CAGCCtCTGC TtGtCGTACA GTTCACCAAc

796-875 a  CTcACCATGG ACACTGAAAT cCGCATAGAG TGTAAGGCGT AtGGTGAGAA CATTGGGTAC AGTGAGAAAG ACCGTTTTCA
      b  CTTACCATGG ACACTGAAAT TCGCATAGAG TGTAAGGCGT ACGGTGACAA CATTGGGTAC AGTGAGAAAG ACCGTTTTCA
      c
      d  CTTACCATGG ACACTGAAAT TCGCcTAG-- TGTAAaGCag AtGGTGAGAA CATTGGGcAC AGTGAGAAAG AtgGTTTTCA

876-923 a  GGGACGcTTT GATGTAAAAA TTGAAGTTAA GAGCTGATCA CAAGCtCt
      b  GGGACGTTTT GATGTAAAAA TTGAAGTTAA GAGCTGATCA CAAGCACA
      c
      d  GGGAtcc
```

Fig.2. Nucleotide sequences of the Na^+,K^+-ATPase β-subunit cDNAs from pig kidney (a) [10], HeLa cells (b) [8], and genomic inserts from phages λNKβRH11 (c) and λNKβRH4 (d). Nucleotides in introns and those differing from HeLa sequence are presented in lower case letters.

line), in carboxy-terminal part of cDNA, there are variable as well as constant (marked with black arrow) regions. In contrast, the distribution of nucleotide substitutions in the gene $\beta\psi$ is more even (fig.4A). For instance, in the same N-terminal region, which is considered to be functionally significant, there are 3 times more nucleotide and amino acid differences between HeLa sequence and $\beta\psi$ than between HeLa and pig kidney cDNAs. Moreover, the 5 nucleotide frame shifting insert (indicated by white arrow) falls into this region. These data indicate a possible absence of natural selection control of the structure of this gene which, in turn, may be a proof of absence of RH4-type gene expression.

On the other hand, there are some similarities in pig/HeLa and $\beta\psi$/HeLa nucleotide substitution profiles (in fig.4 this region is underlined by double line). This suggests that prior to inactivation, i.e. conversion into a pseudogene, the RH4 gene ancestor had already diverged from β1-gene, the divergence being within the

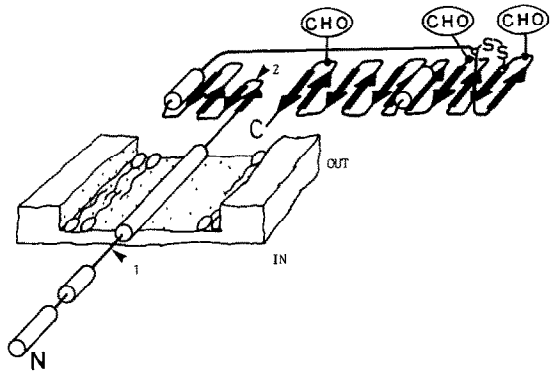

Fig.3. Model for spatial transmembrane organization of Na^+,K^+-ATPase β-subunit [16]. Cylinders and black arrows correspond to α-helical segments and β-pleated sheets, respectively. Arrowheads with numbers indicate intron positions in β1 gene.

limits of natural evolution. Hence, it follows that the family of the Na^+,K^+-ATPase β-subunit genes contains (or contained) other active genes, more homologous to the $\beta 1$ than the recently sequenced human liver βII-subunit cDNA [7]. Based on the estimated neutral rate of evolution in the higher primates which is 1.3×10^{-9} substitutions per site per year [18], it could be hypothesized that this pseudogene has been formed more than 100 million years ago and, therefore, could also be found in the other mammals.

By the nucleotide sequence, human $\beta\psi$ isolated in this work significantly differs from human liver βII-subunit cDNA [7] and is much closer to that of the βI-subunit from HeLa cells [8] and other sources. Thus, it is most probable that hybridization analysis in the work [6] revealed RH4 type pseudogene on human chromosome 4, besides functional gene $\beta 1$ localized on chromosome 1.

The data presented here clearly evidence that a family of gene(s) and pseudogene(s) related to the Na^+,K^+-ATPase β-subunit exists in human genome. What lies ahead is to elucidate how many members of this family there are in the mammalian genome and what is the origin of these genes.

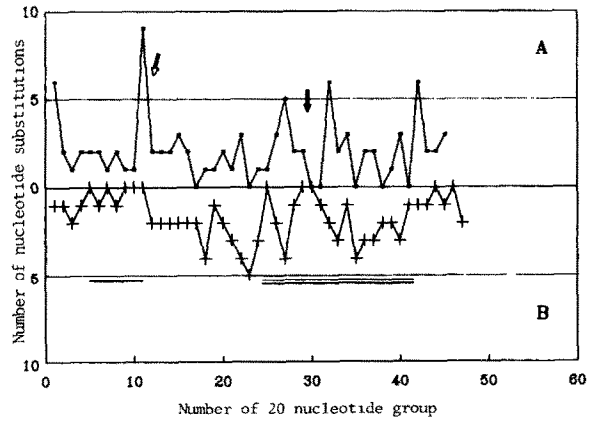

Fig.4. Pairwise comparison of nucleotide substitutions in 3 genes related to Na^+,K^+-ATPase βI-subunit family. (A) HeLa cDNA versus βψ; (B) HeLa cDNA versus pig cDNA. Numbers of substitutions are given for every 20 nucleotides.

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