

Human Na⁺,K⁺-ATPase genes

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

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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 β 1-gene was determined, the coding part of which was completely homologous to cDNA of the Na⁺,K⁺-ATPase β 1-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 β 1-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 β II-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 (α 3) 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 λ EMBL3. 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

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-70-1	a	gacgA-CGCa	AGaGGCaCGC	gGCAaTcACC	CAtCtTCaat	cCCcGCaGCA	GCTGCTGACC	CGCCA---CC
	b	CCTCATCGCC	AGGGGCGCGC	CGCAGCCACC	CACCCTCCGG	ACC-GCGGCA	GCTGCTGACC	CGCCATCGCC
	c							
	d	cagaga	ActacgagGC	tGcCGaAtC	gcgaCcCttc	cgactCGGCA	-CTGCTGcCG	atCtcTgcCG
1-80	a	ATGGCCCCGC	GaAAAGCCAA	GGAGGAGGGC	AGCTGGAAGA	AATTCATCTG	GAACCTcGAg	AAGAAGGAGT
	b	ATGGCCCCGC	GGAAAGCCAA	GGAGGAGGGC	AGCTGGAAGA	AATTCATCTG	GAACCTCAGAG	AAGAAGGAGT
	c	intron 1	----t	tcattccgtgg	gaagattaaa	gtttcattct	gacggatatg	acgtcatcac
	d	ATGaCCtgcg	GGAAAGCCAA	tGAGGAGGGC	AGtTGAAGA	AATTCtTCTG	GAACCTCAGAG	AAGAAGGAGT
81-160	a	GACCGGTGGC	AGTTGGTTTA	AGATCCTTCT	ATTCTACGTt	ATATTTTATG	GCTGCCTGGC	TGGCATCTTC
	b	GACCGGTGGC	AGTTGGTTTA	AGATCCTTCT	ATTCTACGTt	ATATTTTATG	GCTGCCTGGC	TGGCATCTTC
	c	ttctgttctt	cttgcaGTTA	AGATCCTTCT	ATTCTACGTt	ATATTTTATG	GCTGCCTGGC	TGGCATCTTC
	d	GACCGtGGC	AGTTGGTTTA	AGATCaTCT	ATTCTACaT	ATATTTTATG	GCTGCCTGac	TGGCATCTTC
161-235	a	TCCAAGTGAT	GCTGCTCACC	ATCAGTGAAT	TTAAGCCAC	ATATCAGGAC	CGAGT-----	GGCCCCaCCA
	b	TCCAAGTGAT	GCTGCTCACC	ATCAGTGAAT	TTAAGCCAC	ATATCAGGAC	CGAGT-----	GGCCCCGCCA
	c	TCCAAGTGAT	GCTGCTCACC	ATCAGTGAAT	TTAAGCCAC	ATATCAGGAC	CGAGT-----	GGCCCCGCCA
	d	TCCAAGTGt	GCTGCTCACC	ATCAGTGAAT	TTAAaCCAC	ATATCAGaAC	CGcaTatcag	GaCCCCaCCA
236-315	a	AGATTCTCA	GAGCCaAAG	ACTGAAATTT	CtTTTCGTCC	TAATGATCCC	cAaAGCTATG	AatCcTATGT
	b	AGATTCTCA	GATCCAGAAG	ACTGAAATTT	CCTTTCTGTC	TAATGATCCC	AAGAGCTATG	AGGCATATGT
	c	acatgatagc	ttcatttcc	tcagagatag	cat-----	intron 2		
	d	AGATTaCTCA	GATCCAGcAG	ACTGAAATTTg	CCTTTCaTCC	TAATGATCCC	AAGcaCTgTG	AGGCATATGT
316-395	a	GTgAGGTTC	TGGAgAAGTA	CAAAGATTt	GcGcAGaAG	ATGatATGAT	TTTTGAAGAT	TGTGGCaATG
	b	GTTAGGTTC	TGGAAAAGTA	CAAAGATTTC	GCCCAGAGGG	ATGACATGAT	TTTTGAAGAT	TGTGGCAGTG
	c							
	d	GTTAGGTTC	TGGAAAAGTA	CAAAGATTTC	GCCCAGaAG	ATGACATGAT	TTTTGAaAAT	TGTGaCaATG
396-475	a	ActcAAAGAA	CGAGGAGaAT	aTAAcaAcGA	ACGAGGAGAG	CGAAAaGtGt	GCAGgTcCag	GCTcGAATGG
	b	ACCgAAAGAA	CGAGGAGACT	TTAATCATGA	ACGAGGAGAG	CGAAAGGTCT	GCAGATTCAA	GCTTGAATGG
	c							
	d	ACCcAAAGAA	CGAGGAGACT	TTAATCAaGA	AtGAGGtGAG	CGAAAGGTCT	GCAGATTCAA	GCTTgAgTGG
476-555	a	GCTCTGGATT	AAATGATGAA	ACcTATGGCT	ACAAAGAtGG	CAAACcTGt	gTcATTATAA	AGCTCAACCG
	b	GCTCTGGATT	AAATGATGAA	ACTTATGGCT	ACAAAGAGGG	CAAACCGTGC	ATTATTATAA	AGCTCAACCG
	c							
	d	GCTCTGGATT	AAATGAcAAA	ACTTAcGCT	ACgAAGAGGG	CAAACcAc	gTTATTATAA	AGCTCAAGtG
556-635	a	TTCAAACCTA	AGCCTCCCA	GAATGAGTCC	TTGGAGACTT	ACCCAGTGAT	GAAGTATAaT	CCAatATGTCC
	b	TTCAAACCTA	AGCCTCCCA	GAATGAGTCC	TTGGAGACTT	ACCCAGTGAT	GAAGTATAAC	CCAAATGTCC
	c							
	d	TTCAAACCTA	AGtCTCCgA	GAATGAGTCC	TTGGAGACTT	ACCCAGTGAT	GAAGTATAAC	gCAtcTGTgt
636-715	a	tTGCACTGGC	AAGCGtGAcG	AAGATAAGGA	gAAAGTTGGA	AccaTGGAGT	ATTTTGGcCT	GGGCGgCTaC
	b	GTGCATGGC	AAGCGAGATG	AAGATAAGGA	TAAAGTTGGA	AATGTGGGAT	ATTTTGGACT	GGGCAACTCC
	c							
	d	GTGCATGGC	tAGCaAGATG	AAGATAAGGA	TAAaAaTaGA	AATGTGGAGT	ATTTTGGACT	GGGCAACTaC
716-795	a	CTCTaCAGTA	TTAcCCTTAC	TAcGGCAAGc	TCCTGCAGCC	CAAGTACCTG	CAGCCCCTGa	TGGCtGtGCA
	b	CTCTGCAGTA	TTATCCGTAC	TATGGCAAAc	TCCTGCAGCC	CAAATACCTG	CAGCCCCTGc	TGGCCGTACA
	c							
	d	CTCTGCAGaA	TTATCCcTAC	TATGGCAAAc	TCCTGCAGCC	CAAATACCTG	CAGCctCTGc	TtGtCGTACA
796-875	a	CtAcCATGG	ACACTGAAAT	cCGCATAGAG	TGTAAGGCGT	AtGGTGAGAA	CATTGGGTAC	AGTGAGAAAG
	b	CtTACCATGG	ACACTGAAAT	TCGCATAGAG	TGTAAGGCGT	ACGGTGACAA	CATTGGGTAC	AGTGAGAAAG
	c							
	d	CtTACCATGG	ACACTGAAAT	TCGCcTAG--	TGTAAAGcAg	AtGGTGAGAA	CATTGGGcAC	AGTGAGAAAG
876-923	a	GGGACGcTTT	GATGTAAAAA	TTGAAGTTAA	GAGCTGATCA	CAAGCtCt		
	b	GGGACGTTT	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 λ NK β RH11 (c) and λ NK β RH4 (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 β 1-gene, the divergence being within the

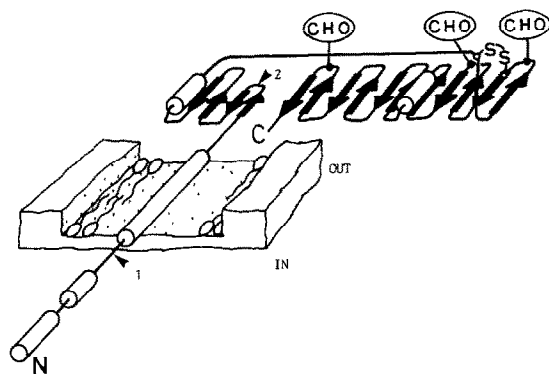


Fig.3. Model for spatial transmembrane organization of Na^+, K^+ -ATPase β -subunit [16]. Cylinders and black arrows correspond to α -helical segments and β -pleated sheets, respectively. Arrowheads with numbers indicate intron positions in $\beta 1$ gene.

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 fami-

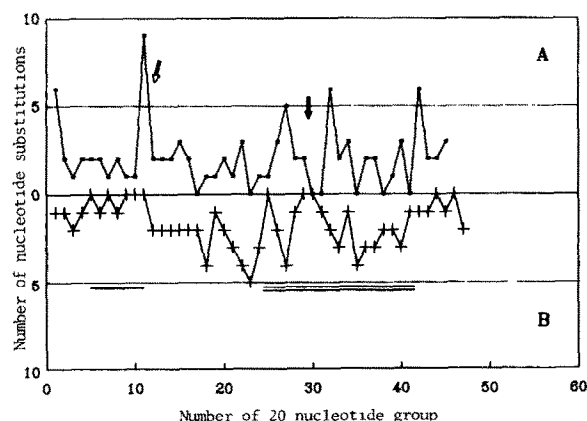


Fig.4. Pairwise comparison of nucleotide substitutions in 3 genes related to Na^+, K^+ -ATPase β -subunit family. (A) HeLa cDNA versus $\beta \psi$; (B) HeLa cDNA versus pig cDNA. Numbers of substitutions are given for every 20 nucleotides.

ly 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.

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