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The family of human Na^+,K^+-ATPase genes

No less than five genes and/or pseudogenes related to the α-subunit

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Five different nucleotide sequences have been found in the human genome homologous to the gene of the α-subunit of Na^+,K^+-ATPase. A comparative analysis of the primary structure of these genes in the region 749–1328 (in coordinates of cDNA from the pig α-subunit) is presented.

Na^+,K^+-ATPase; α-Subunit; Exon/intron structure; Gene family; (Human gene)

1. INTRODUCTION

Recently, we detected in the human genome diverse sequences homologous to the α-subunit of Na^+,K^+-ATPase [1]. At least two of them were shown to be transcribed in adult human brain [1]. This paper demonstrates the existence of at least five different sequences related to the enzyme α-subunit in the human genome.

2. MATERIALS AND METHODS

Human genomic libraries from placenta and adult brain in the EMBL3 vector were constructed according to [1]. Screening of libraries with cDNA probes of the α-subunit from pig kidney Na^+,K^+-ATPase [2], DNA isolation, restriction endonuclease mapping of the recombinant phage DNAs, labeling of DNA fragments, hybridization and DNA sequencing were described in [1].

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3. RESULTS AND DISCUSSION

While analysing human DNA libraries we identified clones which showed strong hybridization in stringent conditions with one DNA probe but had various restriction endonuclease maps (data to be published elsewhere). Fig.1 outlines some nucleotide fragments from the clones λNKαR3-2, λNRαSW3.2, λNKαR15-1, λNKαRD-16 and λNKαTW-4. The various DNA sequences are from 84.6% to 75.8% identical when compared with the sequences of λNKαR3-2; the corresponding amino acid sequences are from 91.2% to 85% identical to the protein fragment coded by λNKαR3-2 (table 1).

Here, we succeeded in determining the complete structure of exon parts and the partial structure of intron parts, of the gene, which comprised the insertion of recombinant phage λNKαR3-2. The largest portion of this sequence was published [1]. Pronounced variations were found upon comparison of the primary structure of the exons of this gene with the structure of the α-subunit cDNA from HeLa cells [3], which appeared shortly before our paper was submitted. This result, as well as the

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Fig.1. Nucleotide sequences of five different gene fragments related to the α-subunit of Na^+,K^+-ATPase from human placental and brain genomic libraries. The region 749–1328 of exons (in coordinates of α-subunit cDNA from pig [2]) is presented. Figures in parentheses denote the size of completed introns in bp. (– – –) Structure not determined; (···) regions of incomplete introns.

Table 1

The differences (%) in nucleotide and amino acid sequences of five different genes in comparison with that of λNKαR3-2

N	Gene	Nucleotide differences (%)	Amino acid differences (%)
1	λNKαSW3.2	17.2 (29/168)	12.7 (7/55)
2	λNKαR15-1	17.0 (24/141)	15.0 (7/47)
3	λNKαRD-16	16.4 (94/572)	8.8 (17/192)
4	λNKαTW-4	24.2 (83/343)	13.1 (15/114)

Figures in parentheses represent the number of substitutions and the length of the fragment, respectively

evident variety of genomic sequences hybridizable to the same probes, prompted us to examine the cDNA library from the adult human brain in order to ascertain whether these sequences are transcribed there. Recently [1] we identified cDNA clones corresponding to exonic parts of λNKαR3-2 as well as to mRNA from HeLa cells of which the structure has been determined [3]. The last sequence is completely identical to the exon sequences of one of the genomic clones listed above, namely λNKαTW-4 (fig.1). So, there exist at least two human genes related to the α-subunit of Na^+,K^+-ATPase and transcribed in human brain.

Primary structures of cDNAs for three different forms of the α-subunit of rat brain Na^+,K^+-ATPase were published [4]. Gene λNKαR3-2 is closely homologous to one of them designated as αIII [4]. The exon sequences of gene λNKαNW-4 (identical to the cDNA sequence of the α-subunit isolated from HeLa cells [3]) are strongly related to the mRNA for the α-form according to the designation used in [4]. We also determined a significant part of exons of another gene; the part is presented in clone λNKαRD-16. This gene differs

Fig.2. Amino acid sequences corresponding to the nucleotide sequences presented in fig.1. The amino acid numbers are according to the α-subunit of pig Na^+,K^+-ATPase (246–437) [2]. Arrows indicate location of introns. Amino acid substitutions when compared with λNKαR3-2 are boxed.

from λNKαR3-2 and λNKαTW-4 and is strongly homologous to the mRNA of the α^+-form of Na^+,K^+-ATPase [4]. Two other sequences, λNKαSW3.2 and λNKαR15-1, differ from each other as well as from λNKαR3-2 and λNKαRD-16. We have not enough data to compare directly these sequences and that of λNKαTW-4, but both sequences differ from the HeLa cell's mRNA sequences [3], completely identical to the exon sequences of λNKαTW-4 in the known regions. These new sequences may represent genes of other isoforms of the α-subunit of Na^+,K^+-ATPase, pseudogenes formed during evolution, of the genes coding for other ion-transporting ATPases strongly homologous to the enzyme α-subunit.

Thus the analysis of restriction endonuclease maps as well as the direct sequencing shows that the human genome includes at least five highly homologous sequences related to the mRNA of the α-subunit of Na^+,K^+-ATPase. This implies one common ancestor sequence for all these sequences.

It is of interest to compare the distribution of conserved and variable regions in the genes. This paper presents sequences containing three exons 749–1017, 1018–1216 and 1217–1328 (in coordinates of the pig kidney α-subunit nucleotide sequence [2]) and four introns. In all the genes studied the localization of introns is the same but their sequences and sizes differ considerably. For instance, intron 1 in gene λNKαR3-2 is 115 bp long; at the same time this intron in gene λNKαTW-4 is over 200 bp in length, and the same intron in gene λNKαRD-16 is over 600 bp. In contrast, the exon sequences of various genes are highly homologous (fig.2). Highly conserved exon 1018–1216 codes for the region of the α-subunit active centre including Asp^{369}, known to form the phosphorylated intermediate during ATP hydrolysis. Practically the entire amino acid sequence coded by this exon is homologous to the corresponding region of Ca^{2+}-ATPase of sarcoplasmic reticulum [5]. In contrast, the most variable exon 1216–1328 probably codes for the protein fragment unimportant for the function. Exon 749–1018 coding for the third and fourth transmembrane segments [2] is moderately conserved and the variable amino acids are as a rule isofunctional. The most homologous regions correspond to the N- and C-termini of the coded polypeptide, and are homologous to the corresponding fragment of Ca^{2+}-ATPase [5]. Thus various genes of the family of ion-transporting ATPases of different specificity preserve the distribution of conserved and variable domains, at least in the region studied.

The discovery of multiple Na^+,K^+-ATPase α-subunit genes poses a number of questions. What are the functions of proteins coded by these genes? Is their expression constitutive and the same in different tissues or is it tissue-specific? Is there time-dependent gene expression during embryo development and cell differentiation? The answers will undoubtedly shed light on the role of ion transport in regulation of the life cycle of cells and organisms.

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