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

A partial nucleotide sequence related to the α-subunit

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$(Na^+ + K^+)$-ATPase; Nucleotide sequence; cDNA

1. INTRODUCTION

The Na^+,K^+-activated adenosine triphosphatase (EC 3.6.1.3) localized in animal cell membranes represents a universal system for the active transport of Na^+ and K^+.

The enzyme is an oligomer composed of two polypeptide chains. Its α-subunit (110 kDa) contains a site for ATP hydrolysis and a site for binding of cardiac glycosides which are specific inhibitors of the enzyme. There is still no evidence of the functional role of the glycosylated β-subunit (44 kDa).

In order to elucidate the molecular mechanism of the active cation transport we have analysed the structural organization of the enzyme from several species. The complete nucleotide sequences of cDNA clones corresponding to the coding regions of appropriate mRNAs were determined, and the primary structure of the α- and β-subunits of the pig kidney Na^+,K^+-ATPase could then be deduced [1–3].

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We are currently studying the structural organization of the genes of the Na^+,K^+-ATPase in the human genome. Screening of human genomic libraries with pig kidney α-subunit cDNA probes revealed several positively hybridizing clones under stringent conditions. Here, we present the results of a structural analysis of two such clones which contain sequences related to the major part of the pig kidney cDNAs.

A further motive for this publication is concerned with a recent paper by Kawakami et al. [4] presenting the sequence determination of the cDNA encoding the Na^+,K^+-ATPase from HeLa cells. Comparison of our genomic sequences with those of Kawakami et al. [4] revealed clear differences. The reasons for such a discrepancy are not obvious, and we therefore felt that we should bring this fact to the attention of scientists involved in investigations of the Na^+,K^+-ATPases. Parts of the present data have been published earlier [5].

2. MATERIALS AND METHODS

2.1. *Construction of genomic libraries*

A human genomic library from human placental DNA was obtained with the use of the vector

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λEMBL3 [6] after partial cleavage of the DNA with *Sau*3AI restriction endonuclease. The resulting library (1.5×10^6 independent clones) was screened with the nick-translated *Pst*I fragment of plasmid pB2801 [1] which codes for the central part of the pig kidney α-subunit (coordinates 1296–2880; henceforth, all coordinates are given in accordance with the appropriate α-subunit cDNA sequence of pig described in [3].)

2.2. *Hybridization procedure*

Restriction endonuclease digestions of the genomic and recombinant phage DNAs were carried out as recommended by the suppliers. Preparation of Southern blots, labelling of DNA fragments, and subsequent hybridization were performed using published procedures [7].

Nick-translated *Pst*I fragments of plasmids pB29 (2432–3405), pB2801 (1296–2880) and pB159 (−216 to 1160) [8] containing C-terminal, middle and N-terminal parts of the pig kidney α-subunit cDNA respectively were used as hybridization probes. Below they are designated as C-, M- and N-probes. A fragment of one of the human genomic clones (λNKα10-3) containing a C-terminal part of the Na^+,K^+-ATPase gene (the exon 2607–2712 and 40 bp of the flanking intron) was recloned into M13mp9, and the phage used further in order to obtain the single-stranded hybridization probe, which is referred to as a human C-probe (fig.1).

2.3. *Restriction endonuclease mapping of the recombinant phage DNAs*

Labelling of the right arm of the recombinant phage DNAs was carried out using the Klenow fragment of *E. coli* DNA polymerase I and [α-^{32}P]dGTP according to Wu [9]. Partial cleavage of the DNAs by restriction endonucleases, subsequent gel electrophoresis and radioautography were carried out according to [10].

2.4. *Nucleotide sequence analysis*

The nucleotide sequences of the three genomic clones λNKα10-3, λNKα3-2 and λNKα15-1 were determined by the method of Sanger et al. [11] with modifications [12] after subcloning of their genomic inserts into the vector M13mp9.

3. RESULTS AND DISCUSSION

With the use of the M-probe ten positively hybridizing clones were selected from the human genomic library.

Considering the Southern blot hybridizations with different α-subunit cDNA parts, two of these clones (λNKα10-3 and λNKα3-2) contain the major part of the gene. Their restriction endonuclease maps were constructed on the basis of partial cleavage of appropriate recombinant phage DNAs which had been labelled at one of the termini with ^{32}P [9] (fig.1). The deduced maps were confirmed by complete restriction endonuclease digests, followed by electrophoresis, Southern blotting and subsequent hybridization using standard techniques [7].

The direct sequencing confirmed the conclusion that these two clones overlap. The overlapping parts of the clones had rather long identical sequences including exonic as well as intronic regions (fig.1).

The nucleotide sequence of 17 exons as well as of 18 intervening sequences which are related to the α-subunit of the human Na^+,K^+-ATPase is presented in fig.2. The sequence of the exons corresponds to coordinates 382–3063 of the pig kidney α-subunit nucleotide sequence. The region flanking the 3′-terminal exon covers 545 bp and has no homology with the 3′-untranslated region of the pig kidney cDNA. The lack of homology in this region might demonstrate the existence of a nonidentified intron.

The DNA sequences at the 5′- and 3′-splice junctions of all exon-intron boundaries correspond to consensus sequences [13].

Comparison of the determined structure with all known amino acid sequences of α-subunits of Na^+,K^+-ATPases from different sources [3,14,15] reveals the non-random distribution of variable amino acids. The major part of the substitutions is observed in the protein region which is coded by the 5′-proximal fragments of exons 1655–1830 and 1968–2118, and a middle part of exon 2567–2712. This might indicate that these parts of the α-subunit are less important for the functioning and structural organization of the enzyme than the highly conserved regions.

On the other hand the amino acid sequences which are involved in the regions of the

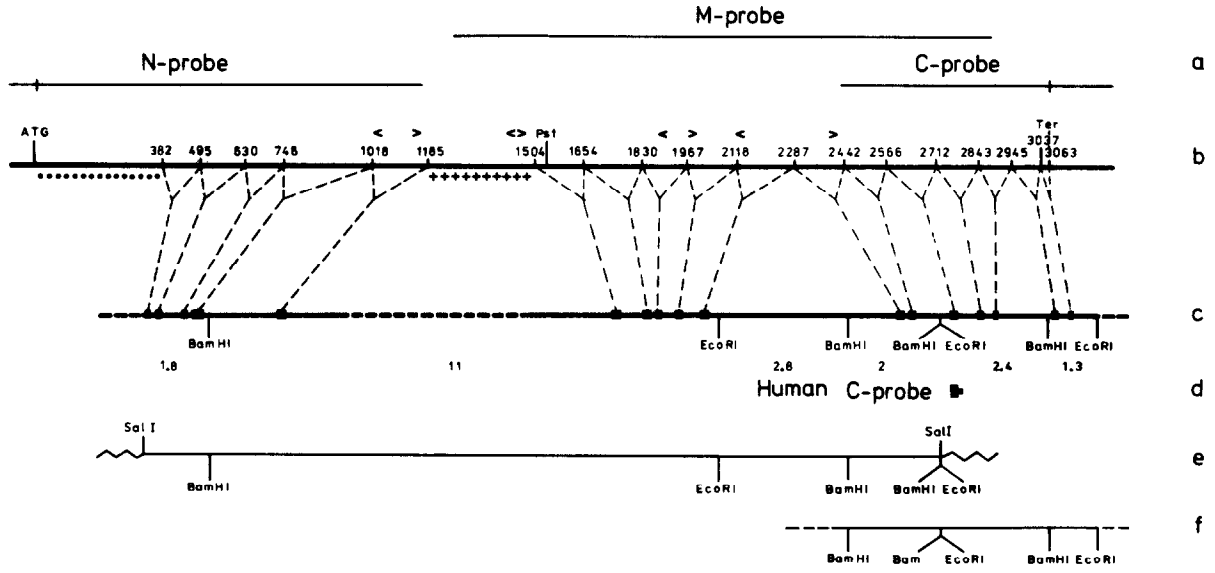


Fig.1. Structural organization of the human genomic sequence related to the α-subunit of the Na^+,K^+-ATPase. (a) The pig kidney cDNA fragments used as hybridization probes. (b) The pig kidney α-subunit cDNA. Indicated are the coordinates (bp) which correspond to the exon boundaries in the human nucleotide sequence. The ATG codon, central *Pst* restriction site and translation termination codon are shown. (+ + +) The fragment under investigation; (···) the human gene fragment which has not been found yet. (≪ ≫) The boundaries of the fragments derived from λNKα15-1, homologous to the determined α-subunit nucleotide sequence. (c) Exon-intron organization of the determined nucleotide sequence. (■) Exons, (▪) introns. Figures below represent the length of restriction fragments in kb. (d) Location of the human C-terminal probe (see section 2). (e,f) Restriction endonuclease maps of overlapping clones λNKα3-2 and λNKα10-3 (fragment), respectively. (∼∼) Phage arms.

hypothetical active centre (Asp^{369}, Lys^{501}, Asp^{710}, Asp^{714}, Lys^{719}) [3,15–17] (exon 1018–1185, 1504–1654, 2119–2287) are highly conserved, and reveal a high extent of homology with respective regions of other ion-transporting ATPases [18,19]. Moreover, the homologous sequences are also coded by exons 496–630, 749–1018, 2443–2566 and 2946–3037. It should be noted that introns 2, 3, 7, 12, 13, 16 and 17 are located within the boundaries of homologous regions. On the other hand, introns 4, 5, 8 and 10 are located in the middle of homologous regions. (A more detailed analysis of the exon-intron structure of the α-subunit gene will be published elsewhere.)

The primary structure of the exons determined here differs from that coding for the HeLa α-subunit [4]. The data on amino acid substitutions for all the known α-subunits are listed in table 1. Thus, as follows from table 1, the evolutionary rate of amino acid substitutions due to divergence of sheep and pig amounts to 0.33×10^{-9} substitutions/site per year. Furthermore, the evolutionary rates of such substitutions between man, pig and sheep according to our data in both cases are about 0.5×10^{-9} substitutions/site per year. These values are in good agreement with each other although they are 2-times higher than the evolutionary rates estimated in the case where all the amino acid sequences had been compared with that of *Torpedo* electroplax (0.176×10^{-9} substitutions/site per year). On the other hand, the evolutionary rate obtained for the α-subunit from HeLa cells is essentially lower than in the case of divergence between sheep and pig. The mean value estimated in the case of HeLa and pig, or HeLa and sheep is about 0.08×10^{-9} substitutions/site per year. This is 2-fold lower than the evolutionary rates due to divergence between ray and mammals. Comparison of the amino acid sequences of the α-subunit of Na^+,K^+-ATPase encoded by the gene in

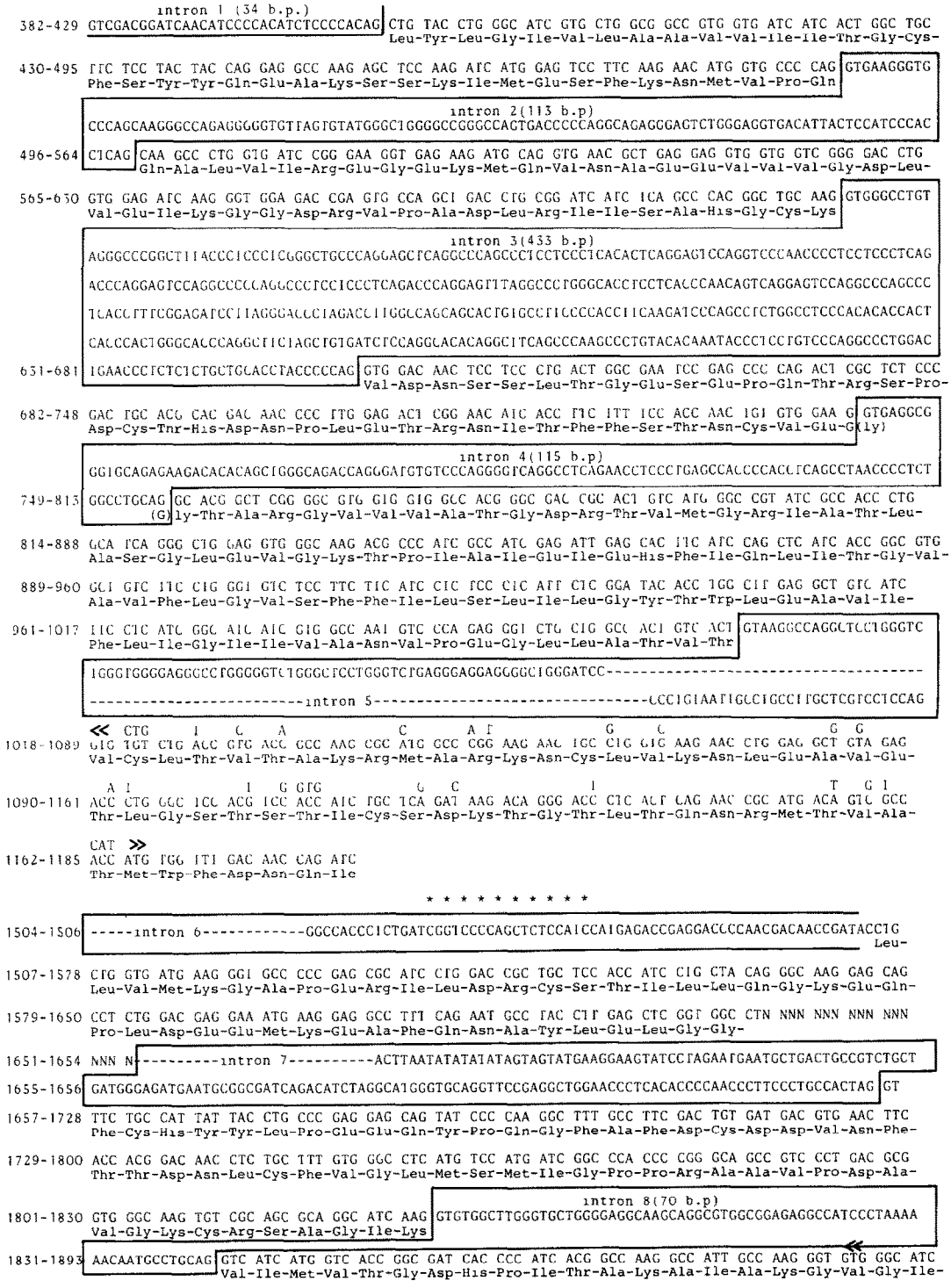
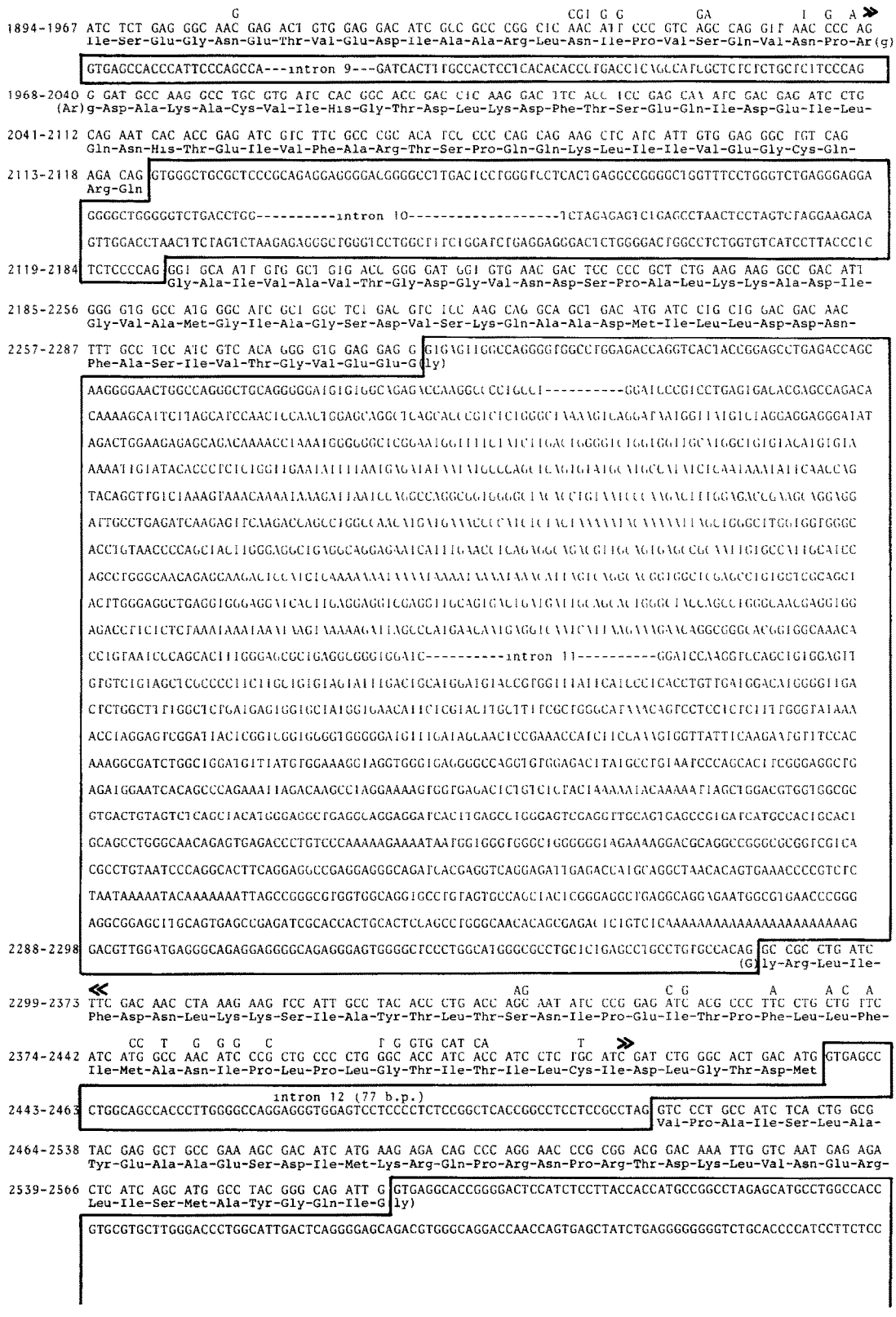


Fig.2. For legend, see p. 78.



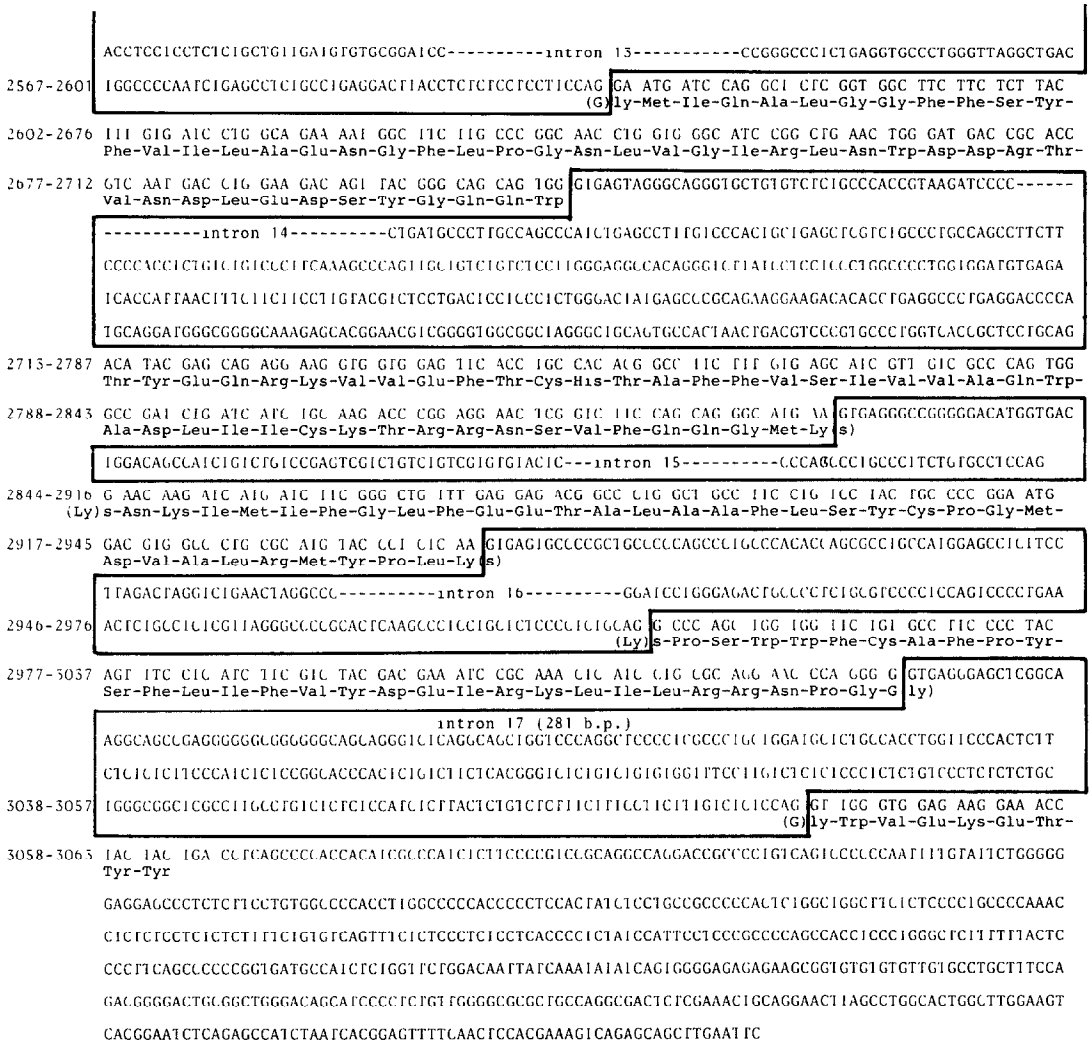


Fig.2. The human genomic nucleotide sequence related to the α-subunit of Na^+,K^+-ATPase. Pig kidney α-subunit coordinates are indicated. Intervening sequences are boxed. Differences in the nucleotide sequence of $\lambda NK\alpha 15\text{-}1$ are shown in the upper line. The gap in the nucleotide sequence is designated by asterisks.

question with the other known amino acid α-subunit sequences reveals the conserved character of substitutions: only 31 of 70 substitutions are nonconserved when human exons are compared with the corresponding sequences of pig.

The picture of nucleotide substitutions in exons is characteristic for coding sequences – the silent substitutions are much more frequent than substitutions leading to amino acid substitutions.

The data on evolutionary rates of amino acid substitutions and the defined correlation between silent and total nucleotide substitutions allow us to suggest that in our case we are dealing with a functional gene, although the final conclusion can only be made on the basis of the whole gene sequence and its transcription evidence.

The discrepancy between our data and those of Kawakami et al. [4] is not clear. One of the explanations could arise from the existence of several genes coding for different forms of α-subunits. In

Table 1

Amino acid substitutions in α-subunits of Na^+,K^+-ATPases[a] from different sources and rates of amino acid substitutions (lower value) ($K_{aa} \times 10^{10}$/site per year)

	Ray [14]	Pig [3]	Sheep [15]	HeLa [4]	Man
Ray		83	85	80	100
		1.41	1.45	1.36	1.76
Pig			10	4	70
			1.43	0.287	5.40
Sheep				10	73
				0.720	5.63
HeLa				0	71
Man					

[a] Amino acid substitutions are calculated for the sequence between coordinates 180 and 1016 of Na^+,K^+-ATPase α-subunit from pig encoded by exons whose structure is presented in this paper

Time of divergence of filetic lines: ray–mammals, 400×10^6 years; sheep–pig, 45×10^6 years; sheep, pig–human, 90×10^6 years [20]

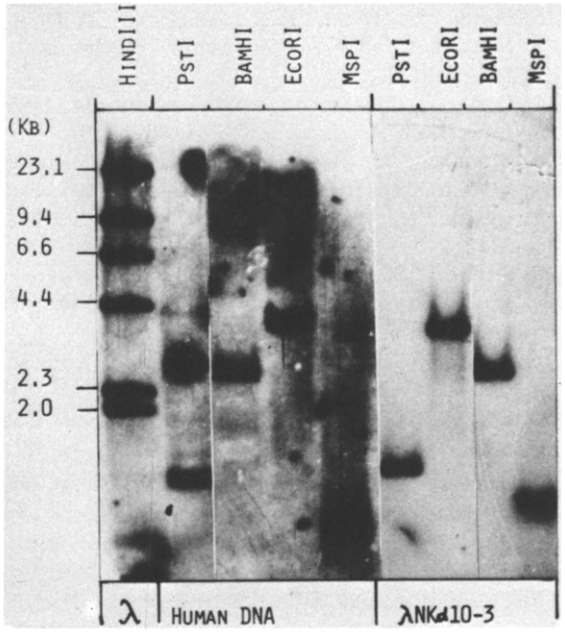


Fig.3. Genomic Southern blot. High-M_r placental DNA and DNA of the clone λNKα10-3 were digested with the indicated restriction endonucleases, electrophoresed through a 0.8% agarose gel, blot transferred to Gene Screen and hybridized to the human C-terminal probe (see section 2).

Table 2

Evolutionary rates for different proteins, including α- and β-subunits of the Na^+,K^+-ATPase ($K_{aa} \times 10^9$/year)

Protein	Amino acids	Nucleotides	
		Synonymous	Total
α-Globin	1.2	2.9	1.6
Myoglobin	0.89	–	–
β-Subunit of Na^+,K^+-ATPase	0.51	0.62	0.52
Insulin A and B chains	0.44	2.4	1.04
Cytochrome *c*	0.3	–	–
α-Subunit of Na^+,K^+-ATPase	0.18	1.69	0.57
Histone H4	0.01	3.7	–
α-Tubulin	0.01	0.88	0.34

fact, it has been shown that in the brain there are two forms of catalytic subunits designated α and α^+ [21,22].

Our data reveal the existence of an additional sequence (human genomic clone λNKα15-1) closely related to that of the α-subunit but still different from the sequence determined in this work as well as from the HeLa cDNA sequence [4] (see fig.1).

Nevertheless, Southern blotting of the human placental DNA digested with several restriction endonucleases, and probed with human C-terminal probe (see section 2), reveals a simple pattern which is similar to that of λNKα10-3 (fig.3).

These data support the assumption that there is a common sequence coding for the 3′-terminal region of the α-subunits of the Na^+,K^+-ATPase. It might also be possible that the variable region of the enzyme is encoded by the N-terminal part(s) of the gene(s).

ADDENDUM

By the time this manuscript was ready for submission we found at least two different nucleotide sequences in the human genome which were closely related to the catalytic subunit of the human Na^+,K^+-ATPase. These data provide evidence for the existence of a family of genes (and pseudogenes?) related to the α-subunit of the Na^+,K^+-ATPase.

In the cDNA library from human adult brain, screening has revealed two types of sequences related to the α-subunit of the pig kidney enzyme. One is identical with the α-subunit cDNA from HeLa cells [4], while the other is identical with the exon parts of the nucleotide sequence in fig.2. Therefore we conclude that, at least in adult human brain, this gene is expressed. It is possible that this sequence codes for the α^+-form of the catalytic subunit of Na,K-ATPase [21,22].

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