Volume 221, number 1, 129–133 FEB 05064 August 1987

Family of Na^+,K^+-ATPase genes

Intra-individual tissue-specific restriction fragment length polymorphism

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Received 7 July 1987

Intra-individual tissue-specific restriction fragment length polymorphism (RFLP) has been demonstrated in DNA isolated from different mammalian tissues using cDNAs of α- and β-subunits of Na^+,K^+-ATPase as hybridization probes. We propose that the RFLPs could result from gene rearrangements in the gene loci for the α- and β-subunits of Na^+,K^+-ATPase. The changes in restriction patterns have been shown to occur during embryonic development and tumor formation. In addition, the tissue specificity of the expression of different genes of the family of Na^+,K^+-ATPase genes and their low expression in tumor cells have been demonstrated.

$(Na^+ + K^+)$-ATPase; Gene family; Genomic blotting; Tissue specificity; Restriction fragment length polymorphism; Gene expression

1. INTRODUCTION

The Na^+,K^+-ATPase of most animal cells is responsible for the active transport of Na^+ and K^+ across the cell membrane. The enzyme contains two types of subunits, a catalytic subunit α (110 kDa) and a glycoprotein β (40 kDa). Recently, cDNAs coding for α- and β-subunits from different sources have been cloned and sequenced [1–4].

Two different molecular forms of the catalytic subunit of the Na^+,K^+-ATPase (α,α^+) have been identified [5]; the N-terminal sequences of these subunits differ considerably and this suggests the existence of different genes coding for these molecular forms. Indeed, recently we have shown the existence of a family of genes containing no less than five members related to the α-subunit of Na^+,K^+-ATPase [6–8], at least two of which are transcribed in human brain [9]. Screening of rat kidney and brain cDNA libraries has led to the isolation of structural genes coding for three molecular forms of the catalytic subunit [10].

Using cloned cDNA of α- and β-subunits of Na^+,K^+-ATPase as hybridization probes, we have unexpectedly found intra-individual restriction fragment length polymorphism in DNA isolated from different mammalian (mouse, rabbit, human) tissues involving restriction sites for *Eco*RI, *Pst*I and *Bgl*II.

These tissue-specific RFLPs could be the result of rearrangements in the gene loci for the α- and β-subunits of Na^+,K^+-ATPase.

2. MATERIALS AND METHODS

Chromosomal DNA was prepared from nuclei [11] isolated from different tissues of mouse, rabbit and man using the standard aqueous-phenol extraction procedure. 5 μg DNA were digested with

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

50 U of various restriction enzymes for 16–20 h at 37°C. DNA fragments were electrophoresed through a 0.8% agarose gel and then blot-transferred to Hybond-H nylon membrane (Amersham) according to the manufacturer's specifications. The filters were prehybridized and hybridized [12] with ^{32}P-labeled cDNA inserts subcloned into the phage cloning vector M13 mp18. The filters were exposed to X-ray film for 12–72 h.

Total cellular RNA was isolated from normal and tumor human tissues by the guanidinium/hot phenol method [13]. Tumor tissues of kidney origin (Wilms' tumor, renal carcinoma) were transplanted into nude mice. The poly(A^+) fraction of total cellular RNA was obtained after two cycles of chromatography on oligo(dT)-cellulose [14]. Poly(A^+) RNA samples (7 μg) were electrophoresed through a 1.5% agarose gel, containing 2.2 M formaldehyde [15], blot-transferred to Hybond-H nylon membrane and hybridized with gene-specific oligoprobes under the conditions described earlier [16].

3. RESULTS AND DISCUSSION

Southern blot analysis of *Eco*RI-digested human genomic DNA isolated from different tissues (some of them from one individual) and probed with a ^{32}P-labeled α-subunit-specific cDNA fragment (coordinates 1545–2880; henceforth, all coordinates are given in accordance with the α- or β-subunit pig cDNA sequences described in [4]) clearly shows the length polymorphism and variation in copy number of DNA fragments (fig.1A). We have found similar tissue-specific RFLPs in the α-subunit genes for the restriction enzymes *Pst*I and *Bgl*II (not shown). Southern blot analysis of restricted DNAs isolated from different tissues of mouse and rabbit also showed tissue-specific polymorphism (not shown). The use of a shorter α-subunit cDNA fragment as hybridization probe (coordinates 1545–1946 [4]) shows the same picture in the case of *Eco*RI fragments. These data as well as the size of hybridization bands allow us to exclude the existence of a polymorphic region upstream or downstream of the gene.

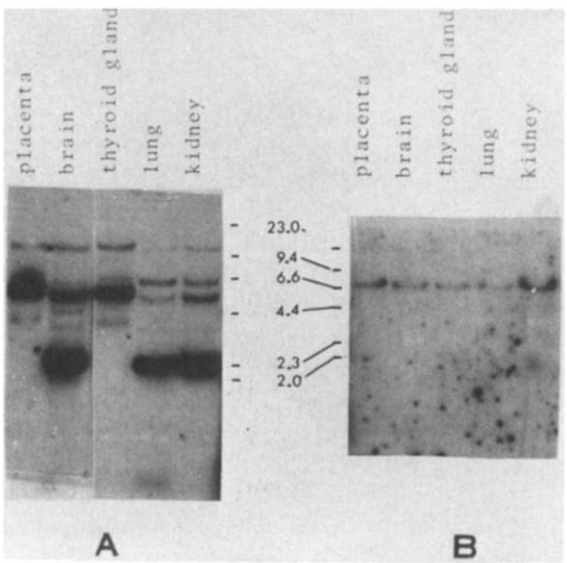

Fig.1. Genomic blotting analysis of human chromosomal DNA from different tissues digested with *Eco*RI restriction endonuclease using as probes (A) the 1300 bp fragment of pig kidney cDNA of the α-subunit isolated from the clone pB2801 [4] and (B) γ-interferon cDNA fragment [17]. Size markers are a *Hin*dIII digest of λ DNA. Brain, lung, kidney and thyroid gland have all been obtained from one individual.

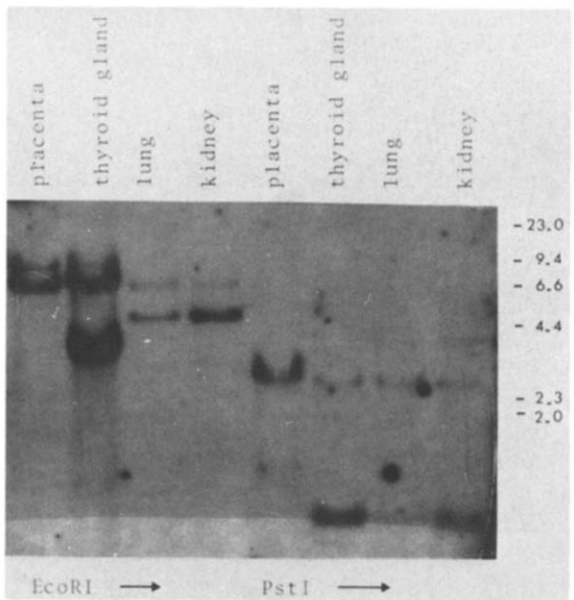

Fig.2. Genomic blotting analysis of human chromosomal DNA from different tissues digested with *Eco*RI and *Pst*I restriction endonucleases using as probe the 1300-bp fragment of pig kidney cDNA coding for the β-subunit of Na^+,K^+-ATPase from the clone pNβ31 [20]. Size markers are shown on the right.

Tissue-specific *Eco*RI and *Pst*I RFLPs have also been observed in the case of hybridization with a β-subunit-specific probe (fig.2).

The expected band pattern obtained after hybridization of the same blot with a γ-interferon-specific probe [17] (fig.1B) excludes incomplete digestion or other artefacts as the cause of the effects observed.

Analysis of the *Eco*RI digests of DNA isolated from mouse embryos of different age and from the adult mouse (fig.3) has demonstrated non-identical hybridization patterns of DNAs from embryos of different stages of prenatal development as well as differences between the embryonic and adult mouse DNA. Comparison of the patterns of α-subunit cDNA hybridization with genomic DNA fragments from normal lung and two different types of lung tumor also revealed polymorphism in restriction fragment length (fig.4). Therefore, we conclude that alterations in the α-subunit genes are likely to be a general feature of differentiation and dedifferentiation.

The question arises as to what are the structural reasons and functional significance of the phenomena observed. One possible explanation for tissue-specific RFLPs in Na^+,K^+-ATPase genes could be connected with different copy number of genes coding for different forms of the α- and β-subunits in different tissues. The possibility that the tissue-specific RFLPs are associated with tissue-specific modification (e.g. methylation) of the genes for the Na^+,K^+-ATPase subunits also cannot be discounted. However, the recurrence of the RFLPs when various restriction endonuclease are used is not consistent with this proposal. The third and from our point of view most probable explanation of the RFLPs in Na^+,K^+-ATPase genes seems to be somatic rear-

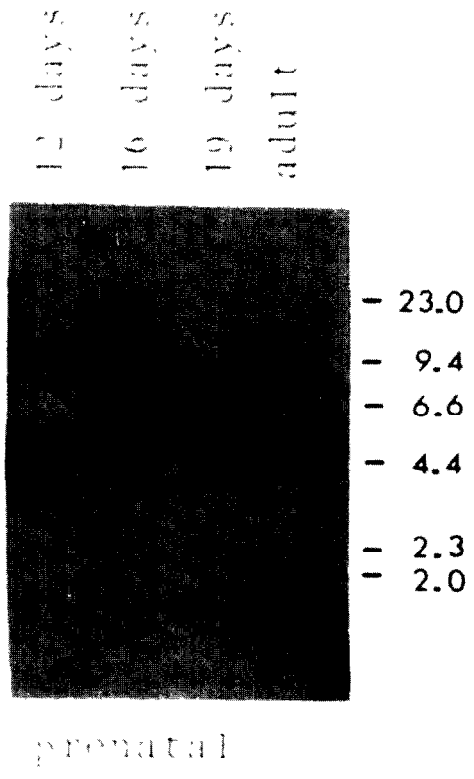

Fig.3. Southern blot analysis of mouse chromosomal DNA digested with *Eco*RI restriction endonuclease. DNA was isolated at different stages of embryonic and postnatal development. Hybridization was performed with the pig kidney α-subunit cDNA probe (see legend to fig.1). Size markers are shown on the right.

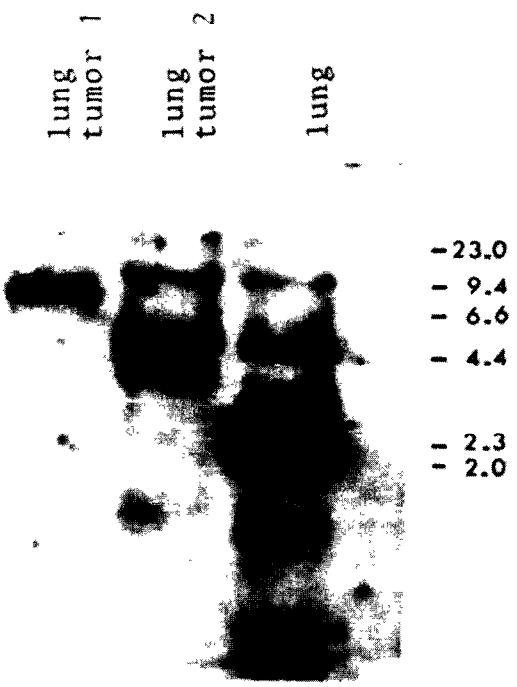

Fig.4.Southern blot analysis of human chromosomal DNA isolated from the lung or different types of lung tumor digested with *Eco*RI restriction endonuclease probed with pig kidney α-subunit cDNA fragment (see legend to fig.1). Size markers are shown on the right.

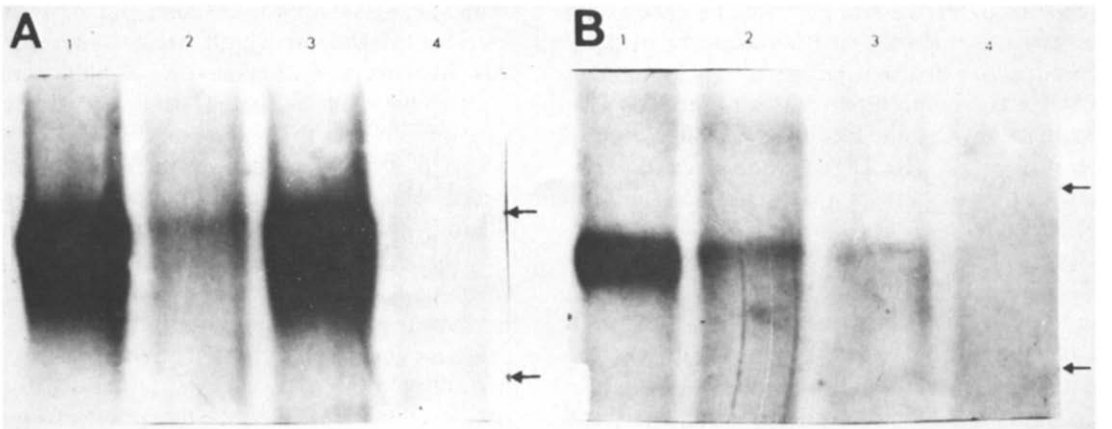

Fig.5. Northrn blot hybridization of poly(A^+) RNA from human kidney, brain, thyroid gland and liver (lanes 1–4, respectively) with (A) NKαTW-4-specific oligoprobe (CATACTTATCACGTCCAACCCCCTT) and (B) NKαR3-2-specific oligoprobe (GTGAGTCCTTGTCATCTTTCTTGTC). NKαTW-4 and NKαR3-2 are members of the α-subunit gene family [6,7] and code for the α and αrp1 molecular forms of the catalytic subunit, respectively (the αrp1 molecular form found in human brain [9] is an equivalent of αIII detected in rat brain [10]. Both gene-specific oligoprobes correspond to the regions of mRNAs coding for the peptides with coordinates 3–11. Arrows indicate the positions of 28 S and 18 S ribosomal RNA.

rangements, which at present are known in mammals only for genes of the immune system [18,19].

As concerns the functional consequences of somatic rearrangements of ATPases genes, it is tempting to propose that there is a connection between the gene structure and the expression of genes in different tissues. Our experiments demonstrated the tissue-specific expression of at least two of the genes from the α-subunit gene family (fig.5). Moreover, their expression is strictly diminished in tumor tissues (fig.6). Therefore we propose that there is a correlation between the

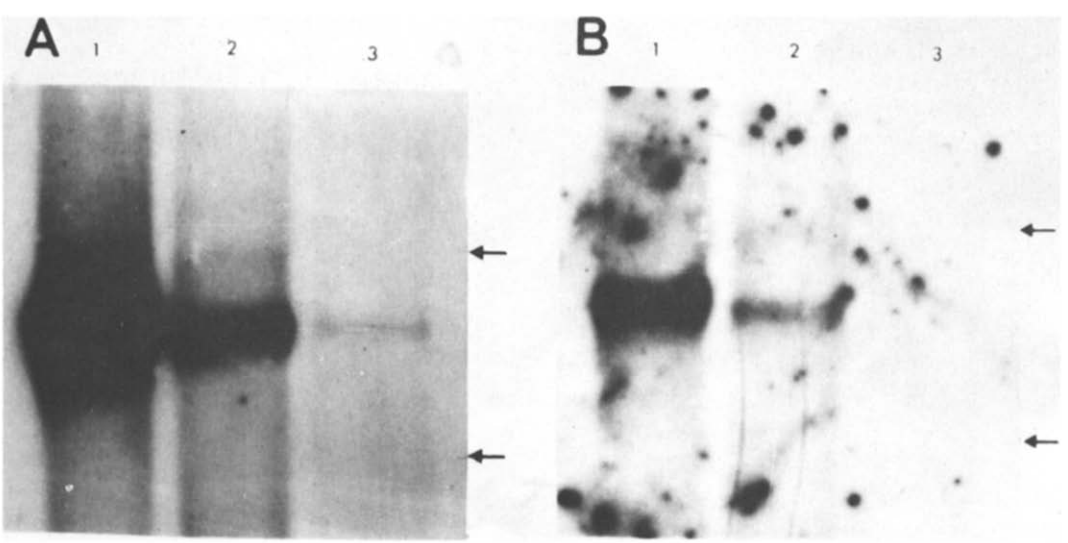

Fig.6. Northern blot hybridization of poly(A^+) RNA from normal human kidney, renal carcinoma and Wilms' tumor (lanes 1–3, respectively). For explanation of (A,B) see legend to fig.5. Positions of 28 S and 18 S ribosomal RNA are indicated by the arrows.

organisation of ATPase genes and the specificity and/or level of their expression. The present data pose the question of whether the role of the Na^+,K^+-ATPases is limited only to ion transport or whether there are some additional functions of the enzyme in the cell which require gene rearrangements.

ACKNOWLEDGEMENTS

We are indebted to V. Gindilis for assistance and helpful discussions throughout the course of this project. We also thank N. Medvedeva, R. Allikments and O. Larionov for assistance in some experiments and E.S. Revasova for providing tumor tissues.

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