cDNA Cloning of Na ,K -ATPase α-Subunit from Embryos of the Sea Urchin, Hemicentrotus pulcherrimus

Authors: Mitsunaga-Nakatsubo, Keiko, Yamazaki, Ken, Hatoh-Okazaki, Midori, Kawashita, Hiroto, Okamura, Chisato, et al.

Source: Zoological Science, 13(6) : 833-841

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.13.833

ZOOLOGICAL SCIENCE 13: 833–841 (1996)

cDNA Cloning of Na^+, K^+-ATPase α-Subunit from Embryos of the Sea Urchin, *Hemicentrotus pulcherrimus*

Keiko Mitsunaga-Nakatsubo[1,*,**], Ken Yamazaki[1,*], Midori Hatoh-Okazaki[1], Hiroto Kawashita[1], Chisato Okamura[1], Koji Akasaka[2], Hiraku Shimada[2] and Ikuo Yasumasu[1,***]

[1]*Department of Biology, School of Education, Waseda University, 1-6-1, Nishiwaseda, Shinjuku-ku, Tokyo 169-50, Japan*
[2]*Graduate Department of Gene Science, Faculty of Science, Hiroshima University, 1-3-1, Kagamiyama, Higashi-Hiroshima 739, Japan*

ABSTRACT—Na^+, K^+-ATPase α-subunit cDNA of the sea urchin, *Hemicentrotus pulcherrimus*, was obtained by twice screening prism and gastrula λgt10 cDNA libraries using an oligonucleotide probe derived from a mostly conserved region, FSBA (5'-*p*-(fluorosulfonyl)-benzoyladenosine) binding site of cation transport ATPases. The 5'-end of the non-coding region was determined by primer extension and the region was amplified by 5'-RACE method. The sea urchin α-subunit cDNA consists of 4401 nucleotides and encodes 1038 amino acid residues (MW, 114 kDa). The predicted primary structure, except N-terminal region, has similar degree of high homology to various metazoan Na^+, K^+-ATPase α-subunits. Alignment of amino acid sequence and a hydropathy profile also predicts eight putative transmembrane segments at least. The phylogenetic tree suspected from alignment of amino acid sequences of 21 species suggests that sea urchin and vertebrate Na^+, K^+-ATPase α-subunits seem to have evolved from a common origin, before vertebrate α-subunit divided into three isoforms.

INTRODUCTION

Na^+, K^+-ATPase is a membrane-bound enzyme responsible for active transport of Na^+ and K^+ across the plasma membrane in most animal cells. The protein is composed of two subunits; a large catalytic α-subunit and a smaller glycosylated β-subunit (Lingrel and Kuntzveiler, 1994). The α-subunit contains an intracellular ATP-binding site (Farley *et al.*, 1984), a phosphorylation site (Post *et al.*, 1973; Walderhaug *et al.*, 1985), and an extracellular binding site for cardiac glycosides such as digoxigenin and ouabain (Schwartz *et al.*, 1975; Lingrel *et al.*, 1990). Three α-isoforms, α1, α2 and α3, in mammals (Shull *et al.*, 1986; Hara *et al.*, 1987; Herrera *et al.*, 1987) and in chicken (Takeyasu *et al.*, 1988, 1990), two in *Artemia* (Baxter-Lowe *et al.*, 1989; Macías *et al.*, 1991), and only one in *Drosophila melanogaster* (Lebovitz *et al.*, 1989), as well as three β-isoforms, β1, β2 and β3 (β3 is only found in *Xenopus laevis* (Good *et al.*, 1990)) have been identified so far (Sweadner *et al.*, 1989). Each subunit isoform of vertebrates shows different tissue-specific and developmentally regulated expressions (Herrera *et al.*, 1987; Orlowski and Lingrel, 1988).

Enzymatic studies revealed that the plasma membrane of sea urchin eggs and embryos contains an ouabain-sensitive Na^+, K^+-ATPase activity (Kinsey *et al.*, 1980; Mitsunaga *et al.*, 1986, 1989), which is stimulated just after fertilization (Ciapa *et al.*, 1984). We have shown the change in the activity of Na^+, K^+-ATPase during early development of the sea urchin *Hemicentrotus pulcherrimus* (Mitsunaga-Nakatsubo *et al.*, 1992a). The activity begins to increase at the mesenchyme blastula stage and reaches the maximum at the gastrula stage. The increase at the gastrula stage is actinomycin D-sensitive and is probably due to the increase in ectoderm cells. Northern blot analysis, using a fragment of the *Hemicentrotus* Na^+, K^+-ATPase α-subunit cDNA, reveals that the mRNA is about 4.6 Kb long and its maximum expression is at the mesenchyme blastula and the gastrula stages (Mitsunaga-Nakatsubo *et al.*, 1992b).

In this study, we have isolated the sea urchin cDNA clone coding Na^+, K^+-ATPase α-subunit, deduced its primary structure from the nucleotide sequence and compared it with those of various metazoans.

* The first and second authors contributed equally to this paper.
** Present address: Graduate Department of Gene Science, Faculty of Science, Hiroshima University, 1-3-1, Kagamiyama, Higashi-Hiroshima 739, Japan.
*** To whom all correspondence should be addressed.

MATERIALS AND METHODS

Culture of embryos

Gametes of the sea urchin, *Hemicentrotus pulcherrimus*, were obtained by an intracoelomic injection of 0.5 M KCl. Eggs were washed with artificial sea water (ASW), inseminated and allowed to develop at 20°C.

Extraction of total RNA from embryos

Total embryonic RNA was extracted from embryos by the guanidium/hot phenol method (Feramisco *et al.*, 1982). Poly(A)$^+$ RNA was fractionated using oligo-(dT) cellulose spun columns (Clontech Lab., Inc.) according to the manufacture's procedure.

Screening

First screening: 10^5 clones of the cDNA library constructed from the poly(A)$^+$ rich RNA of prism stage embryos (Akasaka *et al.*, 1987) were screened with a 47-mer oligonucleotide probe. The probe (5'GCIGGGGAGTCATTGACACCGTCICCIGTTACAGCCACAATGGCACC3') corresponds to a FSBA binding site (GAIVAVTGDGVNDSPALKK) at the amino terminal region of rat Na$^+$, K$^+$-ATPase α-subunit (Shull *et al.*, 1986). This is a highly conserved region among eukaryotes. The oligonucleotide was 5'-end ^{32}P-labeled with T4-polynucleotide kinase and used as the probe for plaque hybridization as described previously (Mitsunaga-Nakatsubo *et al.*, 1992b). Ten positive clones not containing the amino terminal region were obtained.

Second screening: To obtain longer clones carrying the amino terminal region for Na$^+$, K$^+$-ATPase α-subunit, another cDNA library of the gastrula stage was constructed using a 20-base synthetic primer (5'TGCAATGTTCTTGGTCTCCA3') corresponding to the antisense strand (at positions 767–786 in Fig. 2) of SUA5, a clone obtained from the 1st screening. The second library was screened with the *Eco*RI-*Hin*dIII fragment (fragment from position 746 to 1013) of SUA5, which was labeled with [α-^{32}P]dCTP (3000 Ci/mmol) using a random-primed DNA labelling kit (Nippon Gene, Toyama). Filters were prehybridized at 68°C for 1 hr in hybridization buffer containing 5 x SSPE, 5 x Denhardt's, 0.3% SDS and 100 μg/ml denatured salmon sperm DNA. Hybridization was performed in hybridization buffer containing the labeled probe for 12-15 hr. The filters were washed with a buffer containing 1 x SSC and 0.1% SDS for 15 min three times at room temperature and then subjected twice to 30-min washes with 0.1 x SSC and 1% SDS at 68°C. A positive overlapping clone (SUA51) was obtained.

Rapid amplification of cDNA ends (RACE) method

The 5'-noncoding region of cDNA was amplified by the RACE method of Frohman *et al.* (1988) to determine its sequence. First strand cDNA was synthesized using the antisense primer (ISOCHK2; 20 mer from the position 1578 to 1597 in Fig. 2). Deoxycitidine was polymerized to the 3'-end of the first strand, using dCTP and terminal deoxynucleotidyl transferase. PCR was performed with an oligo(dG)$_{15-18}$ primer and Primer4 (at positions 334-353 in Fig. 2) located upstream to the ISOCHK2, which was used for first strand cDNA synthesis. PCR-amplified DNA was extracted and ligated with cosmid vector Charomid 9-42 (Nippon Gene, Toyama), to analyze the insert for sequencing.

Nucleotide sequence of Na$^+$, K$^+$-ATPase α-subunit

The *Eco*RI fragments of the cDNAs (SUA5 and SUA51) were inserted into pUC118 or pUC119 and the overlapping subclones were generated using the stepwise deletion method (Heinkoff *et al.*, 1984). DNA sequencing was performed using the Sequenase version 2.0 7-deaza-dGTP kit (United State Biochemical Corp., USA) and both strands of the cDNA were sequenced.

Sequence alignment

Sequence alignment and numbers of nucleotide substitutions per site were estimated using in algorithm method described by Gotoh (1993). Construction of phylogenetic tree was performed by the neighbor-joining method (Saitou and Nei, 1987).

Chemicals

[γ-^{32}P]ATP and [α-^{32}P]dCTP were purchased from Radiochemical Centre, Amersham, Bucks, UK. Restriction endonucleases and the other enzymes were obtained from Takara Shuzo Co., Ltd., Kyoto, Toyobo Co., Ltd., Japan and Nippon Gene Co., Ltd., Japan. Sequenase version 2.0 7-deaza-dGTP kit and random primer labeling kit were the products of the United States Biochem. Co., USA and Nippon Gene Co. Ltd., Japan, respectively. Artificial sea water was obtained from Jamarin laboratory, Osaka, Japan.

RESULTS AND DISCUSSION

Several conserved regions have been reported in active sites of Na$^+$, K$^+$-ATPase α-subunit: phosphorylation site, FITC-binding site and two FSBA-binding sites. For a probe to screen the cDNA of sea urchin Na$^+$, K$^+$-ATPase α-subunit, one of the FSBA-binding sites located at the C-terminal region was selected and an antisense oligonucleotide (47 mer) was chemically synthesized based on its amino acid sequence reported in mammals (Shull *et al.*, 1986). 10^5 recombinant phages from a λgt10 cDNA library of the prism stage embryos were screened, as described previously (Mitsunaga-Nakatsubo *et al.*, 1992b). Ten positive signals were obtained. They included the nucleotide sequences encoding amino acid sequence of the probe region, whereas none of them had the amino terminal region. Clone SUA5 was selected and its nucleotide sequence was determined (Fig. 1).

To obtain cDNA clones carrying the N-terminal region, a cDNA library of the gastrula stage was constructed using oligonucleotide primer corresponding to the antisense strand sequence near the 5'-end of clone SUA5. 10^5 recombinant phages were screened with the 5' region of SUA5 (268 bp fragment digested with *Eco*RI and *Hin*dIII as shown in Fig. 1). A 779 bp cDNA clone with a 42-bp overlap at the 5'-end of clone SUA5 (SUA51) was obtained (as shown in Fig. 1). cDNA fragment including this overlapping region could be amplified by RT-PCR method using two pairs of primers, which locate opposite sides of the region (HNKUP, 689–710 and HNKDOWN, 1498–1518 or ISOCHK2, 1578–1597; nucleotide numbers correspond to Fig. 2). In addition, Northern blot analysis with probes of specific fragment from each cDNA clone revealed identical size and expression patterns of mRNA during development (data not shown). These results suggest that SUA5 and SUA51 are the cDNA clones coding the same mRNA of Na$^+$, K$^+$-ATPase α-subunit.

The 5'-noncoding region was amplified by the 5'-rapid amplification of cDNA ends (RACE) method. One of clones, R4 had a 346 bp overlapping region of SUA51 and extended 7 bp upstream. The extended length of the clone R4 nucleotide sequence corresponded closely to the major transcription start site determined by primer extension (data not shown). Therefore, the nucleotide sequence combining SUA5, SUA51 and R4 together is shown as a single sea urchin Na$^+$,

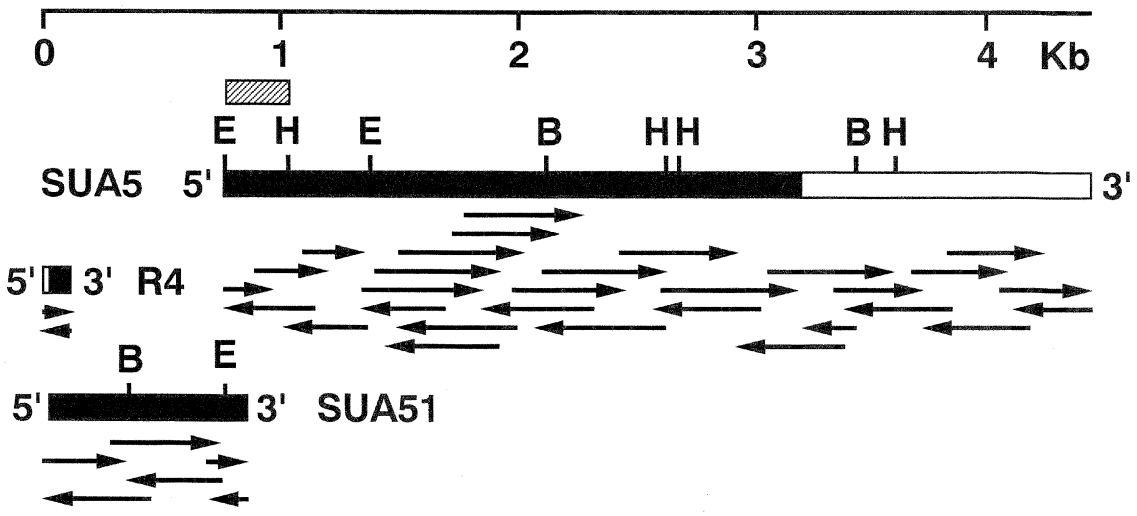


Fig. 1. Restriction maps and sequencing strategy of cDNA clones for Na^+, K^+-ATPase α-subunit. The restriction map is a composite from cDNAs' sequences determined. Darkened and open areas represent the coding and noncoding regions, respectively. Arrows indicate directions of sequencing. SUA5, SUA51 and R4 are cDNA clones. B, E and H are *Bgl*II, *Eco*RI and *Hin*dIII sites, respectively. Shaded box shows 268-bp probe for the second screening.

K^+-ATPase α-subunit sequence (Fig. 2). The total number of nucleotides is 4401.

The cDNA has a long untranslated sequence in the 3' region with a poly(A) additional signal AATAAA approximately 20 nucleotides upstream from the start of the 3'-poly(A) tail. The first methionine codon within the open reading frame is located upstream of a lysine-rich cluster (at positions 115–168, amino acid residues 37–54), which is reported to be a putative ion-selectable domain of Na^+, K^+-ATPase α-subunit. A termination codon is found next to Tyr-1038. The molecular weight of the protein is calculated to be 114 kDa.

As shown in Fig. 3, the overall hydropathy profile of the α-subunit is similar to that of sheep kidney (Shull *et al.*, 1985). Eight major hydrophobic regions are indicated as H1-H8 in the figure. Alignment of amino acid sequences of sea urchin α-subunit with those of diverse species also showed high degrees of sequence similarity in these hydrophobic regions (Fig. 4). Therefore, it is suggested that at least eight putative transmembrane domains also exist in sea urchin Na^+, K^+-ATPase α-subunit. The regions between H1-H2, H3-H4, H5-H6 and H7-H8, respectively, are putative short extracellular loops, whereas the domains between H2-H3 and H4-H5, respectively, are to be longer cytoplasmic loops. The amino acid sequence of the most highly conserved region, H4, matches almost perfectly between sea urchin and other species, whereas the average identity of the entire length is about 76%. Signal peptide could not be identified in the amino terminal region. Like other Na^+, K^+-ATPase α-subunit reported in diverse species (Kawakami *et al.*, 1985, 1986; Ovchinnikov *et al.*, 1986; Shull *et al.*, 1985), the signal peptide is not included in the sea urchin ATPase.

The catalytic sites, reported to be present in the cytoplasmic regions (Lingrel *et al.*, 1990; Lingrel and Kuntzweiler, 1994), were also identified in the region between H4 and H5 transmembrane sites, and are all highly conserved in the amino acid sequences (Fig. 4). Phosphorylation site around the aspartyl residue (no. 393) almost perfectly matches with those of other species. ATP binding-site around the Lys (at 523) probed with fluorescein 5'-isothiocyanate (FITC) is also highly homologous. Binding sites of FSBA, another ATP analogue (AX_1VVHGSDLK (I) and QGAIVAVTGDGVNDS-PALX_2K (II)) (Ohta *et al.*, 1986) are also identified in the sea urchin Na^+, K^+-ATPase α-subunit. Especially, the latter FSBA-binding site FSBA II, used as a probe for screening, matches almost perfectly except one amino acid residue at the N-terminal end of this region. On the contrary, the deduced N-terminal amino acid sequence for sea urchin differs from the sequences reported in vertebrates (Fig. 4) and is comparatively long like that for *Drosophila melanogaster*.

The activity of sea urchin embryonic Na^+, K^+-ATPase is ouabain-sensitive (Kinsey *et al.*,1980; Ciapa *et al.*, 1984; Mitsunaga *et al.*, 1986, 1989). It has been reported that uncharged amino acids are present at the terminus of H1-H2 extracellular domain of every ouabain-sensitive Na^+, K^+-ATPase, and the substitutions of the amino acids into charged amino acids invariably produce a resistant enzyme (Price *et al.*, 1989; Horisberger *et al.*, 1991; Lingrel and Kuntzweiler, 1994). In sea urchin, one of the termini of H1-H2 is negatively charged (Glu at 135), though the other is not (Asn at 146). Relatively high concentration of ouabain necessary for inhibition of sea urchin Na^+, K^+-ATPase activity, may be due to the amino acid residues at the termini of the H1-H2 extracellular domain. In Na^+,K^+-ATPase of *Artemia*, which have low affinity for ouabain, the same amino acids as in sea urchin are present at the termini of H1-H2.

Three kinds of Na^+, K^+-ATPase α-subunit isoforms, which are products of different genes, were demonstrated in chicken (Takeyasu *et al.*, 1988, 1990), rat (Shull *et al.*, 1986) and

Fig. 2. Nucleotide sequence of cDNA for sea urchin Na^+, K^+-ATPase α-subunit and its deduced amino acid sequence. The nucleotide sequence is a combination of SUA5, SUA51 and R4. The number of the nucleotide residues is given at the right end of each line. The deduced amino-acid sequence is shown below the nucleotide sequence. Eight major hydrophobic regions (H1-H8), likely to be transmembrane domains, are boxed. Catalytic sites in the cytoplasmic region were identified by homology with diverse ATPases shown by underlines. P: phosphorylation site, FITC: fluorescein isothiocyanate (FITC) binding region, FSBAI, II: 5'-(*p*-fluorosulfonyl)-benzoyladenosine binding regions.

human (Kawakami *et al.*, 1986; Ovchinnikov *et al.*, 1988; Shull *et al.*, 1989). Two kinds of isoforms were also reported in *Artemia* (Baxter-Lowe *et al.*, 1989; Macías *et al.*, 1991). However, only a single gene for α-subunit was found in *Drosophila melanogaster* (Lebovitz *et al.*, 1989). A region of significantly low homology between the isoforms of vertebrate was reported to be locating 11 amino acid upstream from the lysine residue in the FITC-binding site (Lingrel *et al.*, 1990). This region deduced from the sea urchin cDNA resembles that of α3.

A possible evolutionary relationship of Na^+, K^+-ATPase α-subunit is proposed in Fig. 5. The tree suggests that the sea urchin Na^+, K^+-ATPase α-subunit has evolved from a common ancestor of vertebrates and has diverged before the three vertebrate isoforms (α1, 2 and 3) appeared. Iwabe *et al.* (1996) also reported a phylogenic tree of ion transport ATPases, and also proposed that evolution of these tissue-specific isoforms occurred after the separation of invertebrate and vertebrate. Two isoforms of *Artemia* were suggested to occur after vertebrate-invertebrate divergence (Macías *et al.*,

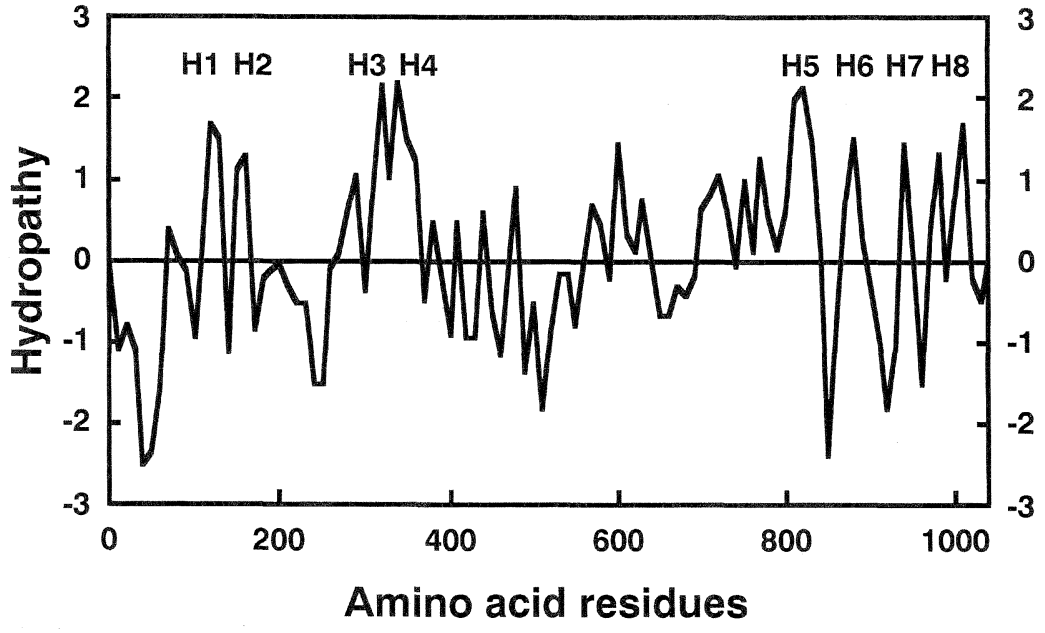


Fig. 3. Kyte-Doolittle hydropathy plot of Na+, K+-ATPase α-subunit (Kyte and Doolittle, 1982). The window was set at 20 amino acids. On the abscissa, the position in the primary amino acid sequence is indicated. The numbers on the ordinate are the relative hydrophobicity values, hydrophobic being positive and hydrophilic being negative. The hydrophobic peaks corresponding to the proposed transmembrane regions are indicated (H1-H8).

1991). However, biochemical analysis of *Hydra* Na+, K+-ATPase reveals the existance of two types of ouabain-sensitive ATPase (Canfield *et al.*, 1992). Analysis of the isoforms in sea urchin embryos is now under investigation.

ACKNOWLEDGMENTS

The authors wish to express their hearty thanks to Dr. O. Gotoh of Saitama Cancer Center for his kind help of constructing the phylogenic tree and alignment of Na+, K+-ATPase. We are also grateful to Dr. H. Yonekawa of the Tokyo Metropolitan Institute of Medical Science for his helpful advice on the construction of phylogenic tree and evolution.

REFERENCES

Akasaka K, Taira M, Shiroya T, Shimada H (1987) Cloning of stage specific gene sequences from a cDNA library representing poly(A)+ RNA of sea urchin prism-embryos. Zool Sci 4: 739–742

Baxter-Lowe LA, Guo JZ, Bergstrom EE, Hokin LE (1989) Molecular cloning of the Na, K-ATPase α-subunit in developing brine shrimp and sequence comparison with higher organisms. FEBS Lett 257: 181–187

Canfield VA, Xu KY, D'Aquila T, Shyjan AW, Levenson R (1992) Molecular cloning and characterization of Na,K-ATPase from *Hydra vulgaris*: Implications for enzyme evolution and ouabain sensitivity. New Biol 4: 339–348

Ciapa B, De Renzis G, Girard JP, Payan P (1984) Sodium-potassium exchange in sea urchin egg. I. Kineteic and biochemical characterization at fertilization. J Cellular Physiol 121: 235–242

Farley RA, Tran C M, Carilli CT, Hawke D, Shively JE (1984) The amino acid sequence of a fluorescein-labeled peptide from the active site of (Na, K)-ATPase. J Biol Chem 259: 9532–9535

Feramisco JR, Smart JE, Burridge K, Helfman DM, Thomas GP (1982) Co-existence of vinculin and a vinculin like protein of higher molecular weight in smooth muscel. J Biol Chem 257: 11024–11031

Frohman MA, Dush MK, Martin GR (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc Natl Acad Sci USA 85: 8998–9002

Good PJ, Richter K, Dawid IB (1990) A nervous system-specific isotype of the β subunit of Na+, K+-ATPase expressed during early development of *Xenopus laevis*. Proc Natl Acad Sci USA 87: 9088–9092

Gotoh O (1993) Optimal alignment between groups of sequences and its application to multiple sequence alignment. Compt Appl Biosci 9: 361–370

Hara Y, Urayama O, Kawakami K, Nojima H, Nagamune H, Kojima T, Ohta T, Nagano K, Nakao M (1987) Primary structures of two types of alpha-subunit of rat brain Na+, K+-ATPase deduced from cDNA sequences. J Biochem 102: 43–58

Heinkoff S (1984) Undirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28: 351–359

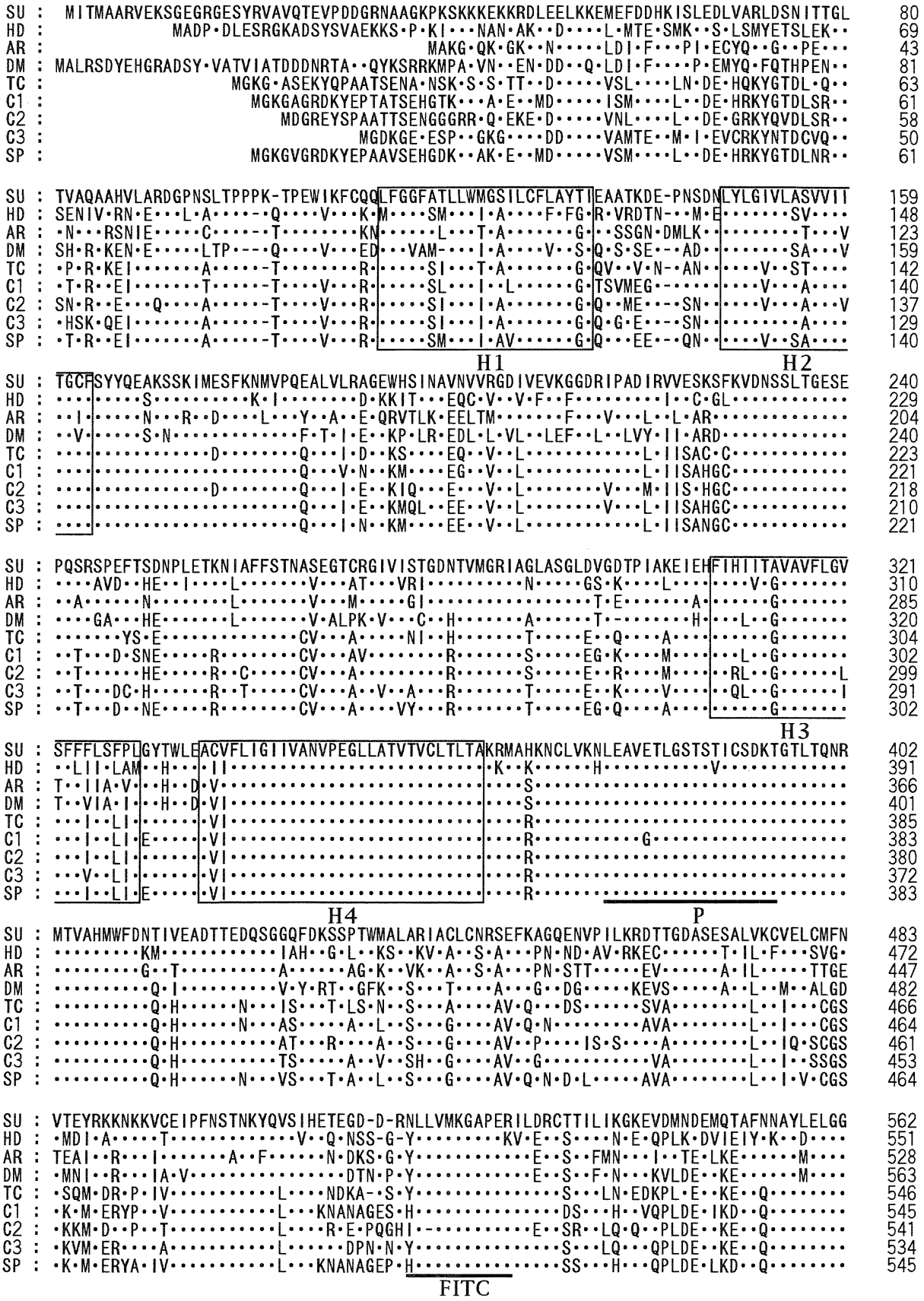
Herrera VLM, Emanuel JR, Ruiz-Opazo N, Levenson R, Nadal-Ginard B (1987) Three differentially expressed Na, K-ATPase α subunit isoforms: structural and functional implications. J Cell Biol 105: 1855–1865

Horisberger J-D, Lemas V, Kraehenbühl J-P, Rossier BC (1991) Structure-function relationship of Na, K-ATPase. Annu Rev Physiol 53: 565–584

Iwabe N, Kuma K, Miyata T (1996) Evolution of gene families and relationship with organismal evolution: rapid divergence of tissue-specific genes in the early evolution of chordates. Mol Biol Evol 13: 483–493

Jaisser F, Canessa CM, Horisberger JD (1992) Primary sequence and functional expression of a novel ouabain-resisitant Na, K-ATPase. The β subunit modulates potassium activation of the Na, K-pump. J Biol Chem 267: 16895–16903

Kano I, Nagai F, Satoh K, Ushiyama K, Nakao T, Kano K (1989) Structure of the α1 subunit of horse Na, K-ATPase gene. FEBS Lett 250: 91–98



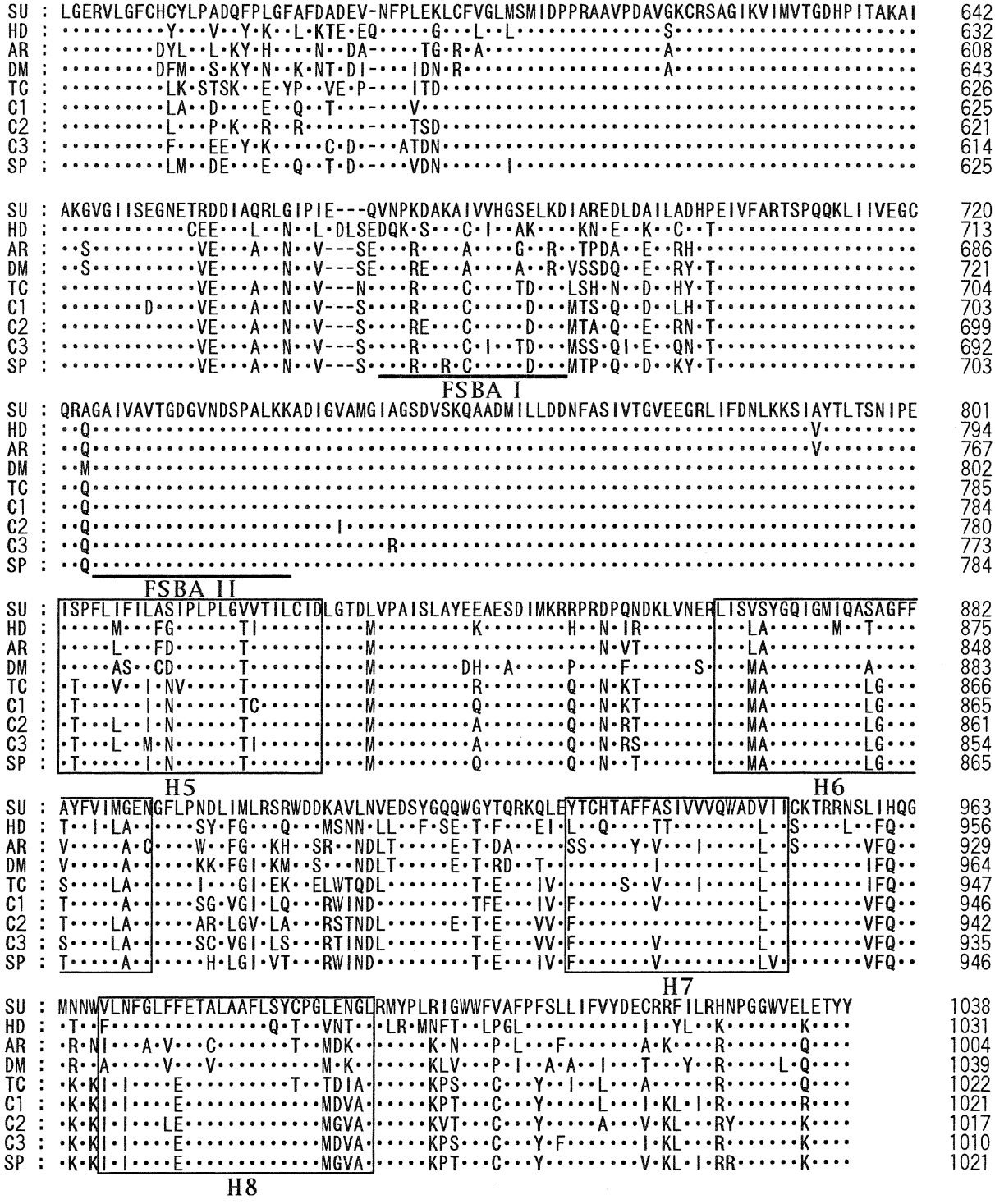


Fig. 4. Alignment of amino acid sequences of α-subunits of the Na^+, K^+-ATPase from the sea urchin *Hemicentrotus pulcherrimus* (SU), *Hydra vulgaris* (HD; Canfield *et al.*, 1992), the brine shrimp *Artemia franciscana* (AR; Macías *et al.*, 1991), *Drosophila melanogaster* (DM; Levobitz *et al.*, 1990), the electric ray *Torpedo californica* (TC; Kawakami *et al.*, 1985), chicken (C1, C2 and C3; α1, α2 and α3 isoforms respectively, Takeyasu *et al.*, 1988, 1990) and sheep (SP; Shull *et al.*, 1985). Sequences are shown in one-letter amino acid code. Gaps (-) are inserted in the sequences to achieve maximum alignment. Identical amino acids, relative to the *H. pulcherrimus* sequence, are indicated by dots. The eight putative transmembrane segments proposed by Shull *et al.* (1985) for the sheep kidney α-subunit are underlined and labeled with H1-H8. The active sites identified by homology with other ATPases are indicated by underlines (P: phosphorylation site, FITC: FITC-binding region, FSBAI, II: FSBA-binding regions).

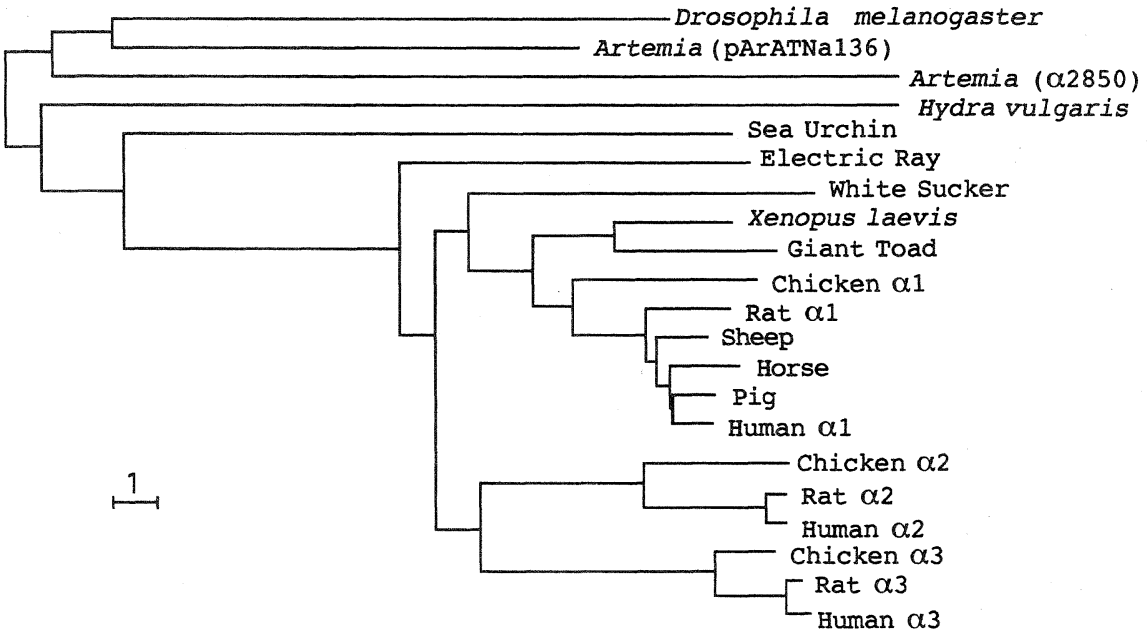


Fig. 5. Phylogenetic analysis of Na^+, K^+-ATPase α-subunit amino acid sequences. The tree was built using the neighbor-joining method applied to a categories distance matrix on the basis of the deduced amino acid sequences coded by sea urchin, *Hydra vulgaris* (Canfield *et al.*, 1992), *Artemia* cDNA clones α2850 and pArATNa136 (Baxter-Lowe *et al.*, 1989; Macías *et al.*, 1991), *Drosophila melanogaster* (Levobitz *et al.*, 1990), electric ray (Kawakami *et al.*, 1985), white sucker (Schönrock *et al.*, 1991), *Xenopus laevis* (Verrey *et al.*, 1989), giant toad (Jaisser *et al.*, 1992) , chicken α1-3 (Takeyasu *et al.*, 1988; 1990), rat α1-3 (Shull *et al.*, 1986), horse α1 (Kano *et al.*, 1989), pig (Ovchinnikov *et al.*, 1986), sheep (Shull *et al.*, 1985) and human α1-3 (Kawakami *et al.*, 1986; Shull *et al.*, 1989; Ovchinnikov *et al.*, 1988). The horizontal bar represents 1 amino acid replacements per 100 sites.

Kawakami K, Noguchi S, Noda M, Takahashi H, Ohata T, Kawamura M, Nojima H, Nagano K, Hirose T, Inayama S, Hayashida H, Miyata T, Numa S (1985) Primary structure of the α-subunit of *Torpedo californica* (Na^+ + K^+) ATPase deduced from cDNA sequence. Nature 316: 733–736

Kawakami K, Ohta T, Nojima H, Nagano K (1986) Primary structure of the α-subunit of human Na, K-ATPase deduced from cDNA sequence. J Biochem 100: 389–397

Kinsey WH, Decker GL, Lennarz WJ (1980) Isolation and partial characterization of the plsma mamebrane of the sea urchin egg. J Cell Biol 87: 248–254

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105–132

Lebovitz RM, Takeyasu K, Fambrough DM (1989) Molecular characterization and expression of the (Na^++K^+)-ATPase alpha-subunit in *Drosophila melanogaster.* EMBO J 8: 193–202

Lingrel JB, Orlowski J, Shull MM, Price EM (1990) Molecular genetics of Na, K-ATPase. In "Prog. Nucleic Acid Res Mol Biol Vol 38" Ed by WE Cohn, K Moldave, Academic Press, New York, pp 37–89

Lingrel JB, Kuntzweiler T (1994) Na^+, K^+-ATPase. J Biol Chem 269: 19659–19662

Macías M-T, Palmero I, Sastre L (1991) Cloning of a cDNA encoding an *Artemia franciscana* Na/K ATPase α-subunit. Gene 105: 197–204

Mitsunaga K, Fujino Y, Yasumasu I (1986) Change in the activity of Cl^-, HCO^-_3-ATPase in microsome fraction during early development of the sea urchin, *Hemicentrotus pulcherrimus.* J Biochem 100: 1607–1615

Mitsunaga K, Fujiwara A, Fujino Y, Yasumasu I (1989) Changes in the activities of H^+, K^+-ATPase and Na^+, K^+-ATPase in cultured cells derived from micromeres of sea urchin embryos with special reference to their roles in spicule rod formation. Develop Growth Differ 31: 171–178

Mitsunaga-Nakatsubo K, Fujiwara A, Yasumasu I (1992a) Change in the activity of Na^+, K^+-ATPase in embryos of the sea urchin, *Hemicentrotus pulcherrimus*, during early development. Develop Growth Differ 34: 379–385

Mitsunaga-Nakatsubo K, Kanda M, Yamazaki K, Kawashita H, Fujiwara A, Yamada K, Akasaka K, Shimada H, Yasumasu I (1992b) Expression of Na^+, K^+-ATPase α-subunit in animalized and vegetalized embryos of the sea urchin, *Hemicentrotus pulcherrimus.* Develop Growth Differ 34: 677–684

Ohta T, Nagano K, Yoshida M (1986) The active site structure of Na^+/K^+ transporting ATPase: Location of the 5'-(*p*-fluorosulfonyl) benzoyladenosine binding site and soluble peptides released by trypsin. Proc Natl Acad Sci USA 83: 2071–2075

Orlowski J, Lingrel JB (1988) Tissue-specific and developmental regulation of rat Na, K-ATPase catalytic α isoform and β subunit mRNAs. J Biol Chem 263: 10436–10442

Ovchinnikov YA, Modyanov NN, Broude NE, Petrukhin KE, Grishin AV, Arzamazova NM, Aldanova NA, Monastyrskaya GS, Sverdlov ED (1986) Pig kidney Na^+, K^+-ATPase. Primary sturcture and spatial organization. FEBS Lett 201: 237–245

Ovchinnikov YA, Monastyrskaya GS, Broude NE, Ushkaryov YA, Melkov AM, Smirnov YV, Malyshev IV, Allikmets RL, Kostina MB, Dulubova IE, Kiyatkin NI, Grishin AV, Modyanov NN, Sverdlov ED (1988) Family of human Na^+, K^+-ATPase genes. Strucure of the gene for the catalytic subunit (αIII-form) and its relationship with structural features of the protein. FEBS Lett 233: 87–94

Post RL, Kume S (1973) Evidence for an aspartyl phosphate residue at the active site of sodium and potassium ion transport adenosine triphosphatase. J Biol Chem 248: 6993–7000

Price EM, Rice DA, Lingrel JB (1989) Site-directed mutagenesis of a conserved extracellular aspartic acid residue affects the ouabain sensitivity of sheep Na, K-ATPase. J Biol Chem 264: 21902–21906

Saitou N, Nei M (1987) The nighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425

Schönrock C, Morley SD, Okawara Y, Lederis K, Richter D (1991) Sodium and potassium ATPase of the teleost fish *Catostomus commersoni*. Sequence, protein structure and evolutionary conservation of the alpha-subunit. Biol Chem Hoppe-Seyler 372: 279–286

Schwartz A, Lindenmayer GE, Allen JC (1975) The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. Pharmacol Rev 27: 3–134

Shull GE, Schwartz A, Lingrel JB (1985) Amino-acid sequence of the catalytic subunit of the (Na^+ + K^+)ATPase deduced from a complementary DNA. Nature 316: 691–695

Shull GE, Greeb J, Lingrel JB (1986) Molecular cloning of three distinct forms of the Na^+, K^+-ATPase α-subunit from rat brain. Biohcemstry 25: 8125–8132

Shull MM, Pugh DG, Lingrel JB (1989) Characterization of the human Na, K-ATPase α2 gene and identification of intragenic restriction fragment length polymorphisms. J Biol Chem 264: 17532–17543

Sweadner KJ (1989) Isozymes of the Na^+/K^+-ATPase. Biochim Biophys Acta 988: 185–220

Takeyasu K, Tamkun MM, Renaud KJ, Fambrough DM (1988) Ouabain-sensitive (Na^+ + K^+)-ATPase activity expressed in mouse L cells by transfection with DNA encoding the α-subunit of an avian sodium pump. J Biol Chem 263: 4347–4354

Takeyasu K, Lemas V, Fambrough DM (1990) Stability of Na^+, K^+-ATPase α-subunit isoforms in evolution. Am J Physiol 259: C619–C630

Verrey F, Kairouz P, Schaerer E, Fuentes P, Geering K, Rossier BC, Kraehenbuhl J-P (1989) Primary sequence of *Xenopus laevis* Na^+-K^+-ATPase and its localization in A6 kidney cells. Am J Physiol 256: F1034–F1043

Walderhaug MO, Post RL, Saccomani G, Leonard RT, Briskin DP (1985) Structural relatedness of three ion-transport adenosine triphosphatases around their active sites of phosphrylation. J Biol Chem 260: 3852–3859

(Received June 24, 1996 / Accepted August 12, 1996)