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Molecular cloning of rat brain Na,K-ATPase α-subunit cDNA

(λgt11/gene amplification/blot hybridization/DNA sequence analysis/synthetic oligonucleotides)

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ABSTRACT **We have isolated a cDNA clone for the rat brain Na,K-ATPase α subunit. A λgt11 cDNA expression library constructed from mRNA of 1- and 2-week-old rat brains was screened with an antibody reactive with rat brain Na,K-ATPase. A positive phage clone, λrb5, containing a 1200-base-pair cDNA insert expressed a β-galactosidase–cDNA fusion protein that was reactive by immunoblotting with the Na,K-ATPase antibody. This fusion protein was also reactive in ELISA with a monoclonal antibody directed against the α subunit of the Na,K-ATPase. A 27S mRNA species exhibiting sequence hybridization to the cDNA insert of λrb5 was identified in rat brain, kidney, and liver, as well as in dog kidney. This 27S mRNA exhibited a tissue-specific pattern of abundance consistent with the relative abundance of Na,K-ATPase polypeptides *in vivo*: kidney > brain > liver. In a ouabain-resistant HeLa cell line, C^+, which contains minute chromosomes and at least a 10-fold greater number of sodium pumps than parental HeLa cells, DNA sequences complementary to λrb5 cDNA were amplified ≈40-fold. Analysis of the λrb5 cDNA sequence demonstrated a perfect nucleotide sequence match between a portion of the cDNA and the amino acid sequence of the Na,K-ATPase α-subunit fluorescein isothiocyanate binding site. Taken together, the data presented here demonstrate that the λrb5 cDNA clone is a portion of the gene coding for the rat brain Na,K-ATPase α subunit. The ATPase gene appears to be present in one or very few copies in the rat and human genomes and to be transcriptionally regulated in different rat tissues. In a ouabain-resistant human cell line, on the other hand, ouabain resistance appears to involve an increase in the number of gene copies coding for the Na,K-ATPase.**

The plasma membrane-associated Na,K-ATPase is an important enzyme found in virtually all animal cells. The Na,K-ATPase maintains the high intracellular levels of K^+ and low intracellular levels of Na^+ characteristic of most living cells by using energy to pump these ions across the cell membrane against their concentration gradients. The known functional capacity of the enzyme appears to reside solely in the α subunit, one of the two polypeptide subunits of the Na,K-ATPase. The α subunit is a polypeptide of M_r ≈100,000 that contains the binding sites for the cardiac glycoside ouabain, a potent inhibitor of the sodium pump, and ATP (1). The β subunit is a glycosidated polypeptide of M_r ≈55,000 whose function remains unknown (1).

The Na,K-ATPase has been shown to play an important role in the regulation of normal cellular homeostasis, cell differentiation (2, 3), and cell proliferation (4, 5). The sodium pump has been extensively characterized at both the enzymatic and biochemical levels. Almost no information is available, however, regarding the regulation of this enzyme at the genetic level. A molecular probe for the Na,K-ATPase would therefore be a valuable reagent that could be used to study the regulation of sodium-pump biosynthesis, assembly, and translocation.

We describe here the results of an antibody screening protocol for cloning Na,K-ATPase α-subunit cDNA. Only a limited amount of amino acid sequence data is currently available for the Na,K-ATPase α subunit. The sequence of 10 amino acids at the amino terminus of the α subunit has been reported (1), as has the sequence of a highly conserved 11-amino acid long tryptic digestion fragment (6). This tryptic fragment has been shown to be specifically labeled by the binding of fluorescein isothiocyanate (FITC) and is a characteristic digestion product of the Na,K-ATPase α subunit. In view of the paucity of amino acid sequence data, we used an antibody directed against rat Na,K-ATPase to probe a rat brain cDNA library constructed in the λgt11 expression system of Young and Davis (7, 8). This approach resulted in the isolation of a recombinant phage clone containing a 1200-base-pair (bp) cDNA insert that encodes a portion of the gene for the Na,K-ATPase α subunit.

MATERIALS AND METHODS

Antibody Screening of λgt11 Library and Protein Blotting. The λgt11 library used in this study was constructed from mRNA isolated from 1- and 2-week-old rat brains and was generously provided by N. Davidson and A. Dowsett (California Institute of Technology). The phage library was plated onto bacterial strain Y1090 and grown for 5 hr at 42°C. After isopropyl β-D-thiogalactoside (IPTG) induction and 2 hr of growth at 37°C, duplicate filters were prepared from each plate and screened with anti-Na,K-ATPase antibody by the method of Young and Davis (7, 8). The antibody used in screening was a rabbit antiserum (K3) raised against rat kidney Na,K-ATPase as described (9). Prior to its use in screening, the antiserum was diluted 1:100 in 50 mM Tris·HCl, pH 8/150 mM NaCl/3% bovine serum albumin and preabsorbed with 0.01 vol of boiled Y1090 bacterial lysate (10). Bacteriophage plaques reactive with the primary antibody were detected by secondary incubation with ^{125}I-labeled *Staphylococcus aureus* protein A (Amersham). The phage clone λrb5 was used to lysogenize the hf1 bacterial strain Y1089 (7, 8). A bacterial lysate was prepared from the λrb5 lysogen after inactivation of the temperature-sensitive *c*I repressor at 45°C and incubation in the presence of IPTG. The cell lysate was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and immunoblotting. Duplicate protein blots reacted first with the Na,K-ATPase antibody or β-galactosidase antibody (kindly provided by Cappel Labora-

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Abbreviations: FITC, fluorescein 5′-isothiocyanate; IPTG, isopropyl β-D-thiogalactoside; bp, base pair(s); kb, kilobase(s).

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tories, Cochranville, PA) and then with protein A alkaline phosphatase (Sigma).

ELISA. Bacterial lysate from IPTG-induced λrb5 lysogen underwent reaction in ELISA (11) with an anti-Na,K-ATPase monoclonal antibody (M. Kashgarian, D. Biemesderfer, M. Caplan, and B. Forbush, personal communication) and peroxidase-conjugated anti-mouse IgG secondary antibody (HyClone, Logan, UT). An anti-β-galactosidase antibody with goat anti-rabbit IgG secondary antibody was used in control experiments.

RNA and DNA Blot Hybridization. RNA was isolated from fresh rat brain, rat kidney, rat liver, and dog kidney outer medulla by the guanidinium isothiocyanate method of Chirgwin *et al.* (12). Total RNA was denatured by heating at 65°C for 10 min in 50% (vol/vol) formamide and was fractionated by electrophoresis through a 1% agarose formaldehyde gel (13). The RNA was transferred to nitrocellulose (Schleicher & Schuell), prehybridized and hybridized as described by Thomas (14), except for the addition of single-stranded poly(A), poly(I), and poly(C) ribonucleotides (each at 100 μg/ml; Boehringer Mannheim) to the hybridization buffer. High molecular weight DNA was prepared from tissue and cells by the method of Gross-Bellard *et al.* (15). DNA was digested with restriction enzymes, fractionated by agarose gel electrophoresis, and transferred to Zetabind (AMF, Meriden, CT) essentially as described by Southern (16). Blots were prehybridized, hybridized, and washed as described (17). Blots were exposed to x-ray film (Kodak XAR-5) at −80°C with a Cronex (DuPont) intensifying screen. [^{32}P]dCTP was incorporated into nucleic acid probes by using a Bethesda Research Laboratories nick-translation kit (18).

Cell Culture. HeLa and HeLa C^+ cells were provided by J. F. Ash (University of Utah Medical School). Cells were maintained in Dulbecco's modified Eagle's medium/10% fetal calf serum (GIBCO). HeLa C^+ cells were grown in medium containing 1 μM ouabain. HeLa C^- cells are HeLa C^+ cells grown in the absence of ouabain for the times indicated in each experiment.

Synthetic Oligonucleotide and DNA Sequence Analysis. A synthetic oligonucleotide was constructed based on the amino acid sequence of the FITC binding-site peptide characteristic of the Na,K-ATPase α subunit (6). This synthetic oligonucleotide consisted of a mixture of eight different 17-mers as shown below. The synthetic oligonucleotide was chemically synthesized by using an Applied Biosystems (Foster City, CA) DNA synthesizer.

NH_2–His–Leu–Leu–Val–Met–Lys–Gly–
Ala–Pro–Glu–Arg–COOH Peptide

5′–AUG–AA$\begin{matrix}G\\A\end{matrix}$–GGC–GCC–CC$\begin{matrix}C\\A\\U\\G\end{matrix}$–GA–3′ mRNA

3′–TAC–TT$\begin{matrix}C\\T\end{matrix}$–CCG–CGG–GG$\begin{matrix}G\\T\\A\\C\end{matrix}$–CT–5′ Oligo

The synthetic oligonucleotide was end-labeled with polynucleotide kinase (Boehringer Mannheim) and [γ-^{32}P]ATP (New England Nuclear), purified by electrophoresis through a 20% polyacrylamide/urea gel, and used to probe various restriction fragments of prb5. The partial DNA sequence of λrb5 cDNA was determined by the dideoxynucleotide chain-termination method (19). DNA sequences were analyzed by using the WISCONSIN program at the Yale Biomedical Computer Center.

RESULTS

Isolation and Characterization of a Rat Brain Na,K-ATPase cDNA Clone. We screened a λgt11 cDNA expression library constructed from the mRNA of 1- and 2-week-old rat brains with an antibody reactive with the rat brain Na,K-ATPase. Several clones were identified as immunoreactive through four successive rounds of antibody screening and plaque purification. One of these phage clones, designated λrb5, has been studied in the greatest detail. DNA was isolated from λrb5, digested with *Eco*RI, and the cDNA insert (1200 bp) was subcloned into the *Eco*RI site of the plasmid cloning vector pAT153 (prb5). The cDNA insert of prb5 was then used as a probe to screen RNAs prepared from various tissues by RNA blot analysis. As shown in Fig. 1, λrb5 cDNA hybridized to a single 27S mRNA in each of several tissues of the rat as well as dog kidney. The 27S mRNA was most abundant in rat kidney and was least abundant in rat liver. This pattern is consistent with the known distribution of Na,K-ATPase polypeptides among different tissues (20). Southern blot analysis of total rat genomic DNA digested with *Eco*RI revealed one band of 4.4 kilobases (kb) (Fig. 2*A*). These results suggest that the gene complementary to λrb5 cDNA is probably represented in only one or very few copies in the rat genome and that the variation in tissue levels of 27S mRNA is likely to be mediated by the regulation of transcription or posttranscriptional RNA metabolism rather than by gene copy number.

The λrb5 cDNA was also used in Southern blotting experiments to probe DNA prepared from HeLa cells, ouabain-resistant HeLa C^+ cells, and revertant HeLa C^- cells. For these studies, HeLa C^- cells were defined as HeLa C^+ cells grown for 2 weeks in the absence of ouabain prior to the preparation of DNA. Previous experiments have shown that growth of C^+ cells in the absence of ouabain leads to a loss of both minute chromosomes and ouabain resistance and to a decrease in the number of Na,K-ATPase polypeptides (21). As shown in Fig. 2*A*, genomic DNA sequences reactive with λrb5 cDNA exhibited a significant degree of amplification in HeLa C^+ cells compared to parental HeLa and revertant HeLa C^- cells. Two additional DNA fragments of 4.6 and 6.6 kb were also generated by *Eco*RI digestion of HeLa C^+ DNA. This result suggests that unstable gene rearrangements occur during amplification of λrb5 cDNA

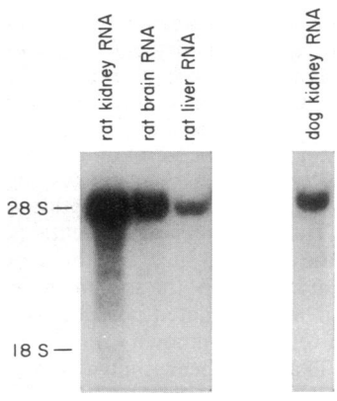

FIG. 1. RNA blot analysis of λrb5 cDNA gene transcription in rat and dog tissues. Total RNA was prepared from various tissues of the rat and dog. Twenty-five micrograms of total cellular RNA was run in each lane. RNA was electrophoresed through a 1% formaldehyde/agarose gel and then transferred to a nitrocellulose filter. The filter reacted with 2×10^7 cpm of radiolabeled λrb5 cDNA probe. The positions of 28S and 18S RNA markers are shown on the left. The mRNA band is clearly distinguishable from 28S RNA and was not seen in parallel blots probed with other rat brain cDNA clones.

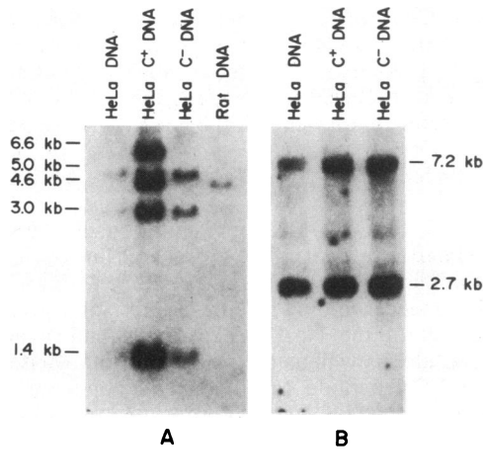

FIG. 2. Southern blot analysis of λrb5 genomic DNA fragments. Total cellular DNA was prepared from various cells and tissues. DNA was digested with *Eco*RI and 25 μg of DNA was loaded in each lane. DNA fragments were electrophoresed through a 0.8% agarose gel and the fragments were transferred to a Zetabind filter. (*A*) The filter reacted with 2×10^7 cpm of radiolabeled λrb5 cDNA probe. (*B*) The filter reacted with 2×10^7 cpm of radiolabeled human γ-globin gene probe. Size markers are shown at the right and left.

genomic sequences in HeLa C^+ cells. A duplicate Southern blot probed for the single-copy human $^A\gamma$- and $^G\gamma$-globin genes revealed bands of equal intensity in all 3 lanes, as shown in Fig. 2*B*. Thus, the differences in sequence content of genes reactive with λrb5 cDNA in HeLa C^+ cells versus HeLa cells appear to be due to amplification of those sequences rather than to any loading artifact. Slot blot analysis of DNA prepared from these cells in separate experiments (unpublished data) indicated that in HeLa C^+ cells λrb5 cDNA genomic sequences were amplified ≈40-fold over the level exhibited in parental HeLa cells and 4- to 10-fold over the level in HeLa C^- cells. These results for the λrb5 cDNA gene correlate well with the increased number of Na,K-ATPase polypeptides present in HeLa C^+ cells and the decline in sodium pumps when C^+ cells are cultured in the absence of ouabain (21). Thus, λrb5 cDNA appears to represent a molecular probe for the Na,K-ATPase.

Antibody Reactivity of λrb5 Fusion Protein. Phage clone λrb5 was used to lysogenize bacterial strain Y1089. A bacterial lysate was prepared from λrb5 lysogen by inactivation of the temperature-sensitive repressor at 42°C and induction of fusion protein production by IPTG. The lysate was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and immunoblotting, as shown in Fig. 3.

The reactivity of lysates with Na,K-ATPase antibody is shown in Fig. 3*C*, and the reactivity of the lysates with β-galactosidase antibody is shown in Fig. 3*B*. The Coomassie blue staining pattern of the lysates is presented in Fig. 3*A*. IPTG induction of λgt11 lysogen results in the increased expression of β-galactosidase (lower arrow in *A*, lanes λgt11). The synthesis of a fusion protein of M_r ≈155,000 was found to be dependent on IPTG induction of the λrb5 lysogen (upper arrow in *A*, lanes λrb5). This fusion protein was immunoreactive with β-galactosidase antibody (Fig. 3 *B*, lanes λrb5) and with Na,K-ATPase antibody (Fig. 3*C*, lanes λrb5). The λgt11 lysogen was not immunoreactive with Na,K-ATPase antibody (Fig. 3*C*, lanes λgt11), and rat brain membranes failed to react with β-galactosidase antibody (Fig. 3*B*). On the other hand, the Na,K-ATPase antibody was reactive with the M_r 100,000 α subunit of rat brain Na,K-ATPase (Fig. 3*C*). These results demonstrate that the Na,K-ATPase antibody is immunoreactive with rat brain Na,K-ATPase α subunit and λrb5 fusion protein, but not with bacterial or phage polypeptides.

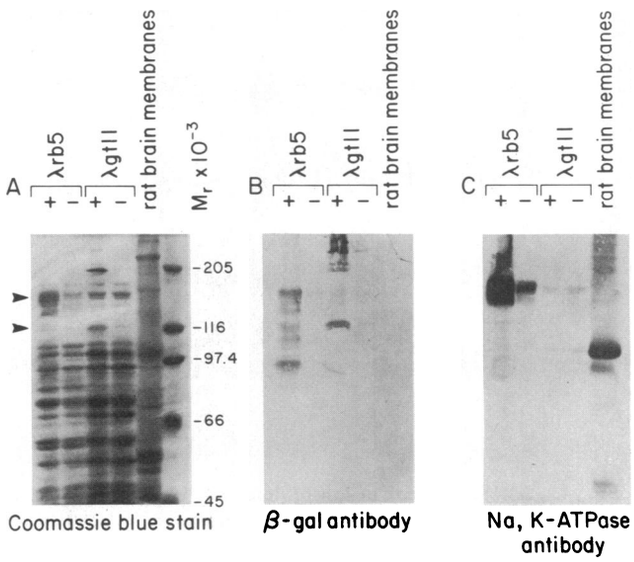

FIG. 3. Immunoblot analysis of λrb5 fusion protein. The phage clones λrb5 and wild-type λgt11 were used to lysogenize bacterial strain Y1089. Lysogens were grown at 32°C, shifted to 42°C, and then induced (+) or not induced (−) with IPTG. Lysogen proteins were fractionated by electrophoresis through a $NaDodSO_4$/7.5% polyacrylamide gel and transferred to nitrocellulose. (*A*) Coomassie blue stain of lysogen proteins. (*B*) Reactivity with β-galactosidase antibody followed by treatment with alkaline phosphatase-conjugated protein A. (*C*) Reactivity with Na,K-ATPase antibody followed by treatment as in *B*. Size markers are shown in *A*. The position of β-galactosidase (lower arrow) was determined from the mobility of purified β-galactosidase. Upper arrow denotes the position of the λrb5 fusion proteins.

To further characterize λrb5 fusion protein, we screened lysates prepared from IPTG-induced and noninduced clones with a monoclonal antibody that had previously been shown (M. Kashgarian *et al.*, personal communication) to be monospecific for Na,K-ATPase α subunit. Lysates from clones were adsorbed onto microtiter plates and screened with the anti-Na,K-ATPase monoclonal antibody or affinity-purified polyclonal rabbit anti-β-galactosidase antibody. As shown in Fig. 4, IPTG-induced λgt11 and phage clone λrb5 were both immunoreactive with anti-β-galactosidase antibody, whereas uninduced clones exhibited no reactivity above the background level. When reacted with the anti-Na,K-ATPase monoclonal antibody, only IPTG-induced λrb5 was immunoreactive. A phage clone isolated from the rat brain λgt11 library, which contains a cDNA insert unrelated to λrb5 (λrb19), was not reactive with the monoclonal antibody. Thus, λrb5 β-galactosidase–cDNA fusion protein appears to contain an epitope that is recognized by a monospecific anti-Na,K-ATPase α-subunit monoclonal antibody. These results are consistent with the view that λrb5 cDNA codes for a portion of the Na,K-ATPase α subunit.

Synthetic Oligonucleotide Hybridization and DNA Sequence Analysis of λrb5 cDNA. We analyzed the reactivity of λrb5 cDNA insert with a synthetic oligonucleotide probe whose sequence was predicted from the Na,K-ATPase α-subunit FITC binding-site amino acid sequence (6). The synthetic oligonucleotide consisted of a mixture of eight different 17-mers. The oligonucleotide mixture was end-labeled with [γ-^{32}P]ATP and hybridized to a Southern blot of various restriction fragments of λrb5 cDNA (data not shown). We found that the synthetic oligonucleotide probe derived from the FITC binding-site sequence hybridized exclusively to a 500-bp *Eco*RI/*Bam*HI restriction fragment of λrb5 cDNA, suggesting that the FITC binding-site sequence was contained within this restriction fragment (data not shown). To

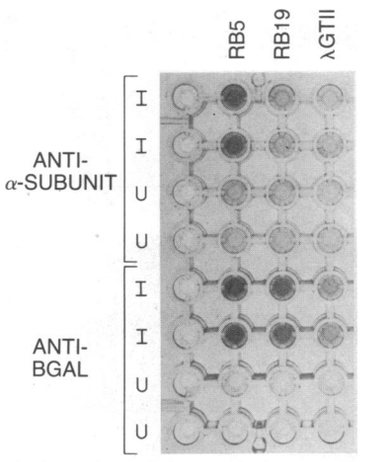

FIG. 4. ELISA of λrb5 fusion protein. The phage clones λrb5, λrb19, and wild-type λgt11 were used to lysogenize bacterial strain Y1089 and the lysogens were grown as described in Fig. 3. Lysogens were adsorbed onto microtiter wells and analyzed by ELISA. The primary β-galactosidase antiserum or Na,K-ATPase α-subunit monoclonal antibody reacted with the lysogens for 60 min, followed by reaction with secondary antibodies for 60 min. The colorimetric reaction products were quantitated by measuring absorbance at 492 nm, using a Titertek (Flow Laboratories) Multiskan. I, IPTG induced; U, uninduced.

test this possibility, the *Eco*RI/*Bam*HI restriction fragment of λrb5 cDNA was subcloned into the phage cloning vector M13 mp8 and sequenced by the dideoxy sequencing procedure. A restriction map of the λrb5 cDNA clone is shown in Fig. 5. The nucleotide sequence of a restriction fragment encompassing the FITC site is also shown. The 11-amino acid sequence of the FITC binding site is depicted below the restriction map. We have found that the nucleotide sequence corresponding to the FITC site begins at the *Bam*HI site of the clone (highlighted nucleotides in Fig. 5). The nucleotide sequence of λrb5 cDNA corresponds exactly for all 11 amino acids of the FITC site. We have also found that the 36 nucleotides immediately adjacent to the FITC binding site correspond to 11 of 12 amino acids in the FITC flanking sequence of the dog kidney α subunit obtained by Kyte and co-workers (J. Kyte and D. Le, personal communication). These results provide direct evidence that the λrb5 cDNA clone is a portion of the gene for the Na,K-ATPase α subunit.

DISCUSSION

We have isolated a cDNA representation of a portion of Na,K-ATPase α subunit mRNA. Several lines of evidence support this conclusion. (*i*) The cDNA clone we have isolated contains sequences coding for a characteristic tryptic digestion fragment of the Na,K-ATPase α subunit, the FITC binding peptide, as well as the sequences immediately adjacent to the FITC peptide. (*ii*) The cDNA clone hybridizes to an mRNA of ≈5000 bp, a size appropriate for a protein such as the ATPase α subunit (M_r ≈100,000). (*iii*) The relative abundance of 27S mRNA in rat kidney, brain, and liver corresponds to the abundance of sodium pump polypeptides expressed in these tissues. The activity of sodium pump in brain and kidney, for example, is 10- to 100-fold higher than the activity in other tissues (20). (*iv*) The fusion protein expressed by the λrb5 phage clone was reactive with a monoclonal antibody directed against the Na,K-ATPase α subunit. (*v*) DNA sequences reactive with λrb5 cDNA probe are far more abundant in HeLa C^+ cells, which are resistant to ouabain, express increased levels of Na,K-ATPase polypeptides, and simultaneously acquired minute chromosomes, than in ouabain-sensitive HeLa cells. Taken together, these findings provide unequivocal evidence that the λrb5 cDNA clone is a portion of the gene coding for the rat brain Na,K-ATPase α subunit.

The data presented here suggest several interesting features of Na,K-ATPase gene expression in different tissues and under different physiological conditions. Southern blot analysis of DNA sequences reactive with λrb5 cDNA in the human and rat genomes is consistent with the view that the sodium pump is coded for by only one or very few genes. On the other hand, Na,K-ATPase α-subunit mRNA levels vary significantly from tissue to tissue in the rat. These results suggest that transcriptional or posttranscriptional control

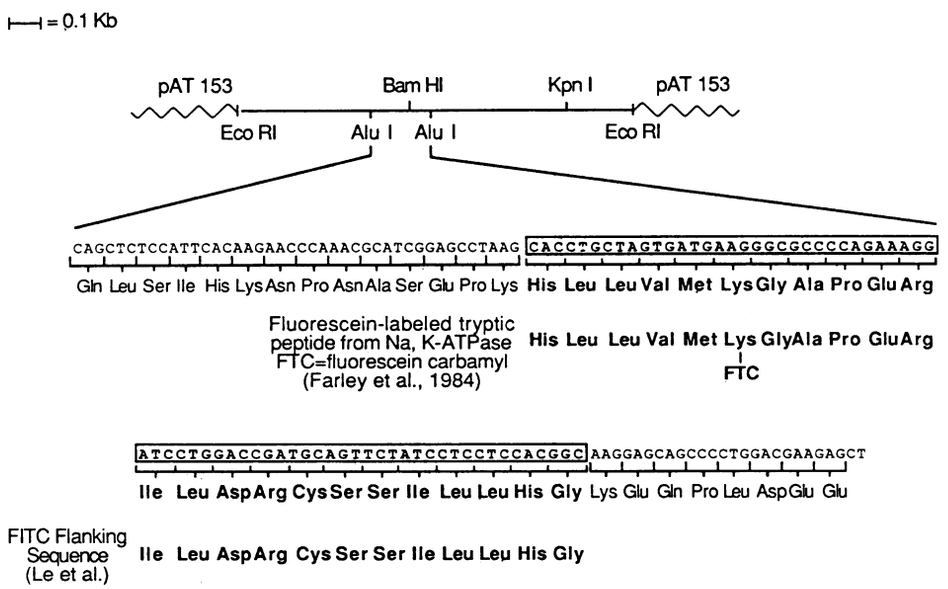

FIG. 5. DNA sequence analysis of λrb5 cDNA. λrb5 cDNA was digested with *Bam*HI and the *Eco*RI/*Bam*HI fragments were subcloned in the phage vector M13 mp8. DNA sequence analysis was carried out by the dideoxy chain-termination method (19). A restriction map of prb5 is shown at the top of the figure, The nucleotide sequence of a portion of λrb5 cDNA corresponding to the Na,K-ATPase α-subunit FITC site and its flanking sequences are shown below the restriction map, as is the predicted sequence of the FITC site (6).

mechanisms may account for the large fluctuations in Na,K-ATPase activity known to occur in different tissues.

Previous experiments have shown that ouabain resistance in the HeLa cell line C^+ occurred in concert with the expression of increased levels of Na,K-ATPase activity and the appearance of minute chromosomes (21). Our data show quite clearly that the gene coding for the α subunit of the Na,K-ATPase is amplified in this cell line. The partial decline in α-subunit gene copy number observed when C^+ cells were removed from ouabain is also consistent with the view that gene amplification is at least one mechanism involved in ouabain resistance in HeLa C^+ cells. It is possible that the amplified sodium pump genes expressed in HeLa C^+ cells may also contain mutations that lead to altered ouabain affinity (21). It is not clear at this time whether the gene coding for the β subunit of the Na,K-ATPase is also amplified in HeLa C^+ cells. Preliminary experiments we have carried out (unpublished) indicate that, in addition to λrb5 cDNA, a second genetic locus is amplified in HeLa C^+ cells. The possibility that this locus contains the gene coding for the Na,K-ATPase β subunit remains to be determined.

We have compared λrb5 cDNA to a previously isolated murine gene that confers ouabain resistance to ouabain-sensitive cells after DNA-mediated gene transfer (17). This ouabain resistance (*oua*^{r}) gene has been shown to hybridize to an mRNA of 1200 bp and is thus too small to be the gene for the Na,K-ATPase α subunit (22). The *oua*^{r} gene also failed to hybridize with λrb5 cDNA (unpublished results). Thus, our data suggest that there are at least two mechanisms by which ouabain resistance can be acquired by cultured cells: (*i*) amplification of the gene for the Na,K-ATPase and (*ii*) acquisition and/or expression of other genes, such as the *oua*^{r} gene.

DNA sequence analysis of the λrb5 cDNA clone provides the most direct evidence that this cDNA clone represents a portion of the gene coding for the Na,K-ATPase α subunit. A 33-nucleotide-long sequence has been localized within λrb5 cDNA that corresponds exactly to the 11-amino acid long segment known to represent the FITC binding site of the α subunit (6). The amino acid sequence of the FITC binding site of another cation transport ATPase, the sarcoplasmic reticulum Ca^{2+}-ATPase, has been shown to be ≈50% homologous to the Na,K-ATPase FITC binding site (6, 23). A five-amino acid long sequence of the Ca^{2+}-ATPase and Na,K-ATPase are, in fact, identical (6, 23). However, the flanking sequences around this region are divergent between the two ATPases. Our results, therefore, rule out the possibility that the cDNA we have cloned codes for the Ca^{2+}-ATPase. While there is a possibility that λrb5 cDNA could code for another transport ATPase, such as the proton ATPase, the fact that λrb5 was isolated by using an antibody directed against the Na,K-ATPase and that the β-galactosidase–λrb5 cDNA fusion protein is immunoreactive with a Na,K-ATPase α-subunit monoclonal antibody make this possibility extremely unlikely.

The Na,K-ATPase α-subunit clone we have isolated is a cDNA representation of a rat brain α-subunit mRNA. Previous experiments have demonstrated that there are two forms of the Na,K-ATPase α subunit in the rat brain, α and α^+ (24, 25). Southern blotting experiments of rat genomic DNA that we have performed cannot resolve the question of whether there are one or two α-subunit genes. Since the antiserum we used recognizes determinants shared by α and α^+, we cannot be certain if λrb5 cDNA codes for the α or α^+ form of the sodium pump. However, since λrb5 cDNA hybridizes to a 27S mRNA in kidney, and there is no α^+ polypeptide detectable in this tissue (25), λrb5 cDNA is likely to be a molecular clone of α and not α^+, if there are indeed two separate genes. The analysis of α-subunit genomic clones should be useful in resolving this issue.

The availability of a molecular probe for the sodium pump should be useful for studies designed to understand the structure, function, assembly, and translocation of the sodium pump. The Na,K-ATPase α-subunit cDNA should also facilitate an understanding of the regulation of expression of the gene for Na,K-ATPase α subunit and the structure of the α-subunit gene itself.

We thank Drs. Norman Davidson and A. Dowsett for providing the rat brain λgt11 library, Rick Ash for HeLa C^+ cells, and M. Kashgarian for the Na,K-ATPase α-subunit monoclonal antibody and Cappel Laboratories for β-galactosidase antibody. We are grateful to Dr. Dan DiMaio for his assistance in constructing the synthetic oligonucleotides used in this study and to Dr. Jack Kyte for providing us with his unpublished Na,K-ATPase amino acid sequence data. We also wish to thank David Geltman, Henry Picozza, and Susan Garetz for their technical assistance and Drs. Alban Linnenbach, Joseph Hoffman, Lew Cantley, David Housman, and Vincent Racaniello for their valuable advice and suggestions. These studies were supported by Grants HL-24385 and AM-28076 from the National Institutes of Health to E.J.B., Jr., and by grants from the National Cancer Institute (CA-38992), American Heart Association, March of Dimes, Cystic Fibrosis Foundation, and Swebilius Foundation to R.L., and Grant AM-17433 from the NIH (to J. Hoffman, P.I.). R.W.M. was supported by a NIH postdoctoral fellowship (NS-07259). J.W.S. was supported by a grant from the NIH MST Program (GM-07205). E.J.B., Jr., is the recipient of RCDA HL-01098 from the NIH.

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