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Control region and gastric specific transcription of the rat H^+,K^+-ATPase α subunit gene

Ko-Ichi Oshiman, Kiyoto Motojima*, Saleh Mahmood, Akira Shimada**, Shigehiko Tamura, Masatomo Maeda and Masamitsu Futai

Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University, Osaka 567, Japan

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The rat gastric H^+,K^+-ATPase α subunit gene was cloned and the nucleotide sequence of its 5'-upstream region was determined. Sequence comparison with the corresponding part of the human gene indicated the presence of highly conserved regions which may be important for specific transcription of the α subunit in gastric parietal cells. The amino-terminal sequence (Met-Gly-Lys-Ala-Glu-) of the rat enzyme was similar to those of the pig and human enzymes. The gene organization of the rat enzyme was also similar to that of the human gene: introns 1, 2 and 9 were located in exactly the same positions as those in the human gene, and, as in the latter, exon 6 was not separated by an intron. The sequences of introns 1 and 2 were highly conserved among the rat, human and pig genes, but were entirely different from those of Na^+,K^+-ATPase catalytic subunit genes. Northern blot hybridization indicated that the gene was transcribed only in gastric mucosa.

H^+,K^+-ATPase; Gastric specific transcription; Control region

1. INTRODUCTION

H^+,K^+-ATPase participates directly in acid secretion into the gastric lumen of the frog and various mammals [1]. The enzyme is a membrane protein with an apparent molecular mass of 100kDa and is phosphorylated during the catalytic cycle [2]. The primary structures of the catalytic α subunits of the pig [3], rat [4] and human [5] enzymes were deduced, respectively, from the sequences of cloned cDNAs [3,4] and gene [5]. The amino acid sequences and gene structures of the H^+,K^+- and Na^+,K^+-ATPase catalytic subunits are highly homologous, suggesting that these two ATPases have evolved from a common ancestral protein [5]. Consistent with these similarities, the β subunit of H^+,K^+-ATPase has been found to be homologous to the corresponding subunit of Na^+,K^+-ATPase [6–9].

In contrast to Na^+,K^+-ATPase, H^+,K^+-ATPase is specifically expressed in parietal cells, as demonstrated by immunochemical analysis [10]. However, the mechanism regulating its gene expression is unknown. The human gene for the α subunit has sequence motifs in its 5'-upstream region that may be recognized by various transcriptional regulatory factors [5]. A practical method for identifying the motifs related to gastric specific transcription is comparison of the 5'-upstream regions of the genes from different mammals, since the expression of H^+,K^+-ATPase seems to be regulated by a common mechanism. In this study, we cloned the gene for the rat H^+,K^+-ATPase α subunit. Sequence comparison of the rat and human genes demonstrated highly conserved regions upstream of the initiation codon. We also obtained evidence that the α subunit gene is transcribed only in gastric mucosa.

Correspondence address: K.-I. Oshiman, Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University, Osaka 567, Japan

**Present address:* Faculty of Pharmaceutical Sciences, Toho University, Chiba 274, Japan

***Present address:* Faculty of Sciences, Hokkaido University, Sapporo 060, Japan

2. MATERIALS AND METHODS

2.1. *Screening of a rat library*

A rat (Sprague-Dawley) liver *Hae*III genomic library (using *Eco*RI linker) constructed with bacteriophage λ Charon 4A, originally made by Drs. L. Jagodzinski and Bonner (Caltech) was kindly donated through Dr. R.D. Andersen (UCLA). Phage plaques propagated in *Escherichia coli* NM539 [11] were transferred to nitrocellulose filters (Schleicher & Schull). The filters were pretreated and hybridized with randomly primed DNA fragments of a pig gastric H^+,K^+-ATPase α subunit cDNA clone (*Nco*I-*Apa*LI positions −1 to 720) [3].

2.2. *Southern hybridization of cloned gene*

Positive clones were digested with *Eco*RI restriction endonuclease and the resulting DNA fragments were separated by agarose (0.7%) gel electrophoresis. The fragments were blotted onto a nitrocellulose filter for Southern hybridization [11]. The 5'-endo-labeled synthetic oligonucleotides (synthesized in an Applied Biosystem Synthesizer 381A) corresponding to rat cDNA [4] were used as region-specific probes: N-probe was used for the amino-terminal region of the coding sequence (sense-strand oligonucleotide corresponding to positions

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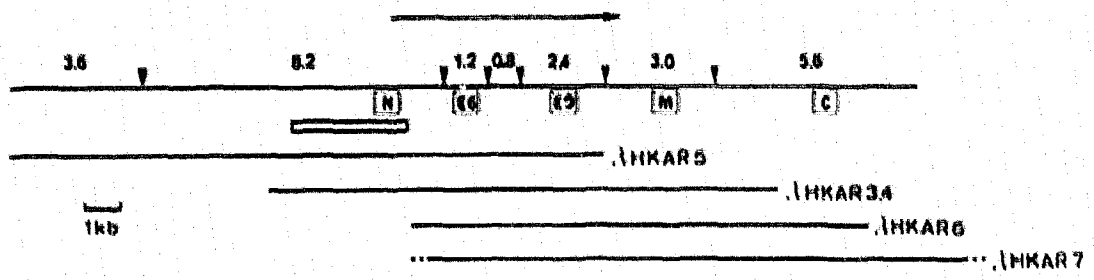


Fig. 1. Restriction map of the rat gastric H^+,K^+-ATPase α subunit gene. Positions of *Eco*RI sites (arrow heads) and sizes of segments (in kilobase pairs) are shown. The regions carried by phage clones are also depicted. The fragments which hybridize with N, E6, E9, M and C probes are shown by corresponding boxes. Open box indicates the region whose sequence is determined (Fig. 2). The arrow indicates the direction of transcription. The terminals of λHKAR7 were not identified.

41-60 from the first letter of the initiation codon), E6-probe (positions 584-603 of the sense-strand) and E9-probe (positions 1257-1276 of the sense-strand) were used for the central part of the coding sequence and C-probe (positions 3019-3054 of the anti-sense strand) was used for the carboxyl-terminal region of the sequence. M-probe (positions 1823-1852 of the anti-sense strand of pig cDNA [3]) was also used. *Eco*RI fragments were subcloned into pUC18 or pBluescript KS(+) and both strands were sequenced by the dideoxy chain-termination method [12].

2.3. *Northern hybridization of total RNA from various tissues*

Total RNA was extracted from the tissues of a 5-week-old Sprague-Dawley rat by the guanidine thiocyanate-CsCl method [11]. An equivalent weight of RNA (20 μg) was denatured using formaldehyde and fractionated by agarose gel (1.0%) electrophoresis. RNA was blotted and hybridized by a published method [11] with randomly primed DNA fragments of a pig cDNA clone (*Nde*I-*Bam*HI positions 2500-2888) [3] of the H^+,K^+-ATPase α subunit as specific probes.

2.4. *Polymerase chain reaction (PCR)*

To clone part of the pig H^+,K^+-ATPase α subunit gene, we synthesized oligonucleotide primers corresponding to the pig cDNA sequence [3] (positions −18 to 11 and 111-138 of the anti-sense strand, and positions 41-66 and 185-212 of the sense strand). PCR was performed using *Taq* DNA polymerase (Perkin-Elmer Cetus) with an ATTO AB-1800 DNA amplification system (Tokyo, Japan) [13]. The clones carrying introns 1 and 2 were then isolated and sequenced.

2.5. *Chemicals*

Restriction enzymes, T4 DNA ligase, T4 polynucleotide kinase and the large fragment of *E. coli* DNA polymerase I were purchased from Takara Shuzo Co. (Kyoto, Japan) and Nippon Gene Co. (Toyama Japan). [α-^{32}P]dCTP (>3000 Ci/mmol) and [γ-^{32}P]ATP (5000 Ci/mmol) were products of the Radiochemical Center Amersham Corp. A randomly primed DNA labeling kit was from Boehringer Mannheim. All other chemicals used were of the highest grade commercially available.

3. RESULTS AND DISCUSSION

3.1. *Isolation of clones carrying the rat H^+,K^+-ATPase α subunit gene*

A phage library constructed from rat genomic DNA was screened with a pig H^+,K^+-ATPase α subunit cDNA as a probe. Five positive clones were obtained from among 2×10^5 plaques. These clones gave positive signals with specific probes for the coding sequence: λHKAR3 and λHKAR4 with the N, E6, E9 and M probes but not with the C probe, λHKAR5 with the N, E6 and E9 probe, and λHKAR6 and λHKAR7 with the E6, E9, M and C probes. From these results and the sizes of the *Eco*RI fragments, a restriction map of the gene was constructed (Fig. 1). The *Eco*RI fragments from λHKAR3 and λHKAR5 (4.8 and 8.2 kb, respectively) were subcloned and a sequence of 3.2 kb around the initiation codon was determined (Fig. 2).

3.2. *Characteristics of the sequence*

Harr plot analysis demonstrated striking similarity (boxed regions A and B) between the rat and human 5′-upstream sequences (Fig. 3), although most of the sequence motifs in the human gene [5] were not found in the rat sequence: 68% and 61% of the residues are identical in regions A and B, respectively. Region B seems to be particularly important for transcriptional regulation, because it contains TATA-like sequence (boxed, at 65 bp upstream of the initation codon) and conserved sequence (tandem repeat at 47 bp upstream of the TATA-like sequence) (Fig. 2). This organization conforms to that of a eukaryotic promoter site forming a transcriptional initiation complex [14]. Other typical sequence motifs as well as a TATA-box were also found in the upstream sequence (Fig. 2).

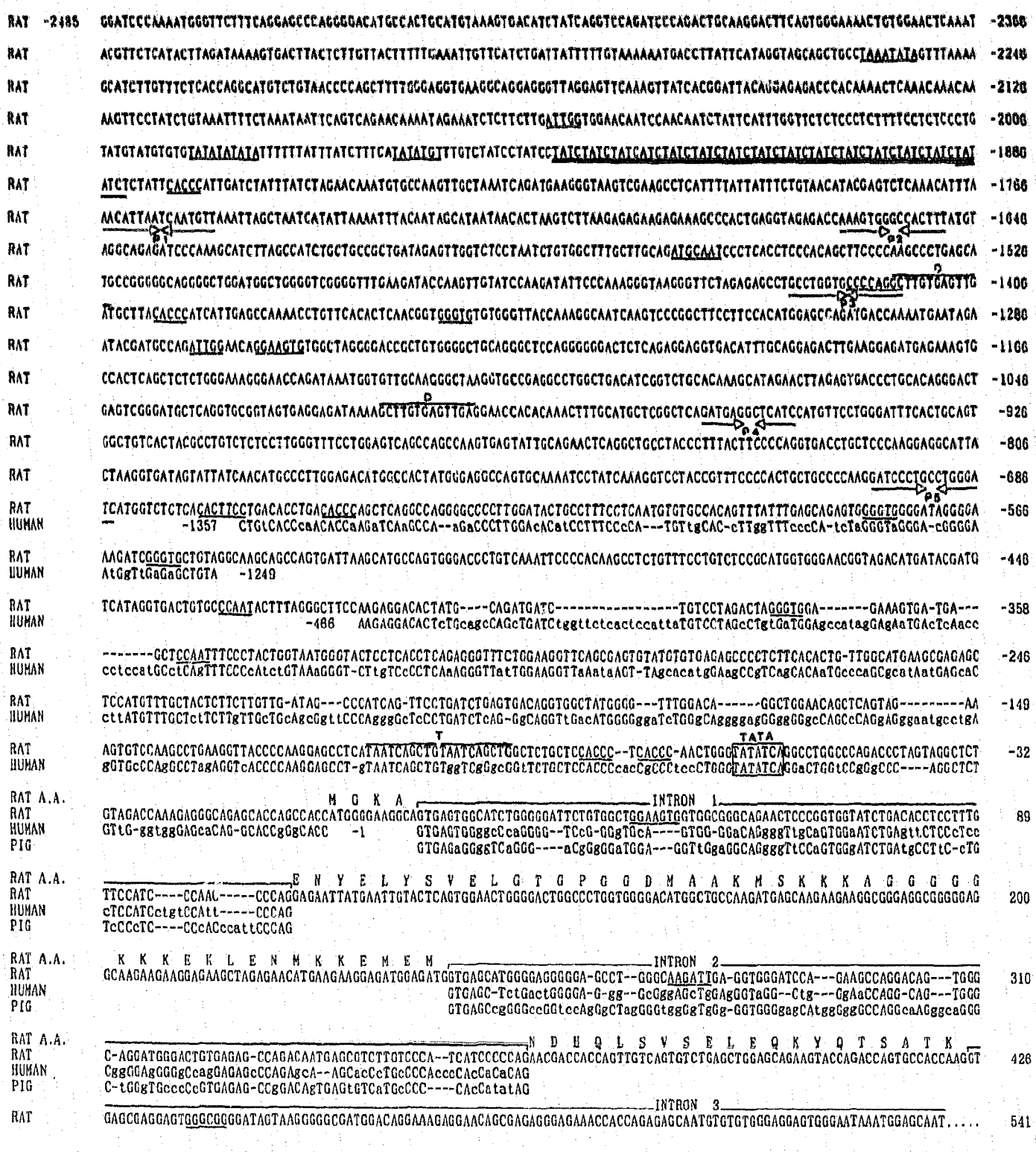
Sequencing of λHKAR3 and λHKAR5 demonstrated that the primary sequence of the rat enzyme from the amino terminus is Met-Gly-Lys-Ala-Glu- (Fig. 2) as in the pig and human enzymes [3,5]. The codon corresponding to the Ala-4 was deleted in the previous rat cDNA clone [4]. Furthermore, the cDNA clone had a 200 base sequence upstream of the initiation codon [4]. However, we could not find this sequence either in the deduced 5′ upstream region of the gene (Fig. 2) or in the further upstream sequence (Fig. 1) using synthetic oligonucleotides for the corresponding part of cDNA as hybridization probes (not shown).

The organization of the rat gene seems to be similar to that of the human gene [5], introns 1, 2 and 9 being in the same positions in the two genes (Fig. 2). Furthermore, as in the latter, exon 6 was not separated by an intron [5]. The sequences of introns 1 and 2 are also conserved in the rat, human and pig genes: pairwise comparison of introns 1 of the rat/human, rat/pig and

human/pig genes demonstrated 62%, 63% and 71% nucleotide identities, respectively, and similar comparisons of the introns 2 showed 63%, 61% and 60% identities, respectively. The sequences of introns 9 of the rat and human genes showed 62% conservation. The corresponding introns of closely related Na^+/K^+-ATPase catalytic subunits (α1, α2 and α3) differed in size and showed no sequence similarities (15-18).

3.3. *Specific expression of the gene in gastric mucosa*

It was important to know whether the mRNA for the α subunit of H^+,K^+-ATPase is transcribed specifically



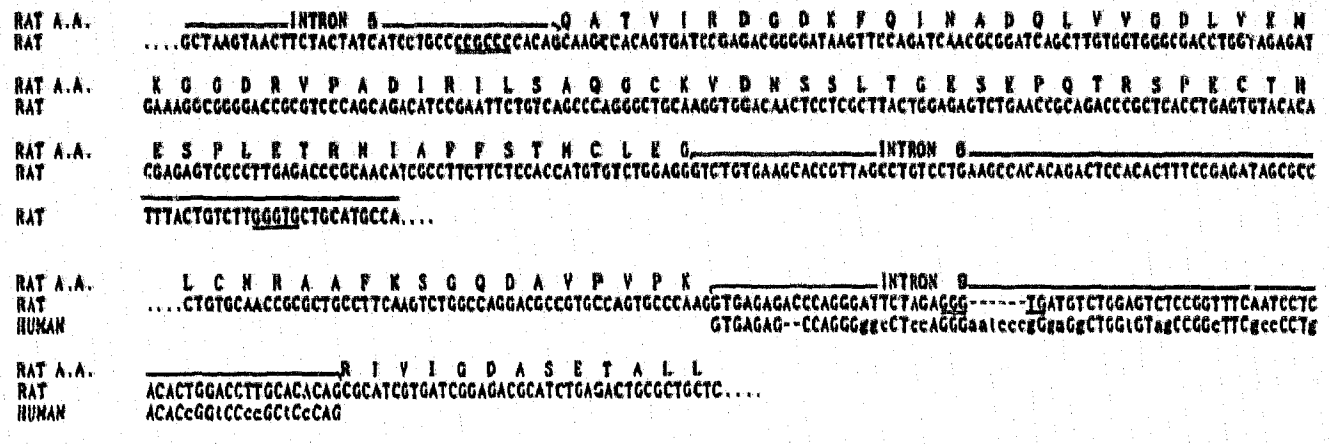


Fig. 2. Nucleotide sequence of the 5'-upstream region of the rat gastric H^+,K^+-ATPase α subunit gene. The nucleotide sequence around the initiation codon of the rat H^+,K^+-ATPase α subunit gene is shown. Nucleotides are numbered on the right of each line from the first letter of the initiation codon. The regions (−1357 to −1249 and −466 to −1) of the human 5'-upstream sequence [5] corresponding to the highly conserved regions A and B, respectively (see Fig. 3) are also aligned. The human and pig sequences corresponding to introns 1, 2 and 9 and introns 1 and 2, respectively, are included. These sequences are aligned with gaps so that the residues are matched as much as possible. The nucleotide residues of the human and pig genes identical to those of the rat gene are indicated by capital letters and the different residues from the latter by small letters. In exon parts, amino acid residues are also indicated. Potential binding sites for RNA polymerase II (TATA box, consensus, TATA(T/A)A(T/A)), Sp1 (GC-box, GGGCGG or CCGCCC), AP2 (CCCCAGGC), NF-Y (ATTGG), OTF1 (ATTGCAT or ATGCAAT), E4TF1 (GGAAGTG or CACTTCC) and STM4 (AATCTT or AAGATT) are underlined [5,19,20]. CACCC box (CACCC and GGGTG) and CAAT box (CCAAT) sequences [20,21] are also underlined. A TATC repeat (double underline), direct repeat (D), tandem repeat (T) and palindrome (P1-P5) sequences are indicated.

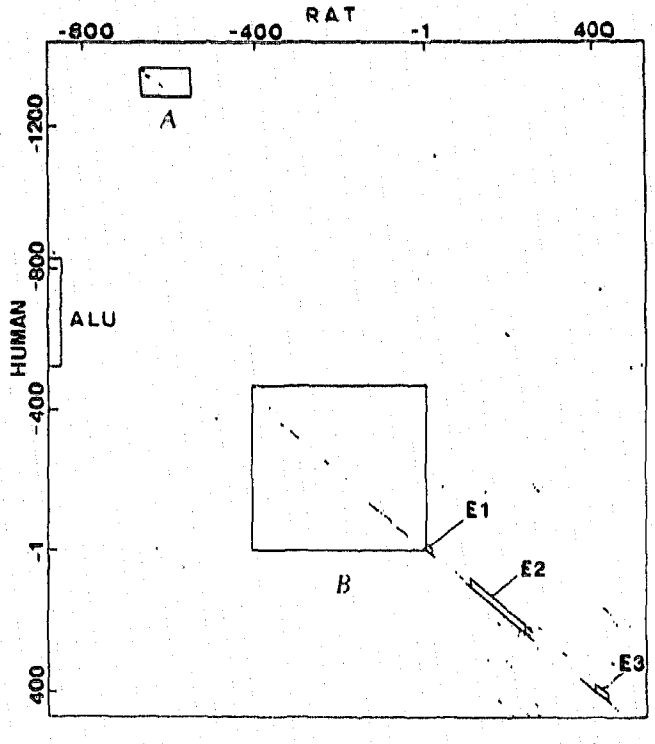


Fig. 3. Similarity of 5'-upstream sequences of the rat and human H^+,K^+-ATPase α subunit genes. The nucleotide sequences of the 5'-upstream regions of the rat (this study) and human [5] genes are compared using a Harr plot program (GENETYX, Software Development Co. Japan). Each dot represents more than 19 identical residues within a span of 30 residues. Highly homologous regions are boxed (A and B). The positions of exons 1-3 (E1-E3) and the *Alu* sequence are also shown.

in stomach. Therefore we examined the mRNA levels in total RNA fractions from various rat tissues. On Northern blot hybridization analysis, the mRNA of the α subunit was detected in the stomach, but not in the brain, lung, heart, liver, kidney, spleen, intestine, or testis (Fig. 4). Furthermore, the mRNA was found in the mucosa layer, but not in muscle from pig stomach (Fig. 4). These results indicated that the α subunit mRNA is transcribed only in gastric mucosa, consistent with immunochemical finding [10]. The sequence similarities between the control regions of the human and rat genes (Figs. 2,3) suggest the presence of com-

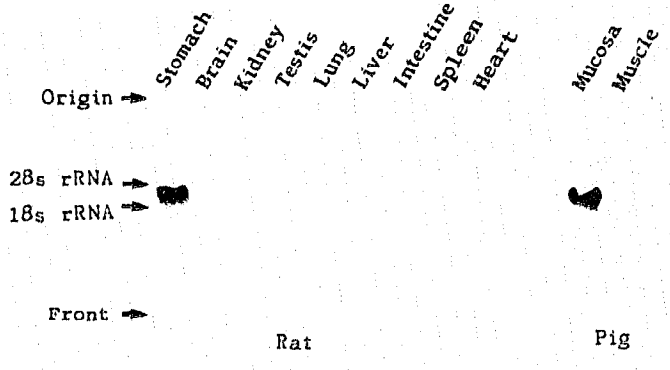


Fig. 4. Tissue distribution of H^+,K^+-ATPase α subunit mRNA. Total RNAs (20 μg) from rat tissues were analyzed by Northern blot hybridization as described in Section 2. The filter was autoradiographed (4 h exposure at −80°C). Samples other than stomach and gastric mucosa did not give signals even after 18 h exposure. The positions of ribosomal RNAs (28 S and 16 S) are shown.

mon gastric specific transcriptional factors recognizing these regions. The β subunit has also been demonstrated to be regulated at the transcriptional level [7]. Thus, it seems important to compare the control regions of α and β subunit genes.

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