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Characterization and expression of the rat heart sarcoplasmic reticulum Ca^{2+}-ATPase mRNA

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Sarcoplasmic reticulum Ca^{2+}-ATPase cDNA clones have been isolated from an adult rat heart cDNA library and the nucleotide sequence of the Ca^{2+}-ATPase mRNA determined. The sequence has an open reading frame of 997 codons. It is identical to a cDNA isolated from a rat stomach cDNA library and 90% isologous to the rabbit and human slow/cardiac cDNAs. Nuclease S_1 mapping analysis indicates that this sequence corresponds to the main Ca^{2+}-ATPase mRNA present in heart and in slow skeletal muscle and that it is expressed in various proportions in smooth and non-muscle tissues, together with another isoform which differs from the cardiac form in the sequence of its 3'-end.

Sarcoplasmic reticulum; ATPase mRNA, Ca^{2+}-; Nucleotide sequence; (Rat heart)

1. INTRODUCTION

Ca^{2+} homeostasis is maintained by pumps, channels and antiporters located in the plasma membrane or in the membrane of intracellular organelles. In muscle cells, the sarcoplasmic reticulum is the main intracellular organelle devoted to the regulation of cytosolic Ca^{2+} concentration. In non-muscle cells, the Ca^{2+} homeostasis is maintained by the rough endoplasmic reticulum, by mitochondria, and by calciosomes, which have been recently identified in the cytoplasm of several non-muscle cells [1,2]. Ca^{2+} influx into the SR and ER is an ATP-supported process mediated by a Ca^{2+}-ATPase (review in [3–6]). The presence of a specific SR Ca^{2+}-ATPase of approximately 110 kDa was first described in skeletal muscle, and its mechanism of action has been widely studied (review in [7,8]). Ca^{2+} transport by the cardiac SR is similar to that of skeletal muscle with the important exception that it is regulated by protein kinase-mediated phosphorylations [9,10]. The evidence for a similar Ca^{2+} transporter in smooth and various non-muscle tissues, is more recent and is based on the presence of a 110 kDa Ca^{2+}-dependent phosphoenzyme and on immunological cross-reactivities with antibodies specific for the SR ATPase [5,6,11–14].

Recently, the structure of the different isoforms of the SR Ca^{2+}-ATPase was intensively studied by cDNA cloning techniques. Four different Ca^{2+}-ATPase mRNAs encoded by two genes were characterized [15–21]. One gene, expressed exclusively in fast skeletal muscle, generates two isoforms by alternative splicing of the penultimate exon: one form is present in the adult and the other in the neonate [16–18]. The other gene also gives rise to two alternatively spliced isoforms [19]: the slow/cardiac and the non-muscle Ca^{2+}-ATPases. cDNAs for the rabbit and human slow/cardiac isoforms were isolated from neonatal skeletal, adult slow skeletal, adult cardiac and kidney libraries [15,17,19]. The corresponding mRNA has been shown to be expressed in adult cardiac, adult slow skeletal and late fetal and neonatal skeletal

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Abbreviations: Ca^{2+}-ATPase, (Ca^{2+}-Mg^{2+})-dependent ATPase; SR, sarcoplasmic reticulum; ER, endoplasmic reticulum; kb, kilobase; bp, base pair

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muscles [17]. In rat, the cDNA identified as the slow/cardiac Ca^{2+}-ATPase isoform based on its homology with the rabbit slow/cardiac cDNA, was isolated from a stomach library [20]. The non-muscle cDNAs were isolated from human kidney and from rat brain, kidney and stomach libraries [19,20]. They differ from the slow/cardiac cDNAs in their 3'-untranslated region and code for a protein identical to the slow/cardiac isoform except for the replacement of four amino acids at the carboxyl-terminal by a 49 amino acids extension. On the other hand we have previously shown, by nuclease S_1 mapping analysis, that in rat a Ca^{2+}-ATPase mRNA which differs for the cardiac mRNA in its 3'-end is expressed in uterus and in intestine [21].

We now report the isolation of cDNA clones specific for a cardiac Ca^{2+}-ATPase isoform from an adult rat heart library. The complete sequence of the main Ca^{2+}-ATPase mRNA expressed in heart was determined and found to be virtually identical to the cDNA isolated from the stomach library. Additionally, we have determined, by nuclease S_1 mapping analysis, the relative level of expression of the Ca^{2+}-ATPase mRNAs present in several striated, smooth and non-muscle tissues.

2. MATERIALS AND METHODS

Recombinant phages from an adult Sprague-Dawley rat heart cDNA library, constructed in λ gt11 (clontech RL 1006 1.3 × 10^6 independent plaque forming units) were plated and transferred to nitrocellulose filters (Millipore HATF) which were prepared for hybridization as described by Maniatis et al. [22]. The filters were hybridized overnight at 42°C to a rabbit Ca^{2+}-ATPase cDNA clone (kindly provided by Dr MacLennan), labelled with [^{32}P]dCTP (3000 Ci/mmol, New England Nuclear) by random priming (Multiprime labelling kit, Amersham) to a specific activity of 1–3 × 10^9 dpm/μg. The hybridization mixture contained 50% formamide, 0.1% bovine serum albumin, 0.1% ficoll, 0.1% polyvinylpyrrolidone, 0.05 M sodium phosphate, pH 6.5, 5 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate), 0.1% SDS and 250 μg/ml salmon sperm DNA. Filters were washed at varying stringencies followed by autoradiography. Some of the positive clones were rescreened and plaque purified a total of three times. The cDNA inserts were characterized by standard restriction endonuclease mapping and their orientation was determined by Southern blot analysis, using probes derived from cDNA clones, whose position along the mRNA was known.

DNA sequencing was performed by the dideoxy chain-termination method of Sanger et al. [23] using [^{35}S]dATP. Appropriate restriction fragments were isolated and subcloned in M_{13} mp18 and mp19 vectors. Single-stranded templates were prepared and sequenced with either the Klenow fragment (M_{13} sequencing kit, Bethesda Research Laboratories) or the T7 DNA polymerase (sequenase-USB) using both dGTP and dITP mixes. The sequence was obtained from several overlapping clones and 90% of the cDNA was sequenced on both strands. A significant amount of the sequence was obtained using template-specific 14- or 15-mer primers synthesized from known DNA sequences, and part of the sequence was obtained using a double-stranded matrix from inserts subcloned into the plasmid pBS (Stratagene).

RNA expression was studied using total RNA from cardiac ventricles, soleus, whole aorta, uterus, liver and kidney of adult Sprague-Dawley rats (180–250 g). RNA was prepared as described [24]. Nuclease S_1 mapping analysis was performed with single-stranded probes obtained by transcribing single-stranded M_{13} templates (500 ng) using as a primer an oligonucleotide synthesized from a known sequence. The reaction was performed for 1 h at 37°C in a mixture containing the Klenow fragment of DNA polymerase I (Boehringer Mannheim), 20 μM of dATP, dGTP, dTTP, 3.5 μM of dCTP plus 3.5 μM of [^{32}P]dCTP (800 Ci/mmol). The labelled M_{13} DNA was then linearized using a restriction endonuclease located 3' to the newly synthesized strand and present only once in the recombinant DNA. The fragment of interest was isolated and recovered from denaturing gels. The single-stranded probe was hybridized to total RNA whose quantity was varied. Hybridization and nuclease S_1 digestion were performed as previously described [25] using 10 U of nuclease S_1 (Biolabs or Amersham) per μg of RNA.

3. RESULTS AND DISCUSSION

400000 recombinant phages from a rat heart cDNA library were screened using a ^{32}P-labelled 742 bp *Pst*I-*Pst*I fragment containing coding and non-coding sequences located at the 3'-end of the rabbit cardiac cDNA pCA3 [15]. 200 positives were obtained after high stringency washing: 0.1 × SSC/0.1% SDS for 1 h at 65°C. Some of these clones were characterized by restriction mapping. Sequence analysis of two of the clones harboring the longest insert (RHCa 39, 2.9 kb and RHCa 23, 2.6 kb) demonstrated that they contained both the 3'-untranslated region and a poly(A) tract of 24 nucleotides (fig.1A). The *Eco*RI fragment from the 5'-end of RHCa 39 was used to rescreen 100000 recombinant phages. Three new clones whose inserts did not hybridize to the 3'-end of the rabbit Ca^{2+}-ATPase cDNA clone were selected. Restriction mapping and sequence analysis of these 5 clones showed that they overlap and cover most of the Ca^{2+}-ATPase mRNA, including 207 bp of the 5'-untranslated region (fig.1A).

The sequence strategy is presented in fig.1B, and the complete nucleotide sequence as well as the

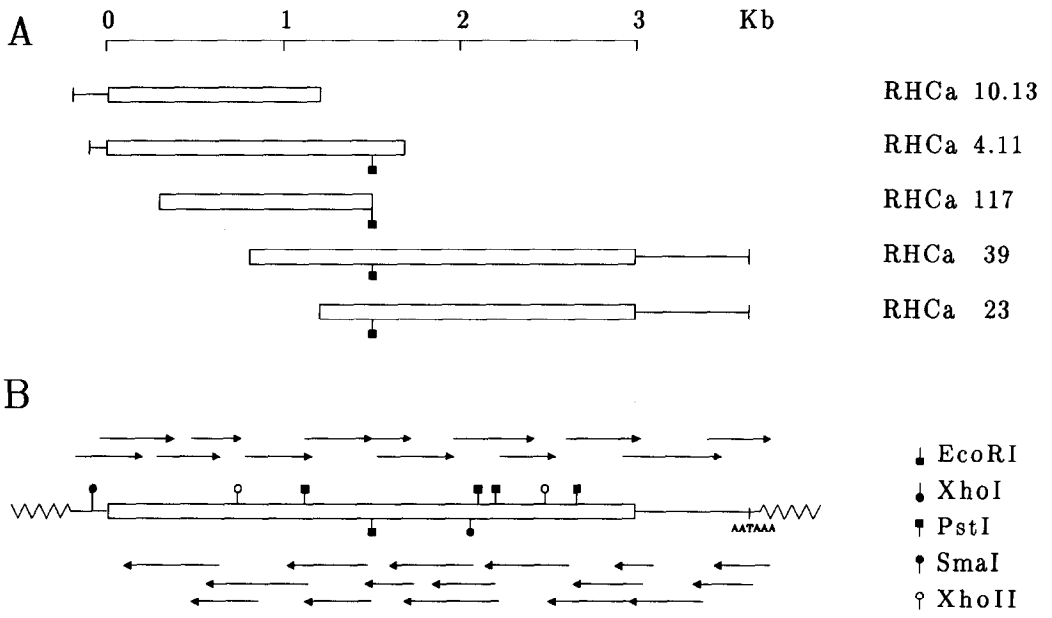

Fig.1. Schematic representation (A) and sequencing strategy (B) of the rat heart Ca^{2+}-ATPase cDNAs. Open boxes represent coding regions and thin lines the untranslated regions. Arrows indicate the direction and extent of sequencing. Zig-zags correspond to the vector.

deduced amino acid sequence is shown in fig.2. The cDNAs cover 3862 nucleotides and have a 997 codon open reading frame which when translated would give a protein with a molecular mass of 109580 Da. Preceding the initiation ATG triplet is a 207 nucleotide 5′-untranslated sequence which is 80% GC rich. The 664 nucleotide 3′-untranslated region terminates with a poly(A) tract, preceded 27 bp upstream by a perfect polyadenylation signal AATAAA. In one of the two clones sequenced, RHCa 39, the polyadenylation site was located 6 nucleotides 5′ to the one presented in fig.2. One substitution is observed within the various clones we sequenced (nucleotide +578 of clone 10.13 and 4.11, C, was substituted in clone 1.17, T) but this substitution did not change the amino acid sequence. This sequence is virtually 100% identical to the cDNA clone, RS8-17, recently isolated from a stomach library [20]. Indeed, only a single nucleotide substitution out of 3000 is observed in the coding region (position +1434 of our sequence, C versus T). This substitution is located in 'wobble' position and the amino acid sequence is thus conserved. The 5′-untranslated region is identical to the corresponding part of the stomach cDNA sequence. The 3′-untranslated region of the cardiac cDNA is identical but 114 nucleotides shorter than the stomach one. Two polyadenylation signals are present in the stomach cDNA while only one was detected in the cardiac clone. In the latter clone, the poly(A) tract is added after the first polyadenylation site located in position 3762 of the stomach sequence. The role of these multiple polyadenylation sites is not known, but, for the skeletal Ca^{2+}-ATPase gene, several poly(A) signals have also been described which did not seem to be either tissue specific or developmentally regulated [17].

Comparison of our rat cardiac Ca^{2+}-ATPase mRNA with the slow isoform isolated from human kidney [19] and rabbit slow skeletal muscle [15,17] reveals a very strong nucleotide conservation. Ten percent of the nucleotides are substituted in the coding region most of which are found in the third position of the codon. Consequently only 8, 7 and 6 amino acid substitutions were observed between rat and rabbit, rat and human, rabbit and human, respectively (table 1). These substitutions do not disturb the overall charge of the molecules at pH 7.0. The presence of a proline in position +538 in rat slow/cardiac muscle instead of an alanine or a serine in rabbit and human, might however change

the conformation of the molecule near the nucleotide-binding site. Compared to rabbit or human hearts, the rat heart is a fast-contracting, fast-relaxing muscle [26]. Interestingly, the sequence of the rat slow/cardiac ATPase in this region (Pro-Gly) resembles more closely that of the fast-twitch skeletal muscle (Gly-Pro) than that of the slow/cardiac ATPase present in the human (Ser-Gly) and rabbit (Ala-Gly) hearts. The amino acid substitutions observed in the slow/cardiac isoforms between species, are mostly located in the α-helix portion of the molecule spanning the phosphorylation and the nucleotide-binding domains. Only one difference is located at position +963 near the last transmembrane domain. None of the substitutions are located in the proposed [16] calcium-binding and transduction domains or in the transmembrane domains, nor are they located in positions where mutations have been shown to alter the function of the fast-skeletal ATPase [27].

We have also studied the level of expression of the slow/cardiac Ca^{2+}-ATPase gene in different striated, smooth and non-muscle tissues by nuclease S_1 mapping analysis. For this purpose, a single-stranded probe, nucleotides +2618 to +3124, was synthesized using a primer located in the untranslated region between nucleotides +3124 and +3111. After digestion with *PstI*, a single-stranded 507 bp fragment was obtained which contains 374 bp of coding and 133 bp of non-coding sequences. Only a fully-protected fragment was observed when total RNA from cardiac and soleus tissues (fig.3A) was hybridized to this probe, which demonstrates that the cDNA we sequenced corresponds to the main Ca^{2+}-ATPase mRNA expressed in slow muscle fibers. These data indicate the presence of the same Ca^{2+}-ATPase mRNA in rat cardiac and slow-twitch muscles and are in full agreement with those of Brandl et al. [17] in the rabbit. A fully protected fragment is also obtained with RNAs from liver, aorta, kidney (fig.3A), and uterus (fig.3A,B), but a second partially protected fragment is also observed with these RNAs. These data indicate that two

Table 1

Localization of the amino acid substitutions in the slow/cardiac SR Ca^{2+}-ATPase between rat, rabbit and human

Amino acid position	Species		
	Rat	Rabbit	Human
371	Lys	Lys	Arg
373	Glu	Asp	Glu
396	Gln	His	His
403	Lys	Lys	Asn
538	Pro	Ala	Ser
644	Asp	Glu	Asp
649	Ser	Ala	Ser
661	Ser	Asn	Asn
713	Ser	Ala	Ala
963	Leu	Val	Val

Ca^{2+}-ATPase mRNAs are expressed in these tissues. The size of the partially protected fragment is 360 ± 3 bp. Since the distance between the *PstI* site and the stop codon is 374 bp, this indicates that the site of divergence is located at 14 ± 3 bp from the stop codon, which corresponds to previously published sequences [19,20]. Similar conclusions were drawn using a probe corresponding to nucleotide +2618 to +3307 (not shown).

The relative proportion of the two mRNA species was determined by densitometric scanning of the autoradiograms. The slow/cardiac mRNA represents a minor proportion of the Ca^{2+}-ATPase mRNA in the two non-muscle tissues (less than 10%) and in uterine smooth muscle (10–15%) while in vascular smooth muscle it is present in larger amounts (30–70%). This demonstrates the expression of the slow/cardiac mRNA in smooth and non-muscle tissues. It seems, however, that its abundance is greater in smooth muscles which have a well developed SR system (e.g. the large arteries), than in other smooth muscle types (e.g. the visceral smooth muscles), or in non-muscle tissues where the reticular system is very poor. The data also indicate that a Ca^{2+}-ATPase mRNA which differs from the cardiac Ca^{2+}-ATPase

Fig.2. Nucleotide and deduced amino acid sequence of the overlapping cDNAs specific for the rat heart SR Ca^{2+}-ATPase mRNA. The nucleotides are numbered beginning at the A of the first apparent initiating ATG triplet. The nucleotides upstream of the initiation codon are numbered negatively while those located downstream are labelled positively. The polyadenylation signal is underlined.

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                                                                GGCGGCGGTTTCTGGGGGAGGGGGCTCGGGTGATTCAGCGCCCGGTCAGGCGGAAGCGGCCGCCGCCGCGGAGGAGGAGGAGAAAGA  -121
GGAGGGGACAGCTGTCCGCGCCCGGGCGCCCGCGGTGGCACGAGCCCGCGGCCCGAGTGCGAGGCGGAGGCGAGGAGGCCGCGGGGACGCGAGGCGAGGCCGGCCGGGGCCCCCGCAGCC   -1

ATGGAGAACGCTCACACAAAGACCGTGGAGGAGGTGCTGGGCCACTTCGGCGTCAACGAGAGCACGGGGCTGAGCCTGGAGCAGGTCAAGAAGCTCAAGGAGAGATGGGGCTCCAACGAA  +120
MetGluAsnAlaHisThrLysThrValGluGluValLeuGlyHisPheGlyValAsnGluSerThrGlyLeuSerLeuGluGlnValLysLysLeuLysGluArgTrpGlySerAsnGlu
1
TTGCCGGCTGAAGAAGGAAAGACCTTGCTGGAACTTGTGATCGAGCAGTTTGAAGACTTACTAGTTAGAATTTTATTGCTGGCAGCATGTATATCTTTCGTTTTGGCTTGGTTCGAAGAA  +240
LeuProAlaGluGluGlyLysThrLeuLeuGluLeuValIleGluGlnPheGluAspLeuLeuValArgIleLeuLeuLeuAlaAlaCysIleSerPheValLeuAlaTrpPheGluGlu
41
GGTGAAGAAACGATTACAGCCTTTGTAGAACCTTTTGTAATTCTGCTTATATTGGTAGCCAATGCAATTGTGGGTGTATGGCAGGAGAGAAACGCTGAAAATGCAATAGAAGCTCTTAAG  +360
GlyGluGluThrIleThrAlaPheValGluProPheValIleLeuLeuIleLeuValAlaAsnAlaIleValGlyValTrpGlnGluArgAsnAlaGluAsnAlaIleGluAlaLeuLys
81
GAGTATGAACCTGAAATGGGCAAGGTGTATCGACAGGACAGAAAGAGTGTGCAGCGGATTAAAGCGAAAGATATAGTTCCTGGGGATATAGTGGAAATTGCTGTTGGTGACAAAGTTCCG  +480
GluTyrGluProGluMetGlyLysValTyrArgGlnAspArgLysSerValGlnArgIleLysAlaLysAspIleValProGlyAspIleValGluIleAlaValGlyAspLysValPro
121
GCTGACATTAGATTGACATCCATCAAGTCTACAACTCTGAGAGTTGACCAGTCGATTCTTACAGGTGAATCTGTCTCGGTCATCAAGCATACTGACCCCGTCCCTGACCCACGAGCTGTT  +600
AlaAspIleArgLeuThrSerIleLysSerThrThrLeuArgValAspGlnSerIleLeuThrGlyGluSerValSerValIleLysHisThrAspProValProAspProArgAlaVal
161
AATCAAGACAAAAAGAACATGCTCTTTTCTGGCACAAACATCGCTGCTGGCAAAGCTATGGGAGTGGTGGTGGCGACTGGAGTCAATACTGAGATCGGCAAGATCCGGGATGAAATGGTT  +720
AsnGlnAspLysLysAsnMetLeuPheSerGlyThrAsnIleAlaAlaGlyLysAlaMetGlyValValValAlaThrGlyValAsnThrGluIleGlyLysIleArgAspGluMetVal
201
GCAACAGAACAGGAGAGAACACCCCTACAGCAGAAGCTGGACGAGTTTGGGGAACAGCTTTCCAAAGTTATCTCCCTCATTTGCATTGCAGTCTGGATCATCAACATCGGGCATTTCAAT  +840
AlaThrGluGlnGluArgThrProLeuGlnGlnLysLeuAspGluPheGlyGluGlnLeuSerLysValIleSerLeuIleCysIleAlaValThrIleIleAsnIleGlyHisPheAsn
241
GACCCAGTTCATGGTGGCTCTTGGATCAGAGGTGCCATCTACTACTTTAAGATTGCAGTGGCCCTGGCTGTTGCTGCCATCCCTGAGGGTCTGCCTGCTGTCATCACCACCTGCTTGGCT  +960
AspProValHisGlyGlySerThrIleArgGlyAlaIleTyrTyrPheLysIleAlaValAlaLeuAlaValAlaAlaIleProGluGlyLeuProAlaValIleThrThrCysLeuAla
281
CTTGGAACTCGAAGGATGGCAAAGAAAAATGCTATTGTTCGAAGTCTGCCTTCTGTGGAAACCCTTGGTTGTACTTCTGTTATCTGCTCAGACAAGACCGGCACACTTACCACAAACCAG  +1080
LeuGlyThrArgArgMetAlaLysLysAsnAlaIleValArgSerLeuProSerValGluThrLeuGlyCysThrSerValIleCysSerAspLysThrGlyThrLeuThrThrAsnGln
321
ATGTCCGTCTGCAGGATGTTCATTCTGGACAAAGTAGAAGGTGATACTTGTTCCCTTAATGAGTTTACCATAACTGGATCAACCTATGCACCCATTGGAGAAGTGCAAAAAGATGACAAG  +1200
MetSerValCysArgMetPheIleLeuAspLysValGluGlyAspThrCysSerLeuAsnGluPheThrIleThrGlySerThrTyrAlaProIleGlyGluValGlnLysAspAspLys
361
CCAGTGAAATGCCATCAGTATGACGGGCTTGTAGAGTTAGCAACGATCTGTGCTCTGTGTAATGACTCTGCTTTGGATTACAATGAGGCGAAGGGTGTGTATGAAAAAGTTGGAGAAGCC  +1320
ProValLysCysHisGlnTyrAspGlyLeuValGluLeuAlaThrIleCysAlaLeuCysAsnAspSerAlaLeuAspTyrAsnGluAlaLysGlyValTyrGluLysValGlyGluAla
401
ACAGAGACTGCTCTCACGTGCCTGGTAGAGAAGATGAATGTATTCGACACGGAGCTGAAGGGACTTTCTAAAATAGAACGCGCCAACGCCTGCAACTCGGTCATAAAACAGCTCATGAAG  +1440
ThrGluThrAlaLeuThrCysLeuValGluLysMetAsnValPheAspThrGluLeuLysGlyLeuSerLysIleGluArgAlaAsnAlaCysAsnSerValIleLysGlnLeuMetLys
441
AAGGAATTCACGCTAGAGTTTTCACGTGATAGAAAATCAATGTCCGTCTACTGTACACCAAACAAACCGAGCCGGACGTCCATGAGCAAGATGTTTGTGAAGGGTGCTCCAGAAGGTGTC  +1560
LysGluPheThrLeuGluPheSerArgAspArgLysSerMetSerValTyrCysThrProAsnLysProSerArgThrSerMetSerLysMetPheValLysGlyAlaProGluGlyVal
481
ATCGACAGGTGCACCCACATCCGAGTTGGAAGTACCAAGGTCCCCATGACGCCTGGTGTTAAACAGAAGATTATGTCTGTCATTCGGGAGTGGGGCAGTGGCAGCGACACACTGCGGTGC  +1680
IleAspArgCysThrHisIleArgValGlySerThrLysValProMetThrProGlyValLysGlnLysIleMetSerValIleArgGluTrpGlySerGlySerAspGlnLeuArgCys
521
CTGGCTCTGGCCACTCATGACAACCCGCTGAGGAGAGAGGAGATGCACCTGGAAGATTCTGCGAACTTCATCAAATATGAGACCAATCTGACTTTCGTTGGCTGTGTGGGCATGCTGGAC  +1800
LeuAlaLeuAlaThrHisAspAsnProLeuArgArgGluGluMetHisLeuGluAspSerAlaAsnPheIleLysTyrGluThrAsnLeuThrPheValGlyCysValGlyMetLeuAsp
561
CCTCCCAGGATTGAAGTGGCCTCTTCTGTGAAGCTGTGCCGGCAAGCGGGCATCCGAGTCATCATGATCACTGGGGATAACAAAGGCACTGCTGTGGCCATCTGTCGCCGCATTGGCATC  +1920
ProProArgIleGluValAlaSerSerValLysLeuCysArgGlnAlaGlyIleArgValIleMetIleThrGlyAspAsnLysGlyThrAlaValAlaIleCysArgArgIleGlyIle
601
TTTGGGCAGGATGAGGATGTGACATCAAAGGCTTTTACAGGGCGAGAATTTGATGAATTAAGCCCCTCAGCCCAGAGAGACGCCTGCTTAAATGCCCGTTGTTTTGCTCGAGTTGAACCT  +2040
PheGlyGlnAspGluAspValThrSerLysAlaPheThrGlyArgGluPheAspGluLeuSerProSerAlaGlnArgAspAlaCysLeuAsnAlaArgCysPheAlaArgValGluPro
641
TCCCACAAGTCTAAGATCGTTGAGTTCCTGCAGTCCTTTGATGAGATCACAGCTATGACTGGTGATGGTGTGAACGACGCGCCCGCTCTGAAGAAGTCGGAAATCGGGATTGCCATGGGC  +2160
SerHisLysSerLysIleValGluPheLeuGlnSerPheAspGluIleThrAlaMetThrGlyAspGlyValAsnAspAlaProAlaLeuLysSerGluIleGlyIleAlaMetGly
681
TCAGGGACTGCAGTGGCTAAGACGGCCTCTGAGATGGTGCTGGCCGACGACAACTTCTCCACCATCGTGGCCGCTGTTGAGGAGGGGCGCGCCATCTACAACAACATGAAGCAGTTCATC  +2280
SerGlyThrAlaValAlaLysThrAlaSerGluMetValLeuAlaAspAspAsnPheSerThrIleValAlaAlaValGluGluGlyArgAlaIleTyrAsnAsnMetLysGlnPheIle
721
CGCTACCTCATCTCCTCCAACGTGGGGGAGGTGGTCTGTATCTTCCTGACGGCAGCCCTTGGGTTTCCTGAGGCTTTGATTCCTGTCCAGTTACTCTGGGTCAATCTGGTGACGGATGGT  +2400
ArgTyrLeuIleSerSerAsnValGlyGluValValCysIlePheLeuThrAlaAlaLeuGlyPheProGluAlaLeuIleProValGlnLeuLeuTrpValAsnLeuValThrAspGly
761
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801
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881
CTGCTGAGGATGCCCCCCCTGGGAGAACATCTGGCTCGTGGGCTCCATCTGCTTGTCCATGTCCCTTCACTTCTTGATCCTCTACGTGGAACCTTTGCCACTCATTTTCCAGATCACACCG  +2880
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921
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961
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ACTGTGTGATGTGGAGGAAATGTGTATTACAAGTGGGGTTTTAGCTGTTGAGTCAAAATAATAACAAGTGTACAATTTAGCATAAGGAATCGGAGAGCCTCTCCAGAGAAGTCGGTTTCT  +3240
TTGCTGCAAGAAGAATGAGGTTCTGAACCCTTATCCAAGAACAGAAGCCATCAGCCAAGTCTCCACATTTCTCTGCAAAATGTTGTAGCCTCTATAACTGTATGATAGTGTAATGCATGC  +3360
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AAGTATTGTGTGCATTTATCGTAATAAAGTGCTCCGCGTCGGTTCAGTTCCTCAC(A)24
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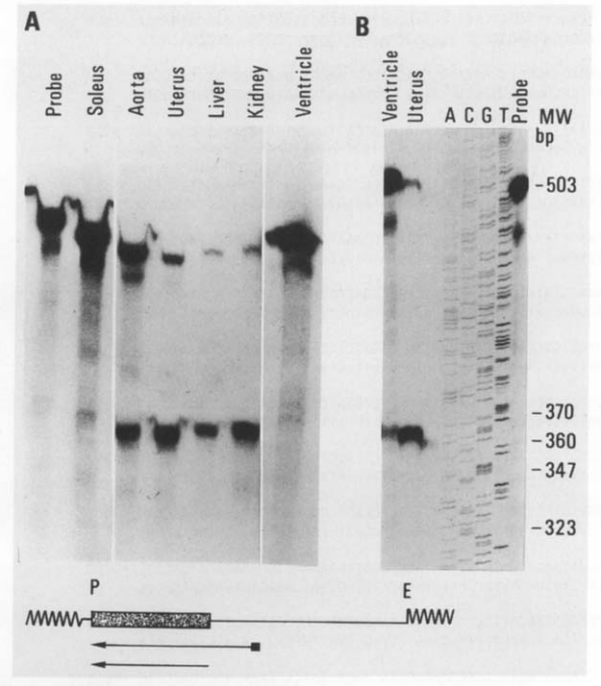

Fig.3. Nuclease S_1 mapping analysis of the Ca^{2+}-ATPase mRNA expressed in several adult tissues. A 3′-end single-stranded and uniformly labelled probe, covering nucleotides +2618 to +3124 was hybridized to 5 μg of total RNA from soleus and ventricle, and 20 μg of RNA from aorta, uterus, liver, and kidney. The probe is represented schematically in the lower part of the figure. The coding region is presented as a dashed box and the untranslated region with a thin line, zig-zags represent M13 sequences. P, *PstI*; E, *Eco*RI. The square black box represents the primer used to initiate the synthesis of the probe, the upper arrow the size of the probe and the fully protected fragment and the lower arrow the partially protected segment. In (B) a sequencing ladder was used as molecular mass markers. The gel was exposed 16 h at −70°C to autoradiography. Part A is a montage of two autoradiograms of the same gel, the probe, soleus and ventricle were exposed for 2 h, whereas the aorta, uterus, liver and kidney were exposed for 16 h.

mRNA in its 3′-end is present in smooth and in non-muscle tissues. The role of these isoforms as well as their precise tissular and cellular localization has yet to be determined.

In conclusion, we have characterized the rat slow/cardiac Ca^{2+}-ATPase mRNA and shown that it is virtually identical to a cDNA clone isolated from stomach and 90% isologous to the rabbit and human isoforms. This Ca^{2+}-ATPase mRNA is the main isoform expressed in heart and slow skeletal fibers and is also present in significant amounts in certain smooth muscles while it is expressed in low amounts in other smooth and in non-muscle tissues. In these two different types of tissues, the slow/cardiac Ca^{2+}-ATPase mRNA is coexpressed together with another one which differs only in the sequence of its 3′-end.

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