Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6908–6912, September 1989
Biochemistry

mRNAs for plasma membrane calcium pump isoforms differing in their regulatory domain are generated by alternative splicing that involves two internal donor sites in a single exon

(skeletal muscle/calmodulin-binding domain/differential splicing)

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Communicated by Harvey F. Lodish, June 19, 1989

ABSTRACT **cDNA clones coding for human plasma membrane Ca^{2+} pump isoforms have been isolated from a fetal skeletal muscle cDNA library. Compared with the sequence of a teratoma cDNA-encoded pump these clones specify isoforms that contain either 29- or 38-amino acid insertions within the calmodulin-binding region. Replacement of two basic arginine residues by an aspartic acid and a glutamine residue could influence the binding of calmodulin to these isoforms. RNase mapping shows that RNA species containing the 29-residue-encoding insertion are particularly abundant in skeletal muscle. The sequences coding for the insertions are present on a single 154-base-pair exon, as demonstrated by an analysis of the corresponding genomic region, and they are included in their respective mRNAs by alternative splicing involving the differential usage of two internal "cryptic" donor splice sites in the presence of a nearby canonical one. Inclusion of the complete 154-base-pair exon results in an mRNA coding for a pump protein with a shorter C-terminal amino acid sequence that lacks a consensus site for phosphorylation by the cAMP-dependent kinase. Exclusion, inclusion, or partial inclusion of the same exon can thus lead to the production of four different mRNAs from a single gene. When expressed as protein, these mRNAs encode Ca^{2+} pump isoforms that differ in their C-terminal regulatory domains.**

Ca^{2+} pumps of plasma membranes play a key role in regulating intracellular free Ca^{2+} concentrations in eukaryotic cells. They belong to the class of P-type ion-motive ATPases that characteristically form a phosphorylated intermediate during the reaction cycle (1). The primary structures of rat and human plasma membrane Ca^{2+} pumps have recently been determined from their cDNAs (2, 3). Besides the highly conserved functional domains involved in ATP binding and in the formation of the phosphorylated intermediate, these pumps also contain, in their C-terminal region, a regulatory domain responsible for mediating the effects of Ca^{2+}-calmodulin and phosphorylation by the cAMP-dependent protein kinase (3–5). This domain encompasses ≈120 C-terminal amino acid residues and accounts for the bulk of the additional molecular mass of plasma membrane Ca^{2+} pumps (M_r = 135,000) when compared to other P-type ion pumps [e.g., the Ca^{2+} pump of sarcoplasmic reticulum (6), the Na^+/K^+ pump (7), H^+ pumps (8, 9); M_r = 90,000–110,000].

Recent evidence shows that several isoforms exist for the plasma membrane Ca^{2+} pump (refs. 2 and 3; E.E.S., P. James, R. Fischer, R. Heim, T. Vorherr, A. G. Filoteo, J. T. Penniston, and E.C., unpublished work) that are encoded by a multigene family. A similar situation exists for the Ca^{2+} pump of the sarcoplasmic (endoplasmic) reticulum and for the Na^+/K^+ pump (7, 10–12). In addition, alternative RNA splicing of a single gene transcript has been shown to augment the number of sarcoplasmic reticulum Ca^{2+} pump isoforms (11, 13, 14). Little is known, however, about the significance of these isoforms with respect to their tissue-specificity and their structural and functional differences.

Here we present evidence for the existence of mRNA species in human skeletal muscle that encode plasma membrane Ca^{2+} pump isoforms containing insertions of either 29 or 38 amino acids* at an identical position within their calmodulin-binding region when compared with the published human Ca^{2+} pump protein sequence (3). These mRNAs are generated by a process of alternative RNA splicing that involves a single 154-base-pair (bp) exon. Differential use of two internal donor splice sites causes the production of mRNAs containing the 29- and 38-codon insertions, whereas inclusion of the entire 154-bp exon leads to a mRNA species that encodes a protein with a completely different C-terminal amino acid sequence due to a shift in reading frame.

MATERIALS AND METHODS

Source of DNA Libraries and of Muscle Tissue. A human fetal skeletal muscle cDNA library (15) and a human leukocyte genomic DNA library (16) were obtained from L. M. Kunkel and S. H. Orkin (Harvard Medical School), respectively. Normal adult human skeletal muscle tissue was provided by H. Eberle and U. Vollenweider (University Hospital, Zurich). Fibers were removed during surgery and were immediately frozen in liquid nitrogen and stored at −80°C.

DNA Library Screening. An aliquot of the original ligation mixture of the cDNA library was packaged into phage heads by using the Gigapack Gold extract (Stratagene) according to the manufacturer's instructions. Approximately 1×10^6 phage plaques were screened as described (17, 18), using as probe a 2.2-kilobase (kb) *Nco* I–*Eco*RI fragment corresponding to nucleotide positions 1395–3578 of the human teratoma Ca^{2+} pump cDNA (3). Screening of $\approx 1 \times 10^6$ phage plaques of the human genomic DNA library in λEMBL3 was carried out with the same cDNA probe and using published procedures (17). Probes were labeled with the random-primed oligolabeling method (19).

DNA Isolation, Subcloning, and Restriction Enzyme Mapping. λ phage DNA was isolated and purified as described (17, 20). Subfragments were cloned into pUC18 and M13mp-18/19 (21) vectors by using standard protocols (17). Clone λhO-2.3 was mapped on partial digests (17) and by Southern blotting procedures (22).

Isolation of RNA and RNase Mapping. Cytoplasmic RNA was isolated by the guanidinium thiocyanate method (23) by

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Abbreviation: nt, nucleotide(s).

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M25824).

using an RNA isolation kit (Stratagene). Plasmid pSP1-4 was constructed by cloning a 386-bp *Dra* I–*Eco*RI fragment [nucleotides (nt) 3198–3583] of the published Ca^{2+} pump cDNA (3) into pSP65 digested with *Sma* I and *Eco*RI. pSP4-7 consists of a 0.6-kb *Hae* III–*Eco*RI genomic fragment containing the alternatively spliced exon, cloned into pSP65 digested with *Sma* I and *Eco*RI. The constructs were linearized with *Bam*HI, and *in vitro* transcription with SP6 polymerase was performed in the presence of [α-^{32}P]CTP ($\geq$ 600 Ci/mmol; 1 Ci = 37 GBq) as described (24). RNase mapping was essentially carried out as described (24), except that hybridizations were performed overnight at 37°C and RNase digestions were at 30°C for 40 min.

Nucleotide Sequence Determination. Sequencing was accomplished with Sequenase (United States Biochemical) according to the manufacturer's instructions. For sequence analysis the University of Wisconsin Genetics Computer Group Software Package (25) was used.

RESULTS

To identify potential tissue-specific isoforms of the plasma membrane Ca^{2+} pump, a human fetal skeletal muscle cDNA library (16) was screened with a probe isolated from a teratoma Ca^{2+} pump cDNA (3). Six independent positive clones (hskm-1.1, 1.2, 1.3, 2.3, 2.4, and 2.5) from the initial screening were analyzed by partial restriction mapping and sequencing (Fig. 1). All of these clones ended with a short poly(A) tail at their 3′ end and contained 150 bp of the 3′ untranslated sequence of the previously characterized Ca^{2+} pump cDNA. With respect to the published cDNA sequence (3), their 5′ ends corresponded to nt 811 (hskm-1.3), 918 (hskm-1.1 and hskm-2.5), 954 (hskm-2.3), 992 (hskm-1.2), and 1076 (hskm-2.4), indicating that none of them were full-length clones (Fig. 1). The partial nucleotide sequences determined for these clones were identical to that of the human teratoma cDNA, except for one region where insertions of either 87 bp (five clones) or 114 bp (one clone) were

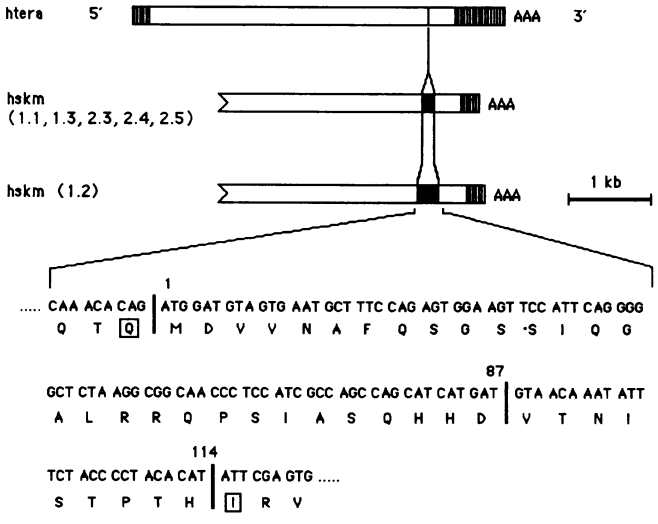


FIG. 1. Schematic representation of human plasma membrane calcium pump cDNAs. (*Top*) Comparison of the cDNA from a human teratoma (htera) library (3) with the two types of cDNAs from a human fetal skeletal muscle library (hskm). Striped boxes correspond to untranslated regions, and AAA indicates the position of poly(A) tails. Black boxes represent insertions in the hskm cDNA clones with respect to the htera cDNA; these boxes are not to scale due to their small size. (*Bottom*) Nucleotide and translated amino acid sequence of the insertions as well as of the codons immediately 5′ and 3′ of this region. Residues 1117 (Q) and 1118 (I) of the htera cDNA-encoded protein are boxed, and the position of divergence between the 87- and the 114-bp insertion is indicated.

detected (Fig. 1). The 114-bp sequence consisted of the same 87 bp as found in the shorter insertion but contained an additional 27 bp at its 3′ end. These insertions code for an integral number of amino acids (29 and 38 residues) and do not change the reading frame as determined for the teratoma Ca^{2+} pump cDNA. The insertions occur precisely after the codon for amino acid residue 1117 of the published sequence, interrupting it within its extended calmodulin-binding domain between subdomains A and B (2–4). Although 6 out of the first 10 amino acid residues of the insertion sequence are identical to the original sequence of residues 1118–1127 (Fig. 2), the insertions may have an effect on the calmodulin-dependent regulation of the enzyme. Replacement of the basic residues Arg-1119 and Arg-1125 by an acidic aspartic acid and a neutral glutamine residue, respectively, could influence the binding of calmodulin to its target domain in the Ca^{2+} ATPase: All calmodulin-binding domains sequenced so far exhibit a strongly basic character, which is assumed to be important for the interaction with calmodulin (4, 26, 27).

The fact that the nucleotide sequence surrounding the insertion region (and extending to the 3′ end of the clones) is identical in the skeletal muscle cDNAs to that of the previously reported sequence (3) indicates that alternative RNA splicing of a common primary transcript is responsible for the generation of the corresponding mRNAs. To prove this hypothesis we isolated the region of the gene that covers the "insertion" region (Fig. 3 *a* and *b*). As predicted, the coding sequence was found to be interrupted by an intron precisely at the point of insertion in the skeletal muscle cDNAs. The exon 5′ of the insertion region codes for amino acids 1057–1117 of the published Ca^{2+} pump sequence. The exon specifying the 87-bp insertion sequence was identified 372 bp further downstream (Fig. 3*b*). It is flanked by a consensus splice acceptor site (28) and a donor site that deviates from the consensus sequence at positions 5 and 6 [gtaaca instead of gt(g/a)agt]. Interestingly, the longer 114-bp insertion sequence is encoded by the same exon; its 27 additional bp correspond to a read-through at the 3′ end of the 87-bp sequence (Fig. 3*b*). The donor splice site after the 114-bp sequence deviates from the consensus sequence by lacking an adenine at position 4. In fact, this donor sequence—like the one following the first 87 bp of this exon—corresponds to an internal splice site. The "complete" exon is 154 bp long (Fig. 3*b*). This surprising finding becomes apparent from an inspection of the cDNA sequence for a rat Ca^{2+} pump isoform (2). This cDNA, RB11-1 (2), corresponds to the human teratoma sequence with the exception of a 154-bp insertion exactly at a position after amino acid codon 1117. The first 114 bp of this sequence differ at only 4 positions from the sequence of the insertion in human cDNA hskm-1.2, and the remaining 40 bp are identical in 39 positions with the sequence after the 114-bp exon region in genomic clone λhO-2.3. At its 3′ end, the full 154-bp exon is flanked by a consensus splice donor site (Fig. 3*b*). The next constitutively spliced exon of the gene lies >6 kb further downstream and

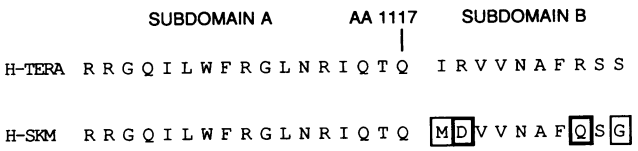
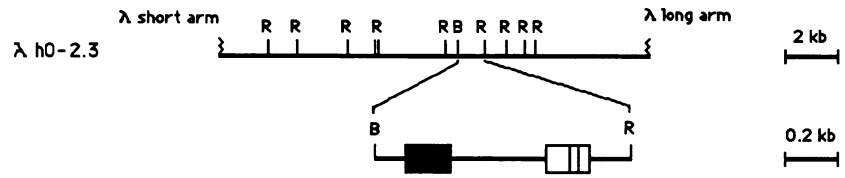


FIG. 2. Sequence comparison of the calmodulin-binding region of Ca^{2+} pump isoforms. Alignment of the calmodulin-binding region sequence as deduced from cloned cDNAs isolated from human teratoma (H-TERA) and fetal skeletal muscle libraries (H-SKM). The position of residue 1117 [numbering of the H-TERA isoform sequence (3)] preceding the insertion region in the H-SKM isoforms is indicated. Residues that differ in the H-SKM isoforms are boxed; nonconservative substitutions are highlighted by boldface boxes.

a



b

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ggatcccattacttcattggtaaagatagtcggatactctcaccacttttaataccttta gtattacagttgatcagctt  80
accttta cttgatatgatttatttctaagttcattcccctgtgttgtag  CTT ATT TCA ACA ATT CCA ACT  150
                                                     L   I   S   T   I   P   T
AGC CGT TTA AAA TTC CTC AAA GAA GCT GGT CAT GGA ACA CAA AAG GAA GAA ATA CCT GAG  210
 S   R   L   K   F   L   K   E   A   G   H   G   T   Q   K   E   E   I   P   E
GAG GAA TTA GCA GAG GAT GTT GAA GAG ATT GAT CAC GCT GAA AGG GAG TTG CGG CGT GGC  270
 E   E   L   A   E   D   V   E   E   I   D   H   A   E   R   E   L   R   R   G
CAA ATC TTG TGG TTT AGA GGT CTG AAC AGA ATC CAA ACA CAG gtatgggcctggtagagaggtag  335
 Q   I   L   W   F   R   G   L   N   R   I   Q   T   Q
gagagatgatgggagccagggctttttgacctgggaggaggttcgagtgtgctccttaaacttcctctctagccttccca  415
acccacaaattgtattctcttgaaaagtaatactggtgcctggaatgagtgagacttttaactgattatgaaagaatgtg  495
tctatatgcccctaagctccttttttcctagctccccatccccacttcatctcaaaatgctattcttgcctatatgct  575
gtgtaaaaagtgccttttttcttttatatgttgatagatggaatggtcctttctcttgttctctctctcttgctgagc  655
aagctgtcacaatctctgattccttgcag  ATG GAT GTA GTG AAT GCT TTC CAG AGT GGA AGT TCC  720
                               M   D   V   V   N   A   F   Q   S   G   S   S
ATT CAG GGG GCT CTA AGG CGG CAA CCC TCC ATC GCC AGC CAG CAT CAT GAT GTA ACA AAT  780
 I   Q   G   A   L   R   R   Q   P   S   I   A   S   Q   H   H   D   V   T   N
ATT TCT ACC CCT ACA CAT GTA GTG TTT TCC TCT TCT ACT GCT TCT ACT ACT GTG GGG T    838
 I   S   T   P   T   H   V   V   F   S   S   S   T   A   S   T   T   V   G
gtgagtgtgtgttcctaagtgcatgaaattaacatttcctacttcacacacctaacgtttctcattttctccttaatgtt  918
tgaattc                                                                          925
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c

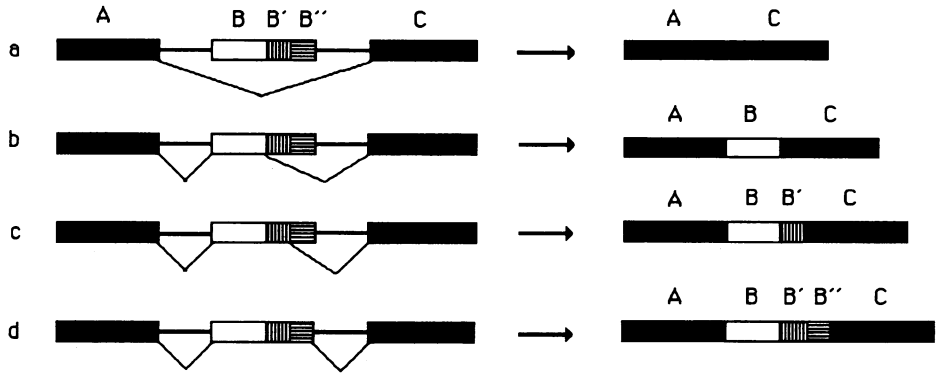

FIG. 3. Structure of the genomic DNA covering the region of alternative splicing. (*a*) Schematic representation of genomic clone λhO-2.3. *Eco*RI(R) and *Bam*HI(B) restriction sites are indicated. The *Bam*HI–*Eco*RI fragment carrying the alternatively spliced exon is shown at an enlarged scale. Solid box, constitutively spliced exon encoding amino acid residues 1057–1117; open box, alternatively spliced exon with the position of internal donor sites indicated by vertical lines. (*b*) Sequence of the genomic *Bam*HI–*Eco*RI fragment. Obligatory intron sequences are displayed in lowercase letters; (potential) exon sequences are printed in uppercase letters, and the encoded amino acids are shown in one-letter code. Consensus intron splice acceptor and donor sites are underlined; the two internal splice donor sites are boxed. The sites where splicing can occur within the exon are indicated by vertical bars. (*c*) Pathways of alternative RNA splicing leading to the production of four different mature mRNAs from a single Ca^{2+} pump gene transcript. Constitutive exons A and C are represented as solid boxes, the alternatively spliced exon B-B′-B″ is displayed in three regions (open, horizontally striped, and vertically striped box), indicating its different subportions.

is not present on clone λhO-2.3 (M.-A.S.-P, unpublished work). Thus, although no human cDNA clone containing the complete 154 bp "alternative" exon sequence has yet been isolated, it is clear that complete exclusion [as in the human teratoma cDNA (3)], complete inclusion [as in rat cDNA RB11-1 (2)] or the use of two different internal splice donor sites (as in clones hskm-1.1 and hskm-1.2) of one single exon can lead to the production of four mature mRNA species from a single gene transcript (Fig. 3*c*).

To prove that alternative RNA splicing is functional in the generation of Ca^{2+} pump mRNAs and to address the potential tissue-specificity of such splicing events, RNase mapping experiments were done. This method was chosen because of its specificity and sensitivity. A high sensitivity of detection is crucial because of the very low abundance of plasma membrane Ca^{2+} pump mRNA (the pump accounts for <0.1% of total membrane protein). Plasmid pSP1-4 generates the antisense RNA to a 386-bp *Dra* I–*Eco*RI fragment of the human teratoma cDNA (3) encoding residues 1067–1194 of that pump isoform. When this probe was used for RNase mapping, a fully protected 386-nt fragment was seen with RNA both from teratoma cells and from adult skeletal muscle (Fig. 4*a*, lanes 2 and 3). However, only skeletal muscle RNA yielded additional prominent bands at 232 and 154 nt (Fig. 4*a*, lane 2). These bands correspond precisely to the partially protected fragments expected for mRNAs containing an insertion at the position described above. The results presented in Fig. 4*a* thus show that mRNA species can be detected in skeletal muscle the sequence of which deviates from that of the teratoma Ca^{2+} pump mRNA (3) at the position suggested to be involved in alternative splicing. The data in Fig. 4*a* also indicate that the level of Ca^{2+} pump mRNA(s) is much higher in adult skeletal muscle than in teratoma cells: even the most prominent band corresponding to the fully protected probe is at least 5-fold weaker with teratoma RNA than with muscle RNA. The additional band seen at ≈184 nt in Fig. 4*a* is probably not due to unspecific background but may correspond to a partially protected fragment ≈30 nt longer than that of 154 nt. The nucleotide sequence encoding the first 9 residues of the "insertion" sequence (specifying subdomain B of the calmodulin-binding region; see Fig. 2) shows 67% identity with the corresponding region of the human teratoma cDNA. Hybridization of this region to the probe under the low-stringency conditions used may thus explain the existence of the ≈184-nt band.

The results obtained with the pSP1-4 probe do not provide clues as to the size of the spliced-in sequence at the insertion position. An antisense RNA probe was therefore used for RNase protection; this probe was derived from plasmid pSP4-7 carrying a 0.6-kb genomic *Hae* III–*Eco*RI fragment

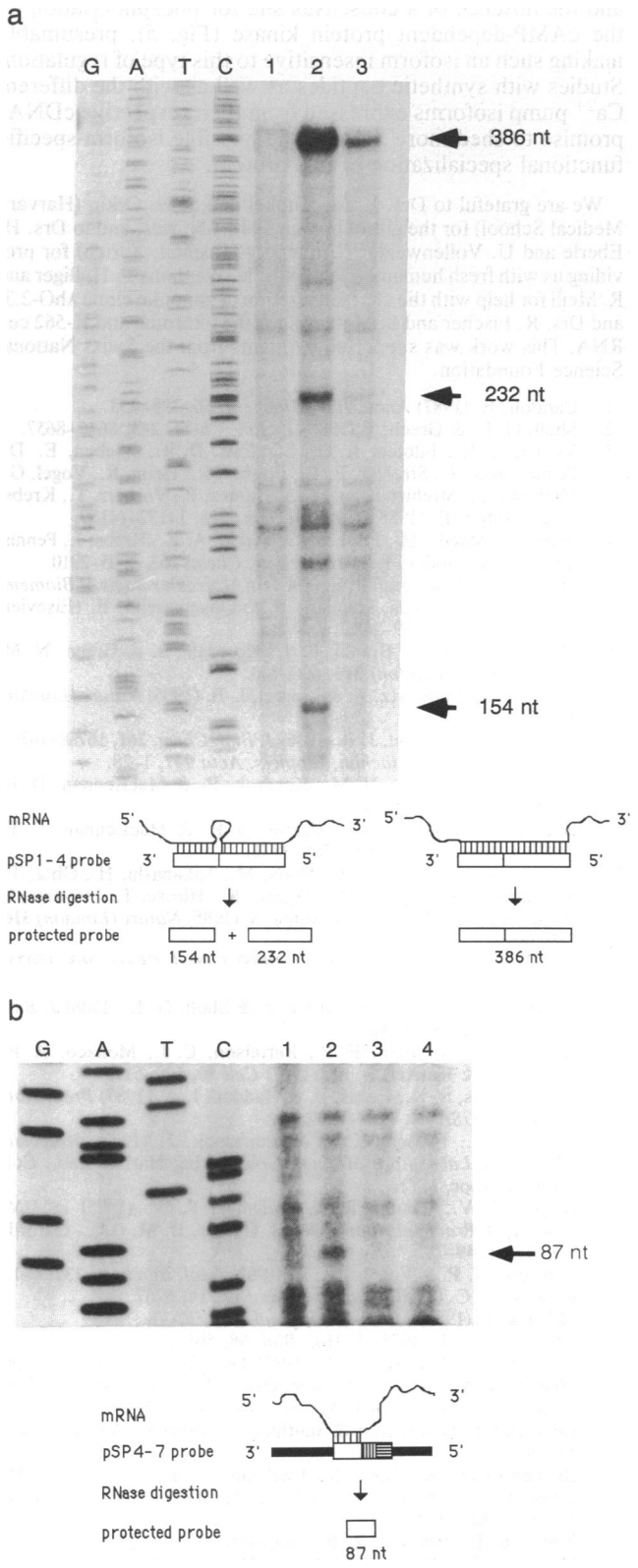

FIG. 4. RNase mapping to detect alternatively spliced Ca^{2+} pump mRNAs. (*a*) (*Top*) RNase protection with 20 μg of tRNA (lane 1, control), 20 μg of adult skeletal muscle RNA (lane 2), and 40 μg of teratoma cell RNA (lane 3). pSP1-4 antisense RNA was used as probe (see text and scheme at bottom). The sequencing ladder (G, A, T, and C) of a known DNA fragment served as size marker. Samples were run on a 6% denaturing polyacrylamide gel. After the gel had been fixed and dried, it was exposed at −80°C to Kodak XAR-5 x-ray film for 2 days with double intensifying screens. Bands appearing in all three lanes are due to background; the positions of bands at 386 nt, 232 nt, and 154 nt are indicated. (*Bottom*) Scheme of RNase protection leading to major fragments of 386, 232, and 154 nt. The position where insertions can occur is indicated by a vertical line in the probe. (*b*) (*Top*) RNase protection with 20 μg of tRNA (lane 1, control), 20 μg of adult skeletal muscle RNA (lane 2), 20 μg of K-562 cell RNA (lane 3), and 40 μg of teratoma cell RNA (lane 4). Labeled pSP4-7 antisense RNA was used as probe (see text and scheme at bottom). The position of the 87-nt band seen only with skeletal muscle RNA is indicated. Experimental conditions were as in *a*. (*Bottom*) Scheme of RNase protection resulting in an 87-nt protected fragment. The different subportions of the alternative exon in the probe are indicated as in Fig. 3*c*; intron sequences are represented by black bars.

that contained the 154-bp "alternative" exon together with adjoining intron sequences (nt 320–925 in Fig. 3*b*). As shown in Fig. 4*b*, an ≈87-nt protected fragment is specifically seen with skeletal muscle RNA, but not with any of the other RNAs tested. Although long exposure times and low-stringency conditions were used (leading to high background, see Fig. 4*b*, lanes 1–4), no specific bands of 114 or 154 nt could be detected. Thus, it appears that pathway b of Fig. 3*c* is the most frequently used alternative splicing event in adult human skeletal muscle (besides pathway a, which cannot be detected with the pSP4-7 probe, but see Fig. 4*a*, 386-nt band in lane 2). The above findings fit well with the distribution of human fetal skeletal muscle cDNA clones as shown in Fig. 1*a*; the cDNAs with the 87-bp insertion are most abundant in the fetal skeletal muscle library.

DISCUSSION

Analysis of cDNA and genomic sequences has shown that a single human plasma membrane Ca^{2+} pump gene can give rise to four different mRNA species by the alternative use of coding information contained within a single 154-bp exon. Although the existence of Ca^{2+} pumps containing the peptide insertions encoded by the alternatively spliced exon has not yet been demonstrated at the protein level, evidence at the mRNA level suggests that they do, indeed, exist. RNase mapping clearly shows the presence of mRNA containing the 87-bp insertion in adult human skeletal muscle, whereas mRNA with the 114-bp insertion could not be detected in the tissues tested. It should be noted, however, that adult human skeletal muscle RNA was used for RNase mapping, whereas the cDNA library represents mRNA from fetal tissue. The occurrence of developmentally and/or tissue-specifically regulated alternative splicing has been well documented in a number of cases (29). It is noteworthy that all clones isolated so far from the human fetal skeletal muscle library contain either the 87-bp or the 114-bp insertion sequence and that no cDNA clone lacking the "alternative" exon was isolated from this library. This is in contrast to the results obtained from RNase mapping experiments that indicate the presence, in adult skeletal muscle, of a significant amount of Ca^{2+} pump mRNA lacking the entire 154-bp exon (as evidenced by the presence of a fully protected fragment in Fig. 4*a*, lane 2). This finding may be interpreted to indicate the possible developmental stage-dependence of the differential splicing pathway used to generate Ca^{2+} pump mRNAs. The only other plausible explanation would be that the "nick" in the RNA–RNA hybrid generated from hybridization of the pSP1-4 probe to a Ca^{2+} pump mRNA with an insertion "loop" sequence (see scheme in Fig. 4*a*) was not efficiently cleaved during RNase treatment. This situation would lead to an overestimate of pump mRNAs that lack the alternatively spliced exon. The fact that no cDNA clone with the 87-bp or the 114-bp insertion could so far be isolated from human cDNA libraries made with RNA from nonmuscle tissues (12 clones have been analyzed: 6 from a teratoma library and 3 each from a colon carcinoma and a small intestinal mucosa library) further indicates the

(skeletal muscle) tissue-specificity of the use of the two internal splice donor sites within the 154-bp exon for the generation of Ca^{2+} pump mRNAs. In contrast to cases where a cryptic internal donor splice site is being used due to mutation(s) within a normal "consensus" donor splice site (30, 31), the alternative splicing pathways described here involve the specific recognition and use of two different internal donor splice sites in the presence of a canonical site in their vicinity.

The potential isoform diversity reported here concerns a particularly interesting region of the molecule. When included, the amino acids encoded by the 154-bp alternative exon lead to a different calmodulin-binding subdomain B structure when compared to that of the published Ca^{2+} pump isoform (3). The more acidic character of subdomain B in isoforms containing sequences encoded by the alternative exon is likely to be of consequence for the interaction of these isoforms with calmodulin, because a strongly basic character is assumed to be characteristic for typical calmodulin-binding target sequences (4, 26, 27, 32). Moreover, the insertions in the skeletal muscle Ca^{2+} pump isoforms move the site of cAMP-dependent phosphorylation, which has been shown to be located C-terminally to the calmodulin-binding domain (33), further away from the latter (Fig. 5). Because the function of the region phosphorylated by the cAMP-dependent kinase appears to be crucially dependent on its direct interaction with the calmodulin-binding domain itself (33), an increase in the spacing between the two domains is likely to alter the regulatory behavior of such a Ca^{2+} pump isoform. The most dramatic change in C-terminal sequence occurs in the Ca^{2+} pump isoform that is generated from a mRNA species that includes the complete 154-bp "alternative" exon because this insertion leads to a change in reading frame. Although the sequence of the calmodulin-binding region itself is the same in all isoforms containing alternative exon sequences (Fig. 5), the isoform generated from a mRNA with the full 154-bp exon is the only one to display a completely different C-terminal protein sequence. The most salient features of this sequence when compared with those of the other isoforms are its shorter length (Fig. 5, and ref. 2)

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        AA 1101          AA 1117
htera     RRGQILWFRGLNRIQTQ  - - - - - - - - - - - - - - - - - - - -
hskm-1.1  RRGQILWFRGLNRIQTQ  MDVVNAFQSGSSIQGALRRQP
hskm-1.2  RRGQILWFRGLNRIQTQ  MDVVNAFQSGSSIQGALRRQP
heqRB11-1 RRGQILWFRGLNRIQTQ  MDVVNAFQSGSSIQGALRRQP

htera     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - I
hskm-1.1  SIASQHHD- - - - - - - - - - - - - - - - - - - - - - - - - I
hskm-1.2  SIASQHHDVTNISTPTH- - - - - - - - - - - - - - - - - - - - I
heqRB11-1 SIASQHHDVTNISTPTHVVFSSSTASTTVGY

htera     RVVNAFRSSLYEGLEKPESRSSIHNFMTHPE
hskm-1.1  RVVNAFRSSLYEGLEKPESRSSIHNFMTHPE
hskm-1.2  RVVNAFRSSLYEGLEKPESRSSIHNFMTHPE
heqRB11-1 SSGECIS AA 1176

htera     FRIEDSEPHIPLIDDTDAEDDAPTKRNSSPP
hskm-1.1  FRIEDSEPHIPLIDDTDAEDDAPTKRNSSPP
hskm-1.2  FRIEDSEPHIPLIDDTDAEDDAPTKRNSSPP

htera     PSPNKNNNAVDSGIHLTIEMNKSATSSSPGS
hskm-1.1  PSPNKNNNAVDSGIHLTIEMNKSATSSSPGS
hskm-1.2  PSPNKNNNAVDSGIHLTIEMNKSATSSSPGS

htera     PLHSLETSL AA 1220
hskm-1.1  PLHSLETSL AA 1249
hskm-1.2  PLHSLETSL AA 1258
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FIG. 5. C-terminal amino acid sequences (in one-letter code) of plasma membrane Ca^{2+} pump isoforms encoded by alternatively spliced transcripts of a single gene. The sequences start with residue 1101 (numbering according to ref. 3); they have been aligned to indicate the regions that are spliced out (dashed lines). The sequences encoded by 87, 114, and 154 bp of the alternatively spliced exon are boxed. The known substrate sequence for the cAMP-dependent protein kinase (33) is underlined. heqRB11-1, Human equivalent of the rat brain isoform RB11-1 (2).

and the absence of a consensus site for phosphorylation by the cAMP-dependent protein kinase (Fig. 5), presumably making such an isoform insensitive to this type of regulation. Studies with synthetic peptides as well as with the different Ca^{2+} pump isoforms expressed from their respective cDNAs promise to shed more light on the possible isoform-specific functional specialization of this protein.

We are grateful to Drs. L. M. Kunkel and S. H. Orkin (Harvard Medical School) for the gift of human DNA libraries, and to Drs. H. Eberle and U. Vollenweider (University Hospital, Zurich) for providing us with fresh human skeletal muscle. We thank P. Holliger and R. Meili for help with the characterization of genomic clone λhO-2.3, and Drs. R. Fischer and S. Mathews for the teratoma and K-562 cell RNA. This work was supported by grants from the Swiss National Science Foundation.

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