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Short sequence-paper

Primary structure of human plasma membrane Ca^{2+}-ATPase isoform 3 [1]

Billie Jo Brown [a], Helene Hilfiker [b,2], Steven J. DeMarco [c], David A. Zacharias [c], Tammy M. Greenwood [a], Danilo Guerini [b], Emanuel E. Strehler [a,c,*]

[a] *Department of Biochemistry and Molecular Biology, Mayo Graduate School, Mayo Clinic / Foundation, 200 First Street S.W., Rochester, MN 55905, USA*

[b] *Laboratory of Biochemistry, Swiss Federal Institute of Technology (ETH), CH-8092 Zurich, Switzerland*

[c] *Program in Molecular Neuroscience, Mayo Graduate School, Mayo Clinic / Foundation, Rochester, MN 55905, USA*

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Abstract

The complete coding sequence of the human plasma membrane calcium ATPase (PMCA) isoform 3 was determined from overlapping genomic and cDNA clones. The cDNAs for the two major alternative splice variants 3a (3CII) and 3b (3CI) code for proteins of 1173 and 1220 amino-acid residues, respectively, which show 98% identity with the corresponding rat isoforms. On a multiple human tissue Northern blot, a major PMCA3 transcript of about 7 kb was detected exclusively in the brain, demonstrating the highly restricted pattern of expression of this isoform to human neuronal tissues. With the elucidation of the human PMCA3 primary structure, complete sequence information is now available for the entire family of human PMCA isoforms.

Keywords: ATPase, Ca^{2+}-; Calcium pump; Isoform; Plasma membrane; (Human)

Plasma membrane calcium pumps are responsible for ATP-driven, active Ca^{2+} extrusion from eukaryotic cells. They belong to a growing family of cation pumps referred to as P-type ATPases based on their formation of a characteristic aspartyl-phosphate intermediate during the reaction cycle [1]. The mammalian plasma membrane calcium ATPases (PMCAs) are encoded by a multigene family comprising four members in rats and humans. Additional isoform diversity is generated from each primary gene transcript by alternative mRNA splicing at two major 'hotspots' called sites A and C [2,3]. Full-length coding sequences have previously been determined for the rat PMCA isoforms 1, 2, 3, and 4 [4–6] and for the human PMCAs 1, 2, and 4[7–9]. By contrast, only two small partial sequences have so far been reported for the human PMCA3, covering the regions of alternative splicing at sites A and C [10]. Studies at both the mRNA and the protein level have shown that PMCA3 expression is highly tissue-specific, essentially being restricted to brain and skeletal muscle [5,10–14]. The absence of PMCA3 mRNA in most tissues and its low abundance even in the brain have hampered previous attempts to obtain the full-length human sequence for this isoform. Here, we report the complete coding sequence for the human PMCA3 as determined by cDNA and genomic cloning as well as by PCR amplification of reverse transcribed mRNA.

Isolation of overlapping clones covering the entire coding region of the human PMCA3. A rat PMCA3 cDNA

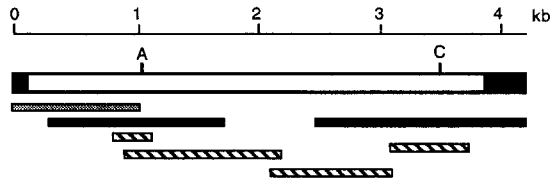

Fig. 1. Scheme of the human PMCA3 cDNA and subclones used for sequencing. A composite of the final PMCA3 cDNA is shown on top; the length (in kb) is indicated. The coding sequence is represented by an open bar, 5′ and 3′ untranslated sequences are shown as black boxes. The positions of alternative splice sites A and C are also indicated. Sequences derived from genomic, cDNA, and RT-PCR clones are indicated by grey, black, and stippled bars, respectively.

* Corresponding author. Fax: +1 (507) 2842384; e-mail: strehler@rcf.mayo.edu.

[1] The sequence data reported in this paper have been submitted to the GenBank Data Library under the accession number U57971.

[2] Present address: Institute of Physiology, University of Zurich-Irchel, CH-8057 Zurich, Switzerland.

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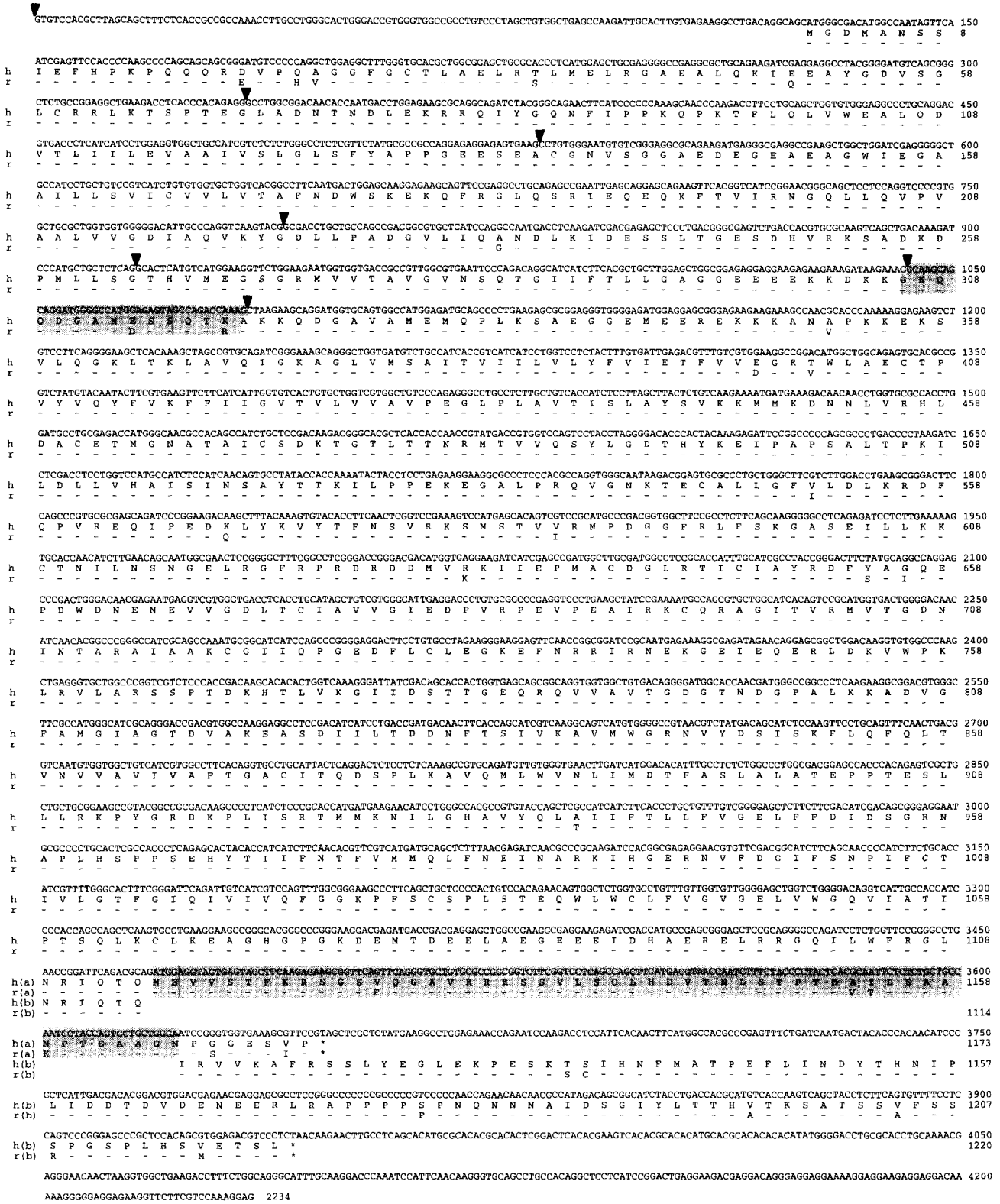

Fig. 2. Nucleotide sequence of the human PMCA3 cDNA and comparison of the deduced amino-acid sequence with that of the rat PMCA3 [5,11]. Nucleotide and deduced amino-acid sequences are numbered on the right, amino acids are shown in single-letter code. The human PMCA3 sequence (h) has been aligned with that of the rat (r). Sequence identity in the rat is indicated by a hyphen, translational stop codons are denoted by asterisks. Alternatively spliced sequences at sites A and C are shaded, the C-terminal amino-acid sequences of the (a) and (b) splice variants are shown separately. The positions of known intron interruptions in the 5'-end region of the human *PMCA3* gene are indicated by black triangles above the nucleotide sequence.

fragment (positions 130 to 680 in [5]), generated by reverse transcriptase PCR (RT-PCR) on rat brain RNA was used initially as probe to screen a human Alzheimer's disease brain cDNA library (Clontech HL1028b). This resulted in the isolation of a 0.7 kb clone (hAlz4.1) specifying a region close to the N-terminus of the human *PMCA3* (nucleotides 241 to 955 in Fig. 2). The insert of this clone was then used to screen a human hippocampus cDNA library (Clontech HL1088a) as well as a human leukocyte genomic DNA library (Clontech HL1006d). In addition, the hippocampus cDNA library was also screened with a RT-PCR-derived fragment covering the C-terminally located splice-site C region [10]. The genomic screening yielded a clone (λH115.2) containing at least 6 exons of the human *PMCA3* gene, including the first coding exon which specifies 126 nucleotides of the 5′ untranslated region and the N-terminal 69 amino acid codons (Fig. 1). The cDNA screenings resulted in the isolation of clones hBr27.1 and hBr1.1. carrying inserts of about 1.5 and 1.6 kb, respectively. The insert of hBr27.1 extended the PMCA3 sequence to position 1857 and that of hBr1.1 provided the 3′-end sequence from position 2549 to 4170. The remaining sequence gap was filled by RT-PCR according to published procedures [15] on RNA from human IMR-32 neuroblastoma cells and using primers derived from the previously determined human or rat [5] PMCA3 sequences. A schematic map of the human PMCA3 cDNA and its coverage by the different overlapping clones is shown in Fig. 1. The complete cDNA sequence of hPMCA3 is displayed in Fig. 2 and its predicted amino-acid sequence is compared with that of the rat PMCA3 isoform.

Comparison of the human and rat PMCA3 isoforms. The comparison in Fig. 2 of both the 3a and the 3b splice variants of PMCA3 (also termed 3CII and 3CI, respectively, in a recently proposed new nomenclature [3]) illustrates the high degree of sequence similarity between the human and rat isoforms (about 98% identity and 99% similarity). These numbers are comparable to the identities between the corresponding PMCA1 and PMCA2 isoforms [6]. By contrast, the rat and human PMCA4 are less well conserved, showing only 87% identity [6]. With the completion of the human PMCA3 cDNA sequence, the entire set of all four rat and human PMCA isoforms has now been characterized at the amino-acid sequence level. At the nucleotide level, pairwise comparisons between the human and rat [5] PMCA3 sequences reveal 89% identity in the coding region, a remarkably high 77% identity in the available 126 nucleotides of 5′ untranslated sequence, and 66% identity in the first 250 nucleotides of the 3′ untranslated region. The gene structure of the rat *PMCA3* has previously been elucidated [11]. The 6 exons of the human *PMCA3* gene identified in clone λH115.2 correspond precisely to exons 3 to 8 of the rat gene: intron interruptions occur at identical positions in the two genes (Fig. 2 and [11]).

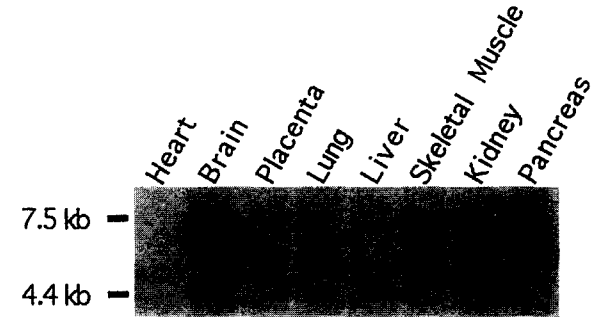

Fig. 3. Analysis of hPMCA3 mRNA expression in human tissues. A poly(A)$^+$ RNA multiple human tissue Northern blot (Clontech) was hybridized with a ^{32}P-labeled hPMCA3-specific probe derived from the 5′ end of the cDNA. The washed blot was exposed for 72 h to a PhosphorImager (Molecular Dynamics) screen. The positions of 7.5 kb and 4.4 kb molecular size standards are indicated on the left, and the tissue source of the RNA is indicated on top of each lane.

Expression of human PMCA3 mRNA. To further confirm the identity of the cloned PMCA cDNA and to obtain information on the size of its nature mRNA, a multiple human tissue Northern blot (Clontech) was hybridized with a radiolabeled probe [16] corresponding to positions 1 to 171 of the hPMCA3 sequence (Fig. 2). The probe fragment was generated by PCR on clone λH115.2 and corresponded to the available 5′ untranslated region and the 15 amino terminal codons. A single, major band corresponding to a mRNA of about 7 kb was detected by this probe on the Northern blot (Fig. 3). Of all tissues tested, only brain expressed significant amounts of PMCA3; no signal was detected in heart and skeletal muscle, placenta, lung, liver, kidney and pancreas (Fig. 3). These data are in agreement with previous findings using RT-PCR [10,15] and Western blotting techniques [13] demonstrating that in humans, PMCA3 expression is essentially restricted to neuronal tissues in the brain as well as to fetal skeletal muscles. By contrast, the rat PMCA3 is also abundantly expressed in adult skeletal muscle, and an alternatively spliced 4.5 kb mRNA is readily detected in addition to the 7.5 kb mRNA in this tissue [5,11]. Thus, despite their very high degree of similarity at the coding and, presumably, the genomic level the expression patterns of the rat and human PMCA3 isoforms do not entirely coincide. It will be of interest to unravel the mechanisms of the highly neuron-specific expression of the human PMCA3 and the significance of this isoform for Ca^{2+} handling in the brain.

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