Expression of two isoforms of the third sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA3) in platelets. Possible recognition of the SERCA3b isoform by the PL/IM430 monoclonal antibody

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Received 10 January 1998

Abstract Human platelets express several sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA) isoenzymes: SERCA2b of 100 kDa apparent molecular mass and two distinct enzymes of 97 kDa, one of them identified as being the SERCA3a isoform. The molecular identity of the third enzyme specifically recognized by the PL/IM430 monoclonal antibody has remained elusive. First, the study of the 3′-end part of platelet SERCA3 mRNA, by means of RT-PCR amplification using sets of primers covering the *N*−3 to *N* (ultimate) exons of the human SERCA3 sequence, revealed the presence of two distinct mRNA sequences, SERCA3a and a longer variant. Second, this additional sequence was identified as SERCA3b and found to refer to the insertion of a new exon of 73 bp, located at bp 349 from the beginning of the intronic sequence, linking the penultimate (*N*−1) exon to the last exon (*N*) of the human SERCA3 gene. Third, a relationship between the expression of this SERCA3b mRNA and the PL/IM430 recognizable SERCA protein was observed. SERCA3b mRNA was found to be absent in epithelial HeLa cells not recognized by the PL/IM430 antibody and the expression of this SERCA3b RNA species correlated with that of the SERCA protein recognized by PL/IM430 which was down-modulated in the platelet precursor megakaryocytic CHRF 288-11 cell line as well as upon in vitro lymphocyte activation. Taken together, these results strongly support the notion of the presence of the SERCA3b protein in human cells by showing SERCA3b mRNA in platelets and the fact that the protein corresponding to this mRNA species is very likely the 97 kDa protein recognized by the PL/IM430 antibody.

Key words: PL/IM430 recognizable SERCA; SERCA3b isoform; Human platelet

1. Introduction

Ca^{2+} ions integrate key events of cell function as a dynamic Ca^{2+} signal which appears in a spatio-temporal manner as Ca^{2+} waves or spikes. Numerous and distinct processes are involved in this Ca^{2+} signalling, including the release of Ca^{2+} from intracellular Ca^{2+} stores. It is now increasingly thought that there are at least two types of intracellular Ca^{2+} pools: inositol 1,4,5 trisphosphate (IP3)-sensitive Ca^{2+} pools and IP3-insensitive Ca^{2+} pools [1,2]. This multiplicity of Ca^{2+} pools may suggest a functional specificity in terms of the control of their Ca^{2+} content, which depends upon the activities of sarco/endoplasmic reticulum Ca^{2+}-transport ATPases (SERCAs). In recent years, evidence has emerged of a diversity of SERCA isoforms which may sustain different Ca^{2+} pools. Indeed, SERCAs are encoded by three distinct genes, termed SERCA1 to 3 which, upon alternative splicing, give rise to various mRNAs and protein isoforms. SERCA1 gives the SERCA1a and SERCA1b isoforms, expressed in fast skeletal muscle [3]. The SERCA2 gene gives rise to the SERCA2a isoform expressed in cardiac muscle and to the ubiquitous SERCA2b isoform found in smooth muscle cells and non-muscle cells [4]. Much less is known concerning SERCA3. A SERCA3a cDNA was cloned from rat kidney [5], mRNA was found to be expressed in various non-muscle cells [5–9] and the corresponding SERCA3a protein was identified in platelets and other non-muscle cells [7,10]. Finally, part of the 3′-end of the human SERCA3 gene, as well as alternative splicing of the SERCA3 primary transcript have been described [11] concomitantly with two (SERCA3a and SERCA3b) mouse cDNAs [12,13]. However, the evidence for a SERCA3b protein remains undescribed.

Intriguingly, using controlled trypsinolysis of human platelet membrane vesicles followed by immunostaining of the resulting fragments by the monoclonal antibody PL/IM430, Kovàcs et al. [14] described a SERCA protein previously unknown in the SERCA family, the so-called PL/IM430 recognizable 97 kDa SERCA. In particular, this SERCA protein was clearly shown to be distinct from the SERCA3a protein by the absence of recognition by the PL/IM430 antibody of the transfected human protein [11]. Moreover, these two SERCA3a and PL/IM430 recognizable 97 kDa SERCA isoforms, which were co-expressed in platelets, had distinct intracellular localizations [15] and were differentially expressed in JUR E6-1 and HeLa cell lines [16].

The aim of the present work was to elucidate the molecular

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Abbreviations: SERCA, sarco/endoplasmic reticulum Ca^{2+}ATPase; PRP, platelet-rich plasma; DMSO, dimethylsulfoxyde; MuLV-RT, murine leukemia-virus reverse transcriptase; RT-PCR, reverse transcription-polymerase chain reaction; PMA, phorbol-12-myristate-13-acetate; PL/IM430, monoclonal anti-Ca^{2+}ATPase antibody raised against highly purified platelet intracellular membranes as immunogen

This work was supported by the Institut National de la Santé et de la Recherche Médicale, Réseau Est-Ouest No. 4E004B, the Groupe d'Etudes sur l'Hémostase et la Thrombose, the Association pour la Recherche sur le Cancer and the Ministère de l'Education Nationale, de l'Enseignement Supérieur, de la Recherche et de l'Insertion Professionnelle (ACC No. 9).

PII S0014-5793(98)00106-9

nature of the PL/IM430 recognizable 97 kDa SERCA isoform based on the hypothesis that this protein may be identical to the SERCA3b protein. The results show the presence of two SERCA3 mRNA species (SERCA3a and SERCA3b) in platelets and demonstrate that the SERCA3b species refers to the presence of a new exon of 73 bp located in the intronic sequence preceding the last exon *N* of the SERCA3 gene. Moreover, a relationship is found between the expression of the SERCA3b mRNA and that of the PL/IM430 recognizable 97 kDa SERCA using different models of expression and regulation of the expression of this protein.

2. Materials and methods

2.1. Cell culture and treatments

The JUR E6-1 (T lymphoblastoid) and HeLa (epithelial) cells were from American Type Culture Collection. The CHRF 288-11 (megakaryocytic) cell line was provided by Dr. M. Lieberman, Cincinnatti, OH, USA. Cells were grown in RPMI 1640 medium (GIBO BRL, Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum, 50 units/ml penicillin, 0.05 mg/ml streptomycin and 2 mM L-glutamine (Boehringer, Mannheim, Germany). HeLa cells were grown in Eagle's minimal essential medium (GIBCO BRL, Paisley, Scotland) with non-essential amino acids and Earle's balanced salt solution. All cell lines were grown at 37°C with 5% CO_2 in a humidified atmosphere. Exponentially growing JUR E6-1 cells were treated by PMA plus ionomycin as described [17].

2.2. Membrane preparation

Cell line membranes were prepared as in [18]. Human platelet membranes were prepared by a similar method, as described previously [14]. The protein concentration of the different membrane preparations was determined by the Bradford method [19] using γ-globulins as standard.

2.3. SDS-PAGE

Proteins were submitted to 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [20] and electrotransferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).

2.4. Antibody preparation and characterization

The monoclonal antibody PL/IM430 [21,22] was purified from culture supernatant of the corresponding hybridoma cell line [14]. This antibody specifically recognized one of the 97 kDa SERCA isoforms in platelets and in different human cell lines [23,24].

2.5. Western blotting

Nitrocellulose membranes were treated for Western blotting using 1 μg/ml of the monoclonal antibody PL/IM430 [14] and incubated with a 1:1000 dilution of the anti-mouse IgG horseradish-peroxidase conjugate (Jackson Immunoresearch, West Grove, PA, USA). Antibody binding was detected using ECL (enhanced chemiluminescence) Western blotting reagents (Amersham International, Life Science, Little Chalfont, UK).

2.6. Isolation of total RNA

For platelet RNA, the isolation of platelets was slightly modified by blood dilution in isotonic saline solution prior to centrifugation steps to isolate platelet-rich plasma (PRP). PRP was withdrawn to within 1 ml of the buffy-coat in order to avoid leukocyte contamination. Under these conditions, the contamination of platelets by other blood cells was less than 1 leukocyte in 4000 platelets. Total RNA was isolated from platelets and cell lines [25], using the RNA Plus solution (Bioprobe, Montreuil sous Bois, France).

2.7. Reverse transcription-PCR analysis

500 ng of total RNA was used as template for first strand-specific cDNA synthesis in a 10 μl reaction mixture containing PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 5 mM $MgCl_2$, 2.5 μM of oligo $d(T)_{16}$, 1 mM of each dNTP, 10 U of RNAse inhibitor and 25 U of MuLV reverse transcriptase (Perkin Elmer, Branchburg, NJ, USA).

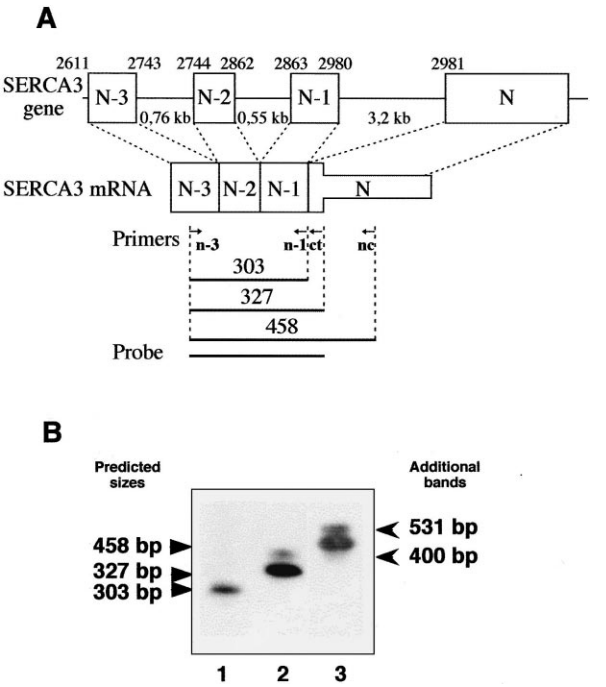

Fig. 1. Evidence for two SERCA3 mRNA species in human platelets. A: Study of the 3′-end part of the SERCA3 mRNA. A schematic representation of the 3′-end of the human SERCA3 gene and SERCA3 mRNA [11] is shown in the upper part of the figure. Boxes represent exons while lines represent introns. The larger and smaller rectangles correspond respectively to the coding and non-coding sequences. This SERCA3 mRNA was studied by RT-PCR (middle part) using different primers located either in the *N*−3 exon n-3, or in the *N*−1 exon n-1, or in the ultimate exon: ct (C-terminal region of the deduced SERCA3 protein) or nc (non-coding region of SERCA3). The predicted sizes of the amplification products and the SERCA3 probe are indicated in the lower part of the figure. B: SERCA3 mRNA amplification in human platelets. 500 ng of total RNA isolated from human platelets was submitted to RT-PCR for 50 cycles using the forward n-3 primers and either the n-1 reverse primers (lane 1), the ct reverse primers (lane 2) or the nc reverse primers (lane 3). Amplification products were analyzed by Southern blotting using the SERCA3 probe indicated above. Numbers indicate the sizes of the amplification products. The figure is representative of four independent experiments.

The reaction mixture was incubated for 30 min at 42°C, then heated for 5 min at 99°C. RT reaction was used as template for PCR in a 50 μl reaction mixture including PCR buffer, 2 mM $MgCl_2$, 0.15 μM of the forward and reverse primers (Eurogentec, Leuven, Belgium) and 1.25 U of *AmpliTaq* DNA polymerase (*Thermus aquaticus*) (Perkin Elmer, Branchburg, NJ, USA). PCR was conducted for 30 s at 94°C, 2 min at 55°C and 2 min at 72°C for 30 or 50 cycles. Amplifications were followed by an extension step for 7 min at 72°C. Amplification products were electrophoresed on 1.5% (w/v) agarose gels stained by ethidium bromide. For Southern blotting [26], the amplification products were blotted onto nylon membranes (Hybond N+ from Amersham International). Prehybridization and hybridization using 5 ng/ml of labeled oligo or DNA probe (3′-oligo or direct nucleic acid labeling kits, Amersham) were performed at 42°C. Membranes were washed and detection was performed using the ECL detection system and Hyperfilms ECL (Amersham).

The SERCA2b-specific cDNA probe was cloned by screening a human platelet cDNA library [27]. The SERCA3-specific probes used were either SERCA3 fragments which had been amplified by PCR and corresponded to nucleotides 2674 to 3000, or 3′-oligolabeled n-1 and ct SERCA3 primers located in positions 2956–2976 and 2981–3000 of the sequence published by Dode et al. [11] respectively.

3. Results

3.1. Evidence for two SERCA3 mRNA species in human platelets

Since alternative splicing may be a general feature in the SERCA family, we looked for differences in the 3′-end of the human SERCA3 mRNA. We used RT-PCR to amplify SERCA3 mRNA and designed different sets of primers allowing the 3′-end region to be covered between the $N-3$ and N exons, according to the published nomenclature [11] of the human SERCA3 gene (Fig. 1A). The forward n-3 primers 5′-GAGTCACGCTTCCCCACCACC-3′ corresponded to nucleotides 2674–2694 and the reverse primers n-1 5′-CATGTGGTTCCGGGACAGGTA-3′, ct 5′-TCACTTCTGGCTCATTTCTT-3′ and nc 5′-GCCTGTCATTTATCCGGCG-3′ corresponded to the inverse sequences of the nucleotide stretches 2956–2976, 2981–3000 and 3113–3131 respectively. The corresponding sizes of the previously known SERCA3a amplification products were 303, 327 and 458 bp. Fig. 1B shows the analysis of the amplification products obtained. Control amplifications in the absence of RT (not shown) did not result in detectable amplification products, in agreement with the absence of DNA contamination in RNA preparations. Two different RNAs were obtained depending upon the sets of primers used (compare lane 1 to lanes 2 and 3). While unique SERCA3 amplification products were obtained with primers covering the region between the $N-3$ and the $N-1$ (penultimate) exons (lane 1), additional products of 400 and 531 bp were detected when using primers covering the region between the $N-3$ and the N (ultimate) exons (lanes 2 and 3), thus presenting an apparent additional sequence of about 70 bp.

This could suggest the presence of two SERCA3 species, SERCA3a and SERCA3b, in platelets and that the latter may include a still unidentified new exon in the intronic sequence linking the penultimate and the ultimate exons.

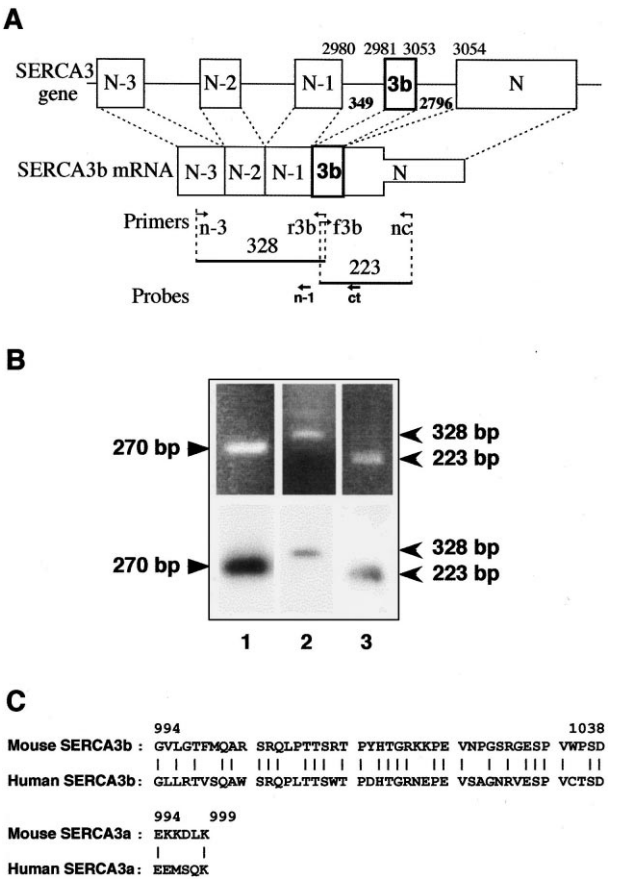

Fig. 2. Evidence for SERCA3b mRNA in human platelets. A: Schematic representation of the human SERCA3 gene and SERCA3b mRNA. The location of the specific SERCA3b exon (see Section 3) and the deduced SERCA3b mRNA are represented in the upper part of the figure. Boxes represent exons while lines represent introns. The larger and smaller rectangles correspond respectively to the coding and non-coding sequences. The forward f3b and reverse r3b SERCA3b-specific primers designed to amplify SERCA3b mRNA, the predicted sizes of the SERCA3b amplification products and the n-1 and ct SERCA3 probes are indicated in the lower part of the figure. B: Specific SERCA3b mRNA amplification. 500 ng of total RNA isolated from human platelets was submitted to RT-PCR for 30 cycles using SERCA2b primers (lane 1), n-3 forward and r3b reverse primers (lane 2), or f3b forward and nc reverse primers (lane 3). Amplification products were analyzed on ethidium bromide stained gels (upper part) and by Southern blotting (lower part) using the SERCA2b probe (lane 1), 3′-oligolabeled n-1 (lane 2) and ct (lane 3) SERCA3 probes. Numbers indicate the sizes of the amplification products. The figure is representative of four independent experiments. C: SERCA3 amino acid similarity comparison. The C-terminal part of the human SERCA3b and SERCA3a amino acid sequences are represented in the lower lines and aligned with the corresponding parts of the mouse SERCA3b and SERCA3a sequences [12,13] represented in the upper lines. Identical amino acids are connected by vertical lines. The numbers on the left indicate the splicing locations and the numbers on the right indicate the ends of the amino acid sequences.

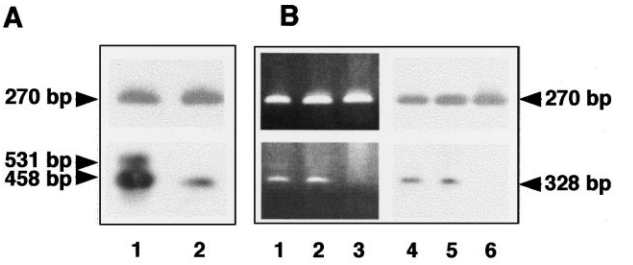

Fig. 3. Cell-dependent expression of the PL/IM430 recognizable 97 kDa SERCA and of the human SERCA3b mRNA. A: Comparative SERCA3 mRNA expression in human platelets and HeLa cells. 500 ng of total RNA was isolated from human platelets (lane 1) and HeLa (lane 2) cells and submitted to RT-PCR for 50 cycles using SERCA2b and SERCA3 n-3 forward and nc reverse primers. The amplification products were analyzed by Southern blotting as in Figs. 1 and 2. Numbers indicate the sizes of SERCA2b (upper part) and SERCA3a and SERCA3b (lower part) amplification products. This figure is representative of three independent experiments. B: Study of SERCA3b mRNA expression in human platelets, JUR E6-1 and HeLa cells. 500 ng of total RNA was isolated from human platelets (lanes 1 and 4), JUR E6-1 (lanes 2 and 5) and HeLa (lanes 3 and 6) cells and was submitted to RT-PCR for 50 cycles using SERCA2b and SERCA3 n-3 forward and r3b reverse primers. Amplification products were analyzed on ethidium bromide stained gels (lanes 1 to 3) and by Southern blottings (lanes 4 to 6), using the SERCA2b (upper parts) and 3′-oligolabeled n-1 SERCA3 probes (lower parts). Numbers indicate the sizes of the amplification products. This figure is representative of four independent experiments.

3.2. Identification of the human SERCA3b mRNA in platelets

To locate this potential exon, we took advantage of the recently published mouse SERCA3a and SERCA3b cDNAs. Both cDNAs were identical between nt 1 and 2980, but diverged downstream of this site. An additional sequence of

73 bp was observed in SERCA3b cDNA, modifying the reading frame and leading to the substitution of the last 6 amino acids of SERCA3a isoform by 45 amino acids in SERCA3b isoform. We searched for a corresponding 73 bp exon in the last intron of human SERCA3 gene. Such a 73 bp exon, presenting canonical exon/intron boundary sites, was found at 349 bp from the beginning of the last human intronic sequence (Fig. 2A), and presented 78% similarity with the mouse SERCA3b exon. Based on the differences of approx. 70 bp observed between the two SERCA3 RNA species in platelets, we thus looked for SERCA3b RNA, by selecting specific human primers to amplify this RNA species. The forward primers n-3 and f3b 5′-CCTTCTCAGGACAGTCTCG-3′ corresponding to nucleotides 2982–3001, the reverse primers r3b 5′-TGCGAGACTGTCCTGAGAAGG-3′ corresponding to the reverse sequence of the nucleotide stretch 2982–3003 and the nc primers were used to amplify both upstream and downstream regions flanking this exon. The corresponding sizes of the SERCA3b amplification products were 328 and 223 bp. Fig. 2B shows the results of these amplifications performed with platelet RNA together with those of SERCA2b (270 bp), used as an internal control (lane 1). As shown either on agarose gels stained by ethidium bromide (upper part of the figure), or by Southern blotting (lower part of the figure), both the expected SERCA3b amplified products were detected (see lanes 2 and 3), thus confirming the presence of this exonic sequence in SERCA3b mRNA. This was further demonstrated by sequencing the 223 bp amplified products which showed 100% identity with the putative human exon (data not shown). Hence, this allowed us to deduce the human SERCA3b amino acid sequence in part C of Fig. 2 which revealed a similarity of 63% with the mouse amino acid sequence.

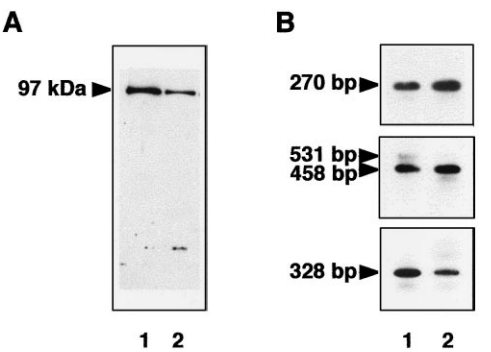

Fig. 4. Comparative expression of the PL/IM430 recognizable 97 kDa SERCA and of the human SERCA3b mRNA in the megakaryocytic lineage. Human platelets (lanes 1) and CHRF 288-11 (lanes 2) cells were either treated for membrane protein preparation or for total RNA isolation. Membrane proteins (50 μg) were electrophoresed and immunoblotted using the PL/IM430 monoclonal antibody (part A). 500 ng of total RNA was submitted to RT-PCR for 30 cycles (part B) using SERCA2b primers (upper part), n-3 forward and either nc (middle part) or r3b reverse primers (lower part) and the amplification products were analyzed by Southern blotting using the SERCA2b (upper part) and the 3′-oligolabeled n-1 SERCA3 probe (middle and lower parts). Numbers indicate the molecular mass of the PL/IM430 recognizable 97 kDa SERCA (part A) or the size of the amplification products (part B). This figure is representative of three independent experiments.

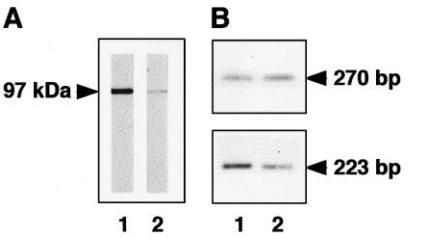

Fig. 5. Comparative regulation of the expression of the PL/IM430 recognizable 97 kDa SERCA and of the human SERCA3b mRNA. JUR E6-1 cells were treated for 4 days with either DMSO as vehicle (lanes 1) or 10 nM PMA and 0.5 mM ionomycin (lanes 2). They were then either treated for whole cell lysate or for total RNA isolations. 25 μg of cell lysate proteins were electrophoresed and immunoblotted using the PL/IM430 antibody (part A). 500 ng of total RNA was submitted to RT-PCR (part B) for 30 cycles using SERCA2b primers (upper part), f3b forward and nc reverse primers (lower part). The amplification products were analyzed by Southern blotting using the SERCA2b (upper part) and the 3′-oligolabeled ct SERCA3 probe (lower part). Numbers indicate the molecular mass of the PL/IM430 recognizable 97 kDa SERCA (part A) or the size of SERCA3b amplification products (part B). This figure is representative of four independent experiments.

3.3. Relationship between the SERCA3b mRNA and the PL/IM430 recognizable 97 kDa SERCA protein

3.3.1. Correlation between the PL/IM430 recognizable 97 kDa SERCA and SERCA3b mRNA expression in different cell lines. We first took advantage of our previous work which showed a differential expression of the SERCA3a and PL/IM430 recognizable 97 kDa SERCA proteins in different cell lines, and in particular, we focussed on the absence of the latter protein in HeLa cells [16]. RNA was isolated from these cells and tested for expression of both SERCA3 mRNA species (lower parts of Fig. 3) as in Fig. 1 as well as that of SERCA2b mRNA as internal controls (upper parts of Fig. 3). Fig. 3A shows the results of this SERCA3 study in HeLa cells (lane 2) compared with platelets (lane 1), which revealed the presence of SERCA3a RNA in these epithelial-like cells, as demonstrated by the presence of the related 458 bp amplified products (compare lanes 1 and 2), but the absence of the additional SERCA3b RNA species (lane 2). This result was further confirmed (Fig. 3B) by studying the specific expression of SERCA3b in platelets (lanes 1 and 4) and HeLa cells (lanes 3 and 6), as well as in another hemopoietic cell line, the lymphoblastoid JUR E6-1 (lanes 2 and 5) previously shown to express the PL/IM430 recognizable SERCA [24]. SERCA3b was detected in the two hemopoietic cells (JUR E6-1 and platelets) when analyzed either on agarose gels stained by ethidium bromide (lanes 1 to 3) or by Southern blotting (lanes 4 to 6), but no expression of SERCA3b was found in HeLa cells.

3.3.2. Correlation between the expression of the PL/IM430 recognizable 97 kDa SERCA and SERCA3b mRNA in the megakaryocytic lineage. Second, we compared the expressions of the PL/IM430 recognizable 97 kDa SERCA and SERCA3b mRNA in the megakaryocytic lineage by studying the cell line CHRF 288-11 described as representative of platelet precursor [28] (Fig. 4). CHRF 288-11 cells were cultured and either treated for membrane protein preparation or for total RNA isolation. Proteins were electrophoresed and immunoblotted using the PL/IM430 antibody (part A). RNA was submitted to RT-PCR as above (part B) using SERCA2b

primers for internal controls (upper part) and SERCA3 primers either to coamplify SERCA3a and SERCA3b (middle part) or to amplify SERCA3b (lower part). The comparative analysis of platelets (lanes 1) and CHRF 288-11 cells (lanes 2) showed a parallel decrease in the expression of PL/IM430 recognizable 97 kDa SERCA (A) and of SERCA3b RNAs (B) as revealed by both the related 531 bp products (middle part) and the specific 328 bp products (lower part).

3.3.3. Correlation between the regulation of the expression of the PL/IM430 recognizable 97 kDa SERCA and SERCA3b mRNA during in vitro lymphocyte activation. Finally, we studied the regulation of the expression of SERCA3b mRNA in JUR E6-1 cells previously shown to exhibit a down-regulation of the PL/IM430 recognizable 97 kDa SERCA upon activation induced by ionomycin plus PMA [17]. Thus, we looked for comparative expression of SERCA3b mRNA in this model (Fig. 5). JUR E6-1 cells were treated as in [17]. Lysates were electrophoresed and immunoblotted using the PL/IM430 antibody (part A) and RNAs were submitted to RT-PCR (part B) using SERCA2b primers for internal controls (upper part) or specific SERCA3b f3b and nc primers (lower part). Comparison of untreated (lanes 1) and treated JUR E6-1 cells (lanes 2) showed a similar down-regulation of the expression of the PL/IM430 recognizable 97 kDa SERCA (part A) and of SERCA3b mRNA (lower part B).

4. Discussion

This study suggests evidence for the SERCA3b protein in human cells by showing SERCA3b mRNA in platelets and that this mRNA species very likely encodes the still unidentified PL/IM430 recognizable 97 kDa SERCA protein.

First, RT-PCR of total platelet RNA demonstrated the presence of the previously known SERCA3a species and of a SERCA3 species about 70 bp larger. This latter mRNA species was further identified as SERCA3b and found to arise from the insertion of a cryptic new exon of 73 bp beginning at position 2981 of the human SERCA3a cDNA. The insertion of this exon modifies the reading frame and results in the suppression of the stop codon used in SERCA3a. This gives rise to SERCA3b which uses an alternative stop codon located at bp 3044 in the non-coding part of the ultimate exon [11]. This resulted in the deduced human SERCA3b protein which differs from SERCA3a in its C-terminal extremity, by the replacement of the last C-terminal amino acids in SERCA3a by 45 amino acids in SERCA3b.

Hence, like SERCA1 and SERCA2 gene products, the SERCA3 gene gives rise to two SERCA3a and 3b isoforms, which result from an alternative splicing process [29–35,11]. Moreover, in a further similarity with SERCA2 proteins, which present two conformational states, thus to the variable carboxy-terminal extremities [36], the structural differences in the two SERCA3 isoforms imply modifications in the three-dimensional membrane organization of the proteins, as revealed by their distinct accessibility to trypsin [14].

Second, by using different experimental models previously tested for their expression of the PL/IM430 recognizable 97 kDa SERCA protein, a relationship was found between the expression of this protein and that of SERCA3b mRNA: (i) in the HeLa cell line no SERCA3b could be detected either by RT-PCR or by using the PL/IM430 antibody; (ii) in the megakaryocytic CHRF 288-11 cell line and platelets, the relative expression of SERCA3b measured by RT-PCR was correlated with SERCA protein levels measured using the PL/IM430 antibody; (iii) in JUR E6-1 cells treated for a down-regulation of the PL/IM430 recognizable 97 kDa SERCA [17], a similar decrease in the SERCA3b mRNA expression was observed. Hence, the most likely interpretation of these findings is that the SERCA3b protein corresponds to the protein recognized by the PL/IM430 monoclonal antibody.

This SERCA3 nature of the PL/IM430 recognizable 97 kDa SERCA protein is consistent with recent data suggesting a similarity of the PL/IM430 recognizable 97 kDa SERCA with the SERCA3a protein. This is based on the recognition of the protein purified by immunoaffinity using the PL/IM430 monoclonal antibody by the anti-SERCA3 N89 antibody, raised against the N-terminal peptide common to both SERCA3 proteins (Kovàcs et al., manuscript in preparation and [37,38]). Moreover, the SERCA3b identity of the PL/IM430 recognizable 97 kDa SERCA3 protein also explains its discrimination from the SERCA3a protein. This is based on the absence of recognition by the PL/IM430 antibody of: (i) the two SERCA3a tryptic fragments of 80 and 25 kDa [14]; (ii) the transfected human SERCA3a protein [11]; and (iii) the N89 recognized SERCA3a protein expressed in epithelial HeLa [16] or endothelial cells [39]. Consequently, one can suggest that the PL/IM430 antibody specifically recognizes the C-terminal extremity of the human SERCA3b protein. So, while the N89 antibody can be used to study both SERCA3 proteins in different species, the PL/IM430 antibody should be useful for specific study of the human SERCA3b isoform.

To conclude, this identification of the third platelet SERCA isoform as the SERCA3b isoform establishes the concept of the presence of a multi-SERCA system [40] in cells such as platelets. This observation sheds new light on the complexity of intracellular Ca^{2+} sequestration of non-muscle cells and opens new avenues in the investigation of the SERCA-associated Ca^{2+} pools organisation and of their functional role in cell Ca^{2+} homeostasis and signalling.

Acknowledgements: We are grateful to Prof. N. Crawford, London, UK for generously supplying the PL/IM430 hybridoma cells, to Dr. M. Lieberman, Cincinnati, USA for the CHRF 288-11 cell line and to C. Mille for technical assistance.

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