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THE ISOFORM- AND LOCATION-DEPENDENCE OF THE FUNCTIONING OF THE PLASMA MEMBRANE CALCIUM PUMP

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Abstract: The plasma membrane is a specialised multi-component structure with inter- and intracellular signalling functions. Ca^{2+} plays a crucial role in cellular physiology, and an ATP-driven plasma membrane calcium pump (PMCA) plays the greatest role in the maintenance of a low free Ca^{2+} concentration in the cytoplasm. The enzyme is coded by four separate genes (PMCA 1-4), and, due to alternative splicing, more than 20 variants can exist. PMCA 1 and 4 isoforms are present in almost all tissues, whereas PMCA 2 and 3 are found in more specialised cell types. The variants differ primarily in their regulatory regions, thus the modulation of calcium pump activity strongly depends on the isoform and the membrane composition. The unique function of PMCA isoforms was confirmed using the practical experimental models – a rat pheochromocytoma cell line, a human neuroblastoma cell line, or, more recently, knockout mice. In addition, based on the finding that PMCA could interact with several specific signaling proteins, it was concluded that its location in defined sites of the cell membrane could be a prerequisite for efficient intercellular communication.

Key Words: Ca^{2+}-ATPase, Isoforms, PC12 Cells, Ca^{2+} Uptake, Antisense Oligonucleotides, Regulation, Plasma Membrane

INTRODUCTION

The regulation of Ca^{2+} homeostasis plays a key role in cellular function and cell-to-cell communication, particularly in nerve tissue. The fundamental parts of the regulatory system are the ATP-driven calcium pumps located in the membranes of the cell. Membrane structure and function depend on protein and lipid

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compounds which are responsible for membrane fluidity, the activity of functional proteins, and finally, the modulation of the signal transduction cascades in the cell. The three types of calcium pump significant in the decreasing of cytosolic Ca^{2+} concentration are the plasma membrane Ca^{2+}-ATPase (PMCA), the endo(sarco)plasmic Ca^{2+}-ATPase (SERCA) and the secretory pathway Ca^{2+}-ATPase (SPCA, PMR) (Tab. 1) [1-3].

Tab. 1. The characteristics of cellular calcium pumps.

Name	Gene	Location	Ion transport
SERCA	ATP2A 1 ATP2A 2 ATP2A 3	sarco/endoplasmic reticulum	$2Ca^{2+}$/ $2H^{+}$
PMCA	ATP2B 1 ATP2B 2 ATP2B 3 ATP2B 4	plasma membrane	Ca^{2+}/ H^{+}
SPCA	ATP2C 1 ATP2C 2	Golgi apparatus	Ca^{2+}, Mn^{2+}/ ?

The plasma membrane Ca^{2+}-ATPase (PMCA) constitutes a high affinity system extruding Ca^{2+} extracellularly, and, in a resting state, maintaining the cytosolic Ca^{2+} concentration at nanomolar levels. The enzyme is coded by four separate genes (PMCA 1-4), and, due to alternative splicing, more than 20 variants can exist [4]. PMCA 1 and PMCA 4 are expressed in almost all tissues, whereas PMCA 2 and PMCA 3 show a cell-type specific pattern of expression. In the erythrocytes of most mammalian species, and in the hair cells of the auditory and vestibular system, PMCA is solely responsible for Ca^{2+} extrusion, whereas in other cells a low affinity Na^{+}/Ca^{2+} exchanger also carries out the removal of Ca^{2+} [5]. Differences in the structure and location of PMCA isoforms are thought to correlate with specific regulatory properties, and may be significant in proper Ca^{2+} signalling during the cell's life.)

THE STRUCTURE OF PMCA

The enzyme contains 10 putative transmembrane regions and two major cytosolic loops – the transduction and catalytic domains (Fig. 1). The first loop hosts the phospholipid-binding (PL) and autoinhibitory regions, and could differ between PMCA variants because of alternative mRNA splicing [4]. Besides, the differentiated sensitivity of the enzyme towards modulation by acidic phospholipids may depend on the phosholipid composition in the membrane.

The site of aspartyl-phosphate formed during the Ca^{2+} transport cycle (PI) and the ATP-regulatory site (ATP) are located in the second loop. Both the N- and C-terminal parts of the PMCA polypeptide chain are also located intracellularly.

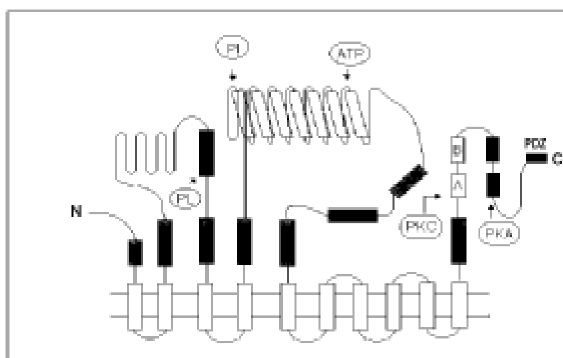

Fig.1. Schematic structure of the plasma membrane calcium pump.

The sites for phosphorylation by protein kinases A and C (PKA, PKC), sequences recognised by proteases (calpains and caspases), and the calmodulin-binding domain (A, B) have been identified at the C-terminus [6, 7]. The binding of calmodulin (or PKC-mediated phosphorylation) reduces the binding of the autoinhibitory domain and increases the Ca^{2+} transporting activity of the pump. The phosphorylation site for PKA is located close but distal to the calmodulin binding domain, but it was suggested that only the PMCA 1 isoform could be a substrate for PKA. The structure of the C-terminus could be significantly affected due to alternative splicing and, as a result, the altered sensitivity of PMCA variants for multiface regulation has been observed. The differences in PMCA isoform composition are believed to be integrated with specific cellular physiology. A cell that responds rapidly to a Ca^{2+} spike requires a “fast” type of PMCA variant, i.e. an isoform that has a high basal activity [8]. By contrast, a cell that requires a sustained Ca^{2+} signal may utilise isoforms with a low basal

Tab. 2. The cell and tissue-dependence of PMCA variants (modified from [8]).

Cell or tissues	Fast variants	Slow variants
heart	2a,	
skeletal muscle	2a, 3f,	
brain	2a, 2b, 4a	
inner ear	2a	
erythrocytes		1b, 4b
Jurkat cells		4b

activity [4, 9]. Both types of response could be differentially modulated by calmodulin. PMCA isoforms also have a potentially wide range of responses to

multiple spikes. Some examples of functionally different variants are shown in Tab. 2 (modified from [8]).
In addition to cellular and tissue-dependent distribution, the specialised location of PMCA in the membranes is also linked with unique protein-protein interaction. PDZ-binding sequences in "b" variants of PMCAs have recently been recognised, suggesting the potential interaction of the enzyme with specific target proteins which possess a characteristic PDZ motif. Direct binding of the PMCA2b and PMCA4b isoforms to membrane-associated guanylate kinase-like proteins (MAGUKs) has been reported [10, 11]. PMCA 4b has been shown to interact with the same clustering proteins (PSD-95, Chapsyn-110) as NMDA receptors. Therefore, the selective and specific interactions of PMCA2b and Na^+/H^+ exchanger regulatory factor 2 (NHERF2) have been added to a growing list of multiprotein complexes [12]. Proteins with the PDZ domain are responsible for clustering and anchoring the structural and functional proteins, i.e. enzymes, receptors or channels, to the membranes or the cytoskeletal compartment. Thus, the specific binding of PMCA to the PDZ-domain of certain proteins could regulate both the concentration of the calcium pump and the intensity of calcium signalling.

PMCA AND CAVEOLAE

The targeting of the PMCA to subcellular membrane compartments may be also responsible for the high local concentrations of the pump within specialised plasma membrane-associated microdomains, known as lipid rafts and caveolae [9, 13]. It could have profound consequences for the local organisation of Ca^{2+} signalling at the plasma membrane, as well as for anchoring Ca^{2+} regulatory complexes to the cytoskeleton. Some other proteins and lipids identified in caveolae are listed in Tab. 3 (modified from [13]). Their common location appears to be a natural place for signal integration. Furthermore, the sequestration of molecules provides an opportunity to modulate spatially and temporally different signalling events.

Tab. 3. Co-location of signalling molecules in caveolae.

Lipids	Acylated protein	Membrane receptor	Signal transducer
sphingomyelin	G proteins	PDGF	MAP kinase
ceramide	e-NOS	EGF	PKC
diacylglycerol	caveolin	insulin	adenylyl cyclase
cholesterol	endothelin	bradykinin	PI3 kinase
			calmodulin
			phosphoinositides

Immunocytochemical analyses revealed that many PMCA isoforms and splice variants, which are expressed in a tissue-and cell type-specific manner, are not

uniformly located in the cell [14-17]. For example, in kidney and intestinal epithelia, the pump is generally located in the basolateral membrane. In cerebellar Purkinje cells, PMCA was detected at the plasma membrane surrounding the soma, and in the dendrites and spines. Moreover, it is co-located with voltage-gated Ca^{2+} channels. For some isoforms, a specific subcellular localization in polarised cells has also been demonstrated.

PMCA ISOFORMS DURING GROWTH AND DIFFERENTIATION

The expression of PMCA isoforms can be selectively modulated during development, and growing evidence indicates that PMCA genes are regulated by agonist- and tissue-specific signalling pathways [4]. The application of knockout strategies revealed the functional consequences of specific mRNA elimination. Reduced expression of certain PMCA isoforms impaired neurite outgrowth, hearing and balance control, and increased cell vulnerability to Ca^{2+}-induced toxicity [18-20]. Recently, after blocking the PMCA 1 isoform with antisense oligonucleotides, an increase in the resting Ca^{2+} concentration in vascular smooth muscle was observed, whereas the knock down of PMCA 1 in human aortic endothelial cells did not affect the resting Ca^{2+} concentration [21]. The inhibition of PMCA 1 expression by the antisense technique in PC6 neuronal cells led to a loss of NGF-induced neurite extension [18].

The NGF-treated rat PC12 pheochromocytoma cell line, which is frequently used as a model for neuron function, possesses all four main PMCA isoforms, and PMCA 4b has been shown to constitute a major isoform. Cell lines with overexpressed PMCA 4 isoforms were less vulnerable to Ca^{2+}-mediated cell death than the clones with suppressed PMCA 4 expression [22]. It has been shown that differentiation can induce a change in the splicing of PMCA mRNAs in muscle and the neuroblastoma IMR32 cell line [23-25]. Conversely, the suppression of endogenous PMCA 4 by the antisense method increased vulnerability to Ca^{2+}-mediated cell death. To evaluate the potential role of other PMCA isoforms in the differentiation process, we transiently blocked the PMCA 2 and 3 isoforms in PC12 cells using phosphothioate antisense oligonucleotides [26].

As shown in Fig. 2, in the control cells, all the main PMCA isoforms were present. These results are consistent with those of Hammes *et al.* [25]. In transiently transfected PC12 cells, we mainly detected PMCA 1 and PMCA 4 products which correspond to the 1b and 4b variants). The kinetic behaviour of the calcium pump was altered in the transfected culture, and the enzyme affinity to Ca^{2+} was 3.5 times lower than that of the control cells (Fig. 3).

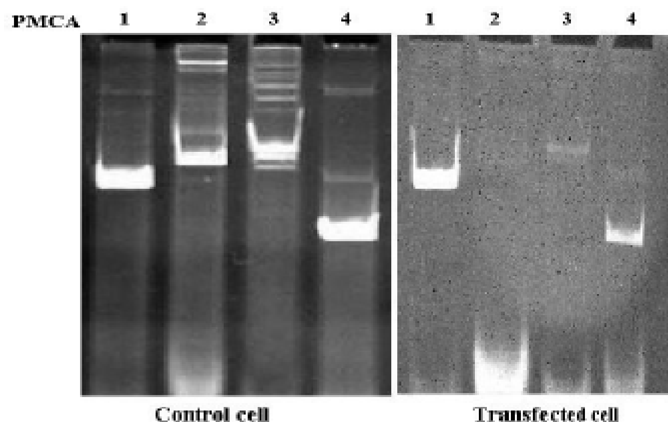

Fig. 2. RT-PCR characteristics of control and transfected PC 12 cells. Transfection in a serum-free medium was conducted using oligofectamine. For RT-PCR analysis, single-stranded cDNA was synthesized from 1µg of total RNA and used directly in the PCR amplification reaction. The PCR products were separated on a 6% acrylamide gel. Unpublished data.

Transfection also brought about morphological changes and increased the mortality of the cells. These finding suggest that PMCA 2 and 3 isoforms may be involved in developmental and differentiation processes, and the regulation of PMCA isoform expression may be critical to cellular survival. In addition, based on a comparison with synaptosomal and erythrocyte membranes (possesing all four PMCA isoforms and PMCA 1 and 4, respectively), we concluded that the activity of the enzyme could be related not only to the presence of the PMCA isoforms, but also to the membrane types.

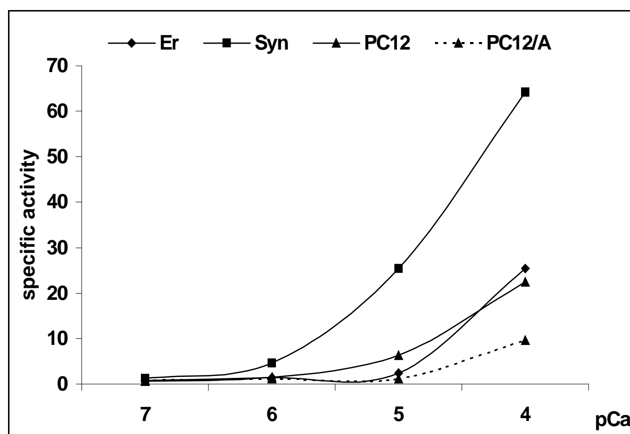

Fig. 3. Ca^{2+}-dependence of Ca^{2+}-ATPase in isolated membranes with different composition of isoforms. Unpublished data.

The transport activity of Ca^{2+}-ATPase was examined under the same conditions using ^{45}Ca with free Ca^{2+} concentrations ranging from 0.1 nM to 100 μM. The results were then plotted using Eadie-Hofstee analysis, and K_{Ca} was calculated. The K_{Ca} values were: 2.96 μM for erythrocyte ghosts, 0.60 μM for rat cortical synaptosomal membranes, 1.64 μM for control PC12 cells, and 5.88 μM for transfected PC12 cells (PC12/A).

CONCLUSIONS

A considerable accumulation of evidence indicates that the structural and functional diversity of PMCAs appears to be fundamental for the modification of calcium signaling in the cell. The most important aspect for future research is an exploration of the relationship between membrane organization and cellular calcium homeostasis under normal and pathological conditions.

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