Neuroscience Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Review article

The PMCA pumps in genetically determined neuronal pathologies

Tito Calì[a], Marisa Brini[b], Ernesto Carafoli[c,*]

[a] Dept. of Biomedical Sciences, University of Padova, 35131 Padova, Italy
[b] Dept. of Biology, University of Padova, 35131 Padova, Italy
[c] Venetian Institute of Molecular Medicine, Via G. Orus, 2, 35129 Padova, Italy

ARTICLE INFO

Keywords:
Ca^{2+} signaling
Plasma membrane calcium ATPases
Deafness
X-linked cerebellar ataxia
Ca^{2+} microdomains

ABSTRACT

Ca^{2+} signals regulate most aspects of animal cell life. They are of particular importance to the nervous system, in which they regulate specific functions, from neuronal development to synaptic plasticity. The homeostasis of cell Ca^{2+} must thus be very precisely regulated: in all cells Ca^{2+} pumps transport it from the cytosol to the extracellular medium (the Plasma Membrane Ca^{2+} ATPases, hereafter referred to as PMCA pumps) or to the lumen of intracellular organelles (the Sarco/Endoplasmatic Reticulum Ca^{2+} ATPase and the Secretory Pathway Ca^{2+} ATPase, hereafter referred to as SERCA and SPCA pumps, respectively). In neurons and other excitable cells a powerful plasma membrane Na^+/Ca^{2+} exchanger (NCX) also exports Ca^{2+} from cells. Quantitatively, the PMCA pumps are of minor importance to the bulk regulation of neuronal Ca^{2+}. However, they are important in the regulation of Ca^{2+} in specific sub-plasma membrane microdomains which contain a number of enzymes that are relevant to neuronal function. The PMCA pumps (of which 4 basic isoforms are expressed in animal cells) are P-type ATPases that are characterized by a long C-terminal cytosolic tail which is the site of interaction with most of the regulatory factors of the pump, the most important being calmodulin. In resting neurons, at low intracellular Ca^{2+} the C-terminal tail of the PMCA interacts with the main body of the protein keeping it in an autoinhibited state. Local Ca^{2+} increase activates calmodulin that removes the C-terminal tail from the inhibitory sites. Dysregulation of the Ca^{2+} signals are incompatible with healthy neuronal life. A number of genetic mutations of PMCA pumps are associated with pathological phenotypes, those of the neuron-specific PMCA 2 and PMCA 3 being the best characterized. PMCA 2 mutations are associated with deafness and PMCA 3 mutations are linked to cerebellar ataxias. Biochemical analysis of the mutated pumps overexpressed in model cells have revealed their decreased ability to export Ca^{2+}. The defect in the bulk cytosolic Ca^{2+} homeostasis is minor, in keeping with the role of the PMCA pumps in the local control of Ca^{2+} in specialized plasma membrane microdomains.

1. Introduction

The Ca^{2+} pumps are present in all animal cells: they maintain the homeostasis of cellular Ca^{2+} by removing it from the cytosol, either by exporting it to the outside medium (the Plasma Membrane Ca^{2+} ATPases, PMCA pumps) or by sequestering it into the endoplasmic reticulum (Sarco/Endoplasmatic Reticulum Ca^{2+} ATPase, SERCA pumps). In excitable cells, e.g., neurons and muscle cells, a powerful Na^+/Ca^{2+} exchanger (NCX) in the plasma membrane also contributes to the clearance of Ca^{2+} from the cytosol. Quantitatively, the contribution of the PMCA pumps to the total clearance of cytosolic Ca^{2+} appears to be minor with respect to that of the SERCA pump, which in all cells is much more abundant. In excitable cells the quantitative role of the PMCAs is further overshadowed by the presence of the NCX. Thus, if only the total quantitative role of the PMCA pumps were considered, the significance of their presence in cells, particularly in excitable cells, would not be easily rationalized. A number of years ago, the demonstration that the sarcolemma of cardiomyocytes contained a PMCA pump in addition to the well-known powerful NCX was indeed completely unexpected [20]. Since the PMCA pumps do nevertheless export Ca^{2+} from cells, their activity, even if of lesser quantitative importance in the overall control of cytosolic Ca^{2+}, must still have some significance: it could for instance play a role in the local control of Ca^{2+} in specific sub-plasma membrane domains, not in the total cytosol at large [65]. A number of years ago evidence was provided for the specific localization of the PMCA pump in caveolae [37,50,78,82], and later work has shown that the microdomain underneath the caveolae contains a number of Ca^{2+} dependent enzymes that are important to cell function [65]. Thus, a novel paradigm of PMCA function has now emerged that would shift its role, and its importance, to the specific

* Corresponding author.
E-mail address: ernesto.carafoli@unipd.it (E. Carafoli).

https://doi.org/10.1016/j.neulet.2017.11.005
Received 23 September 2017; Received in revised form 6 November 2017; Accepted 6 November 2017

control of Ca^{2+} homeostasis in spatially restricted sub-plasma membrane domains [10,60,65].

2. Brief survey of PMCA pump structure, function, and regulation

In mammalian cells, the PMCA pumps are the products of four separate genes. The four basic pump isoforms are differentially distributed in tissues. Two are expressed ubiquitously (PMCA 1 and PMCA 4), two are tissue restricted and especially enriched in neurons. However, PMCA 2 is also abundant in the mammary gland, and PMCA 3 in skeletal muscles. The two ubiquitous isoforms were originally considered to be housekeeping pumps, but later work [70,79] has shown that PMCA 4 also has specialized functions, e.g., in the testis. Thus, only PMCA 1 is now assumed to be the housekeeping pump. In addition to the four basic isoforms, numerous other PMCA pump variants are generated by a complex process of alternative splicing of the primary transcripts: the total number of pump variants now documented as proteins approaches 30 [93]. The splicing processes do not affect the basic catalytic property of the pumps, but may confer to their activity aspects that could be especially important to given cell types.

The PMCA pumps are canonical P-type pumps [73]. They are organized in the plasma membrane with ten transmembrane domains (however, one splice variant has 11) and the canonical 3 main cytosolic domains first documented in the 3D structure of the SERCA pump [101]. The complete 3D structure of the PMCA pump is not yet available, but can by modeled on a SERCA pump structural template (Fig. 1). Basically, it is very similar to the SERCA pump, however the most important difference between the two pumps (which is not reproduced in the 3D model) is the presence in the PMCAs of a long (about 150 residues) C-terminal tail which is the site of its most important regulatory mechanisms.

In addition to mediate the interplay with external regulators, the C-terminal tail has the important function of maintaining the pump in an autoinhibited state in the resting low Ca^{2+}-concentration state. It does so by interacting with two sites in the first and second main cytoplasmic domain of the pump, the latter being close to the catalytic site [31,32]. A third site of interaction of the C-terminal tail has later been found upstream of that in the first cytoplasmic domain [8].

The main regulator of the activity of the pump is calmodulin (CaM), which interacts with a canonical CaM binding domain located about 50 residues downstream in the 10th transmembrane domain of the pump [47,53,103]. As expected, CaM only interacts with the pump in the presence of Ca^{2+}, being optimal at Ca^{2+} concentrations in the vicinity of 0.5 μM. A second, lower Ca^{2+} affinity CaM binding domain has been more recently identified downstream of the first one in some splice isoforms of the pump, [98]. The interplay of CaM with its binding domain is proposed to remove it from the autoinhibitory sites, restoring pump activity. One mechanism of the regulation of the activity of the pump by CaM which has been so far surprisingly neglected is its obligatory oscillatory character: as the pump is released from the autoinhibited state by the increase of Ca^{2+} to levels adequate to trigger the interaction of CaM, it will begin to actively pumping Ca^{2+}, thus impoverishing its concentration in the microenvironment. This will necessarily promote the detachment of CaM from its binding domain, which will then again interact with the autoinhibitory sites, arresting Ca^{2+} pumping activity [18]. Thus, the activation of the pump by CaM cannot be permanent, it necessarily occurs in rapid bursts of short duration.

In spite of the peculiar aspect of its activation, CaM is generally considered to be the most important regulator of the pump (Table 1). A second important mechanism of pump activation is that mediated by phospholipids: experiments on red cell ghosts performed when the pump had not yet been purified showed that the pump was activated by acidic phospholipids [38], but not by zwitterionic phospholipids. Later work demonstrated that the activatory phospholipids interacted with two sites in the cytosolic portion of the purified pump, one being the C-terminal CaM binding domain proper, another a basic sequence in the first main cytosolic unit [12,111]. The activation by acidic phospholipids still has a number of obscure points, however, it has been established that it decreases the K_d Ca^{2+} of the pump to levels that are equivalent (or even lower, [30]) to those induced by CaM. It would be plausible to suggest that acidic phospholipids, since they release the state of autoinhibition, would somehow remove the C-terminal tail of the pump from the autoinhibitory sites, but experimental evidence that this is indeed the case has not been produced as yet. It has been calculated that the amount of acidic phospholipids (essentially,

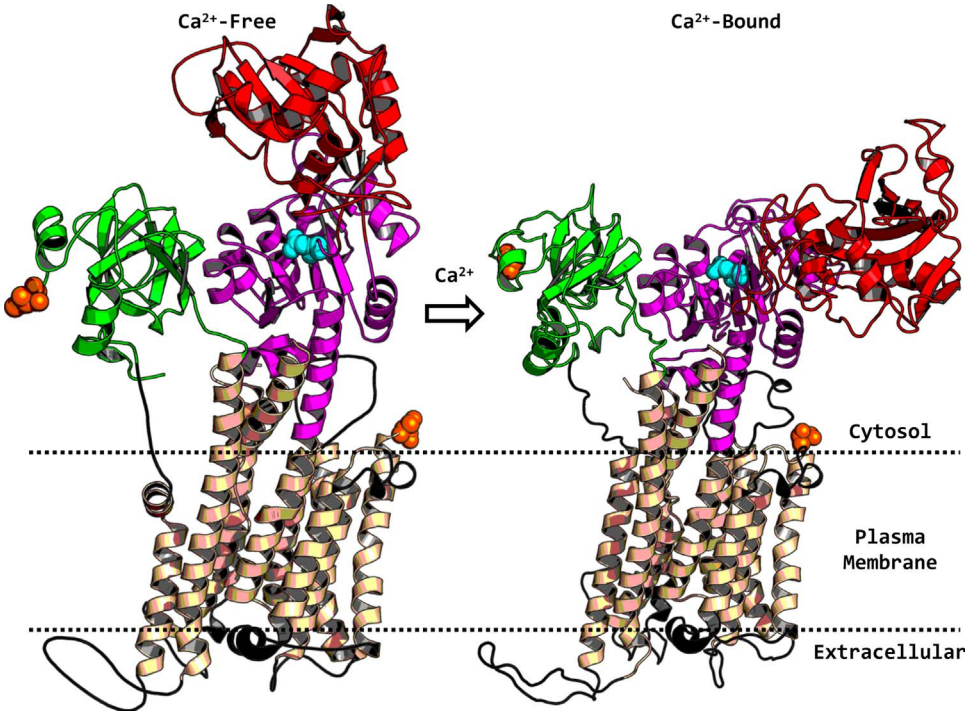

Fig. 1. A 3D model of the PMCA pump in the Ca^{2+}-free (left) and Ca^{2+}-bound (right) states, modeled after the SERCA pump (PDB files 1IWO and 1SU4). The long cytosolic C-terminal tail of the PMCA pump (absent in the SERCA pump) is not included in the superimposition. The highly-conserved D residue is shown in blue, the main domains of the pump, the A-, the P- and the N-domain are shown in green, magenta and red, respectively. The transmembrane domain and the extracellular domain are shown in white and black, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)