

Chapter 2

The Plasma Membrane Ca^{2+} ATPases: Isoform Specificity and Functional Versatility

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Abstract Plasma membrane Ca^{2+} ATPases are single polypeptides of about 1100–1250 amino-acid residues with a molecular mass of 125–140 kDa. They contain ten membrane spanning segments and their N- and C-terminals are both on the cytosolic side. The bulk of their mass is also in the cytoplasm and contains three major intracellular domains: the A (actuator), N (nucleotide-binding), and P (catalytic phosphorylation) domains. Four basic isoforms are encoded by four distinct genes, and their transcripts originated a huge number of alternative splicing variants that in most cases are also translated in the corresponding protein variants. Emerging evidence underlines that PMCA pumps, in addition to maintain resting cytosolic Ca^{2+} levels against a steep concentration gradient (i.e., nM versus mM), play a local control in specific sub-plasma membrane domains by tethering Ca^{2+} -calmodulin-dependent enzymes and reducing their activity, i.e., by decreasing Ca^{2+} concentration in the microenvironment where they are confined. This aspect of pump activity confers to PMCA pump a key role as signal transducer and justifies the existence of so many PMCA variants that could be specialized in tuning the activity of different partners with different Ca^{2+} sensitivity.

Keywords Plasma membrane Ca^{2+} pump • Isoforms • Ca^{2+} signaling

1 Introduction

Ca^{2+} controls the most important cell functions in all eukaryotic organisms. Fertilization, muscle contraction, secretion, several phases of metabolism, gene transcription, apoptotic death, etc. are finely orchestrated by the functional versatility

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of Ca^{2+} signaling and its exquisite spatial and temporal regulation. The specificity of cellular Ca^{2+} signals depends on the coordinated interplay between numerous soluble Ca^{2+} -binding proteins and membrane Ca^{2+} transporters which differ both in their mechanism and sensitivity for Ca^{2+} handling, in their distribution in the intracellular compartments and in their regulation. Ca^{2+} -transporting proteins include ion channels, pumps, and exchangers that drive Ca^{2+} ions across the plasma membrane and across the membranes of intracellular organelles [1].

Three differently located Ca^{2+} ATPase types (pumps) have been described in animal cells: the sarcoplasmic/endoplasmic Ca^{2+} ATPase (SERCA pump) located in the membranes of endo(sarco)plasmic reticulum (including the nuclear envelope), the secretory pathway Ca^{2+} ATPase (SPCA pump) in those of the Golgi network, and the plasma membrane Ca^{2+} ATPase (PMCA pump) in the plasma membrane.

Animal Ca^{2+} pump types belong to the family of P-type ATPases. The name comes from their mechanism for Ca^{2+} transport: the energy from ATP hydrolysis is conserved in the form of a phosphorylated enzyme intermediate (hence P-type) where ATP phosphorylation of an invariant aspartate residue in a highly conserved sequence $\text{SDKTGT}[\text{L/I/V/M}][\text{T/I/S}]$ allows the translocation of Ca^{2+} across the membrane [2]. Structural works on the SERCA pump and the solution of its three-dimensional structure have better elucidated the reaction cycle of P-type ATPases. The polypeptide chain of the pump folds in four main domains: one transmembrane domain M (composed by ten transmembrane helices) and three cytosolic domains—the actuator domain A and the phosphorylation domain P (both connected with the M domain) and the nucleotide-binding domain N, which is connected to the domain P [3]. Upon binding of Ca^{2+} and its translocation, a series of structural changes involving both the protruding cytoplasmic portion and the transmembrane domain results in the “opening” of the “compact” structure of the cytosolic portion (Fig. 2.1). The mechanism of action is the same for all the Ca^{2+} pumps, with the difference that SERCA pump transports two Ca^{2+} ions instead of one and that SPCA is also able to transport Mn^{2+} in addition to Ca^{2+} . Despite of the common mechanism for Ca^{2+} transport, the existence of a multitude of variants for each of the three Ca^{2+} pumps, either encoded by different genes or generated by alternative splicing mechanisms, suggests that the cell needs to differentiate their action in Ca^{2+} extrusion, possibly by activating the proper Ca^{2+} pump in a precise moment or cell district.

In this chapter the focus will be on the plasma membrane Ca^{2+} pumps (PMCA) and the specificity and functional versatility of its isoforms.

2 General Properties of the Plasma Membrane Ca^{2+} Pumps

The PMCA pump has high Ca^{2+} affinity and low transport capacity, with a 1:1 Ca^{2+} /ATP stoichiometry. It was cloned in 1988 [4, 5], and its sequence revealed the same essential membrane organization and topology properties of the SERCA pump. Later, molecular modeling work based on the structure of the SERCA pump predicts the same general features, with ten transmembrane domains and the large cytosolic

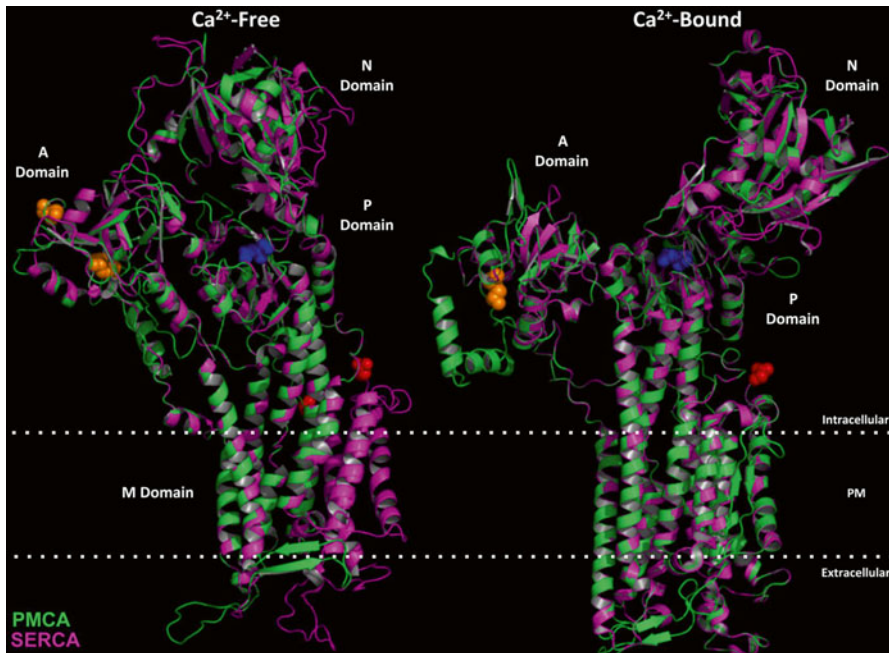


Fig. 2.1 Three-dimensional structure of the PMCA pump. The structure of the PMCA (in *green*) was deduced from that of the SERCA pump (PDB3W5B and 1SU4) and superimposed on it (in *purple*). The structure is shown in the two conformational forms: the Ca^{2+} -free (closed) and the Ca^{2+} -bound (open) form. Upon Ca^{2+} binding, the cytosolic portion of the pump undergoes to a transition from a compact arrangement in the absence of Ca^{2+} to a looser one in its presence. The transmembrane domain M and the three cytosolic domains A, N, and P are shown. The aspartic catalytic residue position is shown as *blue sphere*. The *orange* and the *red spheres* represent the N- and C-terminals, respectively, of the two pumps. In the Ca^{2+} -bound conformation, those of the PMCA and the SERCA pumps perfectly overlap

headpiece divided into the three main cytosolic A, N, and P domains (Fig. 2.2). The catalytic phosphorylation site (**SDKTGLT**) and other important consensus domains are conserved, but the existence of two prominent domains makes the PMCA pump different with respect to the other two Ca^{2+} ATPases. Specifically, a 40-residue-long domain responsible for the binding of activatory phospholipids is present in the first cytosolic loop between transmembrane domains 2 and 3, and a 120-amino-acid-long tail protruding from transmembrane domain 10 and containing the domain that binds calmodulin, i.e., the natural activator of the pump, is present in the C-terminal region [6]. Under nonactivated conditions, the C-terminal tail of the pump is proposed to interact with two sites in the first and second cytosolic loops of the enzyme to maintain the pump auto-inhibited [7, 8]. Calmodulin interacts with its binding domain removing it from the docking sites next to the active center, freeing the pump from autoinhibition [9, 10]. A second calmodulin-binding domain has recently been identified in some splicing variants of the pump [11], and it has been suggested

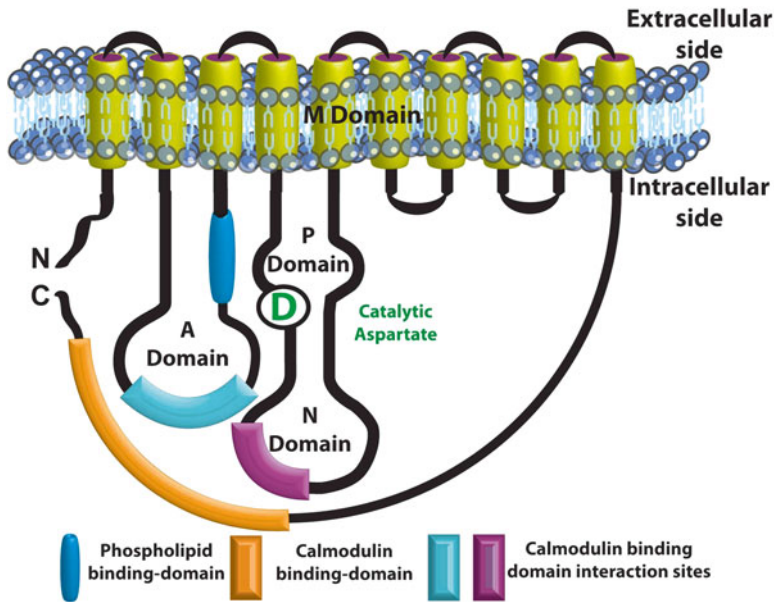


Fig. 2.2 Topology model of PMCA. The pump is organized in the membrane with ten transmembrane domains connected on the external side by short loops. The cytosolic portion of the pump contains the catalytic center and other functionally important domains. The ATP binding site, the acidic phospholipid-binding domain, and the calmodulin-binding domain are shown with different colors

that, together with the original calmodulin-binding domain, it permits the regulation of the pump both in the nanomolar range and in the micromolar range of Ca^{2+} concentration, according to a bi-modular mechanism of control [11].

In addition to calmodulin binding, the PMCA pump has other mechanisms of activation. Among them are the ability of the calmodulin-binding domain to bind also acidic phospholipids [12], the presence of Ca^{2+} -binding motifs upstream and downstream of the calmodulin-binding domain [13], an oligomerization (polymerization) process involving the calmodulin-binding sequence in the C-terminal tail of the pump, the cleavage by calpain, and phosphorylation by protein kinase C and protein kinase A (the latter only occurs in one of the isoforms). The cleavage by calpain occurs immediately upstream of the C-terminal calmodulin-binding domain [14] and activates the pump irreversibly, making it calmodulin insensitive. This irreversible mechanism of activation could become significant in conditions of pathological Ca^{2+} overload that would demand increased Ca^{2+} exporting ability [15]. PKC and PKA consensus sequences have been found in the C-terminal tail of the pump, and regulation of PMCA by PKC has been reported in a variety of cell types [16, 17]. The physiological relevance of the mechanism of phosphorylation is still unclear; however, a number of studies suggest that it could affect various PMCA isoforms and splicing variants in different ways according to their C-terminal sequence characteristics (for a review, refer to [18]).

3 Isoforms of the PMCA Pump

The PMCA pump is the product of a multigene family. In mammals four basic genes (*ATP2B1–ATP2B4*) exist, and their transcripts undergo a complex alternative splicing process that increases the total number of isoforms to about 30 [18–20]. The four gene products (isoforms 1–4) differ in tissue distribution and calmodulin affinity. Pumps 1 and 4 are ubiquitous and have lower calmodulin affinity ($K_d \sim 30\text{--}50$ nM), pumps 2 and 3 have higher calmodulin sensitivity ($K_d \sim 2\text{--}8$ nM), and their expression is restricted to some tissues: PMCA2 is expressed prominently in the nervous system and in the mammary gland, PMCA3 in the nervous and muscle system [18].

All the four PMCA transcripts undergo alternative splicing at two sites (site A and site C), thus originating a large number of variants which differ for distribution, interaction with different proteins, and calmodulin affinity. Site A is located upstream of the phospholipid-binding domain in the first cytosolic loop of the pump, site C in the C-terminal calmodulin-binding domain.

The splicing process at site A leads to the insertion of one exon (in the case of PMCA1, PMCA3, and PMCA4) or up three exons in PMCA2, thus generating variants *w* (three exons included), *x* (two exons included), and *y* (only one exon). The site A inserts are always in frame: they affect the properties of the pumps, but do not substantially alter their structure. The *z* variants display no insertion. Variant *z* is not found in PMCA1, as all mature transcripts of this isoform invariably contain an exon.

The splicing process at site C is characterized by the inclusion of one (in the case of PMCA1, PMCA3, and PMCA4) or two (in the case of PMCA2) full extra exons that results in changes in reading frame and in the introduction of premature stop codons. The mature proteins are truncated and they are designated as *a* variant. The insertion of portions of exon can also occur leading to variants *c*, *d*, *e*, and *f* according to the different isoforms, but their existence and significance at protein levels is not clear. Pumps in which no insertions occur at site C are designated as *b* or full-length variants. In the case of PMCA2 and PMCA3, site C splicing is more complex: two or three, and not only one, novel exons can be included or excluded, thus generating additional C-terminal variants. For a detailed description of splicing variants, the reader could refer to [6].

Differential activity of the splice variants has been studied mainly for those variants generated by the splicing occurring at the C-terminal and focusing on distinguishing the full length from the truncated variants.

Studies on the pump activity performed both on microsomal fraction enriched in specific isoforms or, *in vitro*, on specific PMCA isoforms purified from eukaryotic overexpression systems such as recombinant baculovirus-infected insect Sf9 cells have revealed some differences between the isoforms. These studies are adequate to define isoform functional characteristics with respect to enzyme kinetics ($V_{\max}$, K_m for Ca^{2+}) and regulation by calmodulin, phosphorylation, and phospholipids. They have revealed that the C-terminal truncation determined by the C inserts in the *a* variants lowers, as expected, the affinity of the pumps for calmodulin [21, 22] and that phospholipids mimic the effect of calmodulin [23].

Unfortunately, these studies can provide only limited information concerning the true physiological properties of the isoforms that should be investigated in living cells. One of the major problems in studying PMCA activity in intact cells is that in most cell lines more than one PMCA isoform is expressed (most cells express at least PMCA1 and PMCA4) and multiple splice variants may be present simultaneously. Therefore, assignment of Ca^{2+} extrusion characteristics to a particular isoform or splice variant is not readily possible in vivo. In addition, the absence of specific PMCA inhibitors further complicates this type of analysis. A few studies, however, were informative.

Ca^{2+} measurements performed in intact cells overexpressing the different isoforms and comparing the ability of *a* and *b* variants in counteracting the cytosolic transients generated upon cell stimulation have revealed that the neuron-specific PMCA2 and PMCA3 isoforms were much more effective in counteracting cytosolic transients generated by cell stimulation than the ubiquitously expressed PMCA1 and PMCA4 isoforms [22]. Instead, they have not shown major differences in Ca^{2+} extrusion ability between truncated and full-length variants of PMCA3 and PMCA4 isoforms, thus suggesting that either in intact cells calmodulin was not a limiting factor or their differences in calmodulin affinity were overcome under condition of their maximal activation [22]. The analysis of the joint contribution of site A and site C splicing on the Ca^{2+} -handling ability of PMCA2 pump has instead revealed that *z/a*, *w/b*, and *z/b* splicing variants are all very active, with difference to the doubly inserted *w/a* variant that had only limited ability to rapidly increase activity when challenged with a Ca^{2+} pulse, but had about the same highly non-stimulated (basal) activity of the full-length *z/b* variant [24]. This finding opened the question on whether site A splicing could have an effect in the modulation of phospholipid activation by altering the overall conformation of the second cytosolic loop of the pump. By measuring the ATPase activity in microsomal membranes of transfected CHO cells, we have found that the PMCA2*w/a* variant, as expected, was much less sensitive to calmodulin than the *z/b* and *w/b* isoforms. However, it was also less sensitive to phosphatidylserine, thus underlining the role of the calmodulin-binding domain in the regulation of pump activity by acidic phospholipids. The finding that the *z/b* and *w/b* isoforms had the same response to phosphatidylserine stimulation had indicated that the splicing insertion upstream of the phospholipid-binding domain failed to modify the phospholipid sensitivity of the pump [25].

As to the possible physiological meaning of the splicing at site A, an interesting suggestion comes from the finding that, in the case of PMCA2, the insertion of the three exons at site A (which corresponds to a 45-amino-acid insertion, the *w* form) targets the pump to the apical domain of polarized cells, whereas smaller inserts sort the protein to the basolateral domain of the plasma membrane [26]. Interestingly, it has been later demonstrated that the splicing differentially affects the lipid interactions of PMCA pump with the membrane and that the apical localization of this PMCA variant is lipid raft-dependent and sensitive to cholesterol depletion [27].

A recent elegant analysis performed in intact cells has shed light on the action of three PMCA isoforms (PMCA4a, PMCA4b, and PMCA2b) on regulating the pattern of the store-operated Ca^{2+} entry (SOCE), i.e., the influx of Ca^{2+} from the extracellular ambient induced as a consequence of store depletion [28]. The study has shown that the slow activating PMCA4b isoform produced long-lasting Ca^{2+} oscillations in response to SOCE, whereas the activation of the fast PMCA2b isoform resulted in rapid and highly PMCA abundance-sensitive clearance of SOCE-mediated Ca^{2+} transients. At variance, the activation of the PMCA4a variant reduced cytosolic Ca^{2+} transient induced by Ca^{2+} entry, but resulted in the establishment of a basal cytosolic Ca^{2+} concentration higher than that before SOCE activation, indicating that this isoform is suitable to respond to repeated stimuli. The mathematical modeling indicated that the distinct properties of PMCA isoforms are well suited to differentially affect the shape and the kinetics of the Ca^{2+} transients generated by SOCE activation and thus their relative abundance in different cell types may lead to different activation of downstream signaling pathways [28].

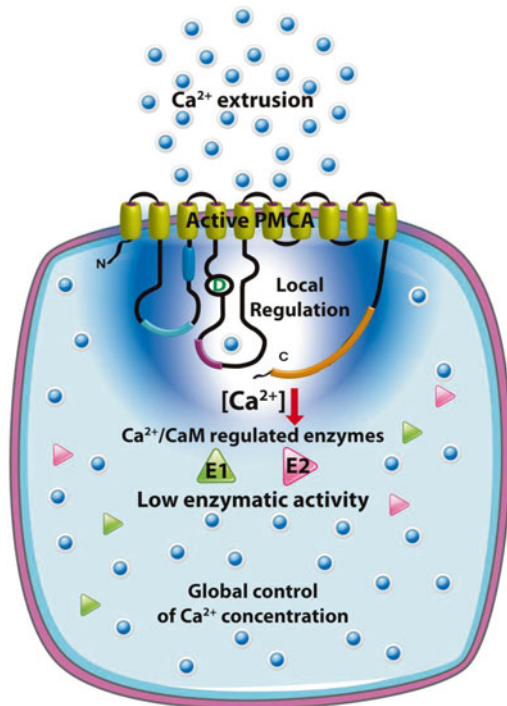
At the end of this paragraph discussing the properties of the different PMCA isoforms and splicing variants, it is important to underline that the expression of some PMCA variants changes during embryonic development and during differentiation both in vivo and in vitro. In muscle, the alternative splicing events occur during myogenic differentiation, and, even in L6 myoblasts cell lines, they can be induced by the application of the muscle differentiation factor myogenin [29]. Interestingly, the induction of the splice form 1c of PMCA1 occurs upon myotube formation [30, 31]. Nerve growth factor treatment of PC12 pheochromocytoma cells leads to the appearance of the “differentiation-specific” splice variants of PMCA1, 2, and 4 (i.e., 1c, 2a, 4a) [29]. Similarly, a marked upregulation of PMCA1a, PMCA2, and PMCA3 at the mRNA and protein level occurs in rat cerebellar granule cells kept under depolarizing conditions for several days (leading to increased Ca^{2+} influx) [32]. In contrast, elevation of intracellular Ca^{2+} resulted in a rapid (within hours) and specific downregulation of the PMCA4a splice variant by a process mediated by the Ca^{2+} -calmodulin-sensitive phosphatase calcineurin [33]. In the human neuroblastoma cell line IMR32, differentiation is accompanied by a marked upregulation of PMCA isoforms 2 and 4 (and to a lesser extent, of PMCA1) which in turn leads to an improved Ca^{2+} extrusion efficiency [34]. Another interesting example is the upregulation of PMCA4b expression occurring during colon and gastric cancer cell differentiation that closely correlates with the induction of established differentiation markers, suggesting that an increase in the PMCA-dependent Ca^{2+} transport activity characterizes the differentiation of these cancer cells [35].

In the last years another important distinguishing aspect of the isoforms has received increasing interest: the identification of a number of protein partners that specifically interact with them has opened the discussion on the possibility that PMCA Ca^{2+} extrusion may be locally tuned to control specific microdomains and thus the activity of the resident enzymes/proteins. These aspects are discussed in detail in the next two paragraphs.

4 Why So Many PMCA Variants?

Emerging evidence suggests that in addition to their function as calcium transporters, PMCA also participate in the regulation of calcium-dependent signal transduction pathways via the interaction with partner proteins. The existence of so many PMCA pump isoforms, including the splice variants, could be rationalized by the finding that they are selectively recruited to plasma membrane compartments/domains by the interaction with specific proteins and that, through a local control of Ca^{2+} concentration, they may regulate the activity of enzymes recruited in functional complexes. Thus, the meaning of the interaction is double: by one side, specific interactors engage PMCA to sub-plasma membrane domains, and by the other, the Ca^{2+} -ejection properties of PMCA, by maintaining intracellular calcium low in cellular microdomains where the tethering with calcium-dependent signaling proteins occurs, negatively modulate Ca^{2+} -sensitive transduction pathways (Fig. 2.3). In agreement with this interpretation, different regulatory interactions have been identified. The identification of some protein partners has, however, only partially reflected differences among isoforms. The preferential site of interaction is the PDZ-binding domain in the C-terminal tail of the *b* variants. PMCA2 and PMCA4 have been shown to interact with several members of the MAGUK (membrane-associated guanylate kinases, or SAP) family of protein kinases which contain PDZ domains and are associated with the cortical actin cytoskeleton [36, 37].

Fig. 2.3 A schematic cartoon showing the double physiological role of PMCA Ca^{2+} extrusion activity. PMCA contributes both to the global regulation of cytosolic Ca^{2+} levels and to the generation of restricted low-calcium microenvironments, where different Ca^{2+} -calmodulin-dependent enzymes can be tethered by the interaction with the PMCA and their activity downregulated by the very low Ca^{2+} levels. *CaM*, calmodulin



Another PDZ domain-containing protein, NHERF2 (Na^+/H^+ exchanger regulatory factor 2), has been shown to interact with PMCA2b, but not with PMCA4b [38]. Interestingly, the specific interaction with NHERF2 enhances the apical concentration of PMCA2w/b by anchoring the pump to the apical membrane cytoskeleton [39]. The PDZ-binding domain of PMCA4b also interacts with neuronal nitric oxide synthase (nNOS, NOS-1) in a complex in which alpha-syntrophin [40] and both the PMCA4 and PMCA2 interact through a portion located between transmembrane domains 4 and 5 with endothelial NOS (eNOS, NOS-3) [41]. The decrease in Ca^{2+} concentrations in the immediate vicinity of the enzyme downregulates the production of NO by synthase, thus playing a crucial role in the pathophysiology of the cardiovascular system.

In addition to the C-terminal domain, other regions of the pumps also interact with protein partners. The main intracellular loop joining transmembrane domains 4 and 5, as mentioned above, interacts with eNOS, but also binds RASSF1 (tumor suppressor RAS-associated factor 1), inhibiting the epidermal growth factor-mediated activation of the RAS signaling pathway [42]. The main intracellular loop interacts with alpha-syntrophin (see above) and with the catalytic subunit of the Ca^{2+} -sensitive signal transduction phosphatase calcineurin. Both PMCA2 and PMCA4 have been shown to functionally interact with it, but the strongest interaction was observed with PMCA2, and it results in inhibition of the calcineurin/nuclear factor of activated T-cell signaling pathway [41]. No interaction was instead detected with PMCA1 [43].

Another important interaction is that occurring between the N-terminal cytosolic region of the PMCA1, PMCA3, and PMCA4, but not of the PMCA2 pump and the epsilon isoform of 14-3-3 protein [44, 45]. This interaction is peculiar since it affects pump activity rather than that of the partner as in the case of the other described interactors.

Finally, it is worth to mention that a novel role for the plasma membrane Ca^{2+} ATPase in the regulation of Ca^{2+} signaling is recently emerged in a paper describing the action of PMCA in the control of phosphatidylinositol 4,5-bisphosphate levels [46]. It has been found that PMCA protect $\text{PtdIns}(4,5)\text{P}_2$ in the plasma membrane from the hydrolysis by phospholipase C (PLC). Two mechanisms have been proposed for this action: the first one is that Ca^{2+} extrusion operated by the PMCA was responsible for limiting Ca^{2+} availability to sustain PLC activity, and the second one is that PMCA binds to $\text{PtdIns}(4,5)\text{P}_2$ and thus reduces the accessibility for PLC and leads to less inositol 1,4,5-triphosphate (InsP_3) production and consequently diminished Ca^{2+} release from intracellular Ca^{2+} stores [46].

5 Functional Versatility

Transgenic animals with altered expression of PMCA are being used to evaluate the physiological significance of the different isoforms. The identification/generation of mice harboring mutations in the gene coding for PMCA pumps has permitted a better understanding of the involvement of specific PMCA isoforms in the regulation of

Ca²⁺ homeostasis of the cells that express them. In the last 10 years, the discovery of human diseases linked to defects in specific isoforms has also contributed to dissect the importance of PMCA isoforms for specific tissues or organs, since the clinical phenotypes linked to PMCA mutations are generally more restricted than their distribution and abundance.

As mentioned, PMCA1 and PMCA4 have wide tissue distribution and have traditionally been considered as the housekeeping enzymes: however, they have now been shown to play a critical and exquisite signal transduction role. For instance, ablation of PMCA4 gene in mice has profound effect on the reproductive function, since it greatly limits sperm motility and generates male infertility [47]. PMCA4 ablation has also a specific effect on the heart-pumping activity: by failing to modulate the activity of NOS-1, it profoundly affects the process of the excitation/contraction coupling of the cardiomyocyte and thus leads to cardiac hypertrophy and alteration in cardiac rhythm [48, 49].

A genome-wide association study aimed at identifying genetic factors that influence blood pressure and hypertension risk has located the most significant single nucleotide polymorphism in the gene for the PMCA1 pump [50, 51], thus ensuring also for this isoform a possible role in sudden cardiac death, blood pressure control, and hypertension.

At variance with PMCA1 and PMCA4, the PMCA2 and PMCA3 pumps have restricted tissue distribution: they are particularly abundant in neurons. PMCA2 ablation in mice generates cerebellar ataxia and hearing loss [52–54]. PMCA3 knockout mice have not been reported so far, possibly because PMCA3 ablation is embryonically lethal.

Several genetic pathologies linked to the dysfunction of the PMCA pumps have now been described in humans. The first described disease phenotype related to a PMCA pump defect is a form of hereditary deafness that involves the PMCA2 isoform of the pump, which is abundantly expressed in the stereocilia of the hair cells of the Corti organ in the inner ear. The tight control of the homeostasis of Ca²⁺ in the endolymph is essential for the functioning of the stereocilia bundle that gates mechano-electrical channels through which K⁺ (and Ca²⁺) flow into the hair cell to generate (or modulate) the acoustic signals. Two human families with a hereditary deafness phenotype caused by two different point mutations in the PMCA2 gene were described [24, 55], and curiously, at variance with mice, in humans the PMCA mutations act as modifier of a phenotype caused by mutations in other genes (i.e., cadherin 23) or through a digenic mechanism, where both mutations in cadherin 23 and in PMCA2 genes are necessary to develop the hearing loss phenotype.

Interestingly, mutations in PMCA3 gene have been recently identified in two families affected by cerebellar ataxia [56, 57]: their mechanism of action is possibly different since in the first family the mutation is responsible per se of the X-linked phenotype [56] and in the second one the phenotype was developed only when the patient also inherited mutations in the *LAMA1* gene, which encodes the extracellular matrix protein laminin subunit 1 α [57].

Molecular studies of the mutant PMCA3 pumps expressed in model cells have shown that the mutations impaired pump ability to extrude Ca²⁺ both in resting condition

and upon cell stimulation [56, 57]. A missense mutation (Tyr543Met) in the PMCA3 pump gene has also been detected in human pancreatic cancer cells [58], but the effects of the mutation on the activity of the pump have not been investigated.

6 Conclusions

The data from several groups largely support the view that the PMCA pumps are not uniquely in place to keep resting cytosolic Ca^{2+} concentration and counteract Ca^{2+} transients generated by cell stimuli and thus turn off activatory signals, but that they can themselves regulate the activity of specific enzymatic complexes by locally controlling Ca^{2+} environments. Surprisingly and interestingly, defects in pump activity, generally, do not lead to global cell impairment that conduces to cell death but originated disease conditions that compromise only specific cell populations or tissues, despite the distribution of mutated isoform is not confined to them. This aspect is particularly fascinating and suggests that the expression of specific PMCA isoforms could be orchestrated by the cell to finely tune Ca^{2+} homeostasis in specific environments and according to specific requirements. A lot of work is still necessary to profoundly understand the complexity of the system and to develop specific drugs that could modulate PMCA activity in an isoform-specific manner and could thus be suitable to therapeutical approaches to target PMCA inactivating mutations.

Acknowledgments We are deeply grateful to Ernesto Carafoli who has greatly contributed during the years to clarify the mechanisms of action and regulation of the plasma membrane Ca^{2+} pump and has trained us in this field of investigation with enthusiasm and passion. T.C is supported by the Scientific Independence of Young Researchers (SIR) grant (Bando SIR 2014 n. RBSI14C65Z) from the Italian Ministry of University and Research (MIUR).

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Chakraborti, S.; Dhalla, N.S. (Eds.)

2016, XIII, 586 p. 101 illus., 26 illus. in color., Hardcover

ISBN: 978-3-319-24778-6