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Plasma membrane calcium ATPases: From generic Ca^{2+} sump pumps to versatile systems for fine-tuning cellular Ca^{2+}

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ABSTRACT

The plasma membrane calcium ATPases (PMCAs) are ATP-driven primary ion pumps found in all eukaryotic cells. They are the major high-affinity calcium extrusion system for expulsion of Ca^{2+} ions from the cytosol and help restore the low resting levels of intracellular [Ca^{2+}] following the temporary elevation of Ca^{2+} generated during Ca^{2+} signaling. Due to their essential role in the maintenance of cellular Ca^{2+} homeostasis they were initially thought to be "sump pumps" for Ca^{2+} removal needed by all cells to avoid eventual calcium overload. The discovery of multiple PMCA isoforms and alternatively spliced variants cast doubt on this simplistic assumption, and revealed instead that PMCAs are integral components of highly regulated multi-protein complexes fulfilling specific roles in calcium-dependent signaling originating at the plasma membrane. Biochemical, genetic, and physiological studies in gene-manipulated and mutant animals demonstrate the important role played by specific PMCAs in distinct diseases including those affecting the peripheral and central nervous system, cardiovascular disease, and osteoporosis. Human PMCA gene mutations and allelic variants associated with specific disorders continue to be discovered and underline the crucial role of different PMCAs in particular cells, tissues and organs.

1. Introduction

Tight regulation of cellular Ca^{2+} is crucial for cell function and survival. Accordingly, cells dedicate a substantial amount of energy to the maintenance of Ca^{2+} homeostasis and the spatial and temporal regulation of changes in [Ca^{2+}]. Membrane-bound Ca^{2+} transporters (channels, exchangers, pumps) and Ca^{2+} buffers are essential components of the cellular Ca^{2+} handling toolbox. Among the transporters, ATP-driven Ca^{2+} pumps are uniquely capable of moving Ca^{2+} across the membrane against a large Ca^{2+} concentration gradient. To maintain long-term homeostasis, a Ca^{2+} pump system is needed in the plasma membrane. The existence of a plasma membrane Ca^{2+} ATPase (PMCA) system was already recognized a half-century ago. Ever since, Ernesto Carafoli has been at the forefront of discoveries related to the PMCAs. As part of this Special Issue of BBRC dedicated to Ernesto, this review recognizes his numerous seminal contributions to the field by providing a compressed history of the major developments over the past 50 years, highlighting the progress that has been made leading to the current realization that the PMCAs are important contributors to human disease and potential targets for therapeutic intervention.

2. Discovery and initial characterization of the PMCA – identification of a cellular sump pump for excess calcium removal?

The discovery of the plasma membrane Ca^{2+} ATPase (PMCA) dates to about 50 years ago, when Schatzmann described the presence of an ATP-dependent active Ca^{2+} pumping system in red blood cell membranes [1]. The choice of (mature) erythrocytes was crucial as these cells lack intracellular membranes and thus allow functional biochemical studies on ion transport in a "pure" plasma membrane system. Studies on resealed erythrocyte "ghosts" were instrumental in determining several important biochemical properties of this calcium pump system, such as its Mg^{2+}, Ca^{2+}, and ATP dependence, inhibition by La^{3+}, and high Ca^{2+} transport specificity (for early reviews see, e.g., [2,3]). Progress in identifying the protein entity and establishing detailed kinetic and regulatory properties of the system was, however, hampered by the

Abbreviations: CaM, calmodulin; PDZ, PSD95/Dlg/ZO-1; MAGUK, membrane-associated guanylate kinase; PMCA, plasma membrane calcium ATPase.

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low abundance of the Ca^{2+} pump in the erythrocyte membrane and by the intrinsic difficulty of working with hydrophobic integral membrane proteins. The finding by the mid-1970s [4,5] that the pump rapidly forms a ~150 kDa phosphorylated intermediate in the presence of Ca^{2+} and ATP facilitated its detection during purification. Equally important was the discovery that the erythrocyte Ca^{2+} pump activity was stimulated, in a Ca^{2+}-dependent manner, by the small heat-stable calcium-modulator protein [6–8] later named calmodulin (CaM) [9]. The first successful partial purification (by gel filtration chromatography) of a ~140 kDa protein from detergent solubilized erythrocyte membranes was reported by Wolf and coworkers [10] but was rapidly superseded by affinity chromatography of the solubilized erythrocyte pump on CaM-Sepharose [11]. This method was jointly developed by the Carafoli and Penniston laboratories and elegantly exploited the high binding affinity of the erythrocyte PMCA for CaM in the presence of (micromolar) Ca^{2+} and its dissociation from CaM upon Ca^{2+} removal (reviewed in Ref. [12]). By the late 1970s and early 1980s, these and other advances in the study of membrane ion transport resulted in much progress in understanding of the biochemical, regulatory, and kinetic properties of the PMCA, including its 1:1 ATP:Ca^{2+} transport stoichiometry, activation by Ca^{2+}-CaM, the effects of different nucleotides, mono- and divalent cations, pH, partial proteolysis, (phospho)lipids and detergents, and its inhibition by La^{3+} and vanadate (for reviews, see Refs. [2,3,13–16].

Although most of the early biochemical and functional data on the PMCA came from work on red blood cells, it was rapidly discovered that a similar Ca^{2+} extrusion system is present in many other cell types and tissues in mammals, and indeed appears to be found in all animal cells as well as in plants [16,17].This could be easily rationalized since all cells need to keep intracellular free [Ca^{2+}] low (generally in the 100 nM range) to maintain viability [18,19]. Due to its high Ca^{2+} transport affinity and selectivity, the pump is ideally suited to maintain the steep Ca^{2+} concentration gradient across the cell membrane and to re-establish the low resting intracellular free [Ca^{2+}] after a temporary surge in $[Ca^{2+}]_i$ [16]. Hence, its primary role in cellular homeostasis was assumed to be that of a sensitive "Ca^{2+} sump pump", turning on when $[Ca^{2+}]_i$ rises above a certain threshold and staying "off" at levels below the set-point. Consistent with such a role, the term "PMCA" or "Ca^{2+} pump" was generally used in the singular form for over 20 years after its discovery, even as protein biochemical and immunological studies indicated that there could be different types of PMCAs [15,20,21]. However, this simple picture of the PMCA was soon undermined by the "molecular biology revolution", which revealed a surprising complexity in the PMCA family and cast doubt on the validity of "the" PMCA as a mere sump pump for calcium clearance from the cell.

3. Things get more complicated – identification and characterization of PMCA isoforms generated from a multigene family and via alternative RNA splicing

The ability to purify sizeable amounts of PMCA by CaM affinity chromatography, together with advances in the isolation, characterization and sequencing of peptide fragments from proteolytic digests opened the door for detailed biochemical, and eventually molecular, characterization of these pumps [22]. In 1988, the advent of "molecular cloning" by recombinant DNA technology resulted in the elucidation of the first full-length amino acid sequence for a rat and a human PMCA, championed by the group of Gary Shull in Cincinnati [23] and the laboratories of John Penniston at the Mayo Clinic and Ernesto Carafoli in Zurich [24]. The following "molecular biology revolution" led to an explosion of new sequence data and the rapid realization that there are, in fact, multiple PMCAs in many species and even within the same tissue of a given species [22,25–27].

The PMCAs comprise a subfamily of the P-type ATPase superfamily (characterized by the formation of a phosphorylated enzyme intermediate during the reaction cycle [28]) and have been classified as P2B subfamily of calcium-transporting ATPases [29–31]. In humans and other mammals, four distinct PMCA isoforms (PMCA 1–4) are encoded by distinct genes (ATP2B1, ATB2B2, ATP2B3 and ATP2B4 in the human genome database nomenclature) located on separate chromosomes (12q21.3, 3p25.3, Xq28, and 1q32.1 for the human; and 10C3, 6E3, XA7.3, and 1E4 for the mouse genes) [32]. In addition, molecular analysis of cDNAs isolated from different tissues revealed the existence of alternative RNA splice variants for each of the primary gene products [33–39]. Two major splice sites (sites "A" and "C") were found to affect the encoded PMCA proteins (see Fig. 1): splicing at site A changes the length of the first intracellular loop, which is part of the A (actuator) domain; whereas splicing at site C impacts the C-terminal tail, altering its length (due to a reading frame shift) and more significantly, leading to very different regulatory and functional properties of the pumps (reviewed in Refs. [40–42]). In addition to some exon splicing combinations that have only been observed in a few species or special tissues [43–45], alternative splicing also occurs in the 5′ untranslated region of the PMCA transcripts and may affect splicing at the downstream sites [46]. Combinatorial use of all available splice options leads to over 30 distinct PMCA variants that could theoretically be generated from the 4 mammalian genes. Although not all of these have been confirmed, and some appear to be expressed only at very low levels, there is robust evidence for the expression of ~20 different PMCA variants at the transcript level [40]. Due to the lack of specific antibodies, not all of these have been confirmed at the protein level; however, proteomic and immunodetection studies suggest that most, if not all, of the expressed transcripts are translated into the respective PMCA isoform [47–55]. Overviews of the different splice options for generating PMCA isoform diversity have been included in many recent reviews [56–58] but new variants are still being added [55]; an updated scheme for the human splice variants is shown in Fig. 2. It should be noted that several splice variants found in other species (e.g., the y-splice form at site A in PMCA2 [35] and c-, d- and f-splices at site C

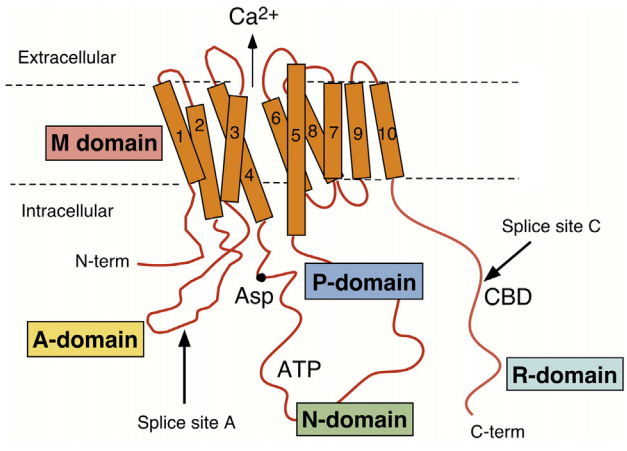

Fig. 1. Schematic overview of the PMCA structure. The membrane-spanning regions are numbered and constitute the M domain. The intracellular amino- (N-term) and carboxy-terminal (C-term) ends are labeled. The conserved aspartate (Asp) forming the phosphorylated intermediate during the enzyme reaction cycle, the ATP binding region (ATP), and the calmodulin-binding domain (CBD) are also indicated. The direction of active Ca^{2+} transport is indicated by an arrow. Major cytosolic protein domains are labeled A (actuator), P (phosphorylation), N (nucleotide-binding), and R (regulatory). Arrows indicate the position where alternative splicing results in isoform variability in the first cytosolic loop (splice site A) and the C-terminal tail (splice site C).

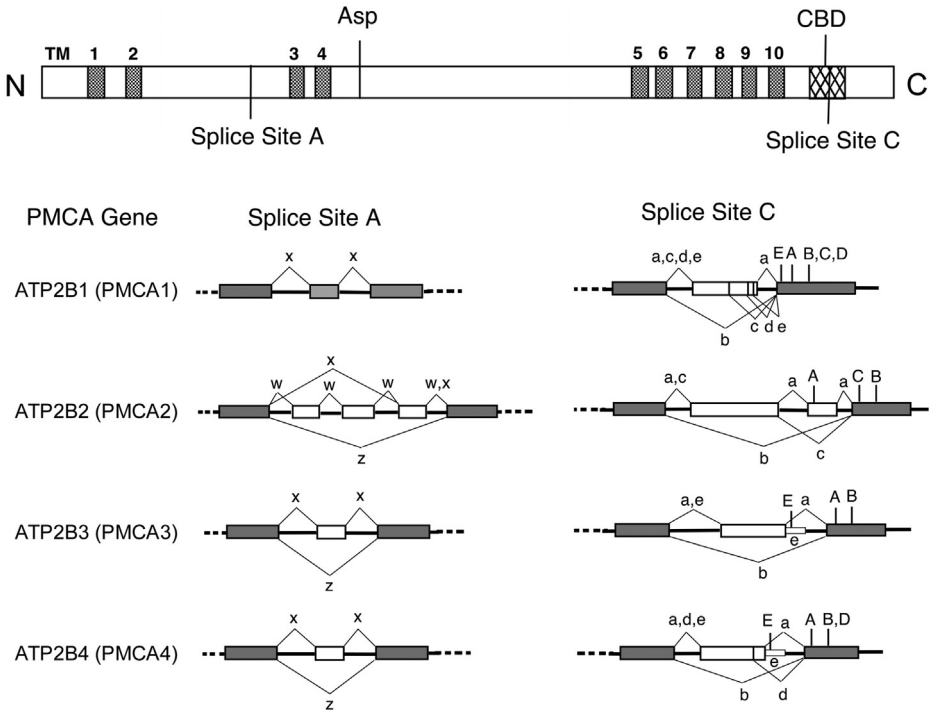

Fig. 2. Major alternative splice options of the human PMCA gene family. The linear scheme of the PMCA at the top shows the location where alternative splicing at sites A and C results in different splice variants. The 10 trans-membrane regions (gray boxes) are labeled TM 1-10, the amino- (N) and carboxyl-terminus (C) are indicated, and the position of the conserved aspartate residue (Asp) and the CaM-binding domain (CBD, hatched box) are labeled as in Fig. 1. The exon arrangement in the regions involved in alternative splicing at sites A and C is shown in the lower part of the figure for each of the four human PMCA (ATP2B) genes. Gray boxes represent constitutively spliced exons, alternatively spliced exons are shown as open boxes. Splicing options are indicated by connecting lines and the resulting splice variants are labeled by lowercase symbols (w, x, z for splice options at site A; a, b, c, d, e for options at site C). The position of the translational stop codon for each of the C-terminal splice variants is indicated by the capital letter corresponding to that splice option (A for splice variant a, B for variant b, etc.). Note that splicing at site A does not change the translational reading frame because all alternatively spliced exons contain integral numbers of triplet codons. Also note that the e-splice variant of PMCA3 and PMCA4 is generated by read-through of the C-terminal alternatively spliced exon into the adjacent intron (shown as thin open box), whereas PMCA1e arises from the use of an internal donor splice site in the corresponding alternatively spliced exon.

in PMCA3 of the rat [38]) have not (yet) been detected in the human transcriptome and may be unique to a given species. Table 1 lists the main human PMCA variants including their length in amino acids (AA) and major sites of expression.

Table 1
Human PMCA isoforms and splice variants.

Isoform	Variant	Size (AA)	Major sites of expression
PMCA1	x/a	1176	Brain
	x/b	1220	Ubiquitous; lung, small intestine, kidney
	x/c	1249	Skeletal muscle; heart
	x/d	1258	Skeletal muscle
	x/e	1171	Brain
PMCA2	w/a	1199	Brain; cochlear outer hair cells
	x/a	1168	Brain; hippocampal presynaptic terminals
	z/a	1154	Brain; excitable tissue
	w/b	1243	Brain; lactating mammary epithelial cells; pancreatic beta cells
	x/b	1212	Brain; cerebellar Purkinje cells; spinal cord
	z/b	1198	Brain; excitable tissue
PMCA3	x/a	1173	Brain; spinal cord
	z/a	1159	Brain; pancreatic beta cells
	x/b	1220	Brain; adrenal gland; skeletal muscle
	z/b	1206	Brain
PMCA4	x/a	1170	Smooth muscle; bladder; uterus; heart
	z/a	1158	Smooth muscle; heart
	x/b	1205	Ubiquitous; heart; kidney
	z/b	1193	Heart
	x/d	1241	Heart
	z/d	1229	Heart
	x/e	1164	Brain; bladder
	z/e	1152	Brain; bladder

The discovery of multiple PMCA isoforms and a plethora of alternative splice variants immediately raises the question of the role of these pumps in cell physiology, and casts doubt on the idea that they merely work as “sump pumps” for restoring and maintaining the low global resting-state intracellular [Ca^{2+}] levels. To be sure, tissue-specific gene expression may explain why some PMCA isoforms are found in certain tissues and not in others, but why would evolution favor such a diversity of splice variants if the only task for the encoded protein was to globally eject “surplus” calcium from the cell?

4. Regulatory and functional differences distinguish different PMCA isoforms and splice variants

Studies using purified PMCAs (mostly PMCA4b) or membrane preparations from eukaryotic cell systems overexpressing specific recombinant PMCAs have provided solid evidence for structural, regulatory and functional differences among PMCA isoforms and their splice variants. One of the most notable differences concerns the regulation (activation) by CaM, which binds to the C-terminal regulatory tail of the PMCAs and thereby releases autoinhibitory interactions of the tail with the catalytic domain of the pump [59–61]. Of interest, the CaM-binding region overlaps splice site C in the PMCAs (see the scheme in Fig. 1). Thus, alternative splicing at site C impacts the affinity of the pumps for Ca^{2+}-CaM as well as the degree and kinetics of their activation [62–65]. In general, the “a” splice variants with a shortened C-terminal tail show lower CaM affinity but increased basal activity and a more moderate CaM stimulation than the “b” splice variants. Pronounced differences in

the basal activity and extent of CaM activation also exist between "b" variants of different PMCA isoforms, notably between PMCA4b and PMCA2b: PMCA4b shows low basal activity and is stimulated several-fold by CaM, whereas PMCA2b displays high basal activity and more modest CaM stimulation [64]. Perhaps more interesting are findings of the different kinetics of (CaM) activation and de-activation of separate isoforms and of different splice variants of the same isoform: These differences result in very different "memories" of individual pumps for previous activation, which may lead to differences in how different pumps deal with intermittent Ca^{2+} spikes and affect the speed of recovery of $[Ca^{2+}]_i$ levels [66,67].

Amino acid sequence differences concentrated mainly in the C-tail of different PMCA isoforms also differentially affect their regulation by phosphorylation (e.g., by protein kinase A and C, or tyrosine kinases such as src), self-association, and different protein–protein interactions [32,42,68–70]. On the other hand, differences in the "actuator" loop region close to splice site A and upstream of the third membrane-spanning domain are thought to be responsible for differences in the regulation of PMCA isoforms by lipids and in particular, by acidic phospholipids [27,71–73]. In addition, differences in the "strength" of auto-inhibition of PMCA isoforms are likely due to sequence (and structural) differences in the intracellular loop regions that interact with the C-terminal regulatory tail [74]; such differences are expected to result in different activation kinetics of the pumps. The picture that has emerged from detailed molecular, biochemical and in vitro functional studies thus clearly shows that the PMCA isoforms and many of their splice variants respond differently to stimuli such as protein kinase activation, changes in the phospholipid environment, or the abundance of Ca^{2+}/CaM and other regulatory proteins. Together with the highly tissue- and cell type-specific expression and variable abundance of multiple isoforms, this strongly suggests the functional division of labor among PMCAs. Recent studies at the cellular level, and increasingly in whole organs and genetically tractable animal models, have borne this out: The PMCAs have emancipated from "simple" gatekeepers of intracellular $[Ca^{2+}]$ to multi-faceted contributors to complex temporal and spatial Ca^{2+} signaling and tightly regulated vectorial Ca^{2+} flux.

5. PMCAs work as members of a team: integration of PMCAs in multiprotein signaling complexes at the membrane

Participation in complex cellular events demands an "awareness" of the local conditions and their temporal changes. To be fully integrated in local signaling events, the PMCAs must be able to cross-talk to the structural and functional components of the signaling network. In hindsight, it is therefore not surprising that yeast two-hybrid and antibody pull-down protein–protein interaction studies yielded many different PMCA-interacting proteins. The b splice variants of all PMCAs contain at their C-terminus a consensus sequence for direct recognition of the PDZ (PSD95/hDlg/ZO-1) protein–protein interaction domain [75]; indeed, numerous PDZ proteins including the membrane-associated guanylate kinases (MAGUKs) PSD95, PSD93/chapsyn-110, SAP97, SAP102 and CASK, as well as other scaffolding and regulatory proteins (CLP-36, NOS-1, NHERFs, Homer-1/Ania3, PISP/AIPP1) have been found to directly bind to the PMCAs [32,76–79]. The ability to bind to PDZ proteins differentiates the b (and c/d) splice variants from the a (and e) splice variants; in addition, some b (c/d) splice variants show selectivity towards several PDZ proteins. For example, while all PMCA b-variants can interact with the MAGUK PSD95 or the single-PDZ protein PISP/AIPP1 [80,81], PMCA2b (and 1b) but not PMCA4b, interacts with NHERFs [82,83]. Thus, specific PDZ protein interaction further differentiates PMCA splice variants and enables their selective incorporation into signaling complexes with cell-type specific functions (e.g., at the postsynaptic membrane in dendritic spines). The functional relevance of PDZ-mediated PMCA recruitment to membrane microdomains has been demonstrated for several PMCA-PDZ protein interactions, e.g., for PMCA4b-NOS in regulating NO production in cardiac function and blood pressure control [76,84–86], for PMCA4b-CLP36 in platelet activation and aggregation [77,87], for PMCA1b-NHERF2 in the response to muscarinic G-protein coupled receptor activation [83], and for PMCA2b-PSD95 in nicotinic acetylcholine receptor-mediated interneuron calcium signaling [88].

Besides PDZ proteins, numerous other proteins have been shown to interact selectively with specific PMCA isoforms or more promiscuously with most PMCAs, depending on whether they interact with a unique or a highly conserved region of the pump. The list of these proteins is still growing and includes signaling and trafficking proteins (RASSF1, 14-3-3e, calcineurin A, syntaxin, Homer 2 [78,89–92]), scaffolding/cytoskeletal proteins (alpha-1-syntrophin, actin [93,94]) and membrane receptors, sensors and channels (alpha-7 nicotinic acetylcholine receptor, glycine transporter 2 (GlyT2), POST, STIM1 [88,95–97]). These diverse protein interactions serve to fine-tune the activity of specific PMCAs directly (e.g., by release of auto-inhibition or by decreasing the affinity for Ca^{2+}, CaM and ATP) or indirectly by altering their abundance at the membrane and their integration into specific signaling complexes. The lipid environment also plays an important role in when and where different PMCA isoforms are most highly active, as differential lipid interactions can result in the partitioning of specific PMCA isoforms into lipid (micro) domains such as glycosphingolipid and cholesterol-rich lipid rafts [98,99]. The differential sorting, trafficking, and recycling of PMCA isoforms and splice variants likely requires additional (transient) associations with specific protein partners; these may include 14-3-3e, Homers, PISP/AIPP1 and scaffolding PDZ proteins, but others clearly remain to be identified. The deployment of the PMCAs to different membrane locales must be carefully orchestrated in response to the changing demands of Ca^{2+} signaling; hence protein–protein interactions of the PMCAs are dynamic and subject to short-term and long-term regulation.

6. Functional specialization of PMCAs as revealed by their involvement in different diseases

Given their distinct pattern of expression and functional properties, as well as their diverse protein partners it is not surprising that different PMCAs are implicated in specific diseases, underlining the functional specialization of PMCA isoforms in controlling different Ca^{2+}-mediated cellular events. Much of the knowledge of the "global" role of each PMCA isoform has been obtained from studies on "knockout" mice first generated in the laboratory of Gary Shull (Pmca1, 2, 4)[100,101] and by the group of Ludwig Neyses (Pmca4)[102], as well as from studies of naturally occurring PMCA mutant mice (for review, see Ref. [103]). In vitro studies using knockdown or overexpression of specific PMCA isoforms have shed further light on their roles in specific cellular events such as differentiation, proliferation or cell death [55,104–111]. Finally, recent large-scale genome-wide association studies and population screening by exome sequencing of families with inherited disease have implicated different PMCAs in various human pathologies. The emerging picture reveals the association of PMCAs with multiple signaling pathways and the consequent complexity of pathological outcomes of the lack or aberrant expression of each pump. For example, transgenic overexpression of PMCA4b in vascular smooth muscles in mice resulted in altered vascular tone and increased blood pressure, which can be explained by the inhibitory effect of

PMCA4b on NOS and the subsequent decrease in NO production [112,113]. However, the regulation of NOS by the PMCA4 is more complex, as the physical tethering of NOS (via the PDZ domain) to PMCA4b is important for maintaining the integrity of the signaling domain at the membrane. Accordingly, PMCA4 depletion (in Atp2b4 null mice) disengages nNOS from its membrane domain and results in increased basal cardiac contractility via a decrease in cGMP and a consequent cAMP-mediated positive inotropic effect [114]. The highly integrated role of the PMCA4 in cardiac physiology was also demonstrated in a recent study where the loss of this pump in a transgenic mouse model of mutant tropomyosin-induced hypertrophic cardiomyopathy was protective, improving left ventricular end-diastolic pressure by upregulating cGMP and preventing the disproportionate increase in heart weight [115].

Mouse knockout studies showed the importance of the ubiquitous PMCA1 in organ and tissue development, revealing that homozygous loss of PMCA1 is not compatible with normal embryonic development [101]. However, because the relative importance of PMCA1 changes during development and in different tissues, mutations in the ATP2B1 gene resulting in loss of expression or function may also cause or contribute to specific diseases. For example, genome-wide association studies in large populations of people from Asian, European and African ancestry showed a highly significant association of specific SNP (single nucleotide polymorphism) alleles in the ATP2B1 locus with increased systolic blood pressure and cardiovascular disease risk [116–119]. Although the precise pathogenic mechanism is not yet clear, a decreased level of PMCA1 and consequent deficient Ca^{2+} handling in vascular smooth muscle are likely involved: Conditional knockout mice in which the Pmca1 was deleted in vascular smooth muscle developed increased blood pressure [120]. Reduction of PMCA1 in other tissues similarly results in specific pathological outcomes, e.g., in altered bone mineralization and remodeling [121] and impaired gastric epithelial wound repair [122]. The latter example is of particular interest as the importance of the PMCA1 appears to be in providing a local pool of extracellular Ca^{2+}. This highlights the multiple functions that PMCA isoforms fulfill depending on the physiological context: In the enterocytes of the small intestine which express primarily PMCA1b, the primary function of the pump is to facilitate hormonally regulated ($1,25(OH)_2$ vitamin D_3-stimulated) trans-cellular Ca^{2+} absorption. Decreased expression of PMCA1b in the small intestine results in impaired dietary calcium absorption and secondary hyperparathyroidism and bone deficits in mice lacking the membrane cytoskeletal protein 4.1R [123]. In this mouse model, protein 4.1R appears to be required for proper stabilization and maintenance of PMCA1b in the basolateral membrane of the enterocytes, reminiscent of the role of NHERF2 in recruiting and stabilizing PMCA1b and 2b in the apical membrane of different epithelial cells [83,124].

What lesson can we learn from these examples? Systemic disease as well as disorders restricted to tissue-specific functional deficits can both be caused by deficiency (due to mutation or aberrant expression) of a single PMCA isoform. As a consequence of the integration of the PMCAs in multi-protein signaling complexes, any disturbance of such complexes may have deleterious consequences. Thus, some mutations in ATP2B genes may manifest as modifier mutations for disorders caused by deficits in functionally linked proteins; conversely, mutations affecting PMCA-associated proteins may impair the normal expression and function of the specific PMCA isoform. The heterogeneity, yet specificity of disease phenotypes caused by mutations of a PMCA are well demonstrated by the example of PMCA2. Several mutations including different single amino acid substitutions and nonsense mutations have been identified in the ATP2B2 gene in mice and humans; these lead to hearing deficits from early onset and severe to late-onset and relatively mild [103,125–128]. Depending on the type of the mutation, other organs and systems may also be affected, such as the vestibular system and spinal cord and cerebellar functions [100,129–132]. Similarly, deficits in PMCA4 are not only associated with male infertility [101,102], cardiovascular problems [133] or altered bone remodeling [121], but may also manifest in additional disease syndromes: A very recent study identified a structure-destabilizing missense mutation (R268Q) in PMCA4 as likely causative for autosomal dominant familial spastic paraplegia in a Chinese family [134].

7. Expanded responsibilities for the PMCAs in cell signaling and cell function

Starting from the relatively vague concept of a plasma membrane calcium pump required in all eukaryotic cells to maintain and restore the low resting intracellular Ca^{2+} levels, the past half-century has seen tremendous progress in our understanding of the biochemical, structural, molecular, and functional characteristics of this versatile molecular machine. In mammals, well over 20 distinct PMCAs are expressed from 4 genes and by alternative RNA splicing; these variants differ substantially in their regulatory and functional properties, their tissue abundance, developmental pattern of expression, and localization in distinct membrane microdomains. Their function is tightly integrated with the cell's physiological Ca^{2+} signaling and Ca^{2+} transport needs: they may be needed for bulk vectorial calcium transport such as in the intestine, kidney and mammary gland, but also for fine-tuning temporally and locally restricted Ca^{2+} signals in response to receptor-mediated cell stimulation. Isoform- and splice variant-specific protein–protein and protein–lipid interactions underlie the specific and dynamically regulated incorporation of PMCAs in multi-component signaling complexes. Reciprocal interactions allow PMCA-mediated regulation of signaling but also feed-back regulation of PMCA function. The diversity of PMCA functions in cell physiology is reflected by the growing number of congenital disorders and disease phenotypes attributed to mutations and aberrant expression of different PMCAs.

Conflict of interest

None.

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Transparency document

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References

[1] H.J. Schatzmann, ATP-dependent Ca^{2+} extrusion from human red cells, Experientia 22 (1966) 364–368.
[2] B. Sarkadi, Active calcium transport in human red cells, Biochim. Biophys. Acta 604 (1980) 159–190.
[3] H.J. Schatzmann, The plasma membrane calcium pump of erythrocytes and other animal cells, in: E. Carafoli (Ed.), Membrane Transport of Calcium, Academic Press, London, 1982.
[4] P.A. Knauf, F. Proverbio, J.F. Hoffman, Electrophoretic separation of different phosphoproteins associated with Ca-ATPase and Na,K-ATPase in human red cell ghosts, J. Gen. Physiol. 63 (1974) 324–336.

[5] S. Katz, R. Blostein, Ca^{2+}-stimulated membrane phosphorylation and ATPase activity of the human erythrocyte, Biochim. Biophys. Acta 389 (1975) 314–324.
[6] R.M. Gopinath, F.F. Vincenzi, Phosphodiesterase protein activator mimics red blood cell cytoplasmic activator of the (Mg^{2+} + Ca^{2+})-ATPase, Biochem. Biopys. Res. Commun. 77 (1977) 1203–1209.
[7] H.W. Jarrett, J.T. Penniston, Partial purification of the (Mg^{2+} + Ca^{2+})-ATPase activator from human erythrocytes: its similarity to the activator of 3':5'-cyclic nucleotide phosphodiesterase, Biochem. Biophys. Res. Commun. 77 (1977) 1210–1216.
[8] H.W. Jarrett, J.T. Penniston, Purification of the Ca^{2+}-stimulated ATPase activator from human erythrocytes: its membership in the class of Ca^{2+}-binding modulator proteins, J. Biol. Chem. 253 (1978) 4676–4682.
[9] W.Y. Cheung, T.J. Lynch, R.W. Wallace, An endogenous Ca^{2+}-dependent activator protein of brain adenylate cyclase and cyclic nucleotide phosphodiesterase, Adv. Cycl. Nucleotide Res. 9 (1978) 233–251.
[10] H.U. Wolf, G. Dieckvoss, R. Lichtner, Purification and properties of high-affinithy Ca^{2+}-ATPase of human erythrocytes, Acta Biol. Med. Ger. 36 (1977) 847–858.
[11] V. Niggli, J.T. Penniston, E. Carafoli, Purification of the (Ca^{2+}-Mg^{2+})-ATPase from human erythrocyte membranes using a calmodulin affinity column, J. Biol. Chem. 254 (1979) 9955–9958.
[12] J.T. Penniston, A.G. Filoteo, C.S. McDonough, E. Carafoli, Purification, reconstitution, and regulation of plasma membrane Ca^{2+}-pumps, Methods Enzymol. 157 (1988) 340–351.
[13] B. Sarkadi, A. Enyedi, G. Gardos, Molecular properties of the red cell calcium pump I Effects of calmodulin, proteolytic digestion and drugs on the kinetics of active calcium uptake in inside-out red cell membrane vesicles, Cell. Calcium 1 (1980) 287–297.
[14] A.F. Rega, Other properties and coupling of Ca^{2+} transport and ATP hydrolysis, in: A.F. Rega, P.J. Garrahan (Eds.), The Ca^{2+} Pump of Plasma Membranes, CRC Press Inc., Boca Raton, 1986, pp. 91–104.
[15] P.J. Garrahan, A.F. Rega, Plasma membrane calcium pump, in: F. Bronner (Ed.), Intracellular Calcium Regulation, Alan R. Liss, 1990, pp. 271–303.
[16] E. Carafoli, Calcium pump of the plasma membrane, Physiol. Rev. 71 (1991) 129–153.
[17] J.T. Penniston, Plasma membrane Ca^{2+}-ATPases as active Ca^{2+} pumps, in: W.Y. Cheung (Ed.), Calcium and Cell Function, Academic Press, New York, 1983, pp. 99–147.
[18] E. Carafoli, Intracellular calcium homeostasis, Annu. Rev. Biochem. 56 (1987) 395–433.
[19] F.C. Mooren, R.K.H. Kinne, Cellular calcium in health and disease, Biochim. Biophys. Acta 1406 (1998) 127–151.
[20] H.J. Schatzmann, The calcium pump of the surface membrane and of the sarcoplasmic reticulum, Annu. Rev. Physiol. 51 (1989) 473–485.
[21] F.F. Vincenzi, The plasma membrane calcium pump the red blood cell as a model, in: P. Agre, J.C. Parker (Eds.), Red Blood Cell Membranes: Structure, Function, Clinical Applications, Marcel Dekker, New York, 1989, pp. 481–505.
[22] E. Carafoli, Biogenesis: plasma membrane calcium ATPase: 15 years of work on the purified enzyme, FASEB J. 8 (1994) 993–1002.
[23] G.E. Shull, J. Greeb, Molecular cloning of two isoforms of the plasma membrane Ca^{2+}-transporting ATPase from rat brain, J. Biol. Chem. 263 (1988) 8646–8657.
[24] A.K. Verma, A.G. Filoteo, D.R. Stanford, E.D. Wieben, J.T. Penniston, E.E. Strehler, R. Fischer, R. Heim, G. Vogel, S. Mathews, M.-A. Strehler-Page, P. James, T. Vorherr, J. Krebs, E. Carafoli, Complete primary structure of a human plasma membrane Ca^{2+} pump, J. Biol. Chem. 263 (1988) 14152–14159.
[25] E.E. Strehler, Recent advances in the molecular characterization of plasma membrane Ca^{2+} pumps, J. Membr. Biol. 120 (1991) 1–15.
[26] E. Carafoli, T. Stauffer, The plasma membrane calcium pump: functional domains, regulation of the activity, and tissue specificity of isoform expression, J. Neurobiol. 25 (1994) 312–324.
[27] J. Lehotsky, Plasma membrane Ca^{2+}-pump functional specialization in the brain. Complex of isoform expression and regulation by effectors, Molec. Chem. Neuropathol. 25 (1995) 175–187.
[28] P.L. Pedersen, E. Carafoli, Ion motive ATPases. I. Ubiquity, properties, and significance to cell function, Trends Biochem. Sci. 12 (1987) 146–150.
[29] J.V. Møller, B. Juul, M. Le Maire, Structural organization, ion transport, and energy transduction of P-type ATPases, Biochim. Biophys. Acta 1286 (1996) 1–51.
[30] M.D. Thever, M.H. Saier, Bioinformatic characterization of P-type ATPases encoded within the fully sequenced genomes of 26 eukaryotes, J. Membr. Biol. 229 (2009) 115–130.
[31] M.G. Palmgren, P. Nissen, P-type ATPases, Ann. Rev. Biophys. 40 (2011) 243–266.
[32] E.E. Strehler, A.J. Caride, A.G. Filoteo, Y. Xiong, J.T. Penniston, Á. Enyedi, Plasma membrane Ca^{2+} ATPases as dynamic regulators of cellular calcium handling, Ann. N. Y. Acad. Sci. 1099 (2007) 226–236.
[33] E.E. Strehler, M.-A. Strehler-Page, G. Vogel, E. Carafoli, mRNAs for plasma membrane calcium pump isoforms differing in their regulatory domain are generated by alternative splicing that involves two internal donor sites in a single exon, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 6908–6912.
[34] R. Heim, M. Hug, T. Iwata, E.E. Strehler, E. Carafoli, Microdiversity of human-plasma-membrane calcium-pump isoform 2 generated by alternative RNA splicing in the N-terminal coding region, Eur. J. Biochem. 205 (1992) 333–340.
[35] H.P. Adamo, J.T. Penniston, New Ca^{2+} pump isoforms generated by alternative splicing of rPMCA2 mRNA, Biochem. J. 283 (1992) 355–359.
[36] S.E. Burk, G.E. Shull, Structure of the rat plasma membrane Ca^{2+}-ATPase isoform 3 gene and characterization of alternative splicing and transcription products, J. Biological Chem. 267 (1992) 19683–19690.
[37] P. Brandt, R.L. Neve, A. Kammesheidt, R.E. Rhoads, T.C. Vanaman, Analysis of the tissue-specific distribution of mRNAs encoding the plasma membrane calcium-pumping ATPases and characterization of an alternately spliced form of PMCA4 at the cDNA and genomic levels, J. Biol. Chem. 267 (1992) 4367–4385.
[38] T.P. Keeton, S.E. Burk, G.E. Shull, Alternative splicing of exons encoding the calmodulin-binding domains and C-termini of plasma membrane Ca^{2+}-ATPase isoforms 1,2,3, and 4, J. Biol. Chem. 268 (1993) 2740–2748.
[39] J. Santiago-García, J. Mas-Oliva, D. Saavedra, A. Zarain-Herzberg, Analysis of mRNA expression and cloning of a novel plasma membrane Ca^{2+}-ATPase splice variant in human heart, Mol. Cell. Biochem. 155 (1996) 173–182.
[40] E.E. Strehler, D.A. Zacharias, Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps, Physiol. Rev. 81 (2001) 21–50.
[41] E.E. Strehler, A.G. Filoteo, J.T. Penniston, A.J. Caride, Plasma-membrane Ca^{2+}-pumps: structural diversity as the basis for functional versatility, Biochem. Soc. Trans. 35 (2007) 919–922.
[42] F. Di Leva, T. Domi, L. Fedrizzi, D. Lim, E. Carafoli, The plasma membrane Ca^{2+} ATPase of animal cells: structure, function and regulation, Arch. Biochem. Biophys. 476 (2008) 65–74.
[43] A. Howard, S. Legon, J.R.F. Walters, Human and rat intestinal plasma membrane calcium pump isoforms, Am. J. Physiol. 265 (1993) G917–G925.
[44] A. Howard, N.F. Barley, S. Legon, J.R.F. Walters, Plasma-membrane calcium-pump isoforms in human and rat liver, Biochem. J. 303 (1994) 275–279.
[45] A. Kamagate, A. Herchuelz, A. Bollen, F. VanEylen, Expression of multiple plasma membrane Ca^{2+}-ATPases in rat pancreatic islet cells, Cell. Calcium 27 (2000) 231–246.
[46] R.S. Silverstein, B.L. Tempel, Atp2b2, encoding plasma membrane Ca^{2+}-ATPase type 2, (PMCA2) exhibits tissue-specific first exon usage in hair cells, neurons, and mammary glands of mice, Neuroscience 141 (2006) 245–257.
[47] T.P. Stauffer, D. Guerini, E. Carafoli, Tissue distribution of the four gene products of the plasma membrane Ca^{2+} pump. A study using specific antibodies, J. Biol. Chem. 270 (1995) 12184–12190.
[48] A.G. Filoteo, N.L. Elwess, A. Enyedi, A. Caride, H.-H. Aung, J.T. Penniston, Plasma membrane Ca^{2+} pump in rat brain. Patterns of alternative splices seen by isoform-specific antibodies, J. Biol. Chem. 272 (1997) 23741–23747.
[49] A. Burette, J.M. Rockwood, E.E. Strehler, R.J. Weinberg, Isoform-specific distribution of the plasma membrane Ca^{2+} ATPase in the rat brain, J. Comp. Neurol. 467 (2003) 464–476.
[50] A. Burette, R.J. Weinberg, Perisynaptic organization of plasma membrane calcium pumps in cerebellar cortex, J. Comp. Neurol. 500 (2007) 1127–1135.
[51] A.C. Burette, E.E. Strehler, R.J. Weinberg, "Fast" plasma membrane calcium pump PMCA2a concentrates in GABAergic terminals in the adult rat brain, J. Comp. Neurol. 512 (2009) 500–513.
[52] T.P. Jensen, L.E. Buckby, R.M. Empson, Expression of plasma membrane Ca^{2+} ATPase family members and associated synaptic proteins in acute and cultured organotypic hippocampal slices from rat, Dev. Brain Res. 152 (2004) 129–136.
[53] J.K. Hill, D.E. Williams, D.E. LeMasurier, R.A. Dumont, E.E. Strehler, P.G. Gillespie, Splice-site A choice targets plasma-membrane Ca^{2+}-ATPase isoform 2 to hair bundles, J. Neurosci. 26 (2006) 6172–6180.
[54] K.A. Kenyon, E.A. Bushong, A.S. Mauer, E.E. Strehler, R.J. Weinberg, A.C. Burette, Cellular and subcellular localizatin of the neuron-specific plasma membrane calcium ATPase PMCA1a in the rat brain, J. Comp. Neurol. 518 (2010) 3169–3183.
[55] T. Afroze, G. Yang, A. Khoshbin, M. Tanwir, T. Tabish, A. Momen, M. Husain, Calcium efflux activity of plasma membrane Ca^{2+} ATPase-4 (PMC4) mediates cell cycle progression in vascular smooth muscle cells, J. Biol. Chem. 289 (2014) 7221–7231.
[56] M. Brini, E. Carafoli, Calcium pumps in health and disease, Physiol. Rev. 89 (2009) 1341–1378.
[57] M. Giacomello, A. De Mario, C. Scarlatti, S. Primerano, E. Carafoli, Plasma membrane calcium ATPases and related disorders, Int. J. Biochem. Cell. Biol. 45 (2013) 753–762.
[58] E.E. Strehler, Plasma membrane calcium ATPases as novel candidates for therapeutic agent development, J. Pharm. Pharm. Sci. 16 (2013) 190–206.
[59] A. Enyedi, T. Vorherr, P. James, D.J. McCormick, A.G. Filoteo, E. Carafoli, J.T. Penniston, The calmodulin binding domain of the plasma membrane Ca^{2+} pump interacts both with calmodulin and with another part of the pump, J. Biol. Chem. 264 (1989) 12313–12321.
[60] R. Falchetto, T. Vorherr, J. Brunner, E. Carafoli, The plasma membrane Ca^{2+} pump contains a site that interacts with its calmodulin-binding domain, J. Biol. Chem. 266 (1991) 2930–2936.
[61] R. Falchetto, T. Vorherr, E. Carafoli, The calmodulin binding site of the plasma membrane Ca^{2+} pump interacts with the transduction domain of the enzyme, Protein Sci. 1 (1992) 1612–1621.

[62] F. Kessler, R. Falchetto, R. Heim, R. Meili, T. Vorherr, E.E. Strehler, E. Carafoli, Study of calmodulin binding to the alternatively spliced C-terminal domain of the plasma membrane Ca^{2+} pump, Biochemistry 31 (1992) 11785–11792.

[63] A. Enyedi, A.K. Verma, R. Heim, H.P. Adamo, A.G. Filoteo, E.E. Strehler, J.T. Penniston, The Ca^{2+} affinity of the plasma membrane Ca^{2+} pump is controlled by alternative splicing, J. Biol. Chem. 269 (1994) 41–43.

[64] N.L. Elwess, A.G. Filoteo, A. Enyedi, J.T. Penniston, Plasma membrane Ca^{2+} pump isoforms 2a and 2b are unusually responsive to calmodulin and Ca^{2+}, J. Biol. Chem. 272 (1997) 17981–17986.

[65] A.J. Caride, N.L. Elwess, A.K. Verma, A.G. Filoteo, Á. Enyedi, Z. Bajzer, J.T. Penniston, The rate of activation by calmodulin of isoform 4 of the plasma membrane Ca^{2+} pump is slow and is changed by alternative splicing, J. Biol. Chem. 274 (1999) 35227–35232.

[66] A.J. Caride, A.G. Filoteo, A.R. Penheiter, K. Pászty, Á. Enyedi, J.T. Penniston, Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca^{2+}: fast pumps reside in fast cells, Cell. Calcium 30 (2001) 49–57.

[67] A.J. Caride, A.R. Penheiter, A.G. Filoteo, Z. Bajzer, Á. Enyedi, J.T. Penniston, The plasma membrane calcium pump displays memory of past calcium spikes. Differences between isoforms 2b and 4b, J. Biol. Chem. 276 (2001) 39797–39804.

[68] K.K.W. Wang, A. Villalobo, B.D. Roufogalis, The plasma membrane calcium pump: a multiregulated transporter, Trends Cell. Biol. 2 (1992) 46–52.

[69] G.R. Monteith, B.D. Roufogalis, The plasma membrane calcium pump – a physiological perspective on its regulation, Cell. Calcium 18 (1995) 459–470.

[70] J.T. Penniston, A. Enyedi, Modulation of the plasma membrane Ca^{2+} pump, J. Membr. Biol. 165 (1998) 101–109.

[71] J. Lehotsky, L. Raeymaekers, L. Missiaen, F. Wuytack, H. DeSmedt, R. Casteels, Stimulation of the catalytic cycle of the Ca^{2+}-pump of porcine plasma-membrane by negatively charged phospholipids, Biochim. Biophys. Acta 1105 (1992) 118–124.

[72] M. Brini, F. Di Leva, C.K. Ortega, T. Domi, D. Ottolini, E. Leonardi, S.C. Tosatto, E. Carafoli, Deletions and mutations in the acidic lipid-binding region of the plasma membrane Ca^{2+} pump: a study on different splicing variants of isoform 2, J. Biol. Chem. 285 (2010) 30779–30791.

[73] R. Lopreiato, M. Giacomello, E. Carafoli, The plasma membrane calcium pump: new ways to look at an old enzyme, J. Biol. Chem. 289 (2014) 10261–10268.

[74] W. Ba-Thein, A.J. Caride, Á. Enyedi, K. Pászty, C.L. Croy, A.G. Filoteo, J.T. Penniston, Chimeras reveal the role of the catalytic core in the activation of the plasma membrane Ca^{2+} pump, Biochem. J. 356 (2001) 241–245.

[75] E. Kim, S.J. DeMarco, S.M. Marfatia, A.H. Chishti, M. Sheng, E.E. Strehler, Plasma membrane Ca^{2+} ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains, J. Biol. Chem. 273 (1998) 1591–1595.

[76] K. Schuh, S. Uldrijan, M. Telkamp, N. Röthlein, L. Neyses, The plasmamembrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase I, J. Cell. Biol. 155 (2001) 201–205.

[77] L.D. Bozulic, M.T. Malik, D.W. Powell, A. Nanez, A.J. Link, K.S. Ramos, W.L. Dean, Plasma membrane Ca($^{2+}$)-ATPase associates with CLP36, alpha-actinin and actin in human platelets, Thromb. Haemost. 97 (2007) 587–597.

[78] M.L. Garside, P.R. Turner, B. Austen, E.E. Strehler, P.W. Beesley, R.M. Empson, Molecular interactions of the plasma membrane calcium ATPase 2 at pre-and post-synaptic sites in rat cerebellum, Neuroscience 162 (2009) 383–395.

[79] W.A. Kruger, G.R. Monteith, P. Poronnik, The plasma membrane Ca^{2+}-ATPase: regulation by PSD-95/Dlg/Zo-1 scaffolds, Int. J. Biochem. Cell. Biol. 42 (2010) 805–808.

[80] S.J. DeMarco, E.E. Strehler, Plasma membrane Ca^{2+}-ATPase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PSD-95/Dlg/ZO-1) domain-containing proteins, J. Biol. Chem. 276 (2001) 21594–21600.

[81] G.M. Goellner, S.J. DeMarco, E.E. Strehler, Characterization of PISP, a novel single-PDZ protein that binds to all plasma membrane Ca^{2+}-ATPase b-splice variants, Ann. N. Y. Acad. Sci. 986 (2003) 461–471.

[82] S.J. DeMarco, M.C. Chicka, E.E. Strehler, Plasma membrane Ca^{2+} ATPase isoform 2b interacts preferentially with Na^+/H^+ exchanger regulatory factor 2 in apical plasma membranes, J. Biol. Chem. 277 (2002) 10506–10511.

[83] W.A. Kruger, C.C. Yun, G.R. Monteith, P. Poronnik, Muscarinic-induced recruitment of plasma membrane Ca^{2+}-ATPase involves PSD-95/Dlg/Zo-1-mediated interactions, J. Biol. Chem. 284 (2009) 1820–1830.

[84] D. Oceandy, E.J. Cartwright, M. Emerson, S. Prehar, F.M. Baudoin, M. Zi, N. Alatwi, L. Venetucci, K. Schuh, J.C. Williams, A.L. Armesilla, N. L, Neuronal nitric oxide synthase signaling in the heart is regulated by the sarcolemmal calcium pump 4b, Circulation 115 (2007) 483–492.

[85] D. Oceandy, P.J. Stanley, E.J. Cartwright, L. Neyses, The regulatory function of plasma-membrane Ca^{2+}-ATPase (PMCA) in the heart, Biochem. Soc. Trans. 35 (2007) 927–930.

[86] T.M.A. Mohamed, D. Oceandy, S. Prehar, N. Alatwi, Z. Hegab, F.M. Baudoin, A. Pickard, A.O. Zaki, R. Nadif, E.J. Cartwright, L. Neyses, Specific role of neuronal nitric-oxide synthase when tethered to the plasma membrane calcium pump in regulating the b-adrenergic signal in the myocardium, J. Biol. Chem. 284 (2009) 12091–12098.

[87] M. Zabe, W.L. Dean, Plasma membrane Ca^{2+}-ATPase associates with the cytoskeleton in activated platelets through a PDZ binding domain, J. Biol. Chem. 276 (2001) 14704–14709.

[88] D. Gómez-Varela, M. Schnidt, J. Schoellerman, E.C. Peters, D.K. Berg, PMCA2 via PSD-95 controls calcium signaling by a7-containing nicotinic acetylcholine receptors on aspiny interneurons, J. Neurosci. 32 (2012) 6894–6905.

[89] A.L. Armesilla, J.C. Williams, M.H. Buch, A. Pickard, M. Emerson, E.J. Cartwright, D. Oceandy, M.D. Vos, S. Gillies, G.J. Clark, L. Neyses, Novel functional interaction between the plasma membrane Ca^{2+} pump 4b and the proapoptotic tumor suppressor Ras-associated factor 1 (RASSF1), J. Biol. Chem. 279 (2004) 31318–31328.

[90] M.H. Buch, A. Pickard, A. Rodriguez, S. Gillies, A.H. Maass, M. Emerson, E.J. Cartwright, J.C. Williams, D. Oceandy, J.M. Redondo, L. Neyses, A.L. Armesilla, The sarcolemmal calcium pump inhibits the calcineurin/nuclear factor of activated T-cell pathway via interaction with the calcineurin A catalytic subunit, J. Biol. Chem. 280 (2005) 29479–29487.

[91] A. Rimessi, L. Coletto, P. Pinton, R. Rizzuto, M. Brini, E. Carafoli, Inhibitory interaction of the 14-3-3e protein with isoform 4 of the plasma membrane Ca^{2+}-ATPase pump, J. Biol. Chem. 280 (2005) 37195–37203.

[92] Y.M. Yang, J. Lee, H. Jo, S. Park, I. Chang, S. Muallem, D.M. Shin, Homer2 protein regulates plasma membrane Ca^{2+}-ATPase-mediated Ca^{2+} signaling in mouse parotid gland acinar cells, J. Biol. Chem. 289 (2014) 24971–24979.

[93] J.C. Williams, A.L. Armesilla, T.M.A. Mohamed, C.L. Hagarty, F.H. McIntyre, S. Schomburg, A.O. Zaki, D. Oceandy, E.J. Cartwright, M.H. Buch, M. Emerson, L. Neyses, The sarcolemmal calcium pump, a-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex, J. Biol. Chem. 281 (2006) 23341–23348.

[94] M.G. Dalghi, M.M. Fernandez, M. Ferreira Gomes, I.C. Mangialavori, E.L. Malchiodi, E.E. Strehler, J.P.F.C. Rossi, Plasma membrane calcium ATPase activity is regulated by actin oligomers through direct interaction, J. Biol. Chem. 288 (2013) 23380–23393.

[95] J. de Juan-Sanz, E. Nunez, F. Zafra, M. Berrocal, I. Corbacho, I. Ibanez, E. Arribas-Gonzalez, D. Marcos, B. Lopez-Corcuera, A.M. Mata, C. Aragon, Presynaptic control of glycine transporter 2 (GlyT2) by physical and functional association with plasma membrane Ca^{2+}-ATPase (PMCA) and Na^+-Ca^{2+} exchanger (NCX), J. Biol. Chem. 289 (2014) 34308–34324.

[96] G. Krapivinsky, L. Krapivinsky, S.C. Stotz, Y. Manasian, D.E. Clapham, POST, partner of stromal interaction molecule 1 (STIM), targets STIM1 to multiple transporters, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 19234–19239.

[97] M.F. Ritchie, E. Samakai, J. Soboloff, STIM1 is required for attenuation of PMCA-mediated Ca^{2+} clearance during T-cell activation, EMBO J. 31 (2012) 1123–1133.

[98] M.R. Sepúlveda, M. Berrocal-Carrillo, M. Gasset, A.M. Mata, The plasma membrane Ca^{2+}-ATPase isoform 4 is localized in lipid rafts of cerebellum synaptic plasma membranes, J. Biol. Chem. 281 (2006) 447–453.

[99] L. Jiang, D. Fernandes, N. Mehta, J.L. Bean, M.L. Michaelis, A. Zaidi, Partitioning of the plasma membrane Ca^{2+}-ATPase into lipid rafts in primary neurons: effects of cholesterol depletion, J. Neurochem. 102 (2007) 378–388.

[100] P.J. Kozel, R.A. Friedman, L.C. Erway, E.N. Yamoah, L.H. Liu, T. Riddle, J.J. Duffy, T. Doetschman, M.L. Miller, E.L. Cardell, G.E. Shull, Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2, J. Biol. Chem. 273 (1998) 18693–18696.

[101] G.W. Okunade, M.L. Miller, G.J. Pyne, R.L. Sutliff, K.T. O'Connor, J.C. Neumann, A. Andringa, D.A. Miller, V. Prasad, T. Doetschman, R.J. Paul, G.E. Shull, Targeted ablation of plasma membrane Ca^{2+}-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4, J. Biol. Chem. 279 (2004) 33742–33750.

[102] K. Schuh, E.J. Cartwright, E. Jankevics, K. Bundschu, J. Liebermann, J.C. Williams, A.L. Armesilla, M. Emerson, D. Oceandy, K.-P. Knobeloch, L. Neyses, Plasma membrane Ca^{2+} ATPase 4 is required for sperm motility and male fertility, J. Biol. Chem. 279 (2004) 28220–28226.

[103] M. Giacomello, A. De Mario, S. Primerano, M. Brini, E. Carafoli, Hair cells, plasma membrane Ca^{2+} ATPase and deafness, Int. J. Biochem. Cell. Biol. 44 (2012) 679–683.

[104] J. Lehotsky, P. Kaplan, R. Murin, L. Raeymaekers, The role of plasma membrane Ca^{2+} pump (PMCA) in pathologies of mammalian cells, Front. Biosci. 7 (2002) d53–84.

[105] S. Sasamura, K.-I. Furukawa, M. Shiratori, S. Motomura, Y. Ohizumi, Antisense-inhibition of plasma membrane Ca^{2+} pump induces apoptosis in vascular smooth muscle cells, Jpn. J. Pharmacol. 90 (2002) 164–172.

[106] A. Bhargava, R.S. Mathias, J.A. McCormick, M.F. Dallman, D. Pearce, Glucocorticoids prolong Ca^{2+} transients in hippocampal-derived H19-7 neurons by repressing the plasma membrane Ca^{2+} ATPase-1, Mol. Endocrinol. 16 (2002) 1629–1637.

[107] D. Fernandes, A. Zaidi, J. Bean, D. Hui, M.L. Michaelis, RNA_i-induced silencing of the plasma membrane Ca^{2+}-ATPase 2 in neuronal cells: effects on Ca^{2+} homeostasis and cell viability, J. Neurochem. 101 (2007) 454–465.

[108] J. Szemraj, I. Kawecka, J. Bartkowiak, L. Zylinska, The effect of antisense oligonucleotide treatment of plasma membrane Ca^{2+}-ATPase in PC12 cells, Cell. Molec. Biol. Lett. 9 (2004) 451–464.

[109] M.C. Curry, N.A. Luk, P.A. Kenny, S.J. Roberts-Thomson, G.R. Monteith, Distinct regulation of cytoplasmic calcium signals and cell death pathways by different plasma membrane calcium ATPase isoforms in MDA-MB-231 breast cancer cells, J. Biol. Chem. 287 (2012) 28598–28608.

[110] J. VanHouten, C. Sullivan, C. Bazinet, T. Ryoo, R. Camp, D.L. Rimm, G. Chung, J. Wysolmerski, PMCA2 regulates apoptosis during mammary gland

involution and predicts outcome in breast cancer, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 11405–11410.
[111] M.P. Kurnellas, H. Li, M.R. Jain, S.N. Giraud, A.B. Nicot, A. Ratnayake, R.F. Heary, S. Elkabes, Reduced expression of plasma membrane calcium ATPase 2 and collapsin response mediator protein 1 promotes death of spinal cord neurons, Cell. Death Diff. 17 (2010) 1501–1510.
[112] K. Schuh, T. Quaschning, S. Knauer, K. Hu, S. Koçak, N. Roethlein, L. Neyses, Regulation of vascular tone in animals overexpresing the sarcolemmal calcium pump, J. Biol. Chem. 278 (2003) 41246–41252.
[113] R. Gros, T. Afroze, X.-M. You, G. Kabir, R. Van Wert, W. Kalair, A.E. Hoque, I.N. Mungrue, M. Husain, Plasma membrane calcium ATPase overexpression in arterial smooth muscle increases vasomotor responsiveness and blood pressure, Circ. Res. 93 (2003) 614–621.
[114] T.M.A. Mohamed, D. Oceandy, M. Zi, S. Prehar, N. Alatwi, Y. Wang, M.A. Shaheen, R. Abou-Leisa, C. Schelcher, Z. Hegab, F. Baudoin, M. Emerson, M. Mamas, G. Di Benedetto, M. Zaccolo, M. Lei, E.J. Cartwright, L. Neyses, Plasma membrane calcium pump (PMCA4)-neuronal nitric-oxide synthase complex regulates cardiac contractility through modulation of a compartmentalized cyclic nucleotide microdomain, J. Biol. Chem. 286 (2011) 41520–41529.
[115] V. Prasad, J.N. Lorenz, V.M. Lasko, M.L. Nieman, M. Jiang, X. Gao, J. Rubinstein, D.F. Wieczorek, G.E. Shull, Ablation of plasma membrane Ca^{2+}-ATPase isoform 4 prevents development of hypertrophy in a model of hypertrophic cardiomyopathy, J. Mol. Cell. Cardiol. 77 (2014) 53–63.
[116] Y.S. Cho, M.J. Go, Y.J. Kim, J.Y. Heo, J.H. Oh, H.-J. Ban, D. Yoon, M.H. Lee, D.-J. Kim, M. Park, S.-H. Cha, J.-W. Kim, B.-G. Han, H. Min, Y. Ahn, M.S. Park, H.R. Han, H.-Y. Jang, E.Y. Cho, J.-E. Lee, N.H. Cho, C. Shin, T. Park, J.W. Park, J.-K. Lee, L. Cardon, G. Clarke, M.I. McCarthy, J.-Y. Lee, J.-K. Lee, B. Oh, H.-L. Kim, A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits, Nat. Genet. 41 (2009) 527–534.
[117] F. Takeuchi, M. Isono, T. Katsuya, K. Yamamoto, M. Yokota, T. Sugiyama, T. Nabika, A. Fujioka, K. Ohnaka, H. Asano, Y. Yamori, S. Yamaguchi, S. Kobayashi, R. Takayanagi, T. Ogihara, N. Kato, Blood pressure and hypertension are associated with 7 loci in the Japanese population, Circulation 121 (2010) 2302–2309.
[118] K.W. Hong, M.J. Go, H.S. Jin, J.E. Lim, J.Y. Lee, B.G. Han, S.Y. Hwang, S.H. Lee, H.K. Park, Y.S. Cho, B. Oh, Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts, J. Hum. Hypertens. 24 (2010) 367–372.
[119] T.I.C.f.B.P.G.-W.A. Studies, Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk, Nature 478 (2011) 103–109.
[120] Y. Kobayashi, N. Hirawa, Y. Tabara, H. Muraoka, M. Fujita, N. Miyazaki, A. Fujiwara, Y. Ichikawa, Y. Yamamoto, N. Ichihara, S. Saka, H. Wakui, S. Yoshida, K. Yatsu, Y. Toya, G. Yasuda, K. Kohara, Y. Kita, K. Takei, Y. Goshima, Y. Ishikawa, H. Ueshima, T. Miki, S. Umemura, Mice lacking hypertension candidate gene Atp2b1 in vascular smooth muscle cells show significant blood pressure elevation, Hypertension 59 (2012) 854–860.
[121] H.J. Kim, V. Prasad, S.-W. Hyung, Z.H. Lee, S.-W. Lee, A. Bhargava, D. Pearce, Y. Lee, H.-H. Kim, Plasma membrane calcium ATPase regulates bone mass by fine-tuning osteoclast differentiation and survival, J. Cell. Biol. 199 (2012) 1145–1158.
[122] E. Aihara, C.L. Hentz, A.M. Korman, N.P.J. Perry, V. Prasad, G.E. Shull, M.H. Montrose, *In vivo* epithelial wound repair requires mobilization of endogenous intracellular and extracellular calcium, J. Biol. Chem. 288 (2013) 33585–33597.
[123] C. Liu, H. Weng, L. Chen, S. Yang, H. Wang, G. Debnath, X. Guo, L. Wu, N. Mohandas, X. An, Impaired intestinal calcium absorption in protein 4.1R-deficient mice due to altered expression of plasma membrane calcium ATPase 1b (PMCA1b), J. Biol. Chem. 288 (2013) 11407–11415.
[124] R. Padányi, Y. Xiong, G. Antalffy, K. Lór, K. Pászty, E.E. Strehler, A. Enyedi, Apical scaffolding protein NHERF2 modulates the localization of alternatively spliced plasma membrane Ca^{2+} pump 2b variants in polarized epithelial cells, J. Biol. Chem. 285 (2010) 31704–31712.
[125] V.A. Street, J.W. McKee-Johnson, R.C. Fonseca, B.L. Tempel, K. Noben-Trauth, Mutations in a plasma membrane Ca^{2+}-ATPase gene cause deafness in deafwaddler mice, Nat. Genet. 19 (1998) 390–394.
[126] K. Takahashi, K. Kitamura, A point mutation in a plasma membrane Ca^{2+}-ATPase gene causes deafness in Wriggle Mouse Sagami, Biochem. Biophys. Res. Commun. 261 (1999) 773–778.
[127] J.M. Schultz, Y. Yang, A.J. Caride, A.G. Filoteo, A.R. Penheiter, A. Lagziel, R.J. Morell, S.A. Mohiddin, L. Fananapazir, A.C. Madeo, J.T. Penniston, A.J. Griffith, Modification of human hearing loss by plasma-membrane calcium pump PMCA2, N. Engl. J. Med 352 (2005) 1557–1564.
[128] M. Giacomello, A. De Mario, R. Lopreiato, S. Primerano, M. Campeol, M. Brini, E. Carafoli, Mutations in PMCA2 and hereditary deafness: a molecular analysis of the pump defect, Cell. Calcium 50 (2011) 569–576.
[129] M.P. Kurnellas, A.K. Lee, H. Li, L. Deng, D.J. Ehrlich, S. Elkabes, Molecular alterations in the cerebellum of the plasma membrane calcium ATPase 2 (PMCA2)-null mouse indicate abnormalities in Purkinje neurons, Mol. Cell. Neurosci. 34 (2007) 178–188.
[130] R.M. Empson, M.L. Garside, T. Knöpfel, Plasma membrane Ca^{2+} ATPase 2 contributes to short-term synapse plasticity at the parallel fibre to Purkinje neurone synapse, J. Neurosci. 27 (2007) 3753–3758.
[131] R.M. Empson, P.R. Turner, R.Y. Nagaraja, P.W. Beesley, T. Knöpfel, Reduced expression of the Ca^{2+} transporter protein PMCA2 slows Ca^{2+} dynamics in mouse cerebellar Purkinje neurones and alters the precision of motor coordination, J. Physiol. 588.6 (2010) 907–922.
[132] A.K. Fakira, S. Elkabes, Role of plasma membrane calcium ATPase 2 in spinal cord pathology, World J. Biol. Chem. 1 (2010) 103–108.
[133] D. Oceandy, T.M.A. Mohamed, E.J. Cartwright, L. Neyses, Local signals with global impacts and clinical implications: Lessons from the plasma membrane calcium pump (PMCA4), Biochim. Biophys. Acta 1813 (2011) 974–978.
[134] M. Li, P.W.-L. Ho, S.Y.-Y. Pang, Z.H.-M. Tse, M.H.-W. Kung, P.-C. Sham, S.-L. Ho, PMCA4 (ATP2B4) mutation in familial spastic paraplegia, PLOS One 9 (2014) e104790.