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Plasma membrane Ca^{2+}-ATPases as dynamic regulators of cellular calcium handling

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Abstract

Plasma membrane Ca^{2+} ATPases (PMCAs) are essential components of the cellular toolkit to regulate and fine-tune cytosolic Ca^{2+} concentrations. Historically, the PMCAs have been assigned a housekeeping role in the maintenance of intracellular Ca^{2+} homeostasis. More recent work has revealed a perplexing multitude of PMCA isoforms and alternative splice variants, raising questions about their specific role in Ca^{2+} handling under conditions of varying Ca^{2+} loads. Studies on the kinetics of individual isoforms, combined with expression and localization studies suggest that PMCAs are optimized to function in Ca^{2+} regulation according to tissue- and cell-specific demands. Different PMCA isoforms help control slow, tonic Ca^{2+} signals in some cells and rapid, efficient Ca^{2+} extrusion in others. Localized Ca^{2+} handling requires targeting of the pumps to specialized cellular locales such as the apical membrane of cochlear hair cells or the basolateral membrane of kidney epithelial cells. Recent studies suggest that alternatively spliced regions in the PMCAs are responsible for their unique targeting, membrane localization, and signaling cross-talk. The regulated deployment and retrieval of PMCAs from specific membranes provides a dynamic system for a cell to respond to changing needs of Ca^{2+} regulation.

Keywords

Calcium homeostasis; calcium pump; calcium signaling; PMCA; splice variant

INTRODUCTION

Ionized calcium (Ca^{2+}) acts as universal messenger controlling cellular processes ranging from fertilization to programmed cell death.[1,2] The signaling function of Ca^{2+} requires an elaborate “toolkit” of proteins to allow Ca^{2+} influx, efflux, and buffering in and between different cellular compartments and among different cells.[3] To achieve spatial and temporal signal sensitivity appropriate types and amounts of the calcium handling proteins must be precisely localized in cellular locales to form tightly connected “calcium signalosomes”. High signal sensitivity is achieved by keeping cytosolic [Ca^{2+}] at very low levels during the resting state. This is accomplished by Ca^{2+}-buffering proteins as well as by membrane-intrinsic Ca^{2+} transport systems capable of removing Ca^{2+} from the cytosol even against a large concentration gradient. ATP-driven calcium pumps and ion gradient-dependent Na^{+}/

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Ca^{2+} exchangers are the major systems responsible for such "uphill" transport of Ca^{2+} across biological membranes. Plasma membrane Ca^{2+} ATPases (PMCAs) are high-affinity Ca^{2+} pumps dedicated to the expulsion of Ca^{2+} from the cytosol into the extracellular space. Because of their ubiquitous expression and low capacity, they have traditionally been thought to act as major housekeeping system responsible for setting and maintaining the normally low cytosolic [Ca^{2+}]. However, the discovery of a multitude of PMCA isoforms and alternative splice variants, as well as recent results on PMCA "knockout" mice and PMCA mutants show that at least some PMCAs have a bigger role in local Ca^{2+} handling. The identification of a growing number of specific PMCA-interacting proteins with regulatory, targeting, and signaling functions further supports the new paradigm that PMCAs are not only responsible for global Ca^{2+} homeostasis but are dynamic participants in spatially defined Ca^{2+} signaling.

MULTIPLE PMCA ISOFORMS AND ALTERNATIVE SPLICE VARIANTS: SPECIFIC ROLES IN CELLULAR CALCIUM REGULATION

PMCAs belong to the type IIB subfamily within the large superfamily of P-type ATPases.[4] In mammals, four separate genes code for the major PMCA isoforms 1–4. In humans, these genes (genome database nomenclature ATP2B1-ATP2B4) are found on chromosomes 12q21.3, 3p25.3, Xq28, and 1q32.1, whereas the mouse genes (database nomenclature Atp2b1-Atp2b4) are located on chromosomes 10C3, 6E3, XA7.3, and 1E4, respectively. Alternative RNA splicing further augments the diversity of PMCA isoforms: splicing at two "hotspots" named site A and site C results in the generation of over 20 PMCA variants.[5] The splice variants differ in the length of the first intracellular loop (site A splicing) and in the C-terminal tail (site C splicing). A scheme of the overall PMCA topology and the location of splice hotspots A and C is shown in Figure 1. Table I lists data on the major PMCA isoforms and splice variants in humans. The various PMCA isoforms and splice variants show developmental-, tissue-, and cell-specific patterns of expression. The ubiquitous PMCA1x/b is detected at the earliest time point in developing mouse embryos and is expressed in most tissues throughout life.[6] By contrast, the splice variants PMCA1x/a and PMCA1x/c are only expressed in specific tissues and cell types and are found mainly in differentiated neurons and striated muscle, respectively.[5,7] Similarly, PMCA isoforms 2 and 3 are almost exclusively expressed in excitable tissues, with some splice forms such as PMCA2w/a being specific for select cell types (e.g., auditory and vestibular hair cells).[8] Multiple splice forms of PMCA isoforms 2 and 3 are also expressed in insulin-secreting β-cells of the endocrine pancreas.[9] Notably, these cells are electrically excitable and display characteristic Ca^{2+} oscillations. The difference in expression pattern and abundance suggests that the different PMCA isoforms and splice variants fulfill different roles in cellular Ca^{2+} regulation. Recent work on mice with targeted deletions or spontaneous mutations of specific PMCA genes supports this notion: homozygous Atp2b1−/− mice are embryonic lethal, Atp2b2-/- mice are ataxic and profoundly deaf, and Atp2b4−/− mice show male infertility.[10–12]

DIFFERENT KINETICS OF REGULATION: PMCA ISOFORMS ARE ADAPTED TO SPECIFIC CALCIUM HANDLING NEEDS

The main regulator of PMCA function is Ca^{2+}-calmodulin.[13] In the absence of calmodulin (CaM), the pumps are auto-inhibited by a mechanism that involves binding of their C-terminal tail to the two major intracellular loops. Activation requires binding of Ca^{2+}-CaM to the C-terminal tail and a conformational change that displaces the auto-inhibitory tail from the major catalytic domain. Release of auto-inhibition may be facilitated by means other than CaM binding, including by acidic phospholipids, protein kinase A- or C-mediated phosphorylation of specific (Ser/Thr) residues in the C-terminal tail, partial proteolytic

cleavage of the tail (e.g., by calpain or caspases), or dimerization via the C-terminal tail.[14,15] Different PMCA isoforms show significant differences in their regulation by kinases and CaM. For example, some isoforms are activated by PKC (PMCA4b) whereas others are unaffected (PMCA4a) or even slightly inhibited (PMCA2a, 3a).[16–18] The CaM affinity and the extent of activation by CaM also vary greatly among PMCA isoforms and splice variants, with the a-splice forms generally showing lower CaM affinity but higher basal (CaM-independent) activity than the b-splice forms.[19,20]

The functional differences among PMCA isoforms are relevant to how the pumps handle temporary changes in $[Ca^{2+}]_i$. Recent work on the rates of activation of individual PMCAs has revealed significant differences in their ability to respond to a sudden increase in Ca^{2+} and to decode the frequency of repetitive Ca^{2+} spikes.[21] The rate for CaM activation of PMCA4b is slow ($t_{1/2}$ ~ 1 min at 0.5 μM Ca^{2+}) compared to PMCA4a ($t_{1/2}$ ~ 20 sec). The inactivation rate (off-rate) for PMCA4b is even slower ($t_{1/2}$ ~ 20 min), indicating that this isoform may be geared towards handling slow, tonic Ca^{2+} changes in non-excitable cells.[22] Because a change in sub-plasmalemmal Ca^{2+} is the crucial physiological event sensed by the PMCAs, their "reaction time" to such a stimulus should be optimal for the desired outcome of Ca^{2+} signaling. Indeed, detailed kinetic studies showed that PMCAs expressed in cells with a need for fast Ca^{2+} responses (e.g., striated muscles, neurons, sensory hair cells) are generally "fast", i.e., they are activated quickly by a rise in $[Ca^{2+}]_i$.[23] Accordingly PMCAs 2a and 3f, which are expressed mostly in excitable tissues, are very fast pumps whilst PMCA4b is a slow pump. The very different off-rates for CaM release (and hence, inactivation) also result in pronounced differences in the "memory" of PMCA isoforms for their previous activation. For example, PMCA2b has a very slow off-rate for CaM dissociation and retains a long memory of its recent activation.[21] In cells with repetitive Ca^{2+} spikes, PMCA2b will thus remain "pre-activated" for an extended time and respond almost immediately to a new Ca^{2+} signal. This facilitation of Ca^{2+} extrusion may be crucial in neurons to maintain sensitivity to signal frequency.

PMCAS ARE INTEGRATED INTO SIGNALING PATHWAYS VIA MULTIPLE PROTEIN-PROTEIN INTERACTIONS

Besides the well-known regulation of PMCAs by CaM, the pumps are affected in less well-studied ways by multiple kinases (both Ser/Thr-specific and Tyr-specific); these probably relay important information about the cell's metabolic state to the calcium pump extrusion system.[15,24] In addition, recent work based on yeast or bacterial two-hybrid screens has identified numerous PMCA-interacting proteins that are likely to connect specific PMCA isoforms to particular signaling pathways in the cell. Figure 2 provides a schematic overview of the major currently known interacting proteins and the site of their interaction with the pump. Many of these protein interactions are specific for a subset of PMCA isoforms and/or splice variants. For example, all b-splice variants, but none of the a-splice variants contain a C-terminal consensus motif for binding PDZ domains. It is perhaps not surprising that specific interacting partners are found for the most divergent regions of the PMCAs, including the alternatively spliced C-tail and the N-terminal tail. On the other hand, some of the interacting proteins are promiscuous and bind to several PMCA isoforms with similar affinity. This includes members of the MAGUK (membrane-associated guanylate kinase) family of PDZ proteins that recognize a short peptide at the C-terminus of PMCA b-splice variants,[25] and α-1 syntrophin, which was recently found to interact with a conserved domain in the major cytosolic loop of PMCA4 and PMCA1.[26] In the following sections, we will briefly summarize the main classes of recently discovered PMCA-interacting proteins and their possible roles in PMCA regulation and signaling cross-talk.

Proteins interacting with the N-terminal tail

Using the N-terminal cytosolic tail of human PMCA4 as bait in a yeast two-hybrid screen, Rimessi and co-workers[27] recently identified the protein 14-3-3ε as a novel interaction partner of PMCA4. This interaction appears to be specific for the N-terminal tail of PMCA4, as the corresponding region of PMCA2 was unable to interact with 14-3-3ε. When over-expressed in HeLa cells, 14-3-3ε had an inhibitory effect on PMCA4 function as measured by the ability of the cells to clear an agonist-induced Ca^{2+} load. While direct regulation (inhibition) of PMCA4 activity is one possibility for the functional role of the 14-3-3ε/PMCA4 interaction, 14-3-3ε is also involved in a variety of other tasks, including protein trafficking and cellular signaling. Given the large number of 14-3-3ε interacting proteins and the diverse tasks already ascribed to 14-3-3ε, a role for this protein in PMCA4 chaperoning and/or trafficking appears plausible.

Proteins interacting with the large cytosolic loops

No interactions with other proteins have yet been described for the first cytosolic loop of the PMCA. However, this region is known to be involved in the acidic phospholipid-sensitivity of the PMCA and to make intra-molecular contacts with the auto-inhibitory C-tail of the pump.[5] This loop also contains the alternative splice site A, where the insertion of extra amino acids affects membrane targeting (Fig. 2):[28] in PMCA2, the w-splice insert directs the pump to the apical membrane of cochlear hair cells.[8,29] This differential membrane targeting may require specific protein interactions of the loop (e.g., with apical/basolateral sorting proteins) to discriminate between the w- and the x/z-splice variants.

At least three different proteins have recently been found to physically interact with the large catalytic loop of PMCA4b (see Fig. 2): the Ras effector protein RASSF1,[30] the catalytic A-subunit of the Ca^{2+}/CaM-dependent phosphatase calcineurin,[31] and α-1 syntrophin.[26] Because these proteins interact with regions in the catalytic domain that are highly conserved among all PMCAs, they are likely promiscuous and affect all PMCA isoforms. Indeed, α-1 syntrophin was shown to bind to both PMCA1 and PMCA4.[26] Functionally, RASSF1 interaction with PMCA4b appears to downregulate Ras-mediated signaling from the EGF receptor to the Erk phosphorylation and transcriptional activation pathway. However, the physiological relevance of this observation remains to be determined as the published data were all obtained in cells over-expressing recombinant proteins.[30] Similarly, the interaction of (over-expressed) PMCA4b with calcineurin A seems to downregulate calcineurin-mediated signaling events (such as NFAT transcriptional activity), perhaps by recruiting calcineurin to the plasma membrane into microdomains of low $[Ca^{2+}]_i$.[31] Alpha-1 syntrophin is thought to form a ternary signaling complex with the PMCA and nitric oxide synthase-1 (which binds PMCA4b independently through a PDZ domain; see below). This complex may tether the PMCA to the dystrophin complex in skeletal and heart muscle.[26] The above examples suggest that PMCAs may be directly involved in the regulation of downstream signaling events, supporting their integration in bi-directional signaling cross-talk from and to the membrane. It must be noted, however, that the impact of any of the above interactions on PMCA function has not yet been studied. Because RASSF1, calcineurin A, and α-1 syntrophin all bind to the large cytosolic loop of the PMCA that is also involved in intra-molecular interactions with the C-tail, the question arises whether these external proteins compete with the C-tail for binding to the catalytic loop, and if and how their binding affects pump activation.

Proteins interacting with the C-terminal tail

Calmodulin is the “prototype” of all PMCA-interacting proteins, having been identified as major regulator of PMCA activity almost 30 years ago.[32] Besides CaM, many additional proteins have now been found to bind to the C-tail of the PMCA, and the list of new

interacting proteins is still growing (Fig. 2). As already mentioned, the b-splice variants of all PMCA isoforms contain the consensus sequence E-T/S-X-L/V for binding type-I PDZ domains at their carboxyl terminus. PDZ proteins shown to interact with the PMCAs include members of the membrane-associated guanylate kinase (MAGUK) family such as the synapse-associated proteins PSD95/SAP90, SAP97/hDlg, SAP102, and PSD93/chapsyn-110,[25] as well as the Ca^{2+}/CaM-dependent serine protein kinase CASK.[33] These protein interactions serve to cluster specific PMCA isoforms into multi-protein signaling complexes at cellular sites such as pre-synaptic nerve terminals or post-synaptic spines. By recruiting PMCAs into close proximity of Ca^{2+} entry receptors/channels (e.g, NMDA receptors), cells are able to generate microdomains of spatially confined Ca^{2+} signaling. Similarly, the interaction with Homer-1/Ania-3 may allow specific PMCAs to be functionally coupled to TrpC and IP_3 receptor Ca^{2+} channels.[34] Consistent with the theme of PMCA-mediated local Ca^{2+} control, the interaction of PMCA4b with nitric oxide synthase-1 (NOS-1) down-regulates NO production presumably due to a PMCA-mediated decrease of local [Ca^{2+}] in the immediate vicinity of NOS-1.[35] By contrast, the functional role of the interaction between the single-PDZ protein PISP/AIPP1[36,37] and the PMCA b-splice variants is less clear. The predominant localization of PISP/AIPP1 in punctuate, presumably vesicular structures suggests that it may be involved in the trafficking of the pumps to or from the plasma membrane. Finally, the interaction of NHERF2 with PMCA2b[38] is of interest. The apically localized NHERF2 may link apically targeted PMCA2b to the underlying actin cytoskeleton (via its ezrin/radixin/moesin-interacting domain) and thereby promote local retention and enhanced apical localization of the pump.

CONCLUSIONS: PMCAS ARE A DYNAMIC SYSTEM TO RESPOND TO CHANGING CA^{2+} HANDLING NEEDS

Work on gene-targeted mice and PMCA mutants, combined with detailed studies of the sub-cellular localization, regulation and kinetics, and novel protein interactions of PMCAs has shown that specific pump isoforms and splice variants fulfill precisely orchestrated roles in handling local Ca^{2+} changes. This requires their integration in multiple intracellular signaling pathways, and mandates that they act as both receiver and sender of signals. Examples include the involvement of specific PMCAs in calcineurin signaling,[31] local NO signaling,[35] and controlling the duration of action potential after-hyperpolarization in sensory neurons.[39] To respond dynamically to the changing needs of Ca^{2+} signaling, cells must be able to precisely control the type, amount, localization, and activation state of each PMCA. This control is largely exerted via proteins that specifically interact with the PMCAs, as discussed throughout this review. As dynamic participants in cellular Ca^{2+} handling, the PMCAs must be regulated at all levels. *Long-term regulation* involves changes of gene transcription, mRNA stability, alternative splicing, and protein translation. This type of regulation is controlled by factors such as cell differentiation and changes in $[Ca^{2+}]_i$ itself. In the *mid-term*, local PMCA availability is regulated by differential membrane targeting, internalization and possibly, recycling. PDZ proteins such as NHERF2, PISP/AIPP1, and MAGUKs are probably involved in this control and provide isoform/splice variant-specificity to the regulation. Finally, *short-term regulation* is provided by calmodulin, differential phosphorylation, interactions with other signaling molecules, and partial proteolysis (e.g., by calpain and caspases). This short-term regulation is highly isoform-specific (as illustrated by the very different CaM activation kinetics of PMCA2b and PMCA4b) and further contributes to the diversity of local Ca^{2+} handling. Multiple levels of control allow the dynamic regulation of PMCA function on time scales ranging from seconds to days, and enable the PMCAs to participate in varying tasks from fast Ca^{2+} signaling in neurons to managing slow Ca^{2+} transients and bulk Ca^{2+} movement in trans-epithelial Ca^{2+} flux.

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REFERENCES

1. Berridge MJ, Bootman MD, Lipp P. Calcium - a life and death signal. Nature. 1998; 395:645–648. [PubMed: 9790183]
2. Carafoli E, et al. Generation, control, and processing of cellular calcium signals. Crit. Rev. Biochem. Molec. Biol. 2001; 36:107–260. [PubMed: 11370791]
3. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. Nature Rev. Mol. Cell Biol. 2003; 4:517–529. [PubMed: 12838335]
4. Axelsen KB, Palmgren MG. Evolution of substrate specificities in the P-type ATPase superfamily. J. Mol. Evol. 1998; 46:84–101. [PubMed: 9419228]
5. Strehler EE, Zacharias DA. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol. Rev. 2001; 81:21–50. [PubMed: 11152753]
6. Zacharias DA, Kappen C. Developmental expression of the four plasma membrane calcium ATPase (Pmca) genes in the mouse. Biochim. Biophys. Acta. 1999; 1428:397–405. [PubMed: 10434059]
7. Kip SN, et al. Changes in the expression of plasma membrane calcium extrusion systems during the maturation of hippocampal neurons. Hippocampus. 2006; 16:20–34. [PubMed: 16200642]
8. Hill JK, et al. Splice-site A choice targets plasma-membrane Ca^{2+}-ATPase isoform 2 to hair bundles. J. Neurosci. 2006; 26:6172–6180. [PubMed: 16763025]
9. Kamagate A, et al. Expression of multiple plasma membrane Ca^{2+}-ATPases in rat pancreatic islet cells. Cell Calcium. 2000; 27:231–246. [PubMed: 10858669]
10. Kozel PJ, et al. Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 1. J. Biol. Chem. 1998; 273:18693–18696. [PubMed: 9668038]
11. Okunade GW, et al. 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. 2004; 279:33742–33750. [PubMed: 15178683]
12. Schuh K, et al. Plasma membrane Ca^{2+} ATPase 4 is required for sperm motility and male fertility. J. Biol. Chem. 2004; 279:28220–28226. [PubMed: 15078889]
13. Penniston JT, et al. Purification, reconstitution, and regulation of plasma membrane Ca^{2+}-pumps. Methods Enzymol. 1988; 157:340–351. [PubMed: 2976465]
14. Carafoli E. Biogenesis: Plasma membrane calcium ATPase: 15 years of work on the purified enzyme. FASEB J. 1994; 8:993–1002. [PubMed: 7926378]
15. Penniston JT, Enyedi A. Modulation of the plasma membrane Ca^{2+} pump. J. Membr. Biol. 1998; 165:101–109. [PubMed: 9744998]
16. Verma AK, et al. Protein kinase C phosphorylates plasma membrane Ca^{2+} pump isoform 4a at its calmodulin binding domain. J. Biol. Chem. 1999; 274:527–531. [PubMed: 9867875]
17. Enyedi A, et al. Protein kinase C activates the plasma membrane Ca^{2+} pump isoform 4b by phosphorylation of an inhibitory region downstream of the calmodulin-binding domain. J. Biol. Chem. 1996; 271:32461–32467. [PubMed: 8943312]
18. Enyedi A, et al. Protein kinase C phosphorylates the "a" forms of plasma membrane Ca^{2+} pump isoforms 2 and 3 and prevents binding of calmodulin. J. Biol. Chem. 1997; 272:27525–27528. [PubMed: 9346883]
19. Enyedi A, et al. The Ca^{2+} affinity of the plasma membrane Ca^{2+} pump is controlled by alternative splicing. J. Biol. Chem. 1994; 269:41–43. [PubMed: 8276828]
20. Elwess NL, et al. Plasma membrane Ca^{2+} pump isoforms 2a and 2b are unusually responsive to calmodulin and Ca^{2+} J. Biol. Chem. 1997; 272:17981–17986. [PubMed: 9218424]

21. Caride AJ, et al. The plasma membrane calcium pump displays memory of past calcium spikes. Differences between isoforms 2b and 4b. J. Biol. Chem. 2001; 276:39797–39804. [PubMed: 11514555]
22. Caride AJ, et al. 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. 1999; 274:35227–35232. [PubMed: 10575008]
23. Caride AJ, et al. Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca^{2+}: fast pumps reside in fast cells. Cell Calcium. 2001; 30:49–57. [PubMed: 11396987]
24. Dean WL, et al. Regulation of platelet plasma membrane Ca^{2+}-ATPase by cAMP-dependent and tyrosine phosphorylation. J. Biol. Chem. 1997; 272:15113–15119. [PubMed: 9182531]
25. DeMarco SJ, Strehler EE. 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. 2001; 276:21594–21600. [PubMed: 11274188]
26. Williams JC, et al. The sarcolemmal calcium pump, a-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. J. Biol. Chem. 2006; 281:23341–23348. [PubMed: 16735509]
27. Rimessi A, et al. Inhibitory interaction of the 14-3-3ε protein with isoform 4 of the plasma membrane Ca^{2+}-ATPase pump. J. Biol. Chem. 2005; 280:37195–37203. [PubMed: 16126729]
28. Chicka MC, Strehler EE. Alternative splicing of the first intracellular loop of plasma membrane Ca^{2+}-ATPase isoform 2 alters its membrane targeting. J. Biol. Chem. 2003; 278:18464–18470. [PubMed: 12624087]
29. Grati M, et al. Molecular determinants for differential membrane trafficking of PMCA1 and PMCA2 in mammalian hair cells. J. Cell Sci. 2006; 119:2995–3007. [PubMed: 16803870]
30. Armesilla AL, et al. Novel functional interaction between the plasma membrane Ca^{2+} pump 4b and the proapoptotic tumor suppressor Ras-associated factor 1 (RASSF1). J. Biol. Chem. 2004; 279:31318–31328. [PubMed: 15145946]
31. Buch MH, et al. 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. 2005; 280:29479–29487. [PubMed: 15955804]
32. Jarrett HW, Penniston JT. 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. 1978; 253:4676–4682. [PubMed: 149133]
33. Schuh K, et al. Interaction of the plasma membrane Ca^{2+} pump 4b/CI with the Ca^{2+}/calmodulin-dependent membrane-associated kinase CASK. J. Biol. Chem. 2003; 278:9778–9883. [PubMed: 12511555]
34. Sgambato-Faure V, et al. The homer-1 protein Ania-3 interacts with the plasma membrane calcium pump. Biochem. Biophys. Res. Commun. 2006; 343:630–637. [PubMed: 16554037]
35. Schuh K, et al. The plasmamembrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase I. J. Cell Biol. 2001; 155:201–205. [PubMed: 11591728]
36. Goellner GM, DeMarco SJ, Strehler EE. 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. 2003; 986:461–471. [PubMed: 12763866]
37. Stephenson SEM, et al. A single PDZ domain protein interacts with the Menkes copper ATPase, ATP7A. J. Biol. Chem. 2005; 280:33270–33279. [PubMed: 16051599]
38. DeMarco SJ, Chicka MC, Strehler EE. Plasma membrane Ca^{2+} ATPase isoform 2b interacts preferentially with Na^{+}/H^{+} exchanger regulatory factor 2 in apical plasma membranes. J. Biol. Chem. 2002; 277:10506–10511. [PubMed: 11786550]
39. Usachev YM, et al. Bradykinin and ATP accelerate Ca^{2+} efflux from rat sensory neurons via protein kinase C and the plasma membrane Ca^{2+} pump isoform 4. Neuron. 2002; 33:113–122. [PubMed: 11779484]

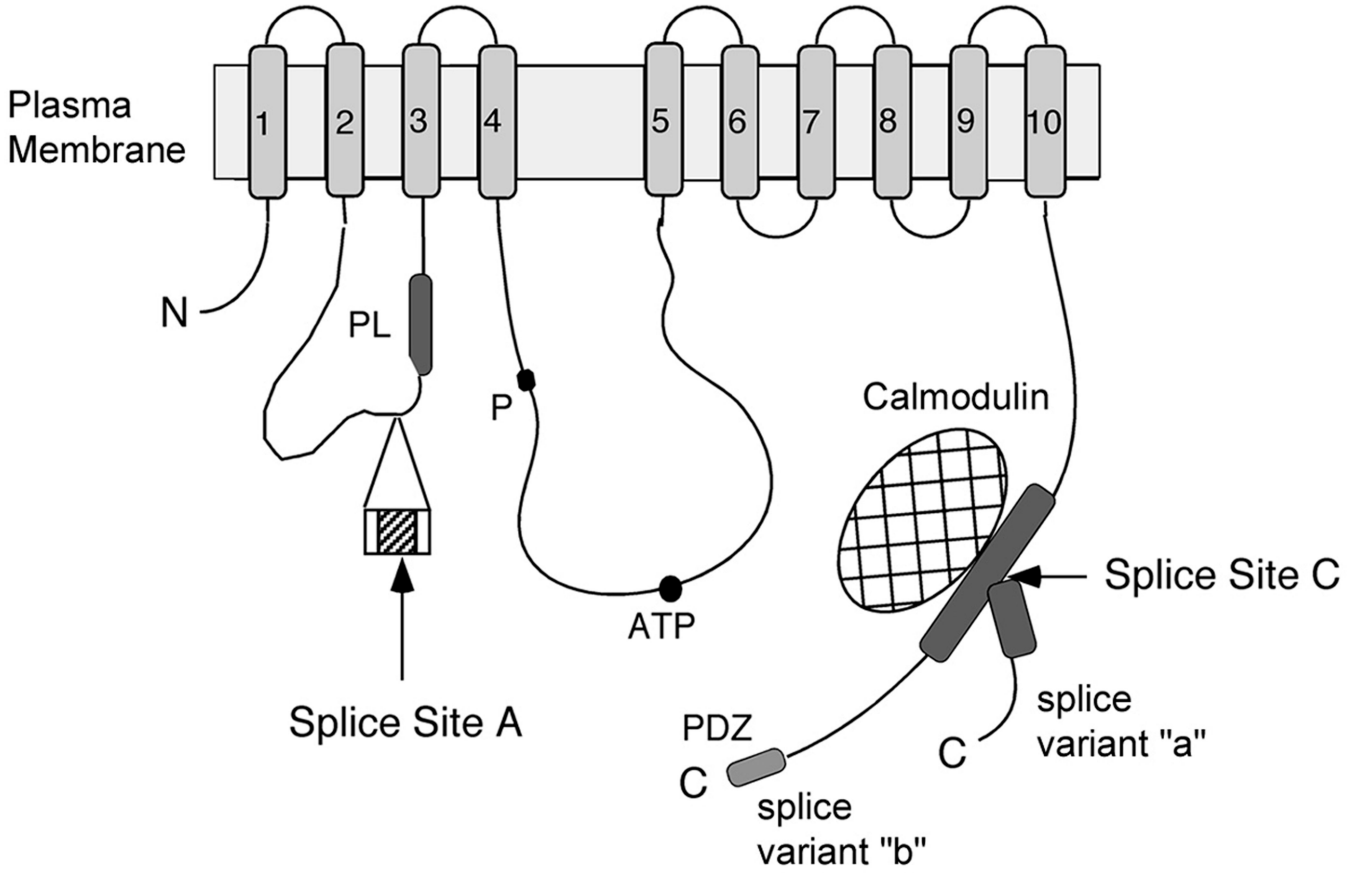

FIGURE 1.

Scheme of the PMCA. The membrane-spanning regions are numbered and shown as shaded boxes. The amino- (N) and carboxyl-terminal (C) ends are labeled, P denotes the obligatory aspartyl-phosphate formed during the reaction cycle, and ATP indicates the region involved in nucleotide (ATP) binding. The phospholipid-sensitive region (PL), the calmodulin-binding region and the PDZ-binding motif at the C-terminus of all PMCA b-splice variants are shown as gray boxes. The PMCA is represented in its activated state with Ca^{2+}-calmodulin (Ca^{2+}-CaM) bound to the C-tail. Arrows labeled “splice site A” and “splice site C” denote the regions affected by alternative splicing. A hatched box indicates the peptide segment encoded by alternatively spliced exon(s) at site A; at site C the two major splice variants (“a” and “b”) are shown as separate tails to indicate their divergent reading frames.

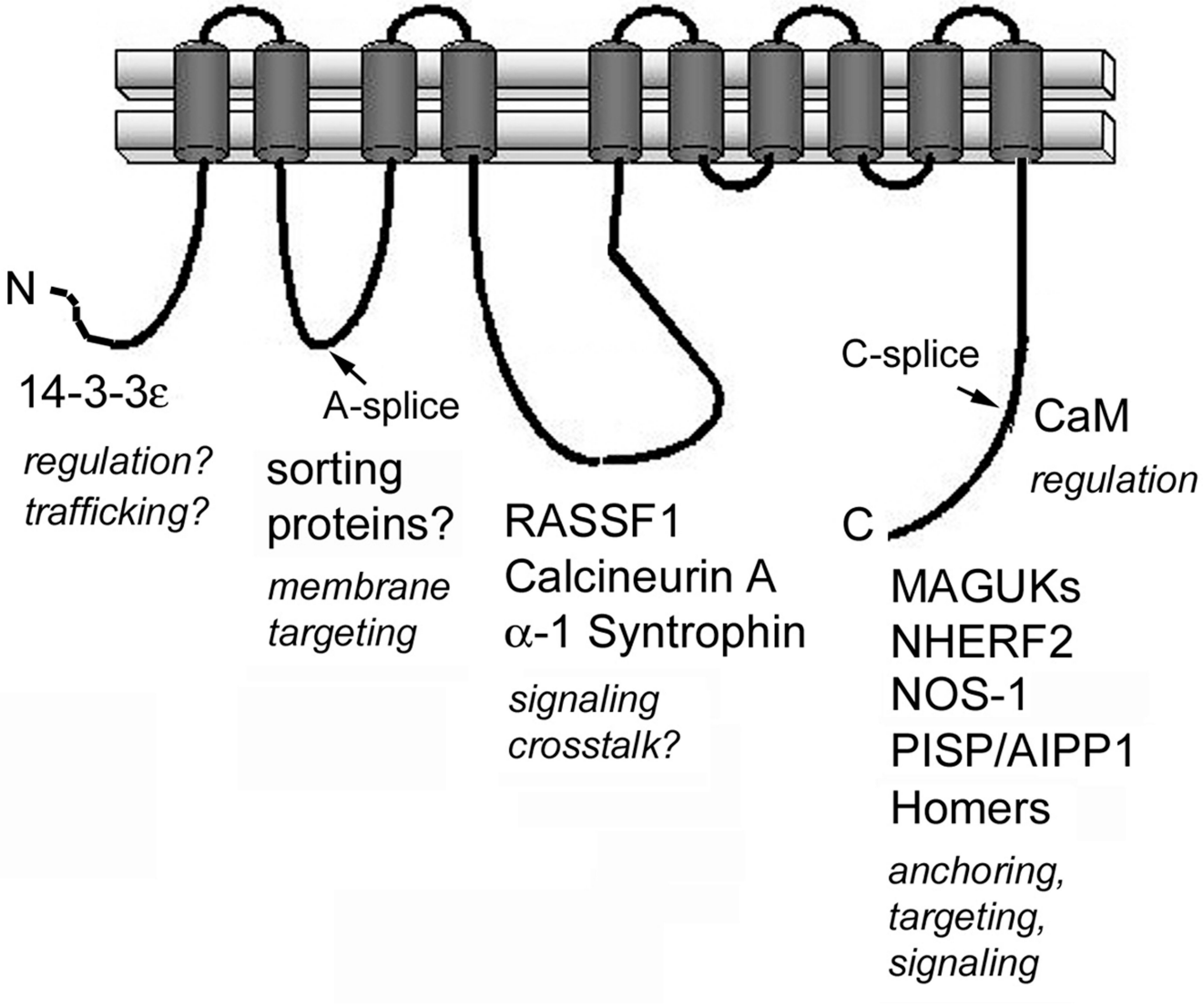

FIGURE 2.

PMCA-interacting proteins and their possible roles in pump regulation and function. PMCA-interacting proteins are listed near the domain of the PMCA to which they bind. Suspected or known functional roles of these different protein interactions are indicated in italics. Note that no binding partners (sorting proteins?) have yet been identified for the fist cytosolic loop of the PMCA carrying the A-splice. Also note that except for calmodulin (CaM), all proteins listed as binding to the C-terminal tail of the PMCA are specific for the b-splice variants and do not interact with the a-splice variants. For details, see the text.

Table I

Human PMCA isoforms and major alternative splice variants

Isoform	Major alternative splice variants	Length in amino acids	GenBank accession numbers	Tissue distribution
PMCA1	PMCA1x/a	1176	NM_001001323	Brain, nervous tissue
	PMCA1x/b	1220	NM_001682	Ubiquitous
	PMCA1x/c	1249		Skeletal muscle, heart
PMCA2	PMCA2w/a	1199	U15688*	Inner ear hair cells
	PMCA2x/a	1168		Brain (relatively rare)
	PMCA2z/a	1154		Brain (generally more abundant than 2x/a)
	PMCA2w/b	1243	NM_001001331	Brain, breast (lactating mammary gland), pancreatic β-cells
	PMCA2x/b	1212		Brain/excitable tissue
	PMCA2z/b	1198	NM_001683	Brain/excitable tissue
PMCA3	PMCA3x/a	1173	NM_021949	Brain
	PMCA3z/a	1159		Brain (cortex, thalamus, substantia nigra), pancreatic β-cells
	PMCA3x/b	1220	NM_001001344	Brain
	PMCA3z/b	1206		Brain (cortex, thalamus, substantia nigra)
	PMCA3x/f	1129		(Fast) skeletal muscle, brain (rare)
PMCA4	PMCA4x/a	1170	NM_001001396	Brain, heart, stomach
	PMCA4z/a	1158		Heart, pancreas (Islet of Langerhans)
	PMCA4x/b	1205	NM_001684	Ubiquitous
	PMCA4z/b	1193		Heart

*Splice site C alternative exon sequence only