The Plasma Membrane Calcium Pump: New Ways to Look at an Old Enzyme

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The three-dimensional structure of the PMCA pump has not been solved, but its basic mechanistic properties are known to repeat those of the other Ca2+ pumps. However, the pump also has unique properties. They concern essentially its numerous regulatory mechanisms, the most important of which is the autoinhibition by its C-terminal tail. Other regulatory mechanisms involve protein kinases and the phospholipids of the plasma membrane in which the pump is embedded. Permanent activation of the pump, e.g. by calmodulin, is physiologically as harmful to cells as its absence. The concept is now emerging that the global control of cell Ca2+ may not be the main function of the pump; in some cell types, it could even be irrelevant. The main pump role would be the regulation of Ca2+ in cell microdomains in which the pump co-segregates with partners that modulate the Ca2+ message and transduce it to important cell functions.

Decades of study on the mammalian Ca2+-ATPase of the plasma membrane (the PMCA pump) (1) have revealed many properties that set it apart from the other members of the superfamily of P-type pumps (2). One property that is immediately obvious is the long C-terminal tail, which is the locale of the numerous regulatory processes. Another distinctive property of the pump is the wealth of interactors with which the pump triggers processes of general significance for the cell. The basic information on these and other general aspects of the PMCA pump has been collected in other comprehensive reviews (3–5) and will not be repeated here. In this minireview, we instead look at the pump in a novel perspective, in which the extrusion of Ca2+ will not be considered as a mechanism to regulate bulk cell Ca2+ but will be integrated in a complex array of processes that modulate Ca2+-dependent processes within the cell (6). We focus on the most important regulatory mechanisms of the pump. We posit that, in many cell types, the quantitative contribution of the pump to the total extrusion of Ca2+ could be minor or even irrelevant. Because the main role of the pump would be to do something else, the conclusion would offer a rationale for the puzzling finding that, in some plasma membranes, the pump coexists with a much more powerful Ca2+ extrusion system, e.g. Na+/Ca2+ exchange in cardiomyocytes (7). Naturally, the function of the pump is still essential to the well being of the cell, as underscored by the causative involvement of its malfunction in disease processes (8).

The pump contains 10 transmembrane domains, two main cytosolic loops, and a long cytosolic C-terminal tail (9, 10). Separate genes encode its four basic isoforms: PMCA1 is ubiquitous and has a housekeeping role; PMCA4 is also ubiquitous but is endowed with tissue-specific roles; and PMCA2 and PMCA3 are tissue-restricted, with high levels of expression in neurons. Complex alternative splicing processes at a site in the first cytosolic loop of the pump (site A) and within the C-terminal calmodulin-binding domain (CaM-BD3; site C) generate numerous pump variants with special properties. The best understood regulatory mechanism of the pump is that by CaM, which interacts with high affinity with the C-terminal tail, reducing the $K_m$ of the pump to submicromolar values. In the absence of CaM, the CaM-BD binds to the main body of the pump to keep it (auto)inhibited (11, 12). CaM removes the domain from these sites, restoring activity. (A second, lower affinity CaM-BD has recently been identified downstream of the first in some splice variants of the pump (13)). The autoinhibition mechanism is unique among P-type pumps: other pumps of the superfamily are also inhibited in the resting state, but the mechanism involves extraneous proteins, e.g. phospholamban in the case of the SERCA pump.

Studies that preceded the purification of PMCA found that it is stimulated by phospholipids (PL). Treatment of red cell ghosts with phospholipases reduced its activity, which was restored by PL. According to one study (14), all PL were effective, whereas another study (15) found that only acidic PL (phosphatidylserine) were effective. The specificity of acidic PL was confirmed on the purified pump (16). They reduced its $K_m$ to even lower values than CaM (17), and it was calculated that the concentration of phosphatidylserine in the inner leaflet of the plasma membrane bilayer was, in principle, sufficient for 50% of maximal pump activity (18). A particularly effective PL was the doubly phosphorylated product of phosphatidylinositol (PI(4,5)P2) (19). The PL effect still has obscure aspects that will be discussed in detail below, but it has always been considered potentially important; for example, a reversible modulation of pump activity was suggested for PI(4,5)P2 (19), which is the only PL that undergoes rapid agonist-induced changes in concentration.

Protein kinases also modulate the activity of the pump by phosphorylating target residues in its C-terminal tail: PKA phosphorylates a Ser residue downstream of the CaM-BD, increasing the Ca2+ affinity of the PMCA1 pump (20). The work on PKC has yielded conflicting results, particularly because the original finding had shown that the effects of phosphorylation and of CaM were additive (21), whereas others had found that the phosphorylation decreased the stimulation by CaM (22). The target residues for PKC are a Thr residue in the

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3 The abbreviations used are: CaM-BD, calmodulin-binding domain; PL, phospholipid(s); PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PI(3)P, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PS, phosphatidylserine.
CaM-BD and a Ser residue farther downstream present in all pump isoforms. Surprisingly, the phosphorylation by PKC was found to increase the $V_{\text{max}}$ of the pump, but not its $K_m$ for Ca$^{2+}$ (22). It was later found that phosphorylation of the Thr residue within the CaM-BD reduced its ability to autoinhibit the pump (23). A complicating aspect of the activation by the kinases is the requirement for the inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG) and thus for the hydrolysis of PIP$_2$, which is itself strongly activatory. Therefore, the activation by the kinases should, in principle, be counteracted by the disappearance of PIP$_2$. Possibly, temporal or/and spatial aspects in the activation could provide a way out of the conundrum.

Activation of the pump by calpain involves the shaving off of the C-terminal tail of the pump (24), which becomes constitutively activated and CaM-independent. This irreversible mechanism could come into play in conditions that demand a permanent increase in the activity of the pump, but it is more likely to have a role in conditions of pathology that produce cytosolic Ca$^{2+}$ overload.

Other described mechanisms of PMCA activity modulation are mechanistically interesting but may not have a physiological role: that by the dimerization (oligomerization) of the pump (25) through its CaM-BD (26); that linked to the membrane protein concentration (27); and that involving the association of the PMCA pump with the actin cytoskeleton (28), with G-actin being activatory and F-actin inhibitory. Interestingly, two possibly allosteric Ca$^{2+}$-binding sites with nanomolar and micromolar affinities, respectively, have been found at the two sides of the C-terminal CaM-BD (29).

**A General Comment on Regulation of the PMCA Pump**

Activation of the pump by protein kinases implies a built-in stop mechanism: the phosphorylation by PKA is activated by the liberation of Ca$^{2+}$ from the endoplasmic reticulum by IP$_3$ because most adenyl cyclase isoforms are Ca$^{2+}$-dependent, and the phosphorylation by PKC is activated by the production of DAG. Both mechanisms demand the hydrolysis of PIP$_2$, and thus, in principle, the removal of its activation of the pump. Phosphatases that dephosphorylate the pump also play a role in reversing the activation. The built-in stop mechanism in the activation by the kinases has more general significance. It also concerns the activation by CaM: once the latter activates the pump, Ca$^{2+}$ in the microenvironment of the enzyme decreases. As a result, CaM will leave the pump, restoring autoinhibition. Thus, one should conclude that the pump can be activated only in temporally limited bursts (apart, of course, from the case of proteolysis or, perhaps, of the long-range effect of the lipid environment; see below). The time factor is relevant; for example, once an IP$_3$-linked agonist provokes the hydrolysis of PIP$_2$, the pump should immediately become less active. Shortly thereafter, however, it will be activated again by Ca$^{2+}$ (directly via CaM and indirectly via PKA) and indirectly by DAG. The spatial factor could also have a role, i.e., the pump could be distributed inhomogenously in the plasma membrane and/or recycled in a regulated way from membrane microdomains by endocytosis. The activation/inhibition effects could thus involve only a fraction of it.

**Regulation of the PMCA Pump by Phospholipids**

Membrane lipids have an obvious structural role, but they are also critical actors in signal transduction: membrane-linked signaling proteins may be sequestered in domains enriched with specific interacting lipids but can also be modulated by lipid-mediated changes in membrane fluidity that could influence their lateral mobility. Special membrane domains such as rafts, caveolae, and dendritic spines are indeed loci of dynamic signal transduction activity involving Ca$^{2+}$. Concerning the response of the PMCA pump to acidic PL, its active core is located closer to the cytosolic side of the membrane. The asymmetric lipid distribution in the bilayer is thus of particular interest. Phosphatidylcholine and sphingomyelin, which do not activate the pump, are located predominantly in the outer leaflet, whereas phosphatidylethanolamine, the acidic phosphatidylserine (PS), phosphatidylinositol, and its two phosphorylated derivatives (PIP$_1$ and PIP$_2$) are located predominantly in the leaflet facing the cytoplasm. This asymmetry is maintained by three classes of enzymes: flippases, floppases, and scramblases (for an extended review, see Ref. 30). Flippases are P-type ATPases that perform the ATP-dependent transfer of amino-PL (especially PS) from the outer to the inner leaflet of the bilayer, whereas floppases catalyze their movement in the opposite direction. Scramblases dissipate the lipid asymmetry: they may reorganize PL in the plasma membrane in response to cell stimulation (31–33). They are specifically important in the externalization of PS on the cell surface, a process that occurs early in apoptosis to make cells recognized by phagocytes (34, 35). Two types of scramblases have been described: one is Ca$^{2+}$-independent (36), and the other is Ca$^{2+}$-dependent (37). A mechanism could thus be envisaged in which scramblases could become activated to externalize PS, e.g., in response to the increase in cell Ca$^{2+}$ (38–40), removing a mechanism for the activation the PMCA pump and thus increasing the Ca$^{2+}$ overload. The mechanism of the flip-flop operation is still poorly understood, but structural information on the enzymes that perform it now begins to shed light (41, 42). Indications have also appeared for a possible role of caspases (43, 44) and of transient receptor potential channels and the Rho kinase family (45, 46) in the PS exposure process.

Acidic PL interact directly with the pump. Two binding sites have been identified: one in the first cytosolic loop and the other in the CaM-BD (47, 48). The contribution of the two sites to activation is still poorly understood (49). However, inhibition of Ca$^{2+}$ efflux has been documented in umbilical vein endothelial cells exposed to treatments that increase PS externalization (50).

Changes in the PL environment of the pump in the rapid time scale of the events linked, for example, to the action of CaM or agonists will probably not occur. Thus, PL probably are longer range “buffers” of pump activity. One exception could be the strongly activatory PIP$_2$ (19), which is the only PL with a concentration that changes rapidly upon cell stimulation. A reversible rapid activation cycle had indeed been proposed for PIP$_2$ (19). However, as mentioned, the role of PIP$_2$ has additional complicating aspects (see below).
In addition to interacting directly with the pump, lipids of the plasma membrane could also influence its activity in indirect ways. Pump units could be sequestered in membrane domains of particular lipid composition and/or fluidity; the fluidity of the lipid environment indeed influences pump activity both in vitro and in vivo (51–53). Plasma membrane domains such as rafts and caveolae (54–56) are enriched in sphingolipids and cholesterol. The plasma membrane of the caveolae concentrates the PMCA pumps, but also PS and many interacting factors that modulate their activity are associated with them (6). Notably, the association of PMCA pumps with caveolae rafts could even be isoform-specific: in intestinal smooth muscle, the full-length isoform 4b is associated with caveolin-1, whereas the truncated splice isoform 4a (see below) is instead associated with the rafts (58). The spines that protrude from neuronal dendrites also compartmentalize multiprotein Ca\(^{2+}\) signaling complexes; their plasma membrane is enriched in lipid rafts (59).

Because the membrane PL are spatially separated from the catalytic core of the pump, it appears to be difficult to involve them directly in the operation of the enzyme cycle. The interaction of PS with cytosolic portions of the pump would demand that the latter come in contact with the surface of the membrane where the polar heads of activatory PL are located. Thus, the effect of PL would be structural: they could somehow affect the access of substrates to the active site, explaining also effects on the enzyme cycle, i.e. acceleration of the dephosphorylation of the pump (60).

**Autoinhibition of the PMCA Pump**

That the PMCA pump at rest is in an autoinhibited state was first indicated by the activatory effect of calpain (18, 61–63). At the time of the finding, the cleavage site by calpain was still undefined. It was then found that the protease removed the entire C-terminal tail of the pump (24), strongly indicating the latter as the portion of the molecule responsible for the autoinhibition. Direct verification was then provided by the finding that synthetic peptides representing the C-terminal CaM-BD inhibited the truncated pump (11, 12). They bound to the two sequences in the main body of the pump: one next to its active site in the main cytosolic unit and the other in the cytosolic unit protruding between transmembrane domains 2 and 3. It was proposed that the C-terminal tail would interact with the main body of the pump with its CaM-BD, somehow “closing” the access route to the active site. CaM would then “open” the pump by detaching its binding domain from the two binding sites. Support for the proposal was then provided by a study in which blue and green fluorescent proteins were fused to the N and C termini of pump 4b (64). In the autoinhibited state, significant FRET was detected in the recombinant pump purified from yeast, consistent with an average distance of 45 Å between the two fluorophores. The FRET intensity decreased when the pump was activated by CaM and also by acidic PL or calpain proteolysis, indicating a rearrangement of the pump structure that separated its two ends. Later studies on the autoinhibition process have shown that even minor alternative splicing differences (see below) in the CaM-BD inhibited the pump to different extents (65, 66). Other elements in the CaM-BD and in the C-terminal tail also interfere with the autoinhibition process (67, 68), e.g. mutation of the CaM anchor residue Trp-1093 of PMCA4 increased its basal activity (69). Other regions outside the CaM-BD also appear to be involved in the maintenance of autoinhibition; in particular, residues in the proposed cytosolic “stalk” regions (Asp-170 in S2) (70) could help in the stabilization of the inactive state, as their mutation decreased the autoinhibition. A recent contribution (71) underlines the role of conserved residues in the stalk region in the formation of four basic pockets, suitable for the binding of the head groups of PIP\(_2\). Remarkably, bound PIP\(_2\) is protected from phospholipase C-mediated degradation.

Structural changes in the transition between the autoinhibited and activated states have also been shown by studies (72, 73) of the incorporation of photoactivatable PL probes into the pump: the incorporation, which would correspond to the exposure of the pump to the surrounding PL, was greatly decreased by CaM and, albeit differently, by acidic PL. The FRET between the pump labeled in the ATP-binding site and a PL included in the micelles of the purified pump was decreased by CaM but not by acidic PL. Differences thus apparently exist between the active conformations of the pump induced by CaM and PL.

**Alternatively Spliced Pump Variants Have Special Properties (and Roles)**

The four basic pumps have a sequence identity of ~80%, but they differ significantly in some regions, very likely reflecting the specific features of each isoform (74). One important feature distinguishes PMCA2 from the other isoforms: its basic activity is nearly as high in the absence of CaM as in its presence. Because the sequence of the CaM-BD of the PMCA2 pump is identical to that of the other isoforms, the difference evidently reflects differences in the portions of the main core of the pump involved in the autoinhibition process and/or differences in the C-terminal tail outside the CaM-BD (75, 76). This property of PMCA2 satisfies the Ca\(^{2+}\) homeostasis demands of cells that require the sustained ejection of Ca\(^{2+}\) even in the absence of activators, e.g. the outer hair cells of the inner ear. The kinetics of activation by Ca\(^{2+}\) also differs in the four isoforms, i.e. the rate of stimulation (and of inactivation upon Ca\(^{2+}\) removal) of PMCA1 and PMCA4 is considerably slower than that of PMCA2 and PMCA3; the first two are thus "slow" and the last two "fast" pumps. Their expression correlates with the requirements of cells in which Ca\(^{2+}\) signals are slow or fast, respectively (77).

The primary transcripts of all PMCA pumps undergo alternative splicing at the two sites mentioned above (5). The splicing at site A is related to the localization of the pump to the apical plasma membrane (78), whereas the splicing at site C, within the CaM-BD, defines the properties of the regulation processes of the pump (74). At site C, exons are inserted in the transcript, producing variants a, c, and d (variant b has no site C inserts) and generating pumps with diverging C-terminal sequences. The insert begins in the middle of the CaM-BD. Interestingly, in variants a, c, and d, five or six of the first 10 residues of the insert that replaces the C-terminal half of the CaM-BD are the same as those of the original non-inserted CaM-BD, i.e. the original full CaM-BD is nearly reconstructed.
in the inserted variance. The insert in variant a is out of frame and generates a truncated pump. The sequence variations produced by the site C inserts affect both the autoinhibitory role of the C-terminal tail and its interaction with CaM. Peptides corresponding to the CaM-BD of variant a have lower affinity for CaM and decreased efficiency as inhibitors of the pump activated by calpain proteolysis than those of variant b (65, 66). The PMCA4a variant is more active in the non-activated state than the PMCA4b variant, i.e. the C-terminal tail of PMCA4a is less autoinhibitory (79).

A recent study (13) on the ortholog of the PMCA pump in *Arabidopsis thaliana* has demonstrated that its autoinhibitory region, which is N-terminal in plant PMCA pumps, contains two (not one) adjacent CaM-binding sites. The second site, which has lower affinity for CaM and is located downstream of the first, plays a role in autoinhibition of the pump, suggesting that the regulation of the pump could be finely tuned by the Ca²⁺ concentration. Nanomolar levels of Ca²⁺ would partially activate the pump via the first CaM-BD, but the pump would become fully activated by the binding of CaM to the second domain only at micromolar levels of Ca²⁺. The presence of the second CaM-binding site has also been found in some splice variants of the animal pumps, notably the PMCA1d and PMCA4d variants. The PMCA4d variant is expressed in adult and fetal human heart cells, where it accounts for ~30% of the PMCA transcripts (80), and the PMCA1d variant is expressed in skeletal muscle and, less prominently, in brain (5). The bimodular regulation mechanism could thus also be operational in some splice variants of the animal pumps. A comprehensive scheme summarizing the major mechanism of PMCA pump regulation is presented in Fig. 1.

**Cellular (Protein) Partners of the PMCA Pump**

Numerous protein partners regulate activity, membrane targeting, and recruitment of the PMCA pump to diverse cell components (e.g. the cytoskeleton). Many studies have dealt with the interplay of the pump with proteins containing PDZ domains; these are 80–90-amino acid domains found in hundreds of signaling proteins that recognize the C terminus of proteins to cluster them with other proteins and “target” them to signaling pathways. The first studied PDZ domain-containing proteins were members of the family of MAGUK kinases (81); the association was proposed to maintain the pumps in specific plasma membrane regions to control Ca²⁺ homeostasis in local microdomains. Other studies have shown that pump 4b is recruited through PDZ domains to the filopodia adjacent to the actin cytoskeleton during platelet activation (82). Another MAGUK kinase, CASK (Ca²⁺/CaM-dependent serine kinase), has also been found to interact with pump 4b (83). CASK is a coactivator of transcription by promoters containing the T-element, which would down-regulate the T-element-dependent reporter activity by depleting Ca²⁺ in its microenvironment. Because CASK is located at synapses, its interplay with the pump could have a role in synaptic regulation. Another PDZ protein, NHERF2 (Na⁺/H⁺ exchanger regulatory factor 2; a member of the family of NHERF proteins, which are implicated in the targeting, retention, and regulation of a number of membrane proteins), has shown specificity for PMCA isoforms, as it interacted with variant 2b, but not variant 4b (84). The interaction was suggested to allow the clustering of the PMCA2b pump in a multiprotein Ca²⁺ signaling complex, facilitating cross-talk among its partners. The finding that variant 4b failed to interact with NHERF2 shows that even minimal
sequence differences, such as those between the C-terminal residues of variants 2b and 4b, could be critical in the interaction with partners. PDZ domains also mediate the interaction of variant 4b of cardiac cells with NOS-1 in a ternary complex with α-syntrophin, which binds to the pump at a site upstream of the C terminus (85). By down-regulating the production of nitrogen monoxide (NO), the interaction would confer to the pump a role in the regulation of Ca\(^{2+}\) related to local signaling that would prevail over its role in the extrusion of Ca\(^{2+}\) (86). It was then found that, in cardiomyocytes of PMCA4 mice, NOS became delocalized from the plasma membrane to the cytosol, leading to a decrease in microdomain cGMP and to elevation of local cAMP. As a result, the L-type channel-mediated Ca\(^{2+}\) influx and contractility were enhanced (87). PMCA4 thus had a structural role in tethering NOS to a selected plasma membrane domain. Importantly, the pump had no influence on global Ca\(^{2+}\) homeostasis in cardiomyocytes, which was unaffected in PMCA4−/− cardiomyocytes.

A number of other PDZ domain-containing proteins also interact with the PMCA pump, recruiting it to selected plasma membrane domains (88, 89) or to actin (90). The recruitment of the pump to restricted domains of the plasma membrane maximizes its effects on the locally restricted Ca\(^{2+}\) signaling but in turn exposes the pump to the

![Diagram](image-url)

**FIGURE 2.** Scheme summarizing the spatial and temporal aspects of PMCA regulation. A, fast and slow regulatory modes are shown. The scheme represents the fast regulation that characterizes the direct interaction of CaM and acidic PL with specific domains of the pump and the slow regulation mediated by changes in the membrane lipid composition, e.g., by variations of the cholesterol content of the lipid bilayer. **B**, spatial regulation of the pump can be performed either by targeting PMCA isoforms and splicing variants to specialized compartments of the cell surface (e.g., stereocilia of outer hair cells of the inner ear) or by confining the pump to specific portions of the plasma membrane (caveolae and lipid rafts) enriched in intracellular signaling complexes.

In addition to the C-terminal domain, other regions of the pump molecule also interact with partner proteins. The main intracellular loop binds the tumor suppressor Ras-associated factor 1, inhibiting the Ras-associated signaling pathway (91), and also interacts with the catalytic subunit of calcineurin, down-regulating its activity (92). The N-terminal cytosolic region, which has a low degree of sequence similarity among pump isoforms, has been used to search for the isoform specificity of interactors. The work has identified protein 14-3-3 as an inhibitory interactor that acts on isoforms 1b, 3b and 4b, but not on isoform 2b; the finding again shows that isoform 2b has properties that set it apart from the other pump isoforms (57, 93).

**Conclusion**

The properties of the PMCA pumps discussed above are not the focus of earlier reviews. Some deal with aspects that are not well understood or not firmly established; they nevertheless offer novel perspectives on the role of the pump. Most of them are related to problems of regulation, which is not surprising, considering the unusually complex set of regulatory mechanisms that act on PMCA pumps. The first important concept to be underscored is the shift in emphasis that is now gaining momentum regarding the primary role of the pump, which is the spatially and temporally restricted control of Ca\(^{2+}\) signaling within cellular microdomains rather than the bulk regulation of cell Ca\(^{2+}\) (Fig. 2). Regulation of the interplay of the pump with partners co-sequestered with it in local microdomains will then transduce the local Ca\(^{2+}\) modulation to important activities elsewhere in the cell.

The recruitment of the pump to restricted domains of the plasma membrane maximizes its effects on the locally restricted Ca\(^{2+}\) signaling but in turn exposes the pump to the
MINIREVIEW: Novel Concepts for the PMCA Pump

potentially important regulatory activity of the PL environment, which could even change relatively rapidly due to the action of the enzymes that move PL across the membrane bilayer. The activatory action of acidic PL is not yet fully understood, but one interesting possibility that is emerging suggests a role of the PS changes in the apoptotic death of cells. It is tempting to suggest that the removal of PS from the inner leaflet of the membrane bilayer, which occurs early in apoptosis, contributes to amplification of the pro-apoptotic Ca\(^{2+}\)-overloading process. The activation by PL is relevant to another aspect of the function of the pump. The action of the classical activator CaM is rapid, and one cannot perhaps expect rapidity also of the action of the kinases primed by the products of PIP\(_2\) hydrolysis. By contrast, the effect of PL (except that linked to the direct binding of PS) is probably slower. Changes in the PL environment, such as fluidity variations that would affect lateral mobility of pump units, could be a means to modulate pump activity in a longer time range.

The matter of pump activation has another interesting facet. As mentioned, fast modulation mechanisms, beginning with CaM, have an internal stop signal. When CaM activates the pump, it decreases Ca\(^{2+}\) in the microenvironment surrounding the active center of the pump. CaM would thus come off the pump, terminating activation. In principle, then, CaM activation is bound to have an oscillatory behavior. The reasoning becomes more complicated in the case of the kinases: their activation demands the hydrolysis of PIP\(_2\), IP\(_3\)/Ca\(^{2+}\) and DAG would then activate the pump, but the pump at the same time had ceased to be activated by PIP\(_2\). Spatial and/or temporal factors in the activation process could possibly provide a reasonable way out of the paradox.

One additional point regarding the activation process must be underscored: activation of the pump is not always in the best interest of cells. If the main role of the pump is the control of Ca\(^{2+}\) signaling in selected cell domains, its permanent activation cannot be tolerated. The correct regulation of cell Ca\(^{2+}\) signaling demands that the activity of PMCA pumps be modulated positively or negatively; their protracted and uncontrolled activation is probably as negative as their continuous inhibition.

The last point that deserves a comment is the autoinhibition process, which is unique among P-type pumps. Some of its molecular aspects are still not completely understood, but it is gradually becoming clear that it may involve not only CaM but also modulatory PL. It is also becoming clear that changes in the autoinhibition process may underlie conditions of pathology in which the PMCA pump has a causative role.

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REFERENCES
1. Schatzmann, H. J. (1966) ATP-dependent Ca\(^{2+}\)-extrusion from human red cells. Experientia 22, 364–365
2. Pedersen, P. L., and Carafoli, E. (1987) Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. Trends Biochem. Sci. 12, 146–150
3. Carafoli, E. (1994) Plasma membrane calcium ATPase: 15 years of work on the purified enzyme. FASEB J 8, 993–1002
4. Penniston, J. T., and Enyedi, A. (1998) Modulation of the plasma membrane Ca\(^{2+}\)-pump. J. Membr. Biol. 165, 101–109
5. Streher, E. E., and Zacharias, D. A. (2001) Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol. Rev. 81, 21–50
6. Holton, M. L., Wang, W., Emerson, M., Neyes, L., and Armesilla, A. L. (2010) Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways. World J. Biol. Chem. 1, 201–208
7. Caroni, P., and Carafoli, E. (1980) An ATP-dependent Ca\(^{2+}\)-pumping system in dog heart sarcolemma. Nature 283, 765–767
8. Brini, M., and Carafoli, E. (2009) Calcium pumps in health and disease. Physiol. Rev. 89, 1341–1378
9. Shull, G. E., and Greeb, J. (1988) Molecular cloning of two isoforms of the plasma membrane Ca\(^{2+}\)-transporting ATPase from rat brain. Structural and functional domains exhibit similarity to Na\(^{+}\)-K\(^{+}\)- and other cation transport ATPases. J. Biol. Chem. 263, 8646–8657
10. Verma, A. K., Filoteo, A. G., Stanford, D. R., Wieben, E. D., Penniston, J. T., Streher, E. E., Fischer, R., Heim, R., Vogel, G., Mathews, S., Streher-Pag, M.-A., James, P., Vorherr, T., Krebs, J., and Carafoli, E. (1988) Complete primary structure of a human plasma membrane Ca\(^{2+}\)-pump. J. Biol. Chem. 263, 14152–14159
11. Falchetto, R., Vorherr, T., Brunner, J., and Carafoli, E. (1991) The plasma membrane Ca\(^{2+}\)-pump contains a site that interacts with its calmodulin-binding domain. J. Biol. Chem. 266, 2930–2936
12. Falchetto, R., Vorherr, T., and Carafoli, E. (1992) The calmodulin-binding site of the plasma membrane Ca\(^{2+}\)-pump interacts with the transduction domain of the enzyme. Protein Sci. 1, 1613–1621
13. Tidow, H., Poulsen, L. R., Andreeva, A., Knudsen, M., Hein, K. L., Wiuf, C., Palmgren, M. G., and Nissen, P. (2012) A bimodal mechanism of calcium control in eukaryotes. Nature 491, 468–472
14. Roelofsen, B., and Schatzmann, H. J. (1977) The lipid requirement of the (Ca\(^{2+}\)+Mg\(^{2+}\))-ATPase in the human erythrocyte membrane, as studied by various highly purified phospholipases. Biochim. Biophys. Acta 464, 17–36
15. Ronner, P., Gazzotti, P., and Carafoli, E. (1977) A lipid requirement for the (Ca\(^{2+}\)+Mg\(^{2+}\))-activated ATPase of erythrocyte membranes. Arch. Biochem. Biophys. 179, 578–583
16. Niggli, V., Penniston, J. T., and Carafoli, E. (1979) Purification of the (Ca\(^{2+}\)+Mg\(^{2+}\))-ATPase from human erythrocyte membranes using a calmodulin affinity column. J. Biol. Chem. 254, 9955–9958
17. Enyedi, A., Flura, M., Sarkadi, B., Gardos, G., and Carafoli, E. (1987) The maximal velocity and the calcium affinity of the red cell calcium pump may be regulated independently. J. Biol. Chem. 262, 6425–6430
18. Niggli, V., Adunyah, E. S., and Carafoli, E. (1981) Acidic PL, unsaturated fatty acids, and limited proteolysis mimic the effect of calmodulin on the purified erythrocyte Ca\(^{2+}\)-ATPase. J. Biol. Chem. 256, 8588–8592
19. Choquette, D., Hakim, G., Filoteo, A. G., Plisker, G. A., Bostwick, J. R., and Penniston, J. T. (1984) Regulation of plasma membrane Ca\(^{2+}\)-ATPases by lipids of the phosphatidylinositol cycle. Biochim. Biophys. Res. Commun. 125, 908–915
20. James, P., Vorherr, T., and Carafoli, E. (1989) Primary structure of the Ca\(^{2+}\)-pumping domain of the plasma membrane calcium pump. Biochemistry 28, 4253–4258
21. Smallwood, J. I., Gi̇gi, B., and Rasmussen, H. (1988) Regulation of erythrocyte Ca\(^{2+}\)-pump activity by protein kinase C. J. Biol. Chem. 263, 2195–2202
22. Wang, K. K., Wright, L. C., Machan, C. L., Allen, B. G., Conigrave, A. D., and Roufogalis, B. D. (1991) Protein kinase C phosphorylates the carboxyl terminus of the plasma membrane Ca\(^{2+}\)-ATPase from human erythrocytes. J. Biol. Chem. 266, 9078–9085
23. Hofmann, F., Anagli, J., Carafoli, E., and Vorherr, T. (1994) Phosphorylation of the calmodulin binding domain of the plasma membrane Ca\(^{2+}\)-pump by protein kinase C reduces its interaction with calmodulin and with its pump receptor site. J. Biol. Chem. 269, 24298–24303
24. James, P., Vorherr, T., Krebs, J., Morelli, A., Castello, G., McCormick, D. J., Penniston, J. T., De Flora, A., and Carafoli, E. (1989) Modulation of erythrocyte Ca\(^{2+}\)-ATPase by selective calpain cleavage of the calmodulin-bind-
ing domain. *J. Biol. Chem.* 264, 8289–8296
25. Kosk-Kosicka, D., and Badega, T. (1988) Activation of the erythrocyte Ca\textsuperscript{2+}-ATPase by either self-association or interaction with calmodulin. *J. Biol. Chem.* 263, 18184–18189
26. Vorherr, T., Kessler, T., Hofmann, F., and Carafoli, E. (1991) The calmo-
dulin-binding domain mediates the self-association of the plasma mem-
brane Ca\textsuperscript{2+} pump. *J. Biol. Chem.* 266, 22–27
27. Vanagas, L., Rossi, R. C., Caride, A. J., Filoteo, A. G., Strehler, E. E., and Rossi, J. P. (2007) Plasma membrane calcium pump activation is affected by the membrane protein concentration: evidence for the involvement of the actin cytoskeleton. *Biochim. Biophys. Acta* 1768, 1641–1649
28. Vanagas, L., de La Fuente, M. C., Dalghi, M., Ferreira-Gomes, M., Rossi, R. C., Strehler, E. E., Mangialavori, I. C., and Rossi, J. P. (2013) Differential effects of G- and F-actin on the plasma membrane calcium pump activity. *Cell Biochem. Biophys.* 66, 187–198
29. Hofmann, F., James, P., Vorherr, T., and Carafoli, E. (1993) The C-termi-
nal domain of the plasma membrane Ca\textsuperscript{2+} pump contains three high affinity Ca\textsuperscript{2+} binding sites. *J. Biol. Chem.* 268, 10252–10259
30. Daleke, D. L. (2003) Regulation of transbilayer plasma membrane PL asymmetry. *J. Lipid Res.* 44, 233–242
31. Zhou, Q., Zhao, J., Stout, J. G., Luhm, R. A., Wiedmer, T., and Sims, P. J. (1996) Molecular cloning of human plasma membrane PL scramblase. A protein mediating transbilayer movement of plasma membrane PL. *J. Biol. Chem.* 272, 18240–18244
32. Wiedmer, T., Zhou, Q., Kwoh, D. Y., and Sims, P. J. (2000) Identification of three new members of the PL scramblase gene family. *Biochim. Biophys. Acta* 1467, 244–253
33. Sun, J., Nanjundan, M., Pike, L. J., Wiedmer, T., and Sims, P. J. (2002) Plasma membrane PL scramblase I is enriched in lipid rafts and interacts with the epidermal growth factor receptor. *Biochemistry* 41, 6338–6345
34. Fadok, V. A., Voelker, D. R., Campbell, P. A., Cohen, J. J., Bratton, D. L., and Henson, P. M. (1992) Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* 148, 2207–2216
35. Verhoven, B., Schlegel, R. A., and Williamson, P. (1995) Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 92, 1759–1763
36. Sepúlveda, M. R., Berrocal-Carrillo, M., Gasset, M., and Mata, A. M. (2006) The plasma membrane Ca\textsuperscript{2+} -ATPase isoform 4 is localized in lipid rafts of cerebellum synaptic plasma membranes. *J. Biol. Chem.* 281, 447–453
37. Linde, C. I., Di Leva, F., Domí, T., Tosatto, S. C., Brini, M., and Carafoli, E. (2008) Inhibitory interaction of the 14-3-3 proteins with ubiquitinous (PMCA1) and tissue-specific (PMCA3) isoforms of the plasma membrane Ca\textsuperscript{2+} pump. *Cell Calcium* 43, 550–561
38. El-Yazbi, A. F., Cho, W. J., Schulz, R., and Daniel, E. E. (2005) Calcium extrusion by plasma membrane calcium pump is impaired in caveolin-1 knockout mouse small intestine. *Eur. J. Pharmacol.* 591, 80–87
39. Hering, H., Lin, C. C., and Sheng, M. (2003) Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. *J. Neurosci.* 23, 3262–3271
40. Schnitzer, J. E., Oh, P., Jacobson, B. S., and Dvorak, A. M. (1984) Caveolae from luminal plasmalemma of rat lung endothelium: microdomains enriched in caveolin, Ca\textsuperscript{2+}-ATPase, and inositol trisphosphate receptor. *Mol. Biol. Rep.* 10, 179–183
41. Sánchez-Magraner, L., Posada, I. M., Andraka, N., Contreras, F. X., Viguera, A. R., Guérin, D. M., Arrondo, J. L., Monaco, H. L., and Gotti, F. M. (2014) The C-terminal transmembrane domain of human PL scramblase I is essential for the protein flip-flop activity and Ca\textsuperscript{2+} binding. *J. Membr. Biol.* 247, 155–165
42. Kipper, L. R., López-Barajas, R. L., and Palmeira, Y. M. (2008) Flip-
es - and -flop in the plasma membrane calcium pump using trypsin proteolysis. *Biochemistry* 47, 10267–10276
43. Minamino, N., and Carafoli, E. (1990) Mapping of functional domains in the plasma membrane Ca\textsuperscript{2+} pump. *Eur. J. Biochem.* 204, 939–946
44. Vanagas, L., Rossi, R. C., Caride, A. J., Filoteo, A. G., Strehler, E. E., and Rossi, J. P. (1996) On the mechanism of activation of PL scramblase by acidic phospholipids. *J. Biol. Chem.* 271, 31075–31085
45. Harper, M. T., Londoño, J. E., Quick, K., Londoño, J. C., Flockerzi, V., Philipp, S. E., Birnbaumer, L., Freichelson, M., and Poole, A. W. (2013) Trans-
sient receptor potential channels function as a coincidence signal detector mediating phosphatidylserine exposure. *Sci. Signal.* 6, r50
MINIREVIEW: Novel Concepts for the PMCA Pump

Principal binding domains from isozymes of the plasma membrane Ca\(^{2+}\) pump have different regulatory properties. J. Biol. Chem. 266, 8952–8956

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

Verma, A. K., Enyedi, A., Filoteo, A. G., and Penniston, J. T. (1994) Regulatory region of plasma membrane Ca\(^{2+}\) pump. 28 residues suffice to bind calmodulin but more are needed for full auto-inhibition of the activity. J. Biol. Chem. 269, 1687–1691

Pászty, K., Penheiter, A. R., Verma, A. K., Padányi, R., Filoteo, A. G., Penniston, J. T., and Enyedi, A. (2002) Alternative splicing of the first intracellular loop of plasma membra-Ca\(^{2+}\) pump isoforms 2b and 4b interact promiscuously and selectively with members of the plasma membrane-associated guanylate kinase family of PDZ (PSD95/Dlg/ZO-1) domain-containing proteins. J. Biol. Chem. 276, 21594–21600

Dean, W. L., and Whiteheart, S. W. (2004) Plasma membrane Ca\(^{2+}\)-ATPase (PMCA) translocates to filopodia during platelet activation. Thromb. Haemost. 91, 325–333

Schuh, K., Uldrijan, S., Gambaryan, S., Roethlein, N., and Neyes L. (2003) Interaction of the plasma membrane Ca\(^{2+}\) pump with the Ca\(^{2+}\)-calmodulin-dependent membrane-associated kinase CASK. J. Biol. Chem. 278, 9778–9783

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

Williams, J. C., Armesilla, A. L., Mohamed, T. M., Hagarty, C. L., McIntyre, F. H., Schomburg, S., Zaki, A. O., Oceandy, D., Cartwright, E. J., Buch, M. H., Emerson, M., and Neyes, L. (2006) The sarco/endo calcium pump, \(\alpha\)-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. J. Biol. Chem. 281, 23941–23948

Cartwright, E. J., Oceandy, D., and Neyes, L. (2009) Physiological implications of the interaction between the plasma membrane calcium pump and nNOS. Pflugers Arch. 457, 665–671

Mohamed, T. M., Oceandy, D., Zia, M., Prehar, S., Alatwi, N., Wang, Y., Shaheen, M. A., Abou-Leisa, R., Schelcher, C., Hegaz, B., Baudoin, F., Emerson, M., Mamas, M., Di Benedetto, G., Zaccolo, M., Lei, M., Cartwright, E. J., and Neyes, L. (2011) 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, 41520–41529

González Flecha, F. L., Bermúdez, M. C., Cézola, N. V., Gagliardino, J. J., and Rossii, J. P. (1990) Decreased Ca\(^{2+}\)-ATPase activity after glycosylation of erythrocyte membranes in vivo and in vitro. Diabetes 39, 707–711

Spamberto-Faure, V., Xiong, Y., Berke, D. J., Hyman, S. E., and Strehler, E. E. (2006) The Homer-1 protein Ania-3 interacts with the plasma membrane calcium pump. Biochem. Biophys. Res. Commun. 343, 630–637

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

Armesilla, A. L., Williams, J. C., Buch, M. H., Pickard, A., Emerson, M., Cartwright, E. J., Oceandy, D., Vos, M. D., Gillies, S., Clark, G. J., and Neyes, L. (2004) 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, 31318–31328

Buch, M. H., Pickard, A., Rodriguez, A., Gillies, S., Maass, A. H., Emerson, M., Cartwright, E. J., Williams, J. C., Oceandy, D., Redondo, J. M., Neyes, L., and Armesilla, A. L. (2005) 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, 29479–29487

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