Chapter 5
The ATP2B Plasma Membrane Ca^{2+} ATPase Family: Regulation in Response to Changing Demands of Cellular Calcium Transport

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Abstract The mammalian ATP2B family of plasma membrane calcium ATPases (PMCAs) consists of over 30 members generated from four genes and via complex alternative RNA splicing. Regulation occurs at the level of ATP2B gene transcription, splicing, translation, and posttranslational modification. PMCA isoforms and splice variants vary in their functional properties and are differentially regulated by intrinsic factors such as calmodulin and lipids, as well as by dynamic interaction with a large number of scaffolding and signaling proteins. A major emerging theme is the functional integration of different PMCAs in multiprotein complexes to allow reciprocal cross talk between localized PMCA-mediated Ca^{2+} control and the function of other members in the complex. The same PMCA isoform may be responsible for bulk calcium export in the cells of one tissue but control the local activity of a signaling microdomain in the cells of another. Regulation of the PMCAs must therefore be understood in the physiological context of the tissues and cells where they are expressed.

Keywords Alternative splicing • ATP2B ion pumps • Calcium microdomain • Calcium signaling • Plasma membrane calcium ATPase • PMCA • Signaling cross talk

1 Introduction

Calcium is an abundant and essential element in all living systems, and is used for both structural (skeleton, teeth) and signaling purposes. Cells expend a large amount of energy to control calcium fluxes and to maintain calcium homeostasis. As an

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important signaling agent, ionized free calcium (Ca^{2+}) must be very tightly regulated. Accordingly, cells have evolved membrane-intrinsic transport systems specifically dedicated to Ca^{2+} transport across biological membranes [1, 2]. Plasma membrane Ca^{2+} ATPases (PMCAs) are present in all eukaryotic cells and belong to the large superfamily of P-type ATP-driven ion pumps characterized by the formation of a phosphorylated enzyme intermediate [3]. Among the P-type ion pumps, the PMCAs are classified as ATP2B subfamily of Ca^{2+} pumps, with ATP2A and ATP2C representing the Ca^{2+} pumps of the sarco/endoplasmic reticulum and the Golgi compartment, respectively [4, 5]. Following the early realization that PMCAs represent a dedicated Ca^{2+} extrusion system capable of removing "excess" Ca^{2+} across the plasma membrane to maintain long-term intracellular Ca^{2+} balance, subsequent studies have shown a remarkable complexity not only in the number of PMCA isoforms expressed in different cells and tissues but also in the mechanisms by which these calcium pumps are regulated. This review will start with an overview of the molecular complexity of the mammalian PMCA family and then highlight different mechanisms of regulation of these proteins and their integration in the physiological demands of cellular Ca^{2+} handling.

2 Overview of the Mammalian ATP2B Gene Family Coding for Plasma Membrane Ca^{2+} ATPases (PMCAs)

The known mammalian genomes contain four separate PMCA genes (nomenclature for the human genes: ATP2B1, ATP2B2, ATP2B3, ATP2B4) located on different chromosomes (12q21.3, 3p25.3, Xq28, and 1q32.1 for human ATP2B1–4, respectively). The genes are large, with ~20–25 protein-coding exons spread over 50 to >100 kb, and additional 5′ untranslated exons separated by large introns in the 5′ region [6]. The human genes, for example, are ~120 kb for ATP2B1, ~380 kb for ATP2B2, ~ 65 kb for ATP2B3, and ~120 kb for ATP2B4 (Fig. 5.1). Several exons are subject to alternative splicing resulting in a multitude of PMCA splice variants differing in specific regions of the protein. The two major regions affected by alternative splicing are located in the first intracellular loop (site A) and the C-terminal tail (site C; see scheme in Fig. 5.2). A remarkable feature of all PMCA genes is the presence of at least one exon containing only 5′ untranslated (5′ UTR) sequence; the ATP2B2 gene has at least four separate upstream exons specifying 5′ UTR sequences (Fig. 5.1). In addition, some of these upstream exons are alternatively spliced and incorporated due to alternative promoters [7]. The complex upstream gene structure and multiple transcriptional start sites are suggestive of complex transcriptional regulation of the ATP2B genes.

The PMCAs contain ~1200 amino acid residues and have molecular masses of ~125–140 kDa [8]. Alternative splicing (at site A) of one to three small exons coding for integer multiples of triplet codons leads to changes in the length of the first intracellular loop, which can thus differ by up to 45 residues (comparing human

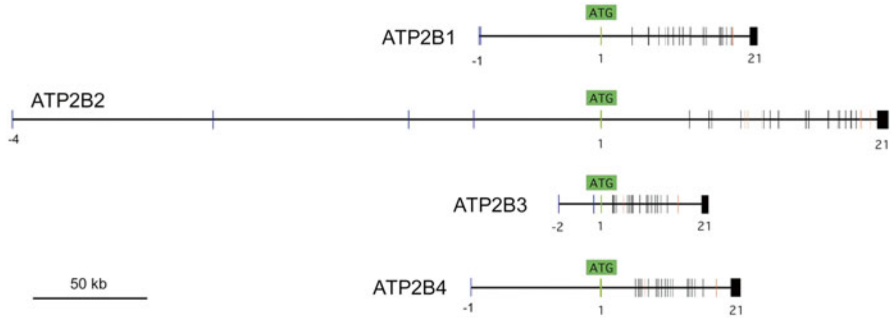

Fig. 5.1 Exon–intron structure of the human ATP2B genes. The four genes are aligned with respect to their ATG initiation codon in exon 1 and their length is shown to scale. Upstream exons (*blue boxes*) have negative numbers, the last exon (exon 21) containing the bulk of the 3′ UTR is also labeled. Constitutively spliced exons are shown as *black boxes*. A scale bar (50 kb) is indicated on the *lower left*

PMCA2 splice variants 2z and 2w). The PMCA site-A variants are indicated by lower case letters w, x, y, z [9]; a splice variant inserting yet a fourth optional exon coding for an additional 12 amino acids has been identified in the bullfrog and has been named variant “v” [10]. The cytosolic loop affected by site-A splicing is part of the A (actuator) domain of the pumps and as such, is intimately involved in the structural transitions that accompany the Ca^{2+} pumping reaction cycle [11]. This loop also contains a region sensitive to acidic phospholipids and participates in the autoregulation (auto-inhibition) of the pump in the basal state, i.e., at low $[Ca^{2+}]_i$ and in the absence of calmodulin (CaM) [12, 13]. Interestingly, however, the site A splice variants have not been found to differ significantly in their in vitro functional properties [14] or acidic lipid sensitivity when over-expressed in transfected CHO cells [15]. The effect of alternative splicing at site A on PMCA regulation may instead be indirect, e.g., by altering membrane targeting [16] and lipid partitioning, or by affecting signaling cross talk via specific lipid sequestration [17] or as yet unknown protein interactions.

Alternative splicing at site C is complex and can either insert additional amino acids into the C-terminal tail (splice variants c, d) or truncate the C-tail due to a shift in reading frame and the earlier occurrence of a stop codon (splice variants a, e, f) [9, 18]. The alternative splicing affects a major regulatory region of the PMCAs, i.e., the CaM-binding domain [19]. In addition, the splice variants with an altered reading frame and earlier stop codon also show major differences in their regulation by other proteins, most notably by PDZ domain-containing signaling and scaffolding proteins (see below) [20]. Because of the potential for combinatorial use of the alternative splice options at sites A and C, over 30 PMCA isoform variants can be generated from the four mammalian genes and >20 of these have been detected at various levels in different tissues [21]. Tables summarizing the various splice options have been published in many recent reviews [18, 19, 21, 22] and the reader is referred to these for further information.

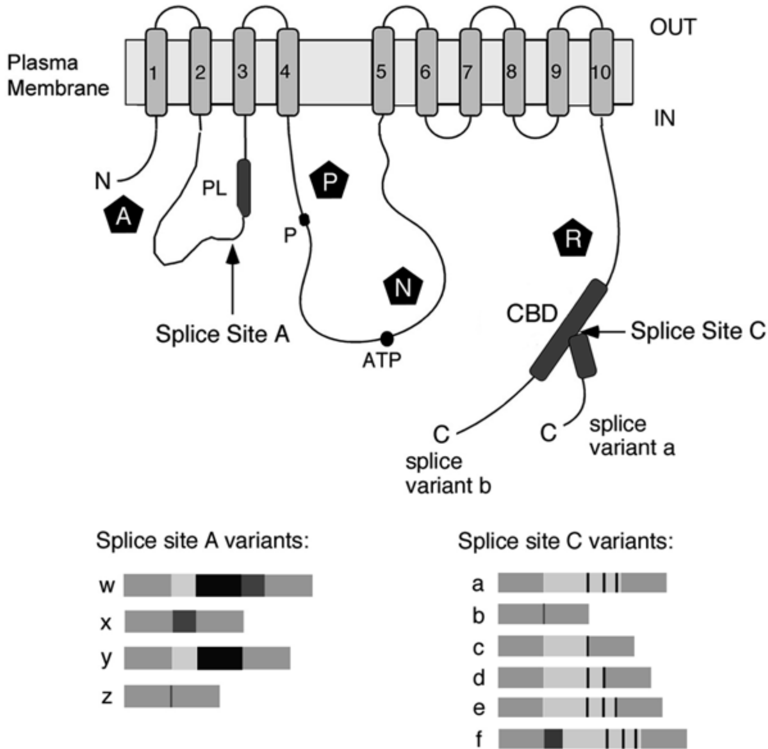

Fig. 5.2 Scheme of the mammalian PMCA and alternative splice options. A model of the PMCA in the plasma membrane is shown on the *top*. The cytosolic (IN) and extracellular (OUT) sides are indicated. The ten membrane-spanning regions of the PMCA are numbered, and the N- and C-terminus are labeled. The phospholipid-binding region in the first cytosolic loop (PL), the obligatory aspartate residue phosphorylated during the enzyme reaction cycle (P), the ATP binding region (ATP), and the CaM-binding domain (CBD) are also indicated. Major functional domains of the PMCA are labeled in *black pentagons* as A (actuator), P (phosphorylation), N (nucleotide-binding), and R (regulatory). *Arrows* indicate where alternative splicing results in isoform variability in the first cytosolic loop (splice site A) and the C-terminal tail (splice site C). Splicing at site C can result in a change in reading frame, this is exemplified by two different C-termini shown for variants a and b. Exon arrangements resulting in splice variants at site A and site C are shown on the *bottom. Flanking gray boxes* represent constitutively spliced exons, alternatively spliced exons are shown as separate *boxes* in different shades of *gray*, and the resulting splice variants are labeled by their *lowercase symbol* on the *left*. Note that splice options c, d, and e use different splice donor sites within the same exon (indicated by *black vertical lines*); complete insertion of this exon gives rise to splice variant a

3 ATP2B Gene Regulation

The transcriptional regulation of ATP2B genes is not well understood, but they are clearly differentially regulated during embryonic development and in different organs and cell types in response to numerous stimuli [23, 24]. In situ hybridization

studies during mouse embryogenesis have shown that *Atp2b1* is transcribed very early on, including in embryonic stem cells [25], and continues to be expressed in virtually all tissues throughout life, albeit at varying levels [26]. It is therefore not surprising that germ-line knockout of both copies of the Atp2b1 gene is embryonic lethal [27]. However, although PMCA1 is often referred to as "housekeeping" isoform, the ATP2B1 gene is subject to tight regulation at the transcriptional and post-transcriptional level. The early response transcription factor c-myb, for example, was shown to repress *Atp2b1* transcription during the G1/S phase of the cell cycle in mouse vascular smooth muscle cells, likely by interacting with myb-binding sites in the promoter region of the gene [28]. Similarly, c-myc can bind directly to a regulatory region in the Atp2b4 gene promoter and mediates transcriptional downregulation of PMCA4 during B lymphocyte differentiation [29]. Early promoter studies on the mouse Atp2b1 gene identified numerous general and specific transcription factor binding sites [30] and showed both protein kinase C (PKC) and protein kinase A (PKA) dependent transcriptional (up)regulation of PMCA1 expression [31]. These kinases are mediators of hormone-induced second messenger (Ca^{2+}, cAMP) regulation of PMCA expression and likely work through transcription factors such as CREB. The ATP2B1 gene is also subject to transcriptional regulation by the active form of vitamin D, 1,25-$(OH)_2$-D3, which strongly induces PMCA1 expression in the small intestine, kidney distal tubules, and osteoblasts [32–36].

The transcriptional regulation of the ATP2B genes is evidently tissue-specific and may change with the differentiation state and in response to external stimuli. Thus, depolarization and a rise in Ca^{2+} in cerebellar granule cells result in opposite regulation of ATP2B1–B3 and ATP2B4: ATP2B4 is downregulated at the transcriptional level in a calcineurin-dependent manner, presumably via calcineurin-mediated increased nuclear translocation of the transcription factor NFAT [37]. By contrast, ATP2B2 is upregulated in cerebellar development by a transcription factor generated from an internal ribosomal entry site in the voltage-gated calcium channel gene CACNA1A [38]. Another striking example of tissue-specific and (likely) hormonal control of transcription of a PMCA gene is that of *Atp2b2* in the lactating mammary gland: the transcript and protein levels of PMCA2 are induced up to 100-fold starting at parturition and during lactation in mice [39, 40]. Interestingly, the mammary gland PMCA2 transcripts are generated by using an alternative promoter and incorporating alternatively spliced 5′ UTR exons of the ATP2B2 gene different from the promoter and 5′ UTR exons used by neuronal cells [7]. As mentioned above, alternative promoters and alternatively spliced 5′ exons may be present in other ATP2B genes (e.g., ATP2B1), further broadening the potential for tissue-specific and physiological context-specific regulation [41].

4 Regulation at the Level of Alternative Splicing and RNA Stability

Alternative splicing of the ATP2B primary transcripts must be carefully regulated because the amino acid changes due to alternative exon usage at sites A and C result in significant changes in the functional properties of the encoded PMCA isoforms.

Evidence for cell-type and differentiation-specific alternative splicing of ATP2B transcripts is widespread. Cochlear outer hair cells almost exclusively express the w/a splice variant of PMCA2, whereas lactating mammary cells express only the w/b variant [10, 40, 42]. In differentiating hippocampal neurons, splicing of ATP2B1, B2, and B3 transcripts shifts from the b- to the a-variant [43], and a recent study in a mouse carotid artery injury model showed that ATP2B4 splicing shifted from the a- to the b-variant in the injured carotids [44]. The factors and mechanisms regulating alternative splicing of ATP2B pre-mRNAs are still incompletely understood. Changes in second messengers, including Ca^{2+} as an important feedback regulator, are clearly playing a role (see for example Ref. [45]), and these likely impact different components of the spliceosome or specific RNA-binding proteins that either promote or repress the use of specific splice sites [46, 47]. In one example Ca^{2+}, working through CaM kinase IV, has been shown to affect alternative splicing in neuronal cells via CaMKIV-responsive RNA elements (CaRREs) [48]. CaRREs are indeed present in relevant regions of ATP2B genes [47] and may be recognized by the RNA binding protein hnRNP-L which is directly phosphorylated by CaMKIV [49]. The transcription factors NFAT1 and NFAT3, possibly working with the histone deacetylase HDAC4, were also recently shown to affect alternative splicing of several ATP2B transcripts in PC12 cells [50]: Inhibition of NFAT1/3 resulted in an increased expression of the "fast" PMCA2x/c, 3x/a, and 4x/a variants primarily at the expense of the "b" variants. For a more detailed discussion of the regulation of alternative splicing in the ATP2B gene family, the reader is referred to a recent review by J. Krebs [18].

The pattern of alternative splicing also depends on the RNA secondary structure, which may be influenced by the length and sequence of the 5′ and 3′ UTR regions. Because several ATP2B genes contain alternative promoters and multiple transcription initiation sites, the promoter and first exon choice could influence the splicing of downstream exons. Similarly, alternative poly-adenylation sites have been documented in the ATP2B genes, resulting in vastly different lengths of 3′ UTR sequences as demonstrated in Northern blots from various tissues [51, 52]. However, no experimental evidence has as yet been provided for a role of the untranslated regions in alternative splicing of the ATP2B pre-mRNAs.

Posttranscriptional regulation of PMCA expression at the level of mRNA stability also deserves attention but has not yet been carefully evaluated. The differences mentioned above in the 3′ UTR regions and poly-adenylation sites of several ATP2B mRNAs may play an important role in the control of their half-lives. In rat aortic and brain vessel endothelial cells ATP2B1 transcripts have been estimated to have a short half-life of 2–3 h [31, 53], a finding that is corroborated by the significant changes in ATP2B1 mRNA levels during the cell cycle in proliferating vascular smooth muscle cells [54]. Thus, when rapid turnover of a specific PMCA isoform is required such as during cell proliferation or in response to external stimuli demanding altered basal Ca^{2+} levels, regulation at the level of ATP2B mRNA stability may be particularly important. However, systematic analyses of the correlation between the 3′ UTR length, poly-A site choice, and mRNA stability have not yet been performed for any of the ATP2B genes.

5 Regulation at the Level of Protein Translation and Stability

The vast majority of studies in which ATP2B expression was determined at the mRNA (Northern blots, in situ hybridization, RT-PCR) and at the protein level (Western blots, immunohistochemistry) report a tight correlation, i.e., PMCA protein levels generally reflect RNA abundance for the particular PMCA. This holds true for most overexpression studies where recombinant cDNAs (usually comprised of only the protein-coding sequence) are transiently or stably transfected into recipient cells. Antisense knockdown (siRNA) studies similarly show a good correlation between decreased RNA levels and loss of the specific PMCA protein, although the analysis of the effects of such treatment requires consideration of the PMCA half-life and is therefore usually performed at least 24–48 h after (transient) mRNA knockdown [55–58]. However, as in the case of ATP2B RNA stability, very little information is available on the half-lives of the endogenous PMCA isoforms in physiological conditions.

The translational regulation of ATP2B mRNAs is another area that has not yet received appropriate attention in the field. The incorporation of alternative 5′ UTR sequences (as well as of alternative exon sequences) may profoundly influence the efficiency of translation initiation due to different secondary RNA structures in the vicinity of the AUG start codon. Specific sequences in the 5′ or 3′ UTR may also play a role in targeting some ATP2B mRNAs to cellular compartments for local translation; this could be of particular relevance for ATP2B2 and ATP2B3, which are mainly expressed in neurons and where specific splice variants are concentrated in membrane microdomains such as presynaptic boutons or postsynaptic spines [59–61].

Information on posttranslational regulation of PMCA stability is also scarce although several recent studies have begun to shed some light on this issue. As already mentioned, early half-life estimates from pulse-chase experiments in endothelial cells showed that the PMCA1 isoform is comparatively unstable. A similar finding was reported by Guerini and coworkers who noted that PMCA1b was remarkably unstable due to its high susceptibility to degradation by the calcium-sensitive protease calpain [62]. Proteolytic cleavage of the PMCA by calpain allows rapid regulation of the Ca^{2+} extrusion capacity of a cell. For example, upon platelet activation by thrombin 50 % of PMCA4b was found to be cleaved by calpain within ~5 min [63]. Initially, this may result in a constitutively active 124 kDa fragment capable of preventing Ca^{2+} overload, but a subsequent reduction in active PMCA at the membrane is required to allow normal progression of clot formation. A similar Ca^{2+}-dependent feedback regulation of the PMCA by calpain appears to be operative in physiological stimulation as well as (NMDA/Ca^{2+}-induced) excitotoxicity in neurons: Limited cleavage of the PMCA is required for normal Ca^{2+} signal control, but excessive calpain cleavage will lead to excitotoxicity and cell death [64–66].

The examples above illustrate instances of the regulated degradation of specific PMCAs. Much less is known about the "constitutive" pathways of PMCA turnover

and the mechanisms of their degradation. Several reports have shown that PMCAs may be “flagged” for destruction, e.g., by posttranslational modifications such as oxidation and glycation, and these modifications result in PMCA inhibition [67–71]. The most plausible routes for the eventual demise of the pumps involve endocytosis followed by lysosomal degradation. Alternatively, PMCA turnover may also occur by shedding of PMCA-enriched membrane vesicles, as appears to be the case at the apical membrane of lactating mammary epithelial cells [40] or in the male and female reproductive tract [72, 73].

6 Regulation by (Reversible) Posttranslational Modification

Besides proteolytic cleavage and generally irreversible modifications such as oxidation and glycation mentioned above, the PMCAs are highly regulated by phosphorylation in an isoform- and splice variant-specific manner. Ser/Thr phosphorylation by prominent signaling kinases including PKA and PKC has been shown to enhance the activity of most isoforms; however, the precise effect (stimulation or decrease of pump activity) is dependent on the isoform and splice variant under study [74–80]. Phosphorylation of a specific tyrosine residue (Tyr-1176) in PMCA4, likely by the focal adhesion kinase FAK, is operative in platelet activation and results in an inhibition of PMCA activity [81–83]. The regulation of different PMCAs by phosphorylation has been extensively covered in many earlier reviews [84–86] and will therefore not be further discussed here. It is worth mentioning, however, that virtually nothing is known about the “off” mechanism of PMCA phosphorylation, i.e., about the specific Ser/Thr- and Tyr-phosphatases that are required for removal of the phosphates to make this type of regulation truly reversible.

7 Regulation by Calmodulin, the Lipid Environment, and Oligomerization

The “mother of all regulation” for the PMCAs is that by CaM, as evidenced by the extensive literature dealing with the affinity, Ca^{2+} dependence, kinetics, mechanism of action, regulation, and functional outcomes of CaM interaction with the PMCAs (see for example Refs. [84, 87–89] for early reviews). All PMCA isoforms and splice variants are sensitive to CaM and in all instances, the binding of Ca^{2+}-CaM activates the pump by releasing auto-inhibitory intramolecular interactions of the C-tail with the two major cytosolic loops in the pump [13, 86]. However, there are large differences in the extent of activation of the basal activity of the various PMCA isoforms by CaM, and perhaps more importantly in the kinetics of CaM regulation. The differences in the rates of activation and termination of CaM regulation profoundly affect how different PMCA isoforms impact the timing and shape of Ca^{2+} signals, and have allowed the distinction of “fast” and “slow” PMCAs with different “memory” for past activation [90–92]. The specific expression pattern of these pumps

reflects the physiological demands and the type of Ca^{2+} signaling in different cells: Fast pumps such as PMCA2x/b are prominent in excitable cells with frequent and rapid Ca^{2+} swings whilst slow pumps (PMCA4x/b) are dominant in non-excitable cells with very different demands on the shape and timing of Ca^{2+} signals.

As integral membrane proteins, the PMCAs are highly sensitive to their lipid environment. Numerous studies have investigated the effect of membrane phospholipids, free fatty acids and cholesterol on the activity and CaM regulation of PMCAs, with most studies focusing on the (erythrocyte) PMCA4x/b [87, 93–96]. Acidic phospholipids, notably the multiply charged phosphatidylinositol bisphosphate (PIP_2), are potent activators of PMCA4b and have been shown to directly bind to two distinct regions in the pump, one within the C-tail overlapping the CaM-binding domain and one in the first intracellular loop close to the third membrane-spanning segment [12]. Although the precise mechanism of direct phospholipid regulation of the PMCA is not fully understood, it likely involves lipid binding to membrane-proximal regions of the C-tail and intracellular loop to "loosen" autoinhibitory interactions, thereby facilitating access of substrates or conformational changes during the reaction cycle [97–99]. The type and shape of lipids surrounding the PMCAs in the lipid bilayer obviously affect pump function; on a longer time-scale PMCAs may thus also be regulated by changes in the lipid composition of the membrane. The fatty acid chain length, degree of unsaturation, type of head groups, as well as the cholesterol content impact the thickness and fluidity of the bilayer, which will impose conformational constraints on the membrane domain of the PMCAs and modulate their activity [100–102]. The membrane lipid composition plays an essential role in the partitioning of different PMCAs into membrane micro- or nanodomains (lipid rafts). Several studies have investigated the relative distribution and activity of different PMCA isoforms in sphingolipid- and cholesterol-rich lipid rafts in various cell types [103, 104]. PMCA4b is specifically concentrated in caveolae, which are specific membrane compartments found in many cell types including cardiomyocytes and endothelial cells [105–108]. The local enrichment of the PMCA in specific lipid microdomains may also facilitate the dimerization/oligomerization of pump molecules, which has been shown to happen via their regulatory C-tails and is thought to result in CaM-independent activation of pump function [109–112]. This could serve to maintain a high constitutive Ca^{2+} efflux activity in these specific membrane domains, allowing the cell to create and maintain local areas of low [Ca^{2+}] without compromising its bulk cytosolic Ca^{2+} level.

8 Integrated Regulation of PMCA Function in Cellular Calcium Signaling

Many of the studies on the regulation and functional properties of individual PMCA isoforms have been carried out on purified or highly enriched preparations of the pump in vitro. While these studies were and still are necessary to gain a detailed understanding of the structural and functional characteristics of each isoform or splice variant, they fail to address the integrated role played by the PMCAs in their

physiological context. This gap is now rapidly being filled, in part due to improved technology to analyze calcium signaling with high spatial and temporal resolution in living cells, but also because of the increased use of cell, organ and animal models amenable to genetic and environmental manipulation. The emerging concept shows that a crucial function of the PMCAs is their tightly integrated participation in all aspects of cellular calcium signaling [102, 113, 114]. In fact, it may be argued that the original role ascribed to the PMCA, i.e., that of maintaining the basal resting level of intracellular [Ca^{2+}], is secondary to their other roles in cellular Ca^{2+} handling. In some physiological contexts such as during lactation, a specific PMCA isoform (PMCA2w/b) has as its main function the massive export of Ca^{2+} into the milk from the apical (luminal) side of breast epithelial cells. Similarly, in enterocytes of the small intestine, PMCA1x/b is responsible for the bulk transport of Ca^{2+} into the blood at the basolateral side to maintain dietary calcium absorption. The expression, splicing, targeting, local regulation, and eventual removal of the PMCA2w/b and PMCA1x/b in these tissues are under tight control by external factors including sex steroids and 1,25-$(OH)_2$ D3, respectively. In other cell types, the same or a different PMCA isoform/splice variant may have an entirely different function, e.g., to control the spiking frequency and signal shape of local Ca^{2+} signals in presynaptic nerve terminals or postsynaptic spines. In these latter cases, the PMCAs have little, if any, effect on bulk cytosolic Ca^{2+}; other PMCA(s) or different calcium extrusion systems such as the Na^+/Ca^{2+} exchangers (NCX) may instead perform this function in the cell.

It is now amply clear that the PMCAs are not working in isolation but are constantly "sensing" the physiological state of the cell by dynamically interacting with other proteins and lipids. Besides CaM, a large and growing number of proteins have been shown to interact with the PMCAs [113–115]. Some of these proteins bind specifically only to a certain isoform or type of splice variant, whereas others interact promiscuously with most isoforms and splice variants. The C-terminus of all b(c/d) splice variants contains a consensus sequence for interaction with PDZ domain proteins, and many different PDZ proteins are now known to bind to the PMCA b splice variants [20, 115]. These PDZ proteins have different functional modalities including scaffolding, membrane trafficking/recycling, and signaling. Other proteins involved in signaling cross talk or direct activation or inhibition regulate several PMCA isoforms by interacting with the N-terminal tail or intracellular loops of the molecule [116]. These include inhibitory interactions of 14-3-3e and Homer-2 with sequences in the N-tail of multiple PMCAs [117–119]. The interaction of a PPXXF motif in the N-tail of PMCA4 with Homer-2 at the apical pole of parotid gland acinar cells illustrates the tight cross talk between a specific PMCA isoform and its signaling partner: Knockdown of Homer-2 results in a parallel increase in PMCA4 expression and Ca^{2+} extrusion activity in acinar cells, whereas increased expression of Homer-2 downregulates PMCA4 expression and activity [119].

Numerous examples of the integrated regulation of PMCA function by differential and dynamic protein–protein interactions have been reported in the recent literature. In rat coagulating (prostate) gland epithelial cells, PMCA1b, but not 4b, was found to be localized in the apical membrane and released by apocrine secretion in a process controlled by androgens: Upon androgen deprivation, the PMCA1b was

no longer released in aposomes and instead accumulated in a cytoplasmic compartment [120]. Rapid agonist-induced recruitment of PMCA1b to the plasma membrane has also been demonstrated in HT29 human colorectal carcinoma cells. Muscarinic G-protein coupled receptor activation by acetylcholine resulted in a significant increase in PMCA1b in the plasma membrane, and this effect was dependent on Ca^{2+} and the PDZ protein NHERF2 [121]. Here, G-protein coupled receptor activation leads to phospholipase C activation, IP3 release, and Ca^{2+} influx from the ER, promoting the targeting of NHERF2 to recruit and retain the PMCA1b in the plasma membrane. The role of NHERF2 as a "recruiter" of specific PMCA isoforms to a particular membrane domain has also been demonstrated for PMCA2w/b: Co-expression of NHERF2 increased the amount of PMCA2w/b in the apical membrane of polarized kidney epithelial cells and reduced its internalization likely via anchoring the pump to the underlying membrane cytoskeleton [122, 123]. Anchoring to the membrane cytoskeleton appears to be important for sustained PMCA function, as actin polymerization increased and disruption of polymerization decreased PMCA-mediated Ca^{2+} efflux during T-cell stimulation [124]. The polymerization state of actin may itself regulate PMCA activity [125], but the major role of anchoring of the PMCA is likely the maintenance of sufficient efflux capacity within a particular membrane domain. During immunological synapse formation preceding T-cell stimulation, the PMCA (4b) is retargeted to a different membrane microdomain by stimulus-induced interaction with other molecules involved in cellular Ca^{2+} signaling, i.e., STIM1 [126, 127], providing an example for Ca^{2+} dependent feedback regulation of PMCA localization and activity.

Integration of specific PMCAs in multi-protein signaling complexes to provide local Ca^{2+} control has also been demonstrated in rod photoreceptor synaptic terminals, where the proper localization and function of PMCA1b was shown to depend on the two PDZ domain containing proteins MPP4 and PSD95 [128, 129]. Recent examples of the importance of the integration of PMCAs in multiprotein complexes for local signaling include the finding of a tight functional coupling of PMCA isoforms 2 and 3 and the Na^+/Ca^{2+} exchanger NCX1 with the presynaptic glycine transporter GlyT2 in lipid raft subdomains in rat brainstem and spinal cord neurons [130]. In the most recent example, PMCA4b was found to cross talk with the G-protein coupled estrogen receptor GPER1/GPR30 in endothelial cells: Receptor stimulation resulted in PMCA4b inhibition by tyrosine phosphorylation and independently by physical interaction mediated by the PDZ protein PSD95. Conversely, the interaction stimulated receptor signaling, demonstrating a tightly connected interplay between Ca^{2+} signaling and GPER-mediated downstream phosphorylation of ERK1/2 via the formation of a heteromeric complex [131].

9 Conclusions

It is ironic that we may have been slow in recognizing the most important functions of the PMCAs because the initial identification and all successful biochemical characterizations were made on the pump from red blood cells, yet in these cells much

of the signaling complexity found elsewhere is absent. It is now abundantly clear that most PMCAs are not acting as "isolated" pumps dedicated solely to maintaining low bulk cytosolic Ca^{2+}; rather they are tightly integrated with scaffolding and other signaling proteins in specific membrane domains to provide "holistic" calcium control in the context of changing cellular demands, which are under external regulation by hormones, neurotransmitters and other factors impacting the cell. The regulation of PMCAs includes dynamic changes in their abundance by (1) changing transcription, splicing, and translation, (2) controlling the forward trafficking, lipid partitioning, anchoring and retention in membrane domains, and (3) recycling, shedding to the extracellular milieu, or removal followed by degradation. In addition, the activity of the PMCAs is regulated by posttranslational modification including phosphorylation, oxidation, and partial proteolysis, by direct interaction with stimulatory or inhibitory proteins and lipids, and by redistribution and association with different protein complexes. Different PMCA isoforms and splice variants are differently impacted by these regulatory mechanisms and show inherent differences in their functional properties including in their basal rates and stimulation kinetics. An important emerging concept is that many PMCAs work as integrated members of teams where they both regulate and are regulated by other members of the signaling/scaffolding protein complex. Thus, replacement of one isoform by another in a specific cell type will generally not correct the pathology caused by the absence or mutation of the former. Systems-level approaches will be needed to fully understand the unique role played by each PMCA isoform in cell physiology and pathophysiology, and may contribute to the development of specific modifiers of the pumps to address the many diseases involving these important calcium transporters.Acknowledgements I am grateful to M.-A. Strehler-Page for help with the preparation of Fig. 5.1. This work was supported in part by the Mayo Foundation for Medical Research.

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