Chapter 5
Molecular Diversity of Plasma Membrane Ca^{2+} Transporting ATPases: Their Function Under Normal and Pathological Conditions

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Abstract Plasma membrane Ca^{2+} transport ATPases (PMCA1-4, *ATP2B1-4*) are responsible for removing excess Ca^{2+} from the cell in order to keep the cytosolic Ca^{2+} ion concentration at the low level essential for normal cell function. While these pumps take care of cellular Ca^{2+} homeostasis they also change the duration and amplitude of the Ca^{2+} signal and can create Ca^{2+} gradients across the cell. This is accomplished by generating more than twenty PMCA variants each having the character – fast or slow response, long or short memory, distinct interaction partners and localization signals – that meets the specific needs of the particular cell-type in which they are expressed. It has become apparent that these pumps are essential to normal tissue development and their malfunctioning can be linked to different pathological conditions such as certain types of neurodegenerative and heart diseases, hearing loss and cancer. In this chapter we summarize the complexity of PMCA regulation and function under normal and pathological conditions with particular attention to recent developments of the field.

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Abbreviations

AD	Alzheimer's disease
ATP	adenosine triphosphate
CaM	calmodulin
CaMKII	calcium/calmodulin-dependent protein kinase II
CASK	calcium/calmodulin-dependent serine protein kinase
CBS	calmodulin binding sequence
ER	endoplasmic reticulum
ERK	extracellular-signal regulated kinase
HDAC	histone deacetylase
HER2	human epidermal growth factor receptor 2
HUVEC	human umbilical vein endothelial cell
IP3	inositol 1,4,5-trisphosphate
IP_3R	inositol 1,4,5-trisphosphate receptor
IS	immunological synapse
MAGUK	membrane-associated guanylate kinase
MLEC	mouse lung endothelial cells
NFAT	nuclear factor of activated T-cell
NHERF2	Na^+/H^+ exchanger regulatory factor 2
nNOS	neural nitric oxide synthase
PIP2	phosphatidylinositol-4,5- bisphosphate
PKC	protein kinase C
PKA	protein kinase A
PMCA	plasma membrane Ca^{2+} ATPases
POST	partner of STIM
PSD-95	post synaptic density protein 95
RANKL	nuclear factor κB ligand
RASSF1	Ras association domain-containing protein 1
RBC	red blood cell
SCD	sickle cell disease
SERCA	sarco/endoplasmic reticulum Ca^{2+} ATPases
SNP	small nucleotide polymorphisms
SOCE	store operated Ca^{2+} entry
SPCA	secretory-pathway Ca^{2+} ATPase
STIM	stromal interacting molecule
TGF	transforming growth factor
TM domain	transmembrane domain

TSA	trichostatin A
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cell

5.1 Introduction

The plasma membrane Ca^{2+} transport ATPase (PMCA protein, *ATP2B gene*) was first described as a Ca^{2+} extrusion pump in red blood cells by Hans J. Schatzmann in 1966 [1]. It became evident that this pump is an essential element of the Ca^{2+} signaling toolkit, and that it plays a vital role in maintaining Ca^{2+} homeostasis in all mammalian cells [2]. After the first discovery of the PMCA many years were spent on identifying its regulators (for example calmodulin and acidic phospholipids) before it was cloned and sequenced at around the time when sequences for many of the other P-type ATPase family members also became available [3, 4]. Further structure-function studies concentrated on the PMCAs unique C-terminal regulatory region (often called the C-tail) and identified there calmodulin and PDZ-domain binding sequence motifs, a built-in inhibitor sequence, phosphorylation sites for protein kinases and a localization signal [5]. It became apparent that PMCAs comprise a P-type ATPase sub-family, encoded by four separate genes *ATP2B1-4* [6, 7] from which alternative splicing generates more than 20 variants with distinct biochemical characteristics that make them suitable to perform specific cellular functions [8, 9]. By now it is well documented that PMCAs are not simply Ca^{2+} extrusion pumps but by changing their abundance and variant composition, having different activation kinetics, locale and partners, they can actively modulate the Ca^{2+} signal in space and time, and hence affect Ca^{2+} mediated signaling events downstream. The PMCA variants are expressed in a tissue and cell type specific manner and many of them have specific function. Although, in the past decades these pumps have been extensively characterized their importance is rather underestimated. This is because only recently we gathered more information on their involvement in diseases such as cancer, neurological disorders, hearing loss and others. In this book chapter, therefore, we will summarize briefly the long known basic characteristics of these pumps paying more attention to the most recent findings on their roles under normal and pathophysiological conditions.

5.2 Structural Features of the PMCA

PMCAs (*ATP2B1-4 gene*) belong to the P-type ATPase family and share basic structural and catalytic features with them. The closest relatives of the PMCAs are the sarco/endoplasmic reticulum type Ca^{2+} pumps (SERCAs, *ATP2A1-3*) with an overall 30 % sequence homology between PMCA4 and SERCA1 [10]. Homology modeling using the SERCA1 structure as a template [11–13] has revealed four major domains shared with SERCA1, and a relatively large unstructured C-terminal region (30–130 residues depending on the isoforms and their variants), which is unique

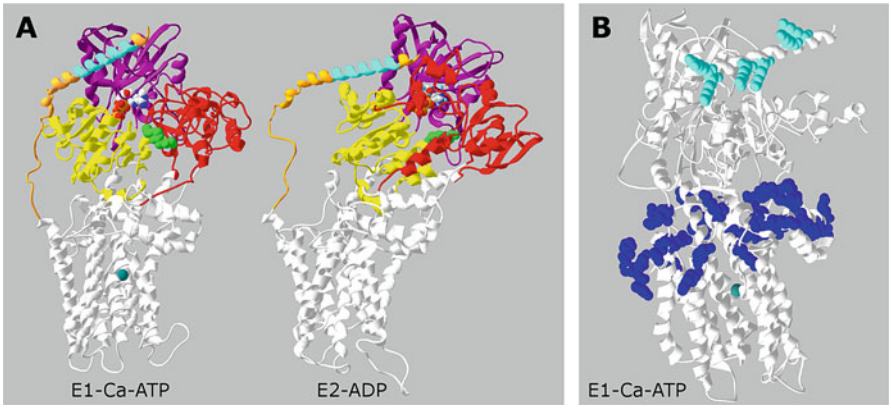

Fig. 5.1 Structural model of the PMCA in the E1-Ca-ATP and E2-ADP conformations. Structures of several intermediates in the enzyme cycle have been determined for SERCA [11–13]. Based on those, models of the intermediates have been constructed for PMCA4b (lacking 90 residues from the C-tail). (**a**): The models show 2 of the intermediates, E1 with Ca and ATP (SERCA PDB 1VFP and 1T5S) and E2 with ADP (SERCA PDB 2C88). In the latter, the Ca has been ejected into the extracellular space. They are colored as follows: A domain *red*; P domain *yellow*; N domain *purple*; stalk; insert and transmembrane domains *white*; C-tail *straw*; Calmodulin-binding domain *cyan*; Ca^{2+}*metallic blue-green*. (**b**): The positively charged residues of the PIP_2 binding regions are colored. The blue collar and the insert *blue*, the calmodulin-binding domain (CBD) *cyan*. The CBD would have the potential of releasing from the conformation shown and lying on the surface of the membrane in a PIP_2-rich region

to the PMCAs (Fig. 5.1a) (for a review see also [14]). The M-domain consists of 10 trans-membrane spanning helices that provide the coordinating ligands for the binding of one cytosolic Ca^{2+} ion to be transported. The N-domain binds an ATP molecule of which the terminal phosphate is transferred to a highly conserved aspartate in the P-domain forming a high-energy acyl-phosphate intermediate. As a result of these events hydrolysis of one ATP molecule provides sufficient energy to translocate one Ca^{2+} ion through the membrane [15] that is coupled to H^+ transport in return with a Ca^{2+}:H^+ ratio of 1:2 [16]. The A-domain coordinates the movements of the other three domains during the E1-E2 transition to complete a full reaction cycle [17]. While the catalytic domains N, P and the M-domain are largely conserved between the PMCAs the C-tail and the A-domain – where alternative splicing generates substantial sequence divergence – vary substantially. These variations in the C-tail and A-domain can generate PMCA proteins with distinct characteristics [18, 19].

The Blue Collar In contrast to the endoplasmic reticulum-resident SERCA pump a cluster of positively charged residues were found at the intracellular near-membrane region of the PMCA forming four binding pockets for the phosphorylated inositol ring of PIP2 (phosphatidylinositol-4,5-bisphosphate) [20], in addition to the previously determined linear PIP2 binding sequences near the A splice-site region at the A-domain [21, 22] and the calmodulin binding sequence at the C-tail [23]. Figure 5.1b shows a blue collar formed from the four PIP2 binding pockets and the

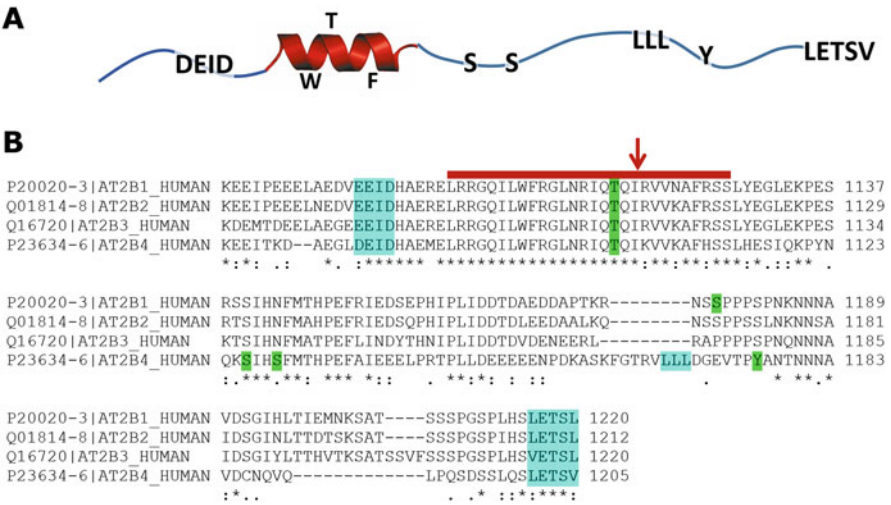

Fig. 5.2 C-tail of the "b" splice variants of PMCA1-4. (**a**) Schematic representation of the C-tail of PMCA4b emphasizing important sequence motifs highlighted below. Calmodulin-binding domain is colored *burgundy*. (**b**) An alignment of C-terminal sequences of "b" splice forms of PMCA1-4 demonstrates that the variants may have distinct regulatory features (i.e. the di-leucine motif in PMCA4b) however; some sequence motifs (caspase 3 sites and the PDZ-binding tails) are relatively conserved. These motifs are colored *cyan*. The PKA, PKC and tyrosine kinase phosphorylation sites are highlighted in *green* and the calmodulin-binding sequence is marked *burgundy*. The arrow indicates where alternative splice changes the sequence in the other splice variants of PMCA1-4

linear lipid binding region of the A domain around the stalk region of the PMCA. This arrangement of positively charged residues follows the positive inside role, which is quite common in plasma membrane proteins and often involved in PIP2 binding [24, 25].

The C-Tail The C-tail, which is also known as the main regulatory unit of these pumps, is the most characterized although the least conserved region of the PMCAs (Fig. 5.2). A major portion of this region is structurally disordered [5], containing multiple recognition sites: a DxxD caspase cleavage site [26, 27], a calmodulin-binding domain (CBD) with an overlapping auto-inhibitory region and acidic lipid binding side chains [3], several protein kinase phosphorylation sites [28, 29], a di-leucine-like localization signal [30] and a PDZ-domain-binding sequence motif at the C-terminus [31]. Some of these motifs are present in nearly all PMCAs (caspase 3 cleavage sites, CBD) while others are specific to certain variants; for instance the di-leucine-like motif is specific to PMCA4b whereas the PDZ-binding motif is present in all "b" splice variants. However, specificity of the PDZ binding may vary because the terminal amino acid is Val in PMCA4b but Leu in PMCA1-3. As an example the sodium-hydrogen exchange regulatory cofactor NHERF2 interacts with PMCA2b but not with PMCA4b [32].

Ca^{2+}-Calmodulin Binding is critical for PMCA function. Early studies identified a 28 residue long sequence at the C-tail of PMCA4b that could bind Ca^{2+}-calmodulin with high affinity. Extensive kinetic [33, 34] and NMR [35] studies with a peptide (c28) representing the complete 28-residue sequence region have revealed two anchor sites Phe-1110 and Trp-1093 in a relative position of 18 and 1, and two steps of Ca^{2+}-calmodulin binding in an anti-parallel manner (Fig. 5.3). In the first step the C-terminal lobe of calmodulin binds the N-terminal Trp-1093, followed by the second step, which is binding of the C-terminal Phe-1110 to the N-terminal lobe of calmodulin. As a result, calmodulin wraps around the c28 peptide that adopts an α-helix with its anchors buried in the hydrophobic pockets of the two distinct CaM lobes. This model correlates well with an earlier NMR structure of Ca^{2+}-calmodulin with a shorter c20 peptide lacking the second anchor Phe-1110 [36]. In that case the peptide could bind only to the C-terminal lobe of calmodulin, which retained its extended structure, as is expected (Fig. 5.3).

The w Insert Another structurally less defined region of the molecule is the sequence that couples the A domain to the third membrane spanning helix. An

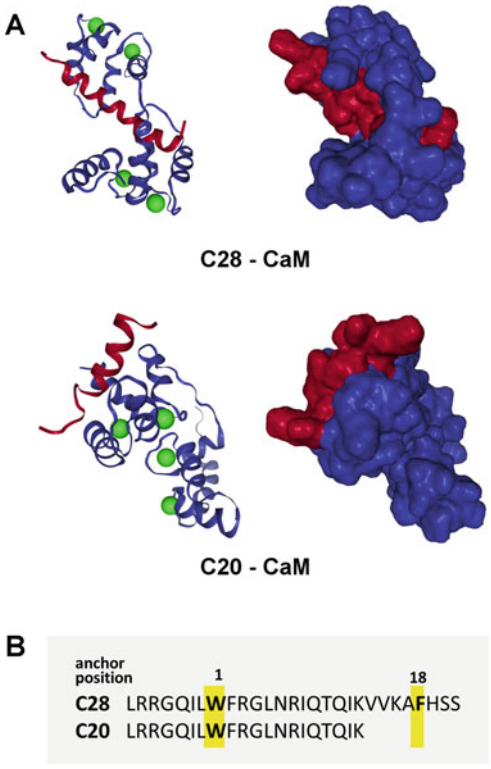

Fig. 5.3 NMR structure of calmodulin in complex with calmodulin binding peptides. (**a**)**:** Structures of C28-calmodulin (https://www.rcsb.org/structure/2KNE) and C20-calmodulin complexes (https://www.rcsb.org/structure/1CFF). C20 and C28 correspond to the appropriate calmodulin binding sequence of PMCA4b. Colors: calmodulin *blue*; peptide *burgundy*; green spheres correspond to the 4 Ca^{2+} bound to CaM. (**b**): Sequences of the peptides C20 and C28 with the anchors 18-1

alternative splice at splice site A changes the structure of this region by including or excluding a single exon, producing the x and z variants of the isoforms [37], however, no functional significance has been linked to these changes. In PMCA2, however, additional variations exist in which two more exons can be inserted generating the PMCA2 y and w forms. Importantly, the w insert – which is a 44-residue long sequence – is essential for targeting PMCA2 to the apical compartment of polarized cells.

5.3 Regulation of PMCA Expression and Function

PMCAs are encoded by four separate genes (*ATP2B1-4*) located at distinct chromosomes: 12q21–23, 3p25.3, Xq28 and 1q25–q32, respectively [8]. Two major alternative splice options at splice sites A and C of the primary transcripts of each *ATP2B* gene have the potential of generating >30 PMCA protein variants, however, only 20 of them have been identified in different tissues [38, 39]. In addition, mutations, single nucleotide polymorphisms and posttranslational modifications further increase PMCA variations. It is not surprising that to keep the level of calcium within a suitable range in the cytoplasm of different cell types with very different function tight regulation of PMCAs is required at the transcriptional, splicing, translational and protein levels.

5.3.1 Regulation at the Transcription Level

Transcriptional regulation of *ATP2B* genes is complex and still not well understood. The intricate regulatory structure of the promoter and enhancer regions of the genes allows the fine-tuning of each PMCA's transcription during embryonic development, in various tissues, as well as upon various stimuli. It has been shown that in mouse smooth muscle cells Atp2b1 expression during G1/S phase is reduced via c-myb binding to the promoter region of the gene [40]. This transcription factor is also involved in the down-regulation of Atp2b1 in differentiating B-lymphocytes [41]. The active form of vitamin D induces the transcription of *ATP2B1* in various tissues and cell types [42–45]. *ATP2B2* gene has four alternative promoters and alternatively spliced 5' exons, which showed higher expression and different promoter usage in mammary gland compared to neuronal cells [46]. EGR1 can bind to a specific region in the CpG island of the *ATP2B2* gene and controls the α-type promoter activity, which is specific to brain and auditory cells [47]. The *ATP2B4* gene contains an enhancer in the intron 1, which has an essential role in the erythroid differentiation, but has no effect in other cell types [48]. From these studies it appears that PMCAs possess general and specific transcription factor binding sites and regions, which only play role under certain conditions, under proper stimulus or differentiation state of the given cell type.

5.3.2 Regulation at the Protein Level

Auto-Inhibition PMCA activity is determined by the presence of an auto-inhibitory unit at the C-tail, which largely overlaps with the calmodulin-binding sequence [49]. This inhibitory unit binds to the N- and A-domains interfering with Ca^{2+} binding to the catalytic sites, and slowing down the reaction cycle by inhibiting the movements of the cytosolic domains [23]. The extent of the auto-inhibition differs from one isoform to the other and is affected by the alternative splice at splice site C [50–52]. As a result, PMCA4b is the only truly inactive pump at resting cytosolic Ca^{2+} ion concentration while all the other pumps are partially active, as determined in cell free systems.

Activation by Caspase 3 The auto-inhibitory C-tail is removed by the executor protease caspase 3 during apoptosis. Caspase 3 cleaves PMCA4b at a canonic caspase 3 cleavage site (DEID) just upstream of the CBD-auto-inhibitory sequence removing the complete auto-inhibitory region [26, 27]. While there has been a long debate on whether caspase 3 activates or inhibits PMCA4b during apoptosis [53] it is conceivable that deleting the auto-inhibitor should result in a gain-of- function pump [54], however, the overall outcome could depend on the given cell type, stimulus and conditions that need further studies.

Activation with Ca^{2+}-Calmodulin A functionally important feature of the PMCA variants is the difference in their activation with Ca^{2+}-calmodulin that determines the rate by which they can respond to the incoming Ca^{2+} signal, and equally important is the length of time during which they remain active after the stimulus [55]. Since pump and calmodulin compete for CBD-autoinhibitor it is expected that a strong pump-CBD-auto-inhibitor interaction will result not only in a low basal activity but also in a slow activation rate. Indeed, PMCA4b has both the lowest basal activity and the slowest activation with calmodulin among the isoforms (slow pump, $T_{1/2}$ is about 1 min) [56]. Although, PMCA4b is activated slowly its inactivation rate is even slower (long memory, remains active for about 20 min) because calmodulin remains bound to the pump for a long period of time [57]. An alternative splice that creates a shorter version of PMCA4 changes the response of the pump to Ca^{2+} completely so that PMCA4a binds Ca^{2+}-calmodulin quickly (fast pump, T1/2 is about 20 s) but then calmodulin dissociates also quickly, resulting in a fast responding pump that remains active for a relatively short period of time (short memory, active for less than a minute) [34]. It is important to note, that PMCA4a also has a relatively high basal activity suggesting weak interaction between pump and auto-inhibitor. All other forms – variants of PMCA2 and PMCA3 – that have been characterized are fast responding pumps having slow inactivation rates (long memory), and as mentioned above they also have relatively high activity even without activators [50, 57].

Activation with Acidic Phospholipids Acidic lipids like PS and the PIPs – PI, PIP and PIP2 – can activate the pump and the amount of activation is augmented as the negative charge of the phospholipid head group increases [58]. It has been

demonstrated that both the CBD and the linear basic sequence in the A-domain are involved in this type of activation [21–23]. It has been suggested that changes in the lipid composition may affect PMCA activity and that PMCAs might be more active in PIP2-rich lipid rafts [59]. Recently, it was demonstrated that the activity of the PMCA is also modulated by neutral phospholipids. The activity of PMCA4b was optimal when it was reconstituted in a 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayer of approximately 24 Å thickness [60]. Molecular simulation studies have revealed that in DMPC several lysine and arginine residues at the extracellular surface are exposed to the medium while in a thicker layer of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) these residues are embedded in the hydrophobic core that could explain the reduced activity observed in DOPC.

Regulation by the Actin Cytoskeleton First it was shown that PMCAs interact with F-actin in activated platelets and they are associated with the F-actin rich cytoskeleton at or near the filopodia [61, 62]. Later it was documented that the purified PMCA4b can bind both monomeric and filamentous actin and while actin monomers activate the pump, F-actin may inhibit its function [63, 64]. These results were confirmed by using live HEK cells expressing isoforms PMCA2 and PMCA4 [65]. Based on these findings it has been suggested that PMCA can regulate actin dynamics through a series of feed-back regulations by lowering Ca^{2+} concentration in its vicinity and promoting actin polymerization, which in turn switches off the PMCA function allowing increase in Ca^{2+} levels and hence actin de-polymerization [66].

5.4 Function of the PMCAs in the Living Cell

It is quite remarkable how the above described diverse structural and biochemical characteristics of the PMCA proteins are translated into specific physiological functions in the different cell types. Distinct kinetics of the PMCAs are transcribed into distinct Ca^{2+} signaling properties while additional structural diversity between the PMCAs determines their localization and interaction patterns with different scaffolding and signaling molecules resulting in unique PMCA variant-specific cellular function (Table 5.2).

5.4.1 PMCAs Shape the Ca^{2+} Signal

It has been widely accepted that PMCAs play a role in the decay phase of the store-operated Ca^{2+} entry (SOCE). However, expression of PMCAs with distinct kinetic properties (see also Table 5.1 and Fig. 5.4) – fast or slow, with or without memories – resulted not only in a faster decay of the signal but also in very different Ca^{2+} signaling patterns in HEK and HeLa cells [67]. While the “slow with

Table 5.1 Distinct kinetic properties and distribution of the PMCAs

Basal activity	Activation with CaM	Memory	Pump variant	Cells, tissues
High	Fast	Short	PMCA4a	Smooth muscle, heart, sperm
High	Fast	Long	PMCA2b	Neuron, mammary gland
			PMCA2a	Neuron, cochlear hair cells
Low	Slow	Long	PMCA4b	Erythrocyte, breast, colon heart, kidney, HUVEC, melanoma

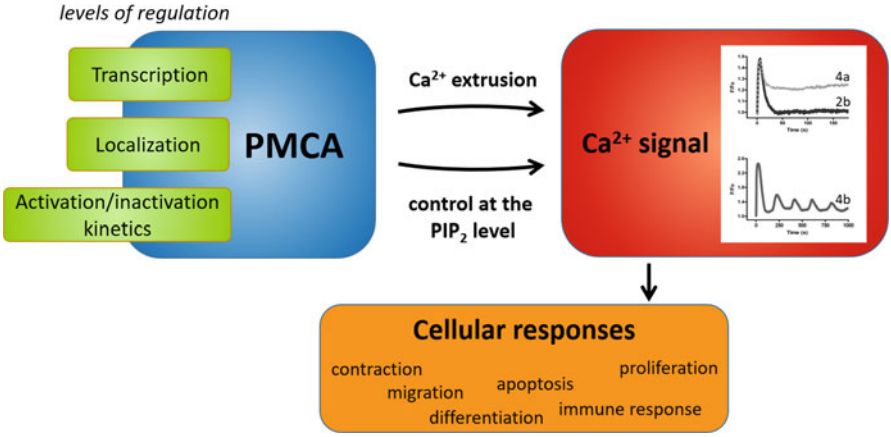

Fig. 5.4 Schematic representation of the role of PMCAs in Ca^{2+}signaling. The abundance of PMCA is regulated at the transcriptional and protein levels. Localization is affected by specific sequence motifs and interaction with other proteins. The activity is regulated by proteins (such as calmodulin) and acidic lipids. This can result in the modulation of the Ca^{2+} signal at two levels: (i) Ca^{2+} extrusion; (ii) IP_3-induced Ca^{2+} release. The resultant Ca^{2+} signal then might be translated to distinct cell responses

memory" PMCA4b induced Ca^{2+} oscillation after the first spike, the C-terminal splice variant of the PMCA4 isoform – the "fast without memory" PMCA4a – responds quickly to the incoming Ca^{2+} but then since it becomes inactivated also quickly the signal returns to an elevated level without oscillation. PMCA2b – a fast pump with memory – allows only short Ca^{2+} spikes and Ca^{2+} concentration always returns to the basal level quickly. It was also demonstrated that in addition to shaping the SOCE mediated Ca^{2+} signal PMCAs also control the formation of IP3 by controlling the availability of the signaling PIP2 molecules, and hence regulate the release of Ca^{2+} from the stores [20] (Fig. 5.4). It is important to note that the Ca^{2+} signal can also be altered through additional cell type-specific regulatory mechanisms of the PMCA. During T-cell activation, for example, it was shown that the activity of PMCA4b is inhibited by the interaction with the ER Ca^{2+} sensor protein STIM1 [68] and its partner scaffold protein POST [69] resulting in a more sustained elevation in intracellular Ca^{2+} concentration.

5.4.2 Cell Type Specific Expression of the PMCAs

Homozygous deletion of the *ATP2B1* gene in mice is lethal suggesting that PMCA1 is the housekeeping isoform [70]. The other isoforms PMCA2-4 are expressed at different stages of development [8]. The slow PMCA4b variant is present in erythrocytes, T lymphocytes and in epithelial cells but also abundantly expressed in the heart and smooth muscle cells [39]. PMCA4a is expressed in the brain and it is the only PMCA isoform present in the sperm tail [71]. Altered expression of *ATP2B4* in mice was associated with arrhythmias, cardiac hypertrophy and heart failure. Deletion of both copies of *ATP2B4* in mice caused male infertility [70, 72]. Interestingly, in activated sperm cells the pattern of the Ca^{2+} signal is similar to that seen in the PMCA4a expressing Hela cells [73]. Ca^{2+} pumps (PMCA1 and PMCA4) were shown to contribute to sustained Ca^{2+} oscillations in human mesenchymal stem cells [74] and airway smooth muscle cells [75].

The fast pumps PMCA2 and PMCA3 are abundantly expressed in excitable tissues such as the brain and skeletal muscle [76, 77]. The PMCA2w/a and PMCA2w/b forms are found in vestibular hair cells and in Purkinje neurons of the cerebellum where they can react quickly to the fast signals induced by the voltage-gated Ca^{2+} channels. A specific form PMCA2w/b is also expressed in the lactating mammary gland. Knock down of the *ATP2B2* gene induced ataxia, deafness [78] and reduced Ca^{2+} concentration in the milk [79]. These are just a few examples demonstrating how variations in PMCA expression contribute to cell-type specific functions (see more details in refs (39, 55, 76, 77) and in Table 5.1.

5.4.3 Polarized Expression of the PMCA

To perform their cellular function it is also important to target PMCA proteins to the appropriate membrane compartment. This is accomplished by intrinsic localization signals and/or by interaction with other proteins in a cell-type specific manner. In many cases these characteristics of the PMCAs are sensitive to alternative splicing. For example, the di-leucine-like localization motif is unique to the "b" splice variant of PMCA4 that was shown to direct this pump to endocytic vesicles in non-confluent epithelial cells [30]. Hence, PMCA4b localizes to the plasma membrane only in fully confluent differentiated cells where it can be stabilized and/or modulated by other interacting molecules [80]. Most recently, basigin/CD147 was identified as a novel interacting protein that may serve as a subunit of the PMCA [81]. It was demonstrated in a variety of cell types that PMCA1-4 interacts with basigin in the ER, which is essentially involved in functional targeting PMCAs to the plasma membrane.

PMCA proteins are localized to specific membrane compartments in polarized cells where they contribute to trans-cellular Ca^{2+} fluxes. While the lateral compartment seems to be the default place, in some cell types PMCAs localize apically.

PMCA2 for example can be directed to the apical compartment by an alternative splice option at site A that introduces a 44-residue long "w" sequence at the region that connects the A and TM domains [37]. The resultant PMCA2w/b and PMCA2w/a variants have very specific functions in the lactating mammary gland [79] and the stereocilia of hair cells [78, 82, 83] where PMCA2w/b is responsible for milk Ca^{2+} while PMCA2w/a contributes to hearing, respectively. The "b" splice variant of PMCA2w might be connected through PDZ-interactions with the scaffold protein NHERF2 to the actin cytoskeleton by which it is immobilized in the apical membrane [84]. In contrast, PMCA2w/a, which is lacking the PDZ-interacting tail, is very mobile, trafficking in and out of the stereocilia of hair cells [85]. In parotid gland acinar cells PMCA4b was found in the apical membrane compartment and its localization was modulated by PDZ-interaction with Homer2 [86]. In the same cells PMCA1 was also found in the apical membrane but only when it was phosphorylated by PKA [87]. PMCA4b plays an important role in the immune synapse where it is targeted to specific signaling micro domains beneath the mitochondria where it is actively involved in Ca^{2+} handling during T-cell activation controlling Ca^{2+} influx through the CRAC channels [88].

Polarized distribution of PMCA was also found in migrating cells. In collectively migrating human umbilical vein endothelial cells (HUVEC) PMCA located to the front of the cells by which it contributed to the front-to-rear Ca^{2+} gradient essential for directed cell migration [89]. In addition, downregulation of PMCA4 increased while its overexpression decreased cell migration in a wound-healing assay of HUVECs [90]. These data are in line with the latest finding demonstrating that PMCA4b interferes with cell migration of a highly motile BRAF mutant melanoma cell line [91]. These examples highlight the importance of PMCA targeting and demonstrate that different interacting partners may change the location of PMCAs resulting in distinct cellular functions (see Table 5.2).

5.4.4 Interaction of PMCAs with Signaling Molecules

Through interactions with other proteins PMCAs can influence downstream signaling events (Table 5.2). In many cases they influence the activity of the interacting signaling molecule by reducing the Ca^{2+} concentration in its vicinity. One example is the interaction of PMCA2 and PMCA4 with the Ca^{2+}-CaM dependent phosphatase calcineurin through their catalytic domain that was found to reduce the activity of the nuclear factor activated T-cell (NFAT) pathway [92, 93]. Inhibition of this interaction increased Fas-ligand expression and apoptosis in breast cancer cells [94], while PMCA4b overexpression in endothelial cells reduced VEGF initiated cell migration and angiogenesis [95]. Another example for this type of interaction was described between PMCA4b and calcium/calmodulin-dependent serine protein kinase (CASK) in rat brain and kidney where PMCA4b binds CASK through its C-terminal PDZ binding motif [96]. CASK together with Tbr-1 induces T-element dependent transcription; however, this is strongly decreased upon interaction with

Table 5.2 PMCA interactions

Interacting partner	Isoform preference	Type/place of interaction	Cell type	Effect	References
Effect of interacting on PMCA					
14-3-3ε	PMCA1, PMCA3, PMCA4	N-terminal	HeLa, CHO	Inhibition of PMCA activity	[233, 234]
G-actin (short oligomers)	PMCA4	Putative actin-binding site	Erythrocyte	Increasing PMCA activity	[63, 64, 235]
F-actin	PMCA4	Putative actin-binding site	Erythrocyte	Inhibition of PMCA activity	[64]
G/F-actin	PMCA4b	Through CLP36 and α-actinin	Platelet		[62, 236]
Calmodulin	All PMCA forms	Calmodulin-binding domain CaMBS1, CaMBS2	All kinds of cells	RELIEVING autoinhibition of the pump, increasing both Ca2+ affinity and pump rate	[237, 238]
CASK	PMCA4b	Via PDZ interaction	Mouse sperm	Reduced PMCA activity	[97]
Homer1	PMCA1-4 b-splice forms	Via PDZ interaction	Hippocampal neurons, MDCK, COS-7	Increasing PMCA activity	[239, 240]
Homer2	PMCA1b	Homer-binding	Mouse parotid gland acinar cells, HEK293	Inhibition of PMCA activity	[86]
	PMCA4b				

(continued)

Table 5.2 (continued)

Interacting partner	Isoform preference	Type/place of interaction	Cell type	Effect	References
Effect of PMCA on the interacting partner					
Calcineurin	PMCA4b	Main intracellular loop (amino acids 501–575)	HEK293, MCF-7, HUVEC, endothelial cells, cardiomyocytes	Inhibition of the calcineurin/NFAT signal transduction pathway	[92, 93, 95, 204]
	PMCA2(b)	Amino acids 462–684	Breast cancer cells, MCF-7, ZR-75-1, MDA-MB-231, T47D,HEK293	Inhibition of the calcineurin/NFAT signal transduction pathway	[93, 94]
CASK	PMCA4b	Via PDZ interaction	Rat brain and kidney, HEK293	Inhibition of CASK function	[96]
CD147 (basigin)	PMCA4		Jurkat E6.1	Negatively regulates IL-2 expression	[231]
nNOS	PMCA4b	Via PDZ interaction	HEK293, neuro-2a, cardiomyocytes, sperm	Decrease nNOS activity, decrease NO production	[197, 241, 242]
eNOS	PMCA4	Catalytic intracellular loop	Endothelial cells, sperm	Decrease eNOS activity, decrease NO production	[243]
Ras-associated factor-1	PMCA4b	Main intracellular loop (region 652–748)	HEK293, cardiomyocytes	Modulating the EGF-mediated Ras signaling pathways	[244]
α1-syntrophin	PMCA1b, PMCA4b	Main intracellular loop (region 652–748)	HEK293, heart	Inhibit NOS-I-mediated NO production	[200]

Changing localization of PMCA					
CLP36	PMCA4b	Via PDZ interaction	Platelet	Connect PMCA to the cytoskeleton	[62]
mGluR1–Homer3–IP3R1	PMCA2	via PDZ interaction	Purkinje neurons	Component of the mGluR1–Homer3–IP3R1 signaling complex	[245]
MAGUK family members: PSD95, PSD93, SAP97 and SAP102	PMCA2b, PMCA4b	Via PDZ interaction	MDCK, hippocampal neurons	Recruit PMCA to specific membrane domains	[31, 80, 246]
	Only PMCA4b	Via PDZ interaction			
neuroplastin or basigin	PMCA1-4	Heteromeric complexes of PMCAs1–4 and either neuroplastin or basigin	Brain, kidney	Stability and effective surface trafficking of PMCA	[81]
NHERF2	PMCA2(w)b	Via PDZ interaction	MDCK	NHERF2 anchors PMCA2w/b to the apical actin filaments via ezrin	[32, 84]
	PMCA1b	Via PDZ interaction	HT-29	Scaffolding and maintaining PMCA at the cell membrane	[247]
PISP	All PMCA b-splice forms	Via PDZ interaction	MDCK	Sorting of PMCAs to and from the plasma membrane	[248]

PMCA4b in HEK cells. Interestingly, CASK and PMCA4b interaction was also found in mouse sperm where CASK inhibited the activity of the pump resulting in an increased Ca^{2+} level and ultimately decreased motility of the sperm [97]. Several other interactions between PMCA proteins and their partners were described that influence downstream signaling events such as interactions with nNOS in the heart, CD147 in T-cells, STIM and POST in the immune synapse or with F- and G-actin. These results demonstrate that besides maintaining the low intracellular calcium level PMCAs are also important signaling molecules modulating the outcome of a variety of cell-type specific functions.

5.5 PMCAs in Disease Pathogenesis

PMCA proteins have been associated with several diseases in humans. Since many isoforms have highly specialized, cell type specific function alterations in their expression, localization, regulation or activity may contribute to the development of distinct pathological conditions (Table 5.3) [98]. Alterations of the PMCAs have been described in cardiovascular diseases, neurodegenerative disorders and cancer [99, 100]. More recently genetic variations in the *ATP2B* genes were also linked to certain pathological conditions.

5.5.1 Diseases Related to Genetic Variations in ATP2B1-4

ATP2B1 Small nucleotide polymorphisms (SNPs) found in the *ATP2B1* gene were associated with hypertension [101, 102], coronary artery disease [103–105] and early onset preeclampsia [106]. Preeclampsia is a disorder during pregnancy and it is characterized by high blood pressure and proteinuria. Reduced Ca^{2+}-ATPase activity of myometrium and the placental trophoblast was described in preeclamptic women [107], and a decreased expression of PMCA1 and PMCA4 in preeclamptic placental tissue was also found [108] suggesting a pivotal role of PMCAs in calcium homeostasis and transport through the placenta. The susceptibility to hypertension resulting in elevated blood pressure was linked to SNP rs11105378 in *ATP2B1* that was suggested to decrease PMCA1 expression in human umbilical artery smooth muscle cells [109]. In patients with chronic kidney disease, SNPs in ATB2B1 were associated with coronary atherosclerosis and myocardial infarction [105].

ATP2B2 SNPs in the *ATP2B2* gene were associated with autism in both European and Chinese population [110, 111]. Also, a missense mutation of PMCA2 (V586M) was shown to exacerbate the effect of the mutation in cadherin-23 leading to hearing loss [112, 113] in good accordance with the finding that ablation or missense mutations in PMCA2 cause deafness in mice [83, 114].

Table 5.3 PMCA related diseases

PMCA/*ATP2B*	Diseases associated with genetic variation in the *ATP2B1-4* genes	Diseases associated with altered expression, localization or activity of the PMCA proteins
PMCA1 (*ATP2B1*)	Hypertension	Multiple sclerosis
	Coronary artery disease	Reduced bone mineral density
	Myocardial infarction	Oral cancer
	Early onset preeclampsia	Ovarian cancer
PMCA2 (*ATP2B2*)	Hereditary deafness	Parkinson's disease
	Autism	Type 1 and type 2 diabetes
		Breast cancer
PMCA3 (*ATP2B3*)	X-linked cerebellar ataxia	Multiple sclerosis
	Aldosterone producing adenomas	
PMCA4 (*ATP2B4*)	Familial spastic paraplegia	Cardiac hypertrophy
	Developmental dysplasia of the hip	Hypertension
	Malaria resistance	Sickle cell disease
		Alzheimer's disease
		Chronic kidney disease
		Diabetes
		Adult idiopathic scoliosis
		Colon cancer
		Breast cancer
		Melanoma

ATP2B3 Missense mutation in the *ATP2B3* gene was found in patients with X-linked congenital cerebellar ataxia in two separate cases, in which the ability of the pump to decrease intracellular Ca^{2+} concentration after stimulation was compromised [115, 116]. Later it was demonstrated that the G1107D replacement altered both activation and auto-inhibition of this pump at low Ca^{2+} levels [117]. Mutations in the *ATP2B3* gene were also identified in some aldosterone producing adenomas (APA), and were linked to elevated aldosterone production compared with wild type APAs [118, 119]. In cellular models it was demonstrated that impaired PMCA3 function resulted in elevated intracellular Ca^{2+} levels and consequently increased aldosterone synthase production in the cells [120].

ATP2B4 Missense mutation in the *ATP2B4* gene was found in one family with familial spastic paraplegia that causes lower limb spasticity and weakness in patients [121]. Later it was shown that overexpression of the mutant PMCA4 protein in human neuroblastoma cells increased the resting cytosolic Ca^{2+} concentration and elevated the maximal Ca^{2+} surge after stimulation relative to the wild type pump [122]. Rear heterozygous variants in the *ATP2B4* and the *HSPG2* genes were described in a family with developmental dysplasia of the hip and based on *in silico* analysis an epistatic interaction was suggested between the genes [123]. SNPs in the *ATP2B4* gene were related to resistance against severe malaria that will be discussed in detail in the next chapter.

5.5.2 PMCAs in Red Blood Cell Related Diseases

PMCAs were among the first proteins described – and later characterized – in the membrane of red blood cells [124–126]. Since mature red cells (RBCs) are easily accessible, and have no internal membrane organelles involved in Ca^{2+} homeostasis, they have become important model cells for the examination of the enzymatic activity and kinetic parameters of the plasma membrane-bound PMCA protein [22, 127, 128]. Two isoforms have been identified in the RBC surface, PMCA1b and PMCA4b, of which PMCA4b appeared to be the most abundant [129–132]. These high affinity calcium pumps are responsible for maintaining the exceptionally low total Ca^{2+} content of red cells [133–135]. They have a crucial role in balancing cell calcium during shear stress in the microcirculation [136], volume control [137, 138] and in senescence and programmed cell death [131, 139, 140] of RBCs. Under certain pathological conditions – such as hereditary hemolytic anemia, malaria and diabetes mellitus – the intracellular Ca^{2+} levels in RBCs are altered [135, 141], therefore, the role of PMCAs in these cases emerges.

In Hereditary Hemolytic Anemia Ca^{2+} transport has a particular importance. In case of sickle cell anemia (SCD) and thalassemia, atypical hemoglobin (such as HbS) polymerization and deoxygenating processes lead to membrane deformation and activation of the mechanosensitive stretch-activated cation channel PIEZO1 [142]. As a result, Ca^{2+} permeability of these atypical RBCs increases. Subsequent stochastic activation of the Gardos or Ca^{2+}-sensitive potassium channel can lead to sickling and dehydration of red cells in SCD patients [131, 138, 143, 144]. It was found that PMCA inhibition is also involved in the maintenance of the high Ca^{2+} concentration needed for sickle cell dehydration [145, 146].

Severe Malaria is one of the most studied infectious diseases worldwide [147, 148]; however, the molecular mechanisms underlying the survival and growth of the parasite in the human body are still not fully understood. As a result of co-evolution of human and *Plasmodium* species, many alleles preserved in our genome, which provide some degree of protection against malaria infection [149, 150]. Majority of these alleles are important in the erythroid stage of the parasite [150] when it binds to the uninfected RBC, invades it and grows inside the red cells. The firstly described genetic factors linked to malaria protection were the hemoglobin genes [151, 152], but there are several other red cell related genetic variants involved in the susceptibility to malaria [148] including ABO blood group [153, 154], G6PD [151, 155], glycophorin genes [156, 157], CR1 [158], band 3 protein (*SLC4A1*) [157], pyruvate kinase (Pklv) [159], basigin [160] and ABCB6 [161]. It was recently discovered that PMCAs present in RBCs are involved in the survival and growth of the parasite and some variations in the *ATP2B4* (encoding PMCA4) gene may lead to malaria resistance [162–165].

The latest genome wide association (GWA) [163, 164] and multicenter [165] studies have shown that the *ATP2B4* gene also carries a haplotype that is involved in malaria protection and this haplotype showed association with red blood cell traits

such as mean corpuscular hemoglobin concentration (MCHC) [166]. According to Lessard et al. [167], this haplotype is located in the enhancer region of the protein, and the complete deletion of this region lead to complete loss of PMCA expression in some erythroid related cell lines, while in case of some other cell lines the deletion does not cause any change in its expression. It is also described [168] that this haplotype leads to reduced expression of PMCA4b in RBCs, but this change is not associated with any additional physiological conditions, probably because this genome region is only essential in erythrocyte development. It is also notable, that this haplotype is much more frequent in malaria-endemic than in malaria-free countries (NCBI and CDC databases). While the relationship between these variations in the *ATP2B4* gene and malaria susceptibility is apparent, the exact function of the PMCA in the parasite's lifecycle within RBCs is still not known [169]. There are controversial data [170] whether the parasitophorous vacuolar membrane (PVM), surrounding the parasite inside the RBCs, contains host membrane proteins [171] or they are excluded from it [172]. Although, the locale of the PMCA during RBC phase of the parasite lifecycle has not been determined, it has been suggested that PMCA remains in the vacuolar membrane, and the parasite may use this protein to maintain a sufficiently high concentration of Ca^{2+} within the vacuolar membrane to proliferate [162]. Thus, selective inhibition of the PMCA may offer a potential new treatment option for malaria in the future.

Diabetes In poorly controlled diabetic patients increased glycosylation and decreased Ca^{2+}-ATPase activity were detected [173]. In another study, oral glucose administration to healthy subjects also decreased the activity of the RBC Ca^{2+}-ATPase [174] Similar results were obtained when protein glycosylation and Ca^{2+}-ATPase activity were measured in membranes from normal erythrocytes pre-incubated with glucose [175]. It has also been shown that the activity of the pump decreases with cell age, however, this effect was independent of the patients' glucose level indicating that glycation could not be responsible for the age dependent decline in pump's activity [176].

5.5.3 PMCAs Linked to Neuronal Disorders and Other Diseases

Although, in several diseases no genetic alterations in the *ATP2B* genes have been identified, modified expression, altered activity or de-regulation of one or more PMCA isoforms could be associated with the disorder. For example, PMCAs have an important role in the brain where they have been linked to certain neurodegen-

erative disorders [100]. In Alzheimer's disease (AD) deposits of amyloid β-peptide are extensively formed and it was suggested that activation of the amyloidogenic pathway was associated with the remodeling of neuronal Ca^{2+} signaling [177]. First it was found that Ca^{2+} dependence of PMCAs was different in membrane vesicles prepared from human AD brains as compared to non-AD brains [178]. Later amyloid β-peptide aggregates were shown to bind to PMCA and inhibit its activity in the absence of calmodulin [179]. Furthermore, microtubule-associated regulatory protein tau, that is hyperphosphorylated and forms neurofibrillary tangles in AD, has been shown to interact with PMCA, as well, and inhibited its activity [180].

Altered activity of PMCA proteins in human brain tissue was also proposed in Parkinson's disease (PD) [181]. In an in vitro model of PD in neuroblastoma cells it was found that the resting cytosolic Ca^{2+} concentration was elevated while PMCA2 expression was decreased leading to decreased cell survival [182]. Alterations in the expression of PMCAs were also found in multiple sclerosis (MS), an inflammatory, demyelinating and neurodegenerative disorder of the central nervous system. In gene microarray analysis of brain lesions from MS patients both PMCA1 and PMCA3 expression was found to be downregulated compared to control [183]. Down-regulation of PMCA2 expression was also described in rats with experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Interestingly, after disease recovery PMCA2 expression was restored in the animals, while in mouse models with chronic EAE PMCA2 level remained low throughout the disease course [184, 185].

Expression of PMCA4b has been shown to be increased in platelets from patient with both type I and type II diabetes compared to control; this might contribute to increased thrombus formation in diabetic patients [186]. In cellular models it was found that PMCA2 plays an important role in the regulation of pancreatic β-cell proliferation, survival and insulin secretion [187–189]. An analysis of PMCA expression in rat pancreatic islets showed that PMCA1 and PMCA4 are expressed in all islet cells while PMCA3 is present only in the β-cells [190]. In fructose rich diet induced insulin resistant rats PMCA expression was altered in the islet cells resulting in reduced total activity. This caused an elevation in the intracellular calcium level that contributes to the compensatory elevated insulin secretion in response to glucose [191]. Alterations in PMCA activity were related to kidney diseases, as well. Decreased PMCA activity and concomitantly increased cytosolic Ca^{2+} concentration was described in red blood cells of children with chronic kidney disease [192]. Furthermore, in patients with idiopathic hypercalciuria PMCA activity of the erythrocytes was increased compared to controls [193].

5.5.4 PMCA4 in Heart Diseases

During cardiac relaxation SERCA and NCX proteins are mainly responsible for Ca^{2+} removal and PMCA4 acts primarily as a signaling molecule in the heart. It plays a role in the regulation of cardiac β-adrenergic response, hypertrophy

and heart failure [194]. β-adrenergic stimulation can initiate neural nitric oxide synthase (nNOS) activity and NO production in cardiac myocytes [195] while nNOS regulates contractility and oxygen radical production [196]. It was demonstrated that PMCA4b can directly interact with the Ca^{2+} sensitive nNOS molecule through its C-terminal PDZ binding motif and it decreases nNOS activity by reducing the Ca^{2+} concentration in its vicinity [197]. In cardiac specific PMCA4b transgenic mice nNOS activity was reduced compared to WT animals and that caused a decreased responsiveness to β-adrenergic stimulation [198]. This interaction might play an important role in remodeling after myocardial infarction (MI). In mice, after induction of MI, nNOS and its adaptor protein CAPON (carboxy-terminal PDZ ligand of NOS1) relocate to caveolae where they make a complex also with PMCA and this way possibly protect the cardiomyocytes from calcium overload. In mice lacking nNOS the redistribution does not happen [199].

PMCA4 also forms a ternary complex in cardiac cells with α-1 syntrophin and nNOS [200]. A mutation in α-1 syntrophin (A390V-SNTA1) was found in patients with long QT syndrome and it was demonstrated that the mutation resulted in the disruption of the interaction with PMCA4. This led to increased nNOS activation and late sodium current causing arrhythmias [201]. Interestingly, in a GWAS study a mutation in CAPON was found to be associated with QT interval variations [202] and variants of the *ATP2B4* gene were associated with congenital ventricular arrhythmia [203].

PMCA4 can also influence cardiac hypertrophy. It is well established that the calcineurin-NFAT pathway is activated during cardiac hypertrophy and it was found that PMCA4 is able to inhibit this pathway through direct binding of calcineurin [92]. In mice overexpressing PMCA4 in the heart both the NFAT-calcineurin signaling and hypertrophy were reduced, while the mice lacking PMCA4 were more susceptible to hypertrophy [204]. Furthermore, after induction of experimental myocardial infarction in mice overexpression of PMCA4 in cardiomyocytes reduced infarct expansion, cardiac hypertrophy and heart failure [205]. However, deletion of PMCA4 in cardiac fibroblasts also prevented cardiac hypertrophy in mice. In the absence of PMCA4, intracellular Ca^{2+} level was elevated in the fibroblasts enhancing secreted frizzled related protein 2 (sFRP2) production and secretion which reduced Wnt signaling in the neighboring cardiomyocytes [206]. Interestingly, overexpression of PMCA4 in arterial smooth muscle cells in mice caused an increase in blood pressure through the inhibition of nNOS [207].

5.5.5 *The Role of PMCAs in the Intestine and Bone Mineralization*

PMCA1 plays a crucial role in the transcellular Ca^{2+} absorption both in the duodenum and in the large bowel. Its expression is induced by vitamin D metabolite 1,25-(OH)2D3 and by estrogens, as well [208]. In mice it was demonstrated that

high bone density correlated with PMCA expression and mucosal to serosal Ca^{2+} transport in the duodenum [209]. Treatment of mice with 1,25-(OH)2D3 strongly increased PMCA1 mRNA level in the duodenum [210] while selective deletion of PMCA1 in the intestinal absorptive cells caused reduced whole body bone mineral density and lower serum Ca^{2+} level [211]. Furthermore, in ovariectomized rats a negative Ca^{2+} balance was induced and this was associated with decreased PMCA1 mRNA expression in an estrogen dependent manner [212], a model for postmenopausal osteoporosis. Interestingly, in biopsies of ulcerative colitis patients reduced PMCA1 expression was also found [213].

PMCAs play an important role in the regulation of bone mineral density already during development. The expression level of PMCA3 in the placenta correlates with neonatal bone mineral content [214] while during lactation PMCA2 expression is strongly induced in the mammary epithelium and it provides Ca^{2+} into the breast milk that is required for the normal bone development of the offspring. In PMCA2-null mice the Ca^{2+} content of the milk was 60% less than in the wild type mice [79]. PMCA isoforms 1, 2 and 4 were described in human osteoblasts, and PMCA1 and PMCA4 in osteoclasts. In osteoblasts of patient with adolescent idiopathic scoliosis expression of PMCA4 was found to be downregulated [215]. During osteoclast differentiation PMCA4 was shown to have an anti-osteoclastogenic effect on one hand by reducing NF-κB ligand–induced Ca^{2+} oscillations, on the other hand by decreasing NO synthesis in the cells [216]. However in mature osteoclast PMCA had an anti-apoptotic effect on the cells. Furthermore, in premenopausal women PMCA4b level showed correlation with high peak bone mass.

5.5.6 Altered PMCA Expression Linked to Tumorigenesis

Ca^{2+} plays an important role in the regulation of many cellular processes such as proliferation, migration or cell death. In tumorous cells these processes are strongly altered and changes in the expression or activity of Ca^{2+} handling molecules in several cancer types have been described. These modifications can result in altered resting Ca^{2+} level in the cellular compartments and can change the spatial and temporal characteristics of the intracellular calcium transients [217].

Alterations in the expression of PMCA proteins have been described in several cancer types. In colorectal cancer a decrease in PMCA4 expression was found during the multistep carcinogenesis of the human colon [218]. In normal human colon mucosa samples PMCA4 was present both at the mRNA and protein levels, however, in high grade adenomas, adenocarcinomas and lymph node metastases the protein expression strongly decreased. Interestingly, the PMCA4 mRNA level was not altered in the samples. Furthermore, after spontaneous differentiation of the colorectal cancer cell line Caco-2 the expression of PMCA4 strongly increased, and treatment with the histone deacetylase (HDAC) inhibitor Trichostatin A induced differentiation and PMCA4 expression in several gastric and colon cancer cell

lines [219, 220]. PMCA1 was also found in colon cancer cells and its expression increased after 1,25-(OH)2D3 treatments, however, this was not accompanied by a change in cellular differentiation [221].

Expression of PMCA proteins was also analyzed in breast cancer. In normal breast epithelium PMCA4 is abundantly present [222], while PMCA2 expression is induced only in the lactating mammary glands. In breast cancer cell lines it was found that the mRNA level of PMCA1 and PMCA2 is increased compared to non-tumorigenic human breast epithelial cell lines [223, 224], while PMCA4 expression is downregulated [222]. In human breast cancer samples PMCA2 mRNA level showed association with higher tumor grade and docetaxel resistance in patients. In a tissue microarray analysis of 652 primary breast tumors PMCA2 expression showed positive correlation with lymph node metastasis and human epidermal growth factor receptor 2 (HER2) positivity. Furthermore, overexpression of PMCA2 in breast cancer cells reduced their sensitivity to apoptosis [225]. It was suggested that PMCA2 regulates HER2 signaling in breast cancer cells and knocking down PMCA2 inhibits HER2 mediated cell growth [226]. In another study PMCA2 expression was found in 9% of 96 breast tumors with various histological subtypes and there was no association with grade or hormone receptor status. However, higher PMCA2 expression was described in samples with basal histological subtype. It was also demonstrated that downregulation of PMCA2 level decreased breast cancer cell proliferation and increased the sensitivity to doxorubicin treatment [227]. While PMCA2 expression is upregulated in certain breast cancer cells, PMCA4 level seems to be downregulated. In MCF-7 breast cancer cells treatment with HDAC inhibitors or with phorbol 12-myristate 13-acetate (PMA) strongly induced PMCA4b expression and this effect was coupled with increased Ca^{2+} clearance from the cells [222].

Altered PMCA protein levels were described in melanomas. In melanoma cell lines with different BRAF and NRAS mutational status PMCA4 and PMCA1 isoforms were detected. Mutant BRAF specific inhibitor treatment selectively increased PMCA4b expression in BRAF mutant melanoma cells and this was coupled with faster Ca^{2+} clearance and strong inhibition of migration [91]. When PMCA4b was overexpressed in a BRAF mutant melanoma cell line A375, it strongly reduced the migratory and metastatic capacity of the cells both in vitro and in vivo, while it did not influence their proliferation rate. Furthermore, HDAC inhibitor treatment increased the expression of both PMCA4b and PMCA1 in melanoma cell lines independently from their BRAF mutational status [228]. Similarly to BRAF inhibitor treatment, HDAC inhibition also increased Ca^{2+} clearance and reduced the migratory activity of the highly motile A375 melanoma cells. These results suggested that PMCA4b plays an important role in the regulation of melanoma cell motility, and its expression is under epigenetic control.

PMCA1 was also found to be epigenetically downregulated in human oral cancer. PMCA1 expression was reduced both in primary oral squamous cell carcinomas (OSCCs) and in oral premalignant lesions (OPLs) compared to normal tissue. In OSCC derived cell lines it was demonstrated that decreased PMCA1 level was caused by the increased DNA methylation in the promoter region of PMCA1 [229].

The emerging role of PMCAs in the regulation of the immune response might also be considered in the treatment of malignant diseases**.** Immune checkpoint inhibitors are relatively new but promising treatment options in cancer therapy that are able to enhance cytotoxic T-cell activation by blocking the negative regulatory signals coming from tumor cells [230]. Recently, it was found that PMCA4 interacts with Ig-like glycoprotein CD147 upon T-cell activation and this interaction is necessary for the immunosuppressive effect of CD147 through the decrease of IL-2 production [231]. CD147 was shown to participate in the development and progression of several cancer types including malignant melanomas, and antibodies targeting CD147 are under development [232]. All these results show that remodeling of the activity and expression of PMCA proteins play an important role in altered cancer cell growth, motility, and in T-cell activation during the immune response to cancer cells that might influence therapy response, as well.

5.6 Conclusion

PMCAs comprise a big family of Ca^{2+} transport ATPases including four separate genes *(ATP2B1-4)* from which more than twenty different protein variants are transcribed. The variants have different regulatory properties, and hence they respond differently to the incoming Ca^{2+} signal, differ in their sub-plasma membrane localization and interact with different signaling molecules. The expression, and thus the abundance of the variants are also tightly regulated in a development and cell-type specific manner, by processes not yet very well understood. In the past we studied many aspects of the biochemical characteristics of these pumps, but we still know very little on how their transcription and translation are regulated and how stable the proteins are in the plasma membrane. Our main goal, therefore, should be to study further these mechanisms particularly because alterations in the PMCA expression and genetic variations in the *ATP2B* genes have been linked to several diseases such as cardiovascular and neurodegenerative disorders, and cancer. Understanding PMCA pathophysiology and learning more about the consequences of PMCA dysfunction may help finding ways to predict, prevent and/or cure such diseases.

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