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Review

The plethora of PMCA isoforms: Alternative splicing and differential expression ☆

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ABSTRACT

In this review the four different genes of the mammalian plasma membrane calcium ATPase (PMCA) and their spliced isoforms are discussed with respect to their tissue distribution, their differences during development and their importance for regulating Ca^{2+} homeostasis under different conditions. This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

1. Introduction

External signals to activate cells often result with a change of the cellular environment. To maintain viability cells must be able to respond. This requires that cells must have the ability to change their functions rapidly due to a given stimulus. Such cellular responsiveness is accomplished through activation of signal transduction cascades transmitting signals from the cell surface to internal cellular machineries, often accompanied by significant alterations of the protein composition of cells. In this respect, calcium is of pivotal importance for many biological processes. As one of the second messengers it participates in many different signal transduction pathways. Therefore, the balance of calcium homeostasis is of central importance controlled by an elaborate integrated system of channels, exchangers and pumps regulating Ca^{2+} fluxes into and out of the cell.

The response to cellular receptor activation can result in up to a 100-fold rise of intracellular free Ca^{2+} concentration which in a resting cell is in the order of 100–200 nM. The uptake of extracellular Ca^{2+} or the release of Ca^{2+} from intracellular stores which thus can serve as an intracellular second messenger leading to signal transduction for a number of different cellular activities such as protein phosphorylation and dephosphorylation, fertilization, cell proliferation, cell division and gene expression, to name a few. Many of these functions are accomplished due to the interaction of Ca^{2+} with specific proteins, mostly so-called EF-hand proteins [1]. This results in specific modulations of protein–protein interactions owing to conformational changes of the Ca^{2+} receptors of which calmodulin is the most dominant regulator [2].

One of the key enzymes controlling the Ca^{2+} level in the cell is the plasma membrane calcium ATPase (PMCA) (for recent reviews see Refs. [3,4]). PMCA is regulated in many ways (see below), and probably the most important regulator is calmodulin which directly interacts with PMCA [5]. In this review the focus is on the diversity of the different spliced isoforms of PMCA and their variation in expression during development, differentiation and disease. Other aspects of PMCA can be taken from Refs. [3,4].

2. General properties of PMCA

The existence of a Ca^{2+}-dependent ATPase in erythrocytes was first described by Dunham and Glynn in 1961 [6]. In 1966 it was Schatzmann who provided evidence that Ca^{2+} is pumped out of the cell on the expense of ATP against a Ca^{2+} gradient across the membrane of human red cells [7]. Like other ion pumps PMCA belongs to the P-type pump family as classified by Pedersen and Carafoli [8,9]. These enzymes are characterized by forming a phosphorylated high-energy intermediate resulting in the formation of an acyl-phosphate, mostly an aspartyl-phosphate, which provides the enzyme with sufficient energy to pump the ion across the membrane against the ion gradient with a 1:1 Ca^{2+}/ATP ratio for PMCA [10]. Therefore, it is thought that during the reaction cycle of the enzyme one can distinguish at least two different conformational states, E1 and E2 [11]. Later, Toyoshima and coworkers described even more different conformations for the related calcium pump of the sarco/endoplasmic reticulum by solving the high-resolution structures at different states of the reaction cycle which defines three different functional domains: the actuator, the

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catalytic or nucleotide-binding and the regulatory domain (for a recent review see Ref. [12]). Similar changes of the conformation may also exist for PMCA as suggested by homology modeling based on the structures of the SERCA pump, even if a high resolution structure of PMCA has not been solved yet [13].

In 1977 two independent reports provided evidence that a Ca^{2+}-binding protein which later became known as calmodulin activated the Ca^{2+} pump of human erythrocytes [14,15]. The direct interaction of calmodulin with the pump was later exploited by Niggli et al. to purify the PMCA pump from human erythrocytes by calmodulin affinity chromatography [16]. In 1988 PMCA was cloned from human [17] and rat [18] tissues and its primary structure was determined. The protein has 10 transmembrane domains, the N- and C-terminus are both located in the cytosol and the major protein mass is protruding into the intracellular space. The enzyme is an essential component of all mammalian plasma membranes, and in mammals four different genes encode the plasma membrane calcium pump which in humans are located on four different chromosomes, i.e. PMCA1 on 12q21–23 [19], PMCA2 on 3p25.3 [20,21], PMCA3 on Xq28 [21] and PMCA4 on 1q25–q32 [19]. Additional isoforms of the protein are produced by alternative splicing of the primary transcripts as first demonstrated by Strehler et al. [22]. For the four genes two splice sites have been characterized which are located either close to or within regulatory regions of the pump giving rise to about 30 spliced isoforms ([23], see Fig. 1). Site "A" is located closely upstream of the phospholipid binding domain, and site "C" is found within the calmodulin binding domain of the enzyme [23]. These splicing variations can lead to proteins of different sizes with molecular weights in the range between 120 and 140 kDa [22–24].

The PMCA Ca^{2+} pump is a low abundant membrane protein, but has a high affinity to Ca^{2+} enabling it to regulate the fine tuning of the calcium homeostasis of the cell. The enzyme can be activated not only by calmodulin, but also by acidic phospholipids or by polyunsaturated fatty acids [25], by oligomerization [26] or by phosphorylation [27,28]. In the absence of calmodulin, the calmodulin-binding domain is interacting with two receptor sites within the catalytic domain of the pump. The variable C-terminal part of the domain interacts with a site upstream of splice site A, and the conserved N-terminal part of the calmodulin-binding domain binds to a site located between the phosphorylation- and the ATP-binding site of the ATPase which keeps the enzyme in an inhibited state ([29,30]; see Fig. 1).

Alternative splicing is a dominant property of higher organisms to produce multiple proteins from a single gene [31,32], but the regulation of alternative splicing is still poorly understood. In order to splice the pre-mRNA, introns must be distinguished from exons. Special sequences at the intron/exon junctions direct the pairing of the splice sites at the 5′ and the 3′ ends of the intron to be spliced which have to be recognized by the spliceosome with high precision. This splicing pattern can be altered in many ways. Next to the frequent observation that exons are constitutively included in the final mRNA, splicing can be regulated in a way that an exon is either included or excluded in the final mRNA, so-called cassette exons which sometimes could lead to mRNAs with mutual exclusive exons [31]. Local *cis*-regulatory sequences such as exonic or intronic enhancers or silencers may either enhance or suppress the usage of a splice site due to its proximity of that site. In order to use these regulatory sequences *trans*-acting factors are binding to those sites to determine the transcription of a specific

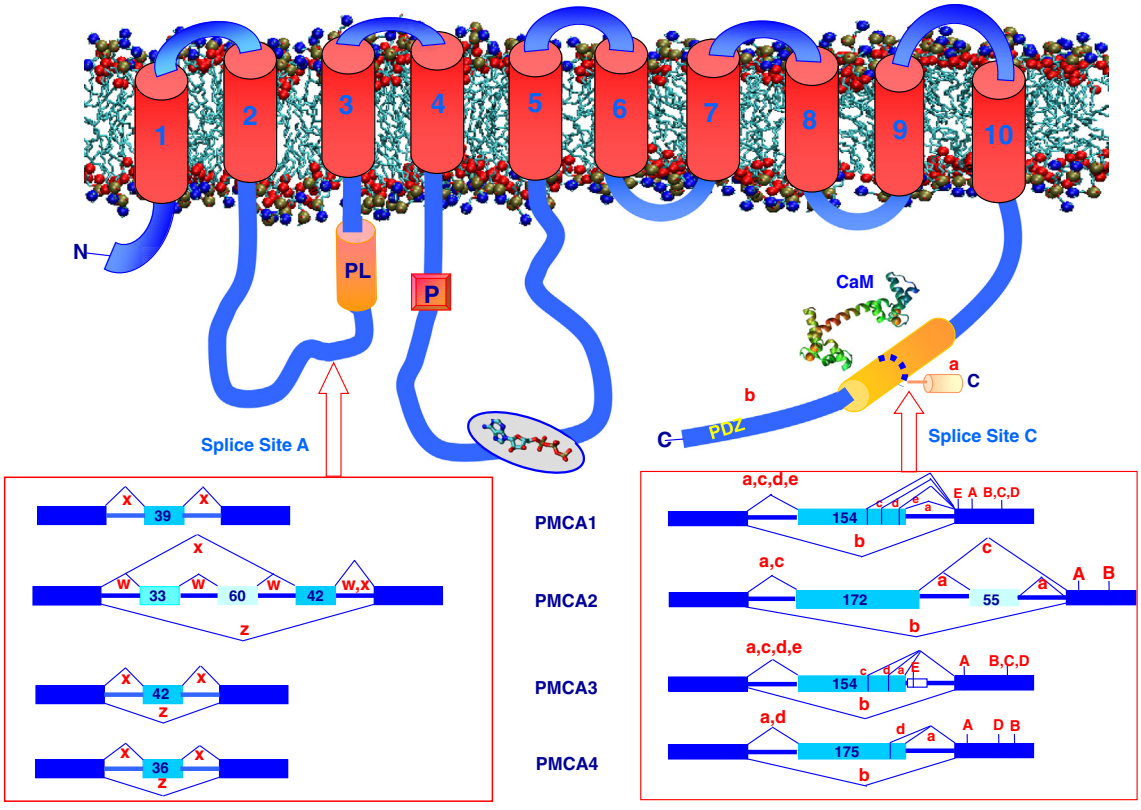

Fig. 1. Topology domains and splicing variants of the human PMCA isoforms. The 10 transmembrane domains of the pump are numbered and indicated by red boxes. Splice sites "A" (first cytosolic loop) and "C" (C-terminal tail) are indicated by red arrows. Splice site "C" lies within the calmodulin-binding domain (yellow cylinder; defined by the structural model of CaM = calmodulin). The exon structure of the different regions affected by alternative splicing is shown for each of the 4 different PMCA genes. Constitutively spliced exons are indicated as dark blue boxes, alternatively inserted exons are shown in light blue; the resulting splice variants are labeled by their lower case symbols, the positions of the translation stop codons for each splice form are indicated by the corresponding capital letters. In PMCA3, splice variant "e" results from a read-through of the 154-nt exon into the following intron (indicated as small open box). The sizes of alternatively spliced exons are given as nucleotide numbers. PL = phospholipid binding domain; P = location of the aspartyl-phosphate formation; PDZ = PSD-95/Dlg/ZO-1 domain. The figure was taken from Fig. 1 of Ref. [24] with permission from the publishers.

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isoform. One of these factors is hnRNPL [33] which is important for the regulation of the expression of PMCA1a as will be discussed below. Thus the expression of a specific isoform can become essential for the development of a tissue or the differentiation of a cell.

3. The different PMCA genes and their spliced isoforms

As mentioned before all 4 PMCA genes contain 2 splice sites, site "A", located closely upstream of the phospholipid binding site, and site "C" which is found within the calmodulin binding domain. This latter splice site creates a highly variable calmodulin binding domain since due to splicing events only the N-terminal part of the domain is conserved in contrast to the C-terminal part which varies considerably. This results in significant differences of the calmodulin affinity for the various spliced isoforms with the exception of the "b" forms for which splicing at the C-site does not occur. Originally it was suggested that there exist 2 additional splice sites, sites "B" and "D" [34], but later these sites turned out to be cloning artifacts [35,36]. As shown in Fig. 1 splicing at site "A" affects an exon of either 36 nt (PMCA4), 39 nt (PMCA1) or 42 nt (PMCA3). These exons can be either spliced in or excluded giving rise to spliced isoforms "x" or "z", respectively. For PMCA2 splicing at site "A" is more complex as can be realized from Fig. 1. In this case either 33, 42, or 60 nucleotides can be either spliced in or are excluded resulting in spliced isoforms "w", "x" or "z". 2 points have to be made here: all additional exons spliced in at site "A" do not interfere with the reading frame, and second, PMCA1 always contains the extra exon of 39 nt spliced in, i.e. only PMCA1x exists.

In contrast to splice site "A" the splicing pattern of site "C" is much more complex as can be realized from Fig. 1. The "a" splicing variant of all 4 genes includes an extra exon (exon 21 for PMCA1). If the entire exon of either 154, 172 or 175 nt was inserted the reading frame was changed resulting in a protein with shorter C-terminal amino acid sequences due to an early stop codon (see Fig. 1). On the other hand, alternative splicing can make use of internal cryptic donor splice sites which results in variants of variable length (forms c–e) [22,37]. This also can lead to a change in the reading frame resulting in a shortened C-terminal sequence. For PMCA2 and 3 the splicing can be even more complex. As can be noticed from Fig. 1 in the case of PMCA2 2 additional exons can be included, i.e. if both are inserted, this leads to variant "a", whereas if only the larger exon of 172 nt is spliced in, it leads to variant "c". The variant "e" of PMCA3 has the special property that 88 nt of the following intron are added to the exon of 154 nt. In contrast, the "b" variant of the 4 human genes do not include exon 21 leading to PMCAs with a highly conserved C-terminal protein sequence which next to the calmodulin-binding domain contains a PDZ domain-interacting sequence [38]. This conserved sequence has been identified in all "b" splicing variants [39] and also in some "c" and "d" isoforms, but not in isoform "a" due to the truncated C-terminal sequence (Fig. 1). PDZ, originally identified in the postsynaptic density protein PSD-95 [40], is known to be important for protein–protein interactions, and thus it has been demonstrated that PMCA can interact with various partners such as PSD-95 of the family of membrane-associated guanylate kinase (MAGUK) and others (for a review see [41]). There is accumulating evidence that such interactions of higher order complexes in Ca^{2+} signaling microdomains favorably occur in lipid rafts as documented in a number of publications (e.g. see [42,43]; for a review see [44]).

4. The influence of splicing on the expression of the different PMCA isoforms

Changes in splice site choice can significantly influence the properties of the encoded protein with respect to its enzymatic activity, its way of regulation or its localization. This is especially common for genes expressed in nervous tissues which can influence the properties of the many different types of neurons [45] which is specifically reflected by the various PMCA isoforms expressed in the brain as will be shown below. In the following the tissue and cell-specific expression of the different PMCA spliced isoforms will be described as well as regulation of expression, localization and their importance for the development of diseases.

4.1. PMCA1

PMCA1 is expressed ubiquitously and is known as a house-keeping form of the plasma membrane calcium pump. This is also demonstrated by the fact that PMCA1 knockout mice are embryonically lethal [46]. Brandt and Neve [47] demonstrated that in the developing rat brain PMCA1b was detected at the earliest embryonic day studied (E10). Surprisingly, by comparing the mRNA levels of PMCA1b with 1a they noticed that not only PMCA1a was faintly visible at E10, but during further development of the rat brain the expression of PMCA1b mRNA declined and the expression of variant 1a continuously increased suggesting that PMCA 1a may be important for the maturation process of neuronal development. This specific splice shift from PMCA1b to 1a was later confirmed by Kip et al. [48] studying rat hippocampal neurons. Furthermore, Kenyon et al. provided evidence that PMCA1a is specifically expressed in the plasma membranes of neurons concentrating in somata, dendrites and spines [49]. The importance of these observations is not only given by the fact that PMCA1a and 1b differ significantly in their activation by calmodulin, but also implicated the question what signal regulates the switch in expression from variant 1b to 1a. Even if this regulation is far from being understood in detail, certain aspects may have become clear recently as will be described below.

In 2001 Xie and Black [50] made the important observation that the Ca^{2+}-calmodulin dependent kinase IV (CaMKIV) known to be essential for calcium-dependent gene transcription using CREB as the transcription factor [51] is directly involved in Ca^{2+}-dependent regulation of alternative splicing. By studying the calcium-dependent potassium channel *slo1* they noticed that the inclusion of exon STREX (stress-axis regulated exon) which confers higher calcium sensitivity to the channel [52] was under the control of alternative splicing. Depolarizing GH_3 pituitary cells or cerebellar neurons resulted in a significant repression of the STREX exon through a CA-rich RNA silencing element (CARRE) which was regulated by CaMKIV [50]. The *trans*-acting factor recognizing the CARRE was later identified as hnRNP L [53]. Furthermore, it could be shown that hnRNP L is phosphorylated at a conserved Ser513 by CaMKIV which resulted in the repression of the STREX exon [54]. By examining the human genome database the group of Black identified a number of exons matching the CARRE motif containing the general sequence CACA(T/C)NNTTAT [55] which later was modified to the minimum consensus sequence CACA(T/C)N_{1-4}A [56]. This permitted to study the CaMKIV dependent splicing of a number of exons carrying this motif which responded to depolarization conditions in P19 cells [56] including exon 21 of PMCA1. As mentioned before the splice site for including exon 21 of PMCA is located within the calmodulin binding domain (see Fig. 1). Exon 21 of PMCA1 contains 2 internal splice donor sites, and it is interesting to note that a CARRE consensus sequence of CACATGTA can be identified at the second splice donor site of exon 21 of PMCA 1 [22]. If PMCA1a should be expressed which includes the total sequence of 154 nt of exon 21 the internal splice donor sites have to be suppressed. This could be achieved on one hand by suppressing the second cryptic splicing site by recognizing the CARRE of that site. On the other hand, it is known that secondary structure of pre-mRNA can influence the regulation of alternative splicing [57] which could contribute to suppress the first internal splicing site of exon 21, thereby resulting in an increase of expressing PMCA1a as observed by depolarizing differentiating granular cells [58], indicating that PMCA1a is a specific neuronal isoform as documented in detail by Kenyon et al. [49]. On the other hand, specific cell type differences in splicing pattern may also exist. In this context it is of interest that Zacharias & Strehler observed a different splicing pattern by studying changes in splicing variants of PMCA in the IMR32 neuroblastoma cells [59]. These authors

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reported that by depolarizing these cells they noticed the additional occurrence of the splice variant PMCA2x next to PMCA2w in contrast to PMCA1, 3 or 4 for which they did not observe a change in splicing pattern. With respect to the discussion before concerning PMCA1a expression it is interesting that Zacharias & Strehler not only noticed that this change of the PMCA2 splice variants was Ca^{2+}-dependent but also that the phosphorylation of probably a splicing regulating factor must be involved in this process [59] as mentioned before for the regulation of PMCA1a expression. In this context it is interesting that Kosiorek et al. recently reported on the regulation of PMCA2 alternative splicing in PC12 cells through the cooperation of NFAT1/3 and HDAC4 [60]. The authors suggested that this influence on the isoform composition may be important for altered dopamine secretion by PC12 cells [60].

One of the functional consequences of including exon 21 in PMCA1a is the considerable difference in the C-terminal amino acid sequence of the calmodulin-binding domain due to the shift of the reading frame. Since the calmodulin-binding domain is responsible for the autoinhibition of the pump due to its interaction with receptor sites close to regulatory domains of the enzyme [29,30] this change in its structural property could influence the basic Ca^{2+}-pumping activity of the enzyme in addition to its loss of calmodulin sensitivity. In fact, Caride et al. [61] studied the difference in calmodulin binding and activation between PMCA4a and 4b and demonstrated that PMCA4a is more efficient than PMCA4b in reducing cytosolic Ca^{2+} concentrations after a Ca^{2+} spike. Similar conclusions could be drawn for the activation kinetics of PMCA1a in comparison to PMCA1b. Another important difference between the "a" and the "b" variant concerns the ability to interact with PDZ-domains which is only observed in the "b" form (and in some "c" and "d" spliced isoforms) but never in the "a" isoform due to the frame shift. Since the PDZ domain is a kind of scaffold for interacting with a number of proteins influencing the activity of the PMCA Kip et al. [48] recently argued that the lack of the PDZ-interaction sequence in PMCA1a could result in a higher frequency of shuttling of this specific isoform between different membrane domains involved in Ca^{2+} signaling. This could enable PMCA1a to control Ca^{2+} homeostasis in different local microdomains. In this context it is of interest that PMCA2 which is specifically abundant in hippocampal neurons does not shift between isoforms "a" and "b" [48] which may be due to the fact that the corresponding exon of PMCA2 does not contain a CARRE domain which could permit CaMKIV to regulate the expression of PMCA2a. This is in contrast to what has been described for the splicing pattern of PMCA2 at splice site A where the x splice variant was concomitantly expressed with w during depolarization of the cells [59].

Another aspect concerning the importance of CaMKIV dependent regulation of the expression of PMCA1a during brain development concerns the finding that the expression of CaMKIV during brain development is tightly controlled by the thyroid hormone [62]. In a detailed study we provided evidence that the expression of CaMKIV is induced by T_3 in a concentration and time dependent manner in a rat fetal telencephalic culture system [62]. These findings have later been supported by similar observations of Liu and Brent in mouse stem cells including the identification of a thyroid hormone receptor recognition sequence in the promoter region of CaMKIV [63] and by the group of Bernal who studied thyroid hormone regulation of gene expression in the developing rat fetal cerebral cortex [64]. Many studies provided evidence that the availability of the thyroid hormone T_3 is absolutely essential for fetal brain development (for recent reviews see [65,66]). It is known that in the first weeks of fetal neurodevelopment the pup is completely dependent on maternal thyroid hormone supply [67]. Maternal thyroid hormone deficiency could therefore result in severe brain impairment leading to mental retardation, cognitive deficits and impaired learning and memory [68,69]. In a recent study by Zhang et al. [70] it was shown that maternal hypothyroidism of rats strongly impaired CREB-depending signaling pathways which is closely associated with synaptic plasticity, learning and memory. Since the transcription factor CREB is mainly controlled by CaMKIV it was interesting to see that Zhang et al. provided evidence that rat pups suffering from maternal hypothyroidism showed a significantly impaired expression of CaMKIV [70]. Therefore it would be of interest to investigate whether the expression of PMCA1a would be impaired in the developing brain during maternal hypothyroidism, and whether this would influence the role of PMCA1a in calcium homeostasis during neuronal development and synaptic formation. A detailed analysis of these implications has to await further experiments.

4.2. PMCA2

Early on it was recognized that PMCA2 and its spliced isoforms are specifically expressed only in highly specialized tissues such as inner hair cells, the nervous system or other excitable tissues. Among the 4 different PMCA genes PMCA2 has the highest binding affinity for calmodulin, but also a very high basal Ca^{2+} activity in the absence of calmodulin [71] which could be important in specialized cells where a continuous Ca^{2+} pumping activity at high rate is demanded. Splicing at site A inserts up to 3 exons, at site C one or two exons (see Fig. 1). PMCA2 was first described by Brandt et al. [72] studying the developing nervous system of the rat, later the human homologue was characterized and the expression of its spliced isoforms have been described [73]. PMCA2 is especially abundant in Purkinje and granular cells of the cerebellum [73], but 2 spliced isoforms of PMCA2 deserve a special discussion here, i.e. the "w/a" variant in which all possible exons of splice sites A and C are spliced in leading to a truncated form of the pump due to the frame shift, and the "w/b" variant in which all 3 exons of site A are included, but at site C no extra exon is inserted. As pointed out by Antalffy et al. [74] splicing at site A determines the final localization of the pump, thus the "w" variant directs the enzyme to the apical membrane of polarized cells. PMCA2w/a is highly expressed in the outer hair cells of the inner ear [75] where it is localized in the stereocilia [76]. Of special interest is the 5′ UTR of PMCA2 since it seems to contain different regulatory domains which direct the enzyme into different cell types by using four different transcriptional start regions [77]. One of these start regions permits the expression of the pump in Purkinje neurons and in the outer hair cells, another in cerebellar granular cells, and 2 in the epithelia of the lactating mammary gland. Reinhardt & Horst [78] identified six calcium pumps in the lactating mammary tissue, i.e. 3 PMCAs, 2 SERCAs and a Golgi Ca^{2+}-ATPase, later known as SPCA (secretory pathway calcium ATPase). Of special interest with respect to the developing lactation process is the observation by Reinhardt and Horst that the most abundant transcript of a calcium pump was that of PMCA2w/b which could increase up to 100 fold at the peak of lactation. As already pointed out by the authors the especially high basic Ca^{2+} activity of PMCA2b is well suited for the maintenance of calcium homeostasis in the lactating gland which was later supported by the observation that the calcium content of the milk of lactating mice lacking PMCA2w/b was reduced by 60% [79]. Another interesting aspect is the modulation of the PMCA2 activity by the calcium-sensing receptor which regulates the calcium level in the milk [80].

As mentioned before PMCA2w/a is selectively expressed in the outer hair cells of the inner ear where it is localized in the stereocilia containing the sensory transduction apparatus [75,76]. Since other Ca^{2+} clearance systems are absent in stereocilia cells it is obvious that PMCA2w/a plays a critical role in maintaining Ca^{2+} homeostasis in those cells. Therefore it is not surprising that loss of function of PMCA2 results in deafness as first observed in deafwaddler mice which also have difficulties in maintaining balance [81]. Similar results have been reported by Kozel et al. [82] who produced PMCA2-deficient mice by gene targeting. Street et al. [81] identified a mutation of a conserved glycine to serine within the transduction domain (close to the phospholipid-binding domain of PMCA) which couples ATP hydrolysis with Ca^{2+} transport. According to the authors this glycine 283 of PMCA2 is conserved among cation transporting ATPases, and has been shown for the SERCA pump that substitution of a homologous glycine impaired calcium transport. A similar G293S mutation of PMCA2w/a downstream of the G283S

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mutation reported for mice has later been demonstrated to occur in humans. However, the reported symptoms were only similar if cadherin 23 was also mutated. The latter is critical for the correct functioning of the transduction complex [83].

4.3. PMCA3

The plasma membrane calcium pump of gene 3 is like PMCA2 mainly restricted to the nervous system, but in contrast to PMCA2 which is mainly found in postsynaptic densities PMCA3 is mainly located at presynaptic terminals, especially in the cerebellum and in the choroid plexus [84–86], but PMCA3 has also been found in skeletal muscles [87]. Even if detailed findings concerning specificity and Ca^{2+} handling of PMCA3, also with respect to the use of spliced isoforms, are still limited, two recent reports of the involvement of PMCA3 mutations in connection with specific human diseases are of interest here. In an extensive clinical study of patients suffering from aldosterone-producing adenomas (APA) which leads to secondary hypertension, it was reported that due to somatic mutations in the Na^+/K^+-ATPase (ATP1A1) and in ATP2B3 in a number of clinical cases the ion homeostasis of the cells were impaired [88,89]. In these studies the authors identified two deletion mutants of PMCA3 within the transmembrane domain M4 (V426, V427/L425, V426), leading to a distortion of the domain which probably affects the Ca^{2+} binding site of the pump. The second study concerning a mutation of PMCA3 was reported by Zanni et al. [90] who found a missense mutation in PMCA3 which is located on the X chromosome [21]. This mutated PMCA3 was discovered in a family with congenital cerebellar ataxia. The identified G1107D mutation is located within the calmodulin-binding domain closely upstream of splice site C. The authors overexpressed the mutated form of the pump in human fibroblasts and by comparing with controls they found that the Ca^{2+}-pumping activity of the mutated form was impaired. These findings can be rationalized on the basis of the NMR structure of calmodulin bound to its binding domain [91] which would predict that replacing gly1107 by asp would perturb the interaction between calmodulin and its binding domain leading to decreased calmodulin-dependent activation of the pump. On the other hand, since it is known that in the absence of calmodulin its binding domain interacts with an internal acceptor site of the pump [29,30] the G1107D mutation could also weaken this interaction leading to an enhanced basal activity of the mutated pump, in other words the functional situation of the mutated Ca^{2+} pump is likely to be more complex.

4.4. PMCA4

PMCA4 is like PMCA1 ubiquitously distributed in all tissues examined, but unlike PMCA1 it is not embryonically lethal. On the other hand, a number of specific phenotypes have been reported indicating that PMCA4 is of critical importance for certain cellular functions. So it has been demonstrated that a plasma membrane calcium pump was critical to maintain a resting Ca^{2+} level in sperm cells [92]. This PMCA was later identified by Neyses and his co-workers as PMCA4b which is highly enriched in the flagellar apparatus of the spermatozoon important for the sperm motility [93]. Therefore the authors decided to target the PMCA4 gene by homologous recombination in embryonic stem cells. They could demonstrate that male homozygous deficient mice became infertile due to impaired sperm motility [93]. However, an interesting observation was recently reported by Brandenburger et al. [94]. They studied PMCA4 splice variants in bovine epididymis and found a switch from PMCA4b to 4a during functional sperm maturation suggesting that the sperm maturation process requires a shift from the slow PMCA4b to the faster 4a variant due to its higher basic Ca^{2+} activity. By contrast, in the female reproductive tract of mice both splice variants of PMCA4, i.e. "a" and "b", can be detected in a vesicular fraction by which PMCA4a, and probably also 4b, can be secreted [95].

Since it has been demonstrated that all "b" variants of PMCA contain a PDZ-interaction sequence at the C-terminus [38,39] Schuh et al. [96] provided evidence that PMCA4b can interact with a calcium/calmodulin-dependent serine protein kinase (CASK) which was also observed by Aravindan et al. [97] in the mouse sperm flagellum. Similar PDZ-dependent interaction of PMCA4b with the neuronal form of NO synthase (nNOS) was shown by Oceandy et al. [98]. By generating transgenic mice overexpressing human PMCA4b it could be demonstrated that due to the interaction with nNOS PMCA4b could regulate the activity of nNOS by controlling the local calcium concentration which is important for the regulation of beta-adrenergic signal transmission [97]. Such interaction could occur in microdomains as organized by lipid rafts which have been demonstrated to be involved in the regulation of various Ca^{2+} signaling pathways [44]. Also a PDZ-independent interaction of PMCA4b with the catalytic subunit of calcineurin, calcineurin A, has been described [99]. The domain of PMCA4b to interact with calcineurin has been located close to the ATP-binding site of the ATPase. This interaction resulted in the inhibition of the calcineurin/NFAT controlled signaling pathway.

Very recently Li et al. [100] reported the interesting observation that a missense mutation of PMCA4 was discovered in a Chinese family with autosomal dominant familial spastic paraplegia (FSP). By whole-exome and Sanger sequencing they detected a R268Q mutation in six family members who suffered from FSP which could not be detected in healthy members of the family, and was also an unknown mutation in any of the databanks. This mutation is located close to splice site A and in the neighborhood of the phospholipid binding domain, 2 important regulatory sites of the enzyme. Computational modeling revealed that this R268Q mutation could lead to a partly misfolded protein supported by the finding of thermodynamic instability of the protein. Since PMCA4 has been reported to be localized in lipid rafts in the cerebellum [101] which are important for postsynaptic signaling complexes Li et al. speculate that the R268Q mutation may be responsible for dysregulation of Ca^{2+} signaling at such sites which may cause neuronal deficits associated with FSP.

In summary, it can be concluded that the proteins translated from the 4 different genes of the mammalian plasma membrane calcium

Table 1
Summary of Distribution of Different PMCA Isoforms and their Link to Different Diseases.

PMCA	Location	Destination	KO	Mutation	Disease	Refs.
1	Ubiquit.		Lethal			[46]
2	Neural	Postsynaptic				[67,84,85]
2w		Apical				[74]
2w/a		Stereocilia	Deafness, unbalanced	G283S G293S	mDeaf hDeaf	[76,81–83]
2w/b		Lact. gland				[78,79]
3	Neural, skelet. muscle	Presynaptic				[86]
3				Deletion of V426, 427/L425, V426	APA	[88,89]
3				G1107D	Ataxia	[90]
4	Ubiquit.		Male infertility			[93]
4b				R268Q	FSP	[100]

mDeaf = Deafness in mice; hDeaf = Deafness in human; APA = aldosterone-producing adenomas; FSP = familial spastic paraplegia.

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pump and their spliced isoforms differ in their tissue distribution, their differences in controlling Ca^{2+} homeostasis and how their expression is regulated. In addition, it becomes increasingly noticeable that mutations at critical sites of PMCA can lead to severe dysfunction and diseases (see Table 1).

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