Primary Active Ca^{2+} Transport Systems in Health and Disease

Jialin Chen,¹ Aljona Sitsel,¹ Veronick Benoy,¹ M. Rosario Sepúlveda,^{2,3}
and Peter Vangheluwe^{1,3}

¹Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven, 3000 Leuven, Belgium

²Department of Cell Biology, Faculty of Sciences, University of Granada, 18071 Granada, Spain

Correspondence: peter.vangheluwe@kuleuven.be

Calcium ions (Ca^{2+}) are prominent cell signaling effectors that regulate a wide variety of cellular processes. Among the different players in Ca^{2+} homeostasis, primary active Ca^{2+} transporters are responsible for keeping low basal Ca^{2+} levels in the cytosol while establishing steep Ca^{2+} gradients across intracellular membranes or the plasma membrane. This review summarizes our current knowledge on the three types of primary active Ca^{2+} -ATPases: the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) pumps, the secretory pathway Ca^{2+} -ATPase (SPCA) isoforms, and the plasma membrane Ca^{2+} -ATPase (PMCA) Ca^{2+} -transporters. We first discuss the Ca^{2+} transport mechanism of SERCA1a, which serves as a reference to describe the Ca^{2+} transport of other Ca^{2+} pumps. We further highlight the common and unique features of each isoform and review their structure–function relationship, expression pattern, regulatory mechanisms, and specific physiological roles. Finally, we discuss the increasing genetic and in vivo evidence that links the dysfunction of specific Ca^{2+} -ATPase isoforms to a broad range of human pathologies, and highlight emerging therapeutic strategies that target Ca^{2+} pumps.

Ca^{2+} signaling is crucial for many physiological processes and is dysregulated in a multitude of pathological conditions. Ca^{2+} influx from outside the cell or Ca^{2+} release from intracellular reservoirs increases cytosolic Ca^{2+} levels in the nano- to micromolar range, leading to a Ca^{2+} signal that can vary in amplitude, frequency, and subcellular localization. Afterward, resting cytosolic Ca^{2+} levels must be restored by primary and secondary active transport systems, which are referred to as Ca^{2+} pumps and exchangers, respectively. In this review, we will fo-

cus on the primary active Ca^{2+} -transporters or Ca^{2+} -ATPases, which are responsible for keeping low basal Ca^{2+} levels in the cytosol while establishing vitally important Ca^{2+} gradients across intracellular membranes or the plasma membrane. All Ca^{2+} -ATPases belong to the family of P-type ATPases: the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA), the Golgi/secretory pathway Ca^{2+} -ATPase (SPCA), and the plasma membrane Ca^{2+} -ATPase (PMCA) (Fig. 1A). SERCA and SPCA share 43% sequence similarity and belong to the P2A sub-

³These authors contributed equally to this work.

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Figure 1. Primary active transporters in the cell. (A) Schematic representation of a cell, depicting the subcellular localization of primary active Ca^{2+} -transporters, which generate steep Ca^{2+} gradients across various cellular membranes (Ca^{2+} concentrations are shown in gray). Sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) isoforms are expressed in the ER, Golgi/secretory pathway Ca^{2+} -ATPase (SPCA) isoforms are expressed throughout the Golgi apparatus and secretory vesicles, and plasma membrane Ca^{2+} -ATPase (PMCA) isoforms are present in the plasma membrane. Although SERCA transports two Ca^{2+} ions per ATP, SPCA and PMCA transport only one Ca^{2+} per ATP. All Ca^{2+} -ATPases present a similar domain organization (one transmembrane [TM] domain, and three cytosolic domains: A, actuator domain; P, phosphorylation domain; and N, nucleotide-binding domain). (B) Post-Albers cycle of SERCA1a is depicted, which serves as the reference Ca^{2+} transporter. The cycle shows four major conformational states of the Ca^{2+} pump (the high Ca^{2+} affinity forms E1, E1 ~ P; and low Ca^{2+} affinity forms E2-P, E2). All Ca^{2+} -ATPases belong to the P-type ATPases that transiently undergo catalytic autophosphorylation during transport. The phosphorylation and dephosphorylation reactions control, respectively, the closure of the cytosolic and luminal gates, resulting in occluded intermediates. SERCA1a is a $2\text{Ca}^{2+}/2-3\text{H}^{+}$ countertransporter.

family, whereas the more distal PMCA shares 33% sequence similarity with SERCA and belongs to the P2B subfamily (Vangheluwe et al. 2009).

The transport process of a P-type Ca^{2+} -ATPase follows the Post-Albers cycle, that is, alternating between a Ca^{2+} -bound E1 state and a Ca^{2+} -free E2 state (Fig. 1B; Albers 1967; Post et al. 1972). During transport, Ca^{2+} -ATPases undergo reversible autophosphorylation on a critically conserved Asp residue in one of the cytosolic domains, which controls the opening and closure of the Ca^{2+} -binding sites in the membrane region. Since 2000, many structures of SERCA1a in various conformations were solved (Toyoshima et al. 2000, 2013; Olesen et al. 2004, 2007; Toyoshima and Mizutani 2004; Jensen et al. 2006; Clausen et al. 2016),

and more recently, also, PMCA1 (Gong et al. 2018) and SERCA2a and SERCA2b structures were reported (Inoue et al. 2019; Sitsel et al. 2019). These structures revealed the Ca^{2+} -transporter architecture, which involves a transmembrane (TM) domain of 10 TM helices and three cytosolic domains (Fig. 2B). The TM region contains the Ca^{2+} -binding sites and ion entrance/exit pathways. Although SERCA pumps contain two Ca^{2+} -binding sites (I and II, formed by helices M4, M5, M6, and M8 in SERCA isoforms), SPCA and PMCA only contain one ion-binding site, closely resembling the Ca^{2+} -binding site II of SERCA (Fig. 2C; Toyoshima 2009; Vangheluwe et al. 2009). The cytosolic nucleotide-binding (N-) domain contains a highly conserved Lys residue for ATP coordination in the KGA motif (Møller et al. 2010). The phosphorylation

(P-) domain carries the Asp acceptor residue for autophosphorylation found in the DKTGT P-type ATPase signature motif. The TGE motif in the actuator (A-) domain regulates the access of water for the dephosphorylation reaction (Fig. 2; Olesen et al. 2004; Møller et al. 2010).

THE Ca²⁺ TRANSPORT MECHANISM EXEMPLIFIED BY SERCA1a

The crystal structures of the skeletal muscle isoform SERCA1a in the major conformational states have been solved. SERCA1a, therefore, became the archetypical Ca²⁺ pump for which the Ca²⁺ transport mechanism is described in great molecular detail, and which is summarized below (Fig. 2A; Toyoshima 2009; Møller et al. 2010; Primeau et al. 2018). In the high Ca²⁺ affinity E1 state, the cytosolic gate of SERCA1a is open, allowing 2–3 H⁺ to be displaced by two Ca²⁺ ions from the cytosol. The two Ca²⁺ ions bind sequentially and cooperatively at the Ca²⁺-binding sites I and II, leading to the stepwise repositioning of the Ca²⁺-binding residues (Fig. 2C). The induced fit following the binding of Ca²⁺ in the TM region is transmitted to the cytoplasmic domain via movement of M1–M4 (Sorensen et al. 2004; Gorski et al. 2017), which triggers ATP binding to a pocket in the N-domain, close to F487, K492, and K515 (Toyoshima et al. 2000). The adenosine of ATP binds at the N-domain and, with the help of the cofactor Mg²⁺, the γ phosphate of ATP is bridged to D351 at the P-domain. The subsequent SN2 nucleophilic reaction generates a high-energy phospho-intermediate of the pump (E1~P) (Sorensen et al. 2004; Toyoshima and Mizutani 2004). At the same time, M1 is lifted toward the cytosolic side of the membrane and forms a kink that closes the cytosolic entry gate, leading to an occluded state. ATP forms a bridge between the N- and P-domains that generates tension, which is relieved by ATP hydrolysis causing the N-domain to move. This, in turn, creates tension between M3 and the A-domain, allowing the A-domain to rotate nearly 90°, which positions the ¹⁸¹TGE loop of the A-domain at the phosphorylation site in the P-domain. This loop pre-

vents ADP or bulk H₂O from reacting with the aspartylphosphate. The major rotation of the A-domain is also transmitted to the TM region, which distorts the high-affinity Ca²⁺-binding sites and creates a luminal gate through which Ca²⁺ can exit. Hence, the low Ca²⁺ affinity E2-P state is formed, which displays open Ca²⁺-binding sites facing the lumen (Olesen et al. 2007). The empty ion-binding sites are stabilized by two to three protons triggering the dephosphorylation reaction in the cytosolic domains and the closure of the luminal pathway in the TM domain. This is caused by a further rotation of the A-domain, which positions the TGE loop so that E183 fixes a water molecule and catalyzes an attack on the aspartyl phosphate. Consequently, phosphate and Mg²⁺ are released from the P-domain, which repositions the membrane helices and renders the occluded E2 state (Toyoshima and Nomura 2002; Toyoshima et al. 2004). Finally, the A-domain rotates away from the P-domain, which repositions the TM helices and recreates the high-affinity Ca²⁺-binding sites, thereby returning the pump to the E1 state (Ma et al. 2003). Although SERCA pumps countertransport protons when importing Ca²⁺ to the ER, it does not lead to a more basic ER luminal store because of the permeability of the ER membrane to small molecules (Le Gall et al. 2004; Bultynck et al. 2014).

An additional, Mg²⁺-bound structure was solved, representing a transition state between the closed Ca²⁺-free E2 and the open Ca²⁺-bound E1 state (Toyoshima et al. 2013; Winther et al. 2013). The high-affinity Ca²⁺-binding sites are only half formed and occupied by one or two Mg²⁺ ions, but the protein is prevented from undergoing autophosphorylation (Toyoshima et al. 2013; Winther et al. 2013). Mg²⁺ binding may temporarily delay the Ca²⁺ transport in muscle (Winther et al. 2013), but because Ca²⁺ entry is not blocked by Mg²⁺, Mg²⁺ ions may actually help to form the high-affinity Ca²⁺-binding sites (Toyoshima et al. 2013). The conformational transitions of SERCA1a are further facilitated by the protein–phospholipid interplay in the membrane. Interactions between phospholipids and specific R/K residues pro-

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Figure 2. Mechanism of Ca^{2+} transport. (A) Schematic overview of the catalytic transport cycle of the sarco(endo)plasmic reticulum Ca^{2+} -ATPase 1a (SERCA1a), which is also used as the reference mechanism for other Ca^{2+} -ATPases. The cycle is accompanied by the description in the main text. Note that after ADP release, a new ATP can immediately bind to the N-domain. (B) Structure of SERCA1a (PDB 3N8G; Bublitz et al. 2013) depicting the major domains in various colors: A, actuator domain (yellow); P, phosphorylation domain (blue); N, nucleotide-binding domain (red), and transmembrane (TM) domain (M1–M10: M1–M2, wheat; M3–M4, brown; M5–M6, dark gray; M7–M10, light gray). Ca^{2+} ions are depicted in dark blue spheres and ATP in green spheres. (C) Detailed image showing the side chains of the coordinating residues in the two Ca^{2+} -binding sites in the SERCA1a TM domain (PDB 3N8G). Note that, in total, 10 residues situated on M4, M5, M6, and M8 contribute either via their side chains (depicted) or their backbone oxygen atoms (not shown). Moreover, two H_2O molecules are also involved in Ca^{2+} coordination in site I (not shown). E309 in red is the gating residue. Only the common site II is highly conserved between Golgi/secretory pathway Ca^{2+} -ATPase (SPCA), plasma membrane Ca^{2+} -ATPase (PMCA), and SERCA.

mote conformational transitions, whereas phospholipid interactions with W residues determine the protein tilt in the membrane. Together, lipid interactions lower the energy cost of the major movements of TM helices during the transport cycle (Norimatsu et al. 2017).

Besides crystallography, biochemical and biophysical approaches, such as fast kinetics and the intramolecular FRET method, provided insights on the dynamics of the Post-Albers cycle, revealing valuable information such as the rate-limiting steps (Dyla et al. 2017) and

Figure 3. Comparison of a sarco(endo)plasmic reticulum Ca²⁺-ATPase 1a (SERCA1a) crystal structure (A) (PDB: 3W5B; Toyoshima et al. 2013), a homology model of SPCA1a (B) (Chen et al. 2017), and a cryo-electron microscopy (cryo-EM) structure of PMCA1 (C) (PDB 6A69; Gong et al. 2018). In the three enzymes, the major domains are presented in the same colors: A, actuator domain (yellow); P, phosphorylation domain (blue); N, nucleotide-binding domain (red); transmembrane domain (M1–M10: M1–M2, wheat; M3–M4, brown; M5–M6, dark gray; M7–M10, light gray). The protein neuroplastin (NPTN) subunit in the plasma membrane Ca²⁺-ATPase (PMCA) structure is shown in orange.

transition of conformations (Raguimova et al. 2018).

CONSERVATION AND MODULATION OF THE Ca²⁺ TRANSPORT MECHANISM

All reported P-type ATPase structures, including the Ca²⁺-ATPases SERCA1a (Toyoshima et al. 2000), SERCA2a (Sitsel et al. 2019), SERCA2b (Inoue et al. 2019), and PMCA (Gong et al. 2018), display an identical domain organization (Fig. 3A–C) and contain the key signature motifs for ATP hydrolysis and coupled Ca²⁺ transport (Vangheluwe et al. 2009; Palmgren and Nissen 2011). Although this indicates that the Ca²⁺ transport mechanism is highly conserved among Ca²⁺-ATPases (Møller et al. 2010; Bublit et al. 2011), all Ca²⁺ pumps also present distinct properties that depend on isoform-specific sequences. Indeed, SERCA1a and the cardiac muscle isoform SERCA2a display different kinetic properties (Dode et al. 2003), but present strikingly similar structures (Inoue

et al. 2019; Sitsel et al. 2019). The discrete properties of the different Ca²⁺-ATPases likely arise from isoform-specific residues that alter the intramolecular network of salt bridges and hydrogen bonds, which may change the molecular dynamics of the pump and affect the rate of conformational transitions (Sitsel et al. 2019). Other Ca²⁺-ATPase isoforms, like SERCA2b, SPCA1-2, and PMCA1-4, contain extra protein stretches, mainly at the amino and/or carboxyl terminus, which provide additional regulatory control (Chen et al. 2016). Finally, isoform-specific residues participate in regulation by including sites for protein interactions or post-translational modifications ([PTMs]; Sitsel et al. 2019). In conclusion, each Ca²⁺-pump isoform presents a unique dynamic behavior and regulatory control. Together with a cell-type-specific expression profile and/or limited subcellular distribution, this ensures that each Ca²⁺-ATPase fulfils a distinct physiological role, and when dysregulated, may lead to specific pathological conditions, which will be further reviewed.

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SERCA

The SERCA pump was identified and purified from skeletal muscle (Hasselbach 1964; MacLennan 1970), in which it plays an important role in muscle relaxation. Mammals contain three genes (*ATP2A1–3*) that express SERCA isoforms (SERCA1–3), which comprise around 1000 amino acids. Interestingly, no SERCA pumps are found in yeast, whereas invertebrates express only one SERCA gene, which corresponds to the mammalian SERCA2 isoform. In humans, alternative splicing of the messenger RNA (mRNA) transcripts of the three genes introduces additional variations in the carboxyl terminus rendering, in total, more than 10 SERCA protein variants. These variants are differently regulated and display distinct enzymatic properties and expression profiles, thereby fulfilling tissue-specific functions (Table 1; Periasamy and Kalyanasundaram 2007). SERCA proteins are selectively inhibited by the plant extract thapsigargin or its derivatives, by the synthetic compound 2,5-di(tert-butyl)-hydroquinone (BHQ), and the mycotoxin cyclopiazonic acid (CPA). Structural complexes of SERCA1a with these inhibitors show that thapsigargin binds to a pocket formed by M3, M5, and M7, whereas BHQ and CPA bind to overlapping pockets occupying the Ca^{2+} access channel delimited by TM segments M1–M4 (Toyoshima and Nomura 2002; Obara et al. 2005; Moncoq et al. 2007).

SERCA Isoforms

The two major skeletal muscle variants, SERCA1a and SERCA1b, are encoded by the *ATP2A1* gene. The splice variant SERCA1b is expressed during embryonic myogenesis and in the neonatal stage in myotubes and myoblasts, as well as in regenerating adult muscles (Brandl et al. 1987; Zádor et al. 2007). Conversely, SERCA1a is the adult variant, which replaces SERCA1b during development (Brandl et al. 1987; Periasamy and Kalyanasundaram 2007). Its expression is highest in the fast twitch skeletal-muscle fibers, in which it serves as the relaxing factor by removing all Ca^{2+} from the myofilaments at the end of contraction. Functionally, SERCA1a displays a twofold higher

maximal activity as compared with SERCA1b at high luminal Ca^{2+} concentrations (Zhao et al. 2015), which is a consequence of the SERCA1b-specific carboxy-terminal tail ($^{994}\text{DPEDERRK}$ in SERCA1b, instead of ^{994}G in SERCA1a). Interestingly, a truncated variant of SERCA1 was identified and named S1T (Chami et al. 2001). It lacks a large part of the N- and P-domain, as well as M5–M10, hence, is inactive in Ca^{2+} pumping (Chami et al. 2001). The presence of S1T in ER-mitochondria contact sites increases Ca^{2+} leakage through ER, which results in increased Ca^{2+} transfer into mitochondria inducing apoptosis (Chami et al. 2008).

The housekeeping *ATP2A2* gene generates three variants (SERCA2a–c) that were confirmed at the protein level and differ at their carboxyl termini (Vandecaetsbeek et al. 2009). SERCA2a shares 84% sequence identity with SERCA1a, and is found in the heart, slow-twitch skeletal muscle, and smooth muscle cells (Lyttton et al. 1989; Zarain-Herzberg et al. 1990). In humans, SERCA2a removes between 70% and 90% of the elevated cytosolic Ca^{2+} after cardiomyocyte contraction (Bers 2002), which is stored inside the sarcoplasmic reticulum (SR) for the next contraction. Therefore, SERCA2a is a major determinant of cardiomyocyte relaxation, but it also determines the SR Ca^{2+} content that controls the Ca^{2+} release for contraction (Periasamy et al. 2008).

SERCA2b is ubiquitously expressed in the ER and is the main SERCA isoform in the brain (Miller et al. 1991; Baba-Aissa et al. 1998; Mata and Sepúlveda 2005). SERCA2b keeps cytosolic Ca^{2+} levels in the submicromolar range while filling the ER with 0.5–1 mM Ca^{2+} (Suzuki et al. 2016), which is important for ER homeostasis and protein maturation such as N-linked glycosylation (Helenius and Aebi 2001) and disulfide bridge formation (Michalak et al. 2002). In addition, SERCA2b mRNA levels increase 3- to 4-fold when ER stress is induced (Caspersen et al. 2000). The four amino acids that comprise the short SERCA2a-specific carboxyl terminus ($^{994}\text{AILE}$) are replaced by 49 amino acids in SERCA2b, which is referred to as the 2b-tail. The 2b-tail consists of an additional TM helix (M11) and a luminal extension (Lyt-

Table 1. Overview of the expression profile and disease links of Ca^{2+} -ATPases

Gene	Isoform	Tissue distribution	Human disease and associated alterations on genetic/protein level	OMIM
ATP2A1	SERCA1a	Fast twitch skeletal muscle (adult)	Brody disease (autosomal recessive inheritance): splice site mutations, premature stop codons, missense mutations	108730
	SERCA1b	Fast twitch skeletal muscle (fetal)	Myotonic dystrophy type 1: SERCA1b alternative mRNA splicing and dysregulated expression	
ATP2A2	SERCA2a	Highly expressed in cardiac and slow twitch skeletal muscle, smooth muscle, neuronal cells	Darier–White disease (autosomal dominant): acrokeratosis verruciformis	108740
	SERCA2b	Ubiquitous	Heart failure: impaired SERCA2a protein expression and ATPase activity, mutations in PLB and reduced DWORF expression	
	SERCA2c	Cardiac muscle (slow and fast twitch) skeletal muscle, myeloid and nonmyeloid cells, primary blood monocytes	Cancer: dysregulated protein expression, targeted SERCA inhibition as cancer therapy	
ATP2A3	SERCA3a–f	3a, d, f: cardiac muscle; nonisoform specific: smooth muscle and other nonmuscle cells (endothelial, epithelial cells, lung, and pancreas)	Gastric carcinomas, colon and lung cancer: dysregulated protein expression Diabetes: dysregulated protein expression	601929
ATP2C1	SPCA1a–d	Ubiquitous	Hailey–Hailey disease (autosomal inheritance): nonsense, splice-site, and nonconservative missense mutations; frameshift insertion and deletions Breast cancer: up-regulated protein expression	604384
ATP2C2	SPCA2	Gastrointestinal tract, trachea, thyroid gland, salivary gland, mammary gland, prostate, brain (hippocampal neurons), keratinocytes (mRNA level)	Breast cancer: up-regulated protein expression	613082
ATP2B1	PMCA1a–e	Ubiquitous (1b: fetal; 1a: adult) 1a, 1c, 1e: brain; 1c in skeletal muscle	Cardiovascular disease risk, preeclampsia, salt sensitivity: associated SNPs	108741
ATP2B2	PMCA2a–f	Brain (fetal and adult), cerebellar Purkinje cells, hair cells in the inner ear, mammary gland	Autosomal-recessive deafness: heterozygous point mutation (one case report) Autism: associated SNPs	108733
ATP2B3	PMCA3a–c	Widely expressed in the embryo Brain: cerebellum	Early-onset spinocerebellar ataxia-1 (X-linked): point mutation Aldosterone-producing adenomas: somatic point mutation	300014
ATP2B4	PMCA4a–g	Ubiquitous	Familial spastic paraplegia: point mutation Malaria resistance: associated SNPs	108732

Overview of the specific characteristics of expression profile and disease links of Ca^{2+} -ATPases. SERCA, SPCA, and PMCA are closely related based on phylogeny and their function as active Ca^{2+} -transporters. However, differences in physiological function between these proteins arise through the expression of multiple isoforms, their structural differences, and specific tissue distribution. Additionally, many of these genes/proteins are linked to several human diseases and therefore may serve as interesting therapeutic targets. OMIM, Online Mendelian Inheritance in Man; mRNA, messenger RNA; PLB, phospholamban; DWORF, DWARF open reading frame; GWAS, genome-wide association studies; SNPs, single-nucleotide polymorphisms.

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ton et al. 1992; Verboomen et al. 1992; Vandecaetsbeek et al. 2009). The luminal extension is predicted to interact near luminal loop L7/8 and M11 near M10 (Vandecaetsbeek et al. 2009). Both regions independently alter the kinetic properties of the pump. The luminal extension increases the intrinsic Ca^{2+} affinity and slows the E1~P to E2-P transition, whereas M11 lowers the maximal turnover rate, mainly by reducing E2-P to E2 and E2 to E1 conversion rates (Verboomen et al. 1992; Dode et al. 2003; Vandecaetsbeek et al. 2009; Clausen et al. 2012; Gorski et al. 2012). Recently, a first structure of SERCA2b was solved that depicts the position of M11 on M10, that is, isolated from the other TM segments (Inoue et al. 2019), at a similar position as neuroligin (NPTN) binds to PMCA1 (Gong et al. 2018). The high B-factor values of M11 in SERCA2b and the missing electron densities in the cytosolic and luminal extensions may suggest that the 2b-tail interaction is flexible and may be prone to regulation (Inoue et al. 2019).

SERCA2c (with the carboxy-terminal tail ⁹⁹⁴VLSSEL) mRNA was detected in epithelial, mesenchymal, hematopoietic cell lines, and primary human monocytes (Gélébart et al. 2003), and the protein form is confirmed in the human heart (Dally et al. 2010). It displays a lower Ca^{2+} affinity than SERCA2a and SERCA2b, and a similar V_{max} compared with SERCA2b (Dally et al. 2006).

In humans, six different SERCA3 variants (SERCA3a–f) have been identified. SERCA3 is expressed at high levels in the intestine and lymphatic tissue, platelets (Wuytack et al. 1994; Bobe et al. 2004), Purkinje neurons (Baba-Aissa et al. 1996), hematopoietic cell lineages, epithelial cells, fibroblasts, and endothelial cells (Anger et al. 1994). SERCA3 has also been found in low levels in smooth muscle cells (Wu et al. 2001). Together with SERCA2c, SERCA3a, -3d, and -3f were described in cardiomyocytes (Dally et al. 2009, 2010). In contrast to the more uniform distribution of SERCA3a, SERCA3d and SERCA3f displayed restricted localizations around the nucleus and in the subplasmalemmal area, respectively (Dally et al. 2009, 2010). Although the SERCA3 sequence is about 75%

similar to other SERCA isoforms, all three isoforms displayed a lower apparent Ca^{2+} affinity than SERCA1/2 isoforms, turning them only active at high cytosolic Ca^{2+} concentrations. This is because of a decreased E2 to E1 transition rate and an increased rate of Ca^{2+} dissociation in E1 as compared with SERCA1a (Wuytack et al. 1995; Dode et al. 2002; Chandrasekera et al. 2009). SERCA3 also displays an increased rate of E2-P dephosphorylation and a higher pH optimum at 7.5–9.0 (Dode et al. 2002; Periasamy and Kalyanasundaram 2007). A potential role of SERCA3 in cell differentiation associated with remodeling of ER Ca^{2+} homeostasis was suggested by several studies on endothelial, myeloid, and colon epithelial cells (Launay et al. 1999; Mountian et al. 1999; Gélébart et al. 2002). However, the specific function of the individual SERCA3 variants in many other tissues remains incompletely understood.

Regulation of SERCA Isoforms

As a major regulator of cytosolic and ER/SR luminal Ca^{2+} concentrations, SERCA isoforms are extensively regulated to fine-tune their activity according to the physiological requirements. This tight regulation takes place both at the expression and Ca^{2+} transport level (see detailed reviews elsewhere; Vangheluwe et al. 2005a; Vandecaetsbeek et al. 2011; Stammers et al. 2015). Here, we will focus on an emerging general concept of SERCA regulation by a family of small TM proteins that regulate SERCA Ca^{2+} affinity in an isoform- and/or cell-type-specific manner (Primeau et al. 2018).

Historically, phospholamban (PLB) was identified as the first and main small TM protein regulator of SERCA2a that is part of the β -adrenergic control system in the heart (Wegener et al. 1989). PLB is a 52-amino-acid, single-span integral membrane protein that is highly expressed in cardiac muscle together with SERCA2a, and at a lower level in smooth and slow-twitch skeletal muscles. The monomeric PLB forms a reversible one-to-one complex with SERCA by binding to a groove involving M2, M4, M6, and M9. This direct interaction reduces the apparent Ca^{2+} affinity of the pump

(Periasamy and Kalyanasundaram 2007). The SERCA2a inhibition is reversed at high cytosolic Ca²⁺ concentrations (>1 μ M) or during β -adrenergic stimulation via phosphorylation of PLB by protein kinase A and/or Ca²⁺/calmodulin (CaM) kinase II. Subsequently, PLB congregates into pentamers, serving mainly as a reserve pool of the protein (Kimura et al. 1997). The higher SERCA2a activity improves cardiac relaxation and, via its impact on the SR Ca²⁺ load, also the contraction (for reviews, see Simmerman and Jones 1998; MacLennan and Kranias 2003; Kranias and Hajjar 2012; Akin et al. 2013).

The small integral membrane protein sarcolipin ([SLN], 31 residues) appeared as a functional and structural homolog of PLB (reviewed in MacLennan and Kranias 2003) that interacts with SERCA at the same binding site as PLB (Toyoshima et al. 2013; Winther et al. 2013), lowering both the apparent Ca²⁺ affinity and $V_{\max}$ of the pump (Gorski et al. 2013; Sahoo et al. 2013). SLN contains a shorter amino terminus that holds a regulatory phosphorylation site, and a longer carboxy-terminal extension that is functionally important by docking SLN to the luminal side of the SERCA protein (Gorski et al. 2012). SLN appears more stably associated with the pump than PLB because high Ca²⁺ concentrations do not dissociate SLN (Shaikh et al. 2016). SLN is coexpressed with SERCA1a and SERCA2a in skeletal muscle or with SERCA2a in atrial tissue, but is absent in the ventricles of the heart (Vangheluwe et al. 2005b; Babu et al. 2007a). SLN modifies the atrial contractility and β -adrenergic response (Babu et al. 2007b), which differ from ventricles (Vangheluwe et al. 2005b). Moreover, SLN knockout mice are susceptible to atrial arrhythmias and remodeling dependent on aging (Xie et al. 2012), further showing the physiological relevance of SLN in the atria. In HEK-293 cells coexpressing SLN, PLN, and SERCA, SLN and PLB potentially form a ternary, superinhibitory complex with SERCA (Asahi et al. 2002), whereas SLN directly binds to PLN, inhibiting the formation of PLN pentamers, which may contribute to the superinhibitory effect on SERCA (Asahi et al. 2004). However, physiological evidence showing that SERCA would be superinhibited in atria is not

yet available. In skeletal muscle with a high SLN/SERCA1a coexpression, SLN may uncouple SERCA1a's ATPase activity from Ca²⁺ transport (Sahoo et al. 2013), resulting in energy dissipation and heat production without transporting Ca²⁺. This uncoupling behavior appears physiologically important for muscle-based nonshivering thermogenesis in mammals. Interestingly, nonshivering thermogenic mechanisms through SLN-mediated uncoupling of SERCA1a may be considered as a target for obesity treatment (Bal et al. 2012; Periasamy et al. 2017).

Solved complexes of SERCA1a with SLN (Toyoshima et al. 2013; Winther et al. 2013) or PLB4, a gain-of-function mutant of PLB (Akin et al. 2013), provided structural insights into the modulation of Ca²⁺ transport by small TM regulators. Both regulators bind in the same TM region of the pump, but the observed structural differences may indicate that PLB and SLN stabilize a distinct conformation of SERCA, suggesting that the two regulators present a different mode of action. Indeed, the SERCA1a-SLN complex adopts an E1-like conformation with Mg²⁺ at the ion-binding sites (Toyoshima et al. 2013; Winther et al. 2013), whereas the SERCA1a-PLB4 structure represents an E2-like structure in which Ca²⁺ and Mg²⁺ binding is precluded (Akin et al. 2013).

More recently, several other related small TM proteins were discovered in annotated long noncoding RNAs such as myoregulin (MLN), DWARF open reading frame (DWORF), endoregulin (ELN), and another-regulin (ALN). These proteins present a similar primary and secondary structure as PLB and SLN, contain a conserved LFxxF sequence in the TM region, presumably bind to the same pocket in the SERCA TM region, and regulate the apparent Ca²⁺ affinity of the Ca²⁺ pump. ELN and ALN are mainly expressed in nonmuscle tissue (Anderson et al. 2016), in which they diminish the apparent Ca²⁺ affinity of SERCA2b and SERCA3a. MLN appears as a skeletal muscle-specific regulator of SERCA1a and SERCA2a (Anderson et al. 2015). DWORF is expressed in soleus, the ventricles of the heart, and the diaphragm, in which it may regulate SERCA1a, SERCA2a/b, and SERCA3a/b isoforms (Nelson

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et al. 2016). Interestingly, DWORF exerts no direct effect on the apparent Ca^{2+} affinity of the pump, but instead may stimulate SERCA activity by displacing other regulators like PLB (Makarewich et al. 2018). This points to a complex, tissue-specific interplay between various regulatory proteins, but how this dynamically adapts the SERCA activity to the local physiological demand and how this is modified in disease conditions remains to be further elucidated.

Many other regulators of SERCA have been documented, which directly interact with the Ca^{2+} pump and regulate its activity (e.g., Atrap, sarcalumenin, S100A1, histidine-rich Ca^{2+} -binding protein, ERdj5; reviewed in Vangheluwe et al. 2005a; Vandecaetsbeek et al. 2011). Other interactions control organelle contact sites at the ER. The ER-localized metazoan-specific autophagy protein VMP1 prevents the PLB/SLN inhibition of SERCA, which activates SERCA and reduces contact formation between the ER and isolation membranes (autophagosome precursors), mitochondria, lipid precursors, and endosomes (Zhao et al. 2017). The importance of SERCA in ER-mitochondria contact sites has been recently reviewed in Krols et al. (2016), Chemaly et al. (2018), and Gutiérrez and Simmen (2018). The role of SERCA pumps in ER-mitochondria communication and cell death is also emerging as SERCA is regulated by mitochondrial antiapoptotic proteins HS1-associated protein HAX-1 (Vafiadaki et al. 2009; Bidwell et al. 2018) and B-cell lymphoma 2 (Bcl-2) (Dremina et al. 2004), proapoptotic protein p53 (Giorgi et al. 2015), and palmitoylated calnexin during short-term ER stress (Lynes et al. 2013).

SERCA in Disease

SERCA isoforms and their regulators preserve the required Ca^{2+} balance in cells, whereas mutations and dysregulation of these proteins are implicated in a variety of pathological conditions.

Homozygous or compound heterozygous loss-of-function mutations in the skeletal muscle isoform SERCA1 are associated with autosomal recessive Brody myopathy (Odermatt et al.

1996; Guglielmi et al. 2013), an exercise-induced impairment of fast-twitch skeletal muscle relaxation (Odermatt et al. 1996). Brody myopathy was also described in cattle (Charlier et al. 2008; Drögemüller et al. 2008) and zebrafish (Hirata et al. 2004), whereas loss of SERCA1 in mice is lethal as a result of the respiratory failure and hypercontracture injury of the diaphragm (Pan et al. 2003). *ATP2A1* is also one of the genes that is aberrantly spliced in myotonic dystrophy type 1 muscle, leading to expression of the SERCA1b splice variant instead of SERCA1a in normal muscle (Zhao et al. 2015). Also, improving SERCA1a activity is considered for therapy to restore abnormalities of Ca^{2+} homeostasis in Duchenne muscular dystrophy patients (Morrine et al. 2010; Goonasekera et al. 2011).

Homozygous SERCA2 knockout mice are not viable, whereas heterozygous SERCA2 mice appeared healthy, but showed reduced cardiac muscle contractility (Periasamy et al. 1999). Also, heterozygous SERCA2 mice develop squamous cell tumors more frequently at older age (Liu et al. 2001). When put on a high-fat diet, SERCA2 heterozygous mice develop glucose intolerance, diminished insulin secretion, and elevated β -cell ER stress and death, suggesting that restoring SERCA2 activity may represent a viable strategy to improve glucose homeostasis (Kang et al. 2016; Tong et al. 2016). In humans, the absence of one functional allele of SERCA2 triggers an inherited, dominant skin disorder called Darier disease, which is characterized by distinctive nail abnormalities, warty papules, and plaques mainly on the chest, neck, back, ears, forehead, and groin (Sakuntabhai et al. 1999; Dhitavat et al. 2003; Engin et al. 2015). Most disease mutations display a loss-of-function phenotype causing haploinsufficiency, although a gain-of-function because of a leaky Ca^{2+} pump was also proposed for some Darier mutants (Kaneko et al. 2014).

A reduced expression and activity of SERCA2a strongly contributes to the poor cardiac contractility in patients with end-stage heart failure (for review, see Lipskaia et al. 2010). However, Darier disease patients show no predisposition to develop cardiomyopathy (Mayosi et al. 2006). Although this observation

may raise the question on the causality of SERCA2a dysfunction in heart failure, cardiac-specific SERCA2a knockout in adult mice induces heart failure within weeks (Andersson et al. 2009). Moreover, heterozygous SERCA2 mice are predisposed to some, but not all forms of heart failure (Prasad et al. 2015). In humans, several disease-causing PLB mutations were identified in heritable forms of dilated cardiomyopathy that lead to a chronic inhibition of SERCA2a (MacLennan and Kranias 2003). DWORF expression was also reduced in ischemic failing human hearts, which may lead to a stronger PLB inhibition (Nelson et al. 2016). Consequently, restoring SERCA2a activity is considered as a key therapeutic strategy for end-stage heart failure. Indeed, increasing DWORF expression (Makarewich et al. 2018) or lowering PLB expression (Minamisawa et al. 1999) enhances contractility and prevents heart failure in mouse models of dilated cardiomyopathy. Moreover, adeno-associated viral gene transfer of SERCA2a has beneficial effects on the contractility and remodeling in small and large animal models of heart failure (for review, see Lipskaia et al. 2010; Park and Oh 2013; Gorski et al. 2015). However, in patients, the outcome of the clinical trials was disappointing, presumably caused by an inadequate gene delivery in the diseased human heart (Greenberg et al. 2016). Alternative strategies are currently explored to achieve SERCA2a activation, for example, via small activator compounds or better viral gene delivery methods (Samuel et al. 2018).

A strong reduction in SERCA2 is also observed in limb-girdle muscular dystrophy type 2A, suggesting that restoring SERCA2 activity may also be of therapeutic interest (Toral-Ojeda et al. 2016). In contrast, SERCA inhibition by analogs of the highly potent and selective SERCA inhibitor thapsigargin is considered for prostate cancer treatment. This approach relies on an inactive thapsigargin prodrug that is cleaved by a prostate-specific antigen in the malignant tissue environment, leading to local SERCA2b inhibition and apoptosis of the cancer cells (Denmeade et al. 2012; Mahalingam et al. 2016).

Altered SERCA3 expression levels are reported in diabetes and cancer (Varadi et al.

1999; Xu et al. 2012). Also, SERCA3f mRNA is up-regulated in idiopathic forms of dilated cardiomyopathy, which may be a marker of ER stress induction (Dally et al. 2009). SERCA3 further plays a role in progesterone-triggered Ca²⁺ signaling in the MCF-7 breast cancer cell line, modulating cell proliferation and death (Azeez et al. 2018). However, SERCA3 knockout mice do not show structural malformations and grow normally into adulthood without a clear disease phenotype. Instead, they show an extended bleeding time, defective platelet adhesion, and thrombus growth as a consequence of reduced ADP secretion (Elaiib et al. 2016).

SPCA

SPCA is the most recently identified active Ca²⁺-transporter and was first described in *Saccharomyces cerevisiae*, named plasma membrane-related Ca²⁺-ATPase (PMR1) (Rudolph et al. 1989). Later, the mammalian SPCA isoforms were cloned and characterized (Guntowski-Hamblin et al. 1992; Wootton et al. 2004; Vanoevelen et al. 2005; Xiang et al. 2005). In humans, two genes (*ATP2C1* and *ATP2C2*) code for SPCA proteins that share 63% sequence identity. SPCA1 represents evolutionarily the older and most widespread isoform, whereas SPCA2 emerged later in vertebrate evolution at the rise of tetrapods (Table 1; Vangheluwe et al. 2009; Pestov et al. 2012).

SPCA proteins contain about 950 residues and most likely present a similar domain organization and Ca²⁺ transport mechanism as SERCA1a (Fig. 3B). However, SPCA isoforms are also structurally and mechanistically different from SERCA1a. In addition to Ca²⁺, SPCA proteins also transport Mn²⁺ ions, which depend on structural elements in the amino terminus and TM domain that regulate ion selectivity (Wei et al. 1999; Mandal et al. 2003; Vangheluwe et al. 2009). Also, SPCA isoforms contain a single Ca²⁺-binding site that corresponds to site II of SERCA proteins (Fig. 2C). SPCA pumps transport Ca²⁺ at lower maximal turnover rates, but with much higher apparent affinities than

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SERCA1a (Van Baelen et al. 2001) because of a slower conversion of E1~P(Ca^{2+}) to E2-P (Dode et al. 2005). Different from SERCA, SPCA isoforms present an enhanced rate of E2-P hydrolysis, which is pH independent, suggesting that SPCA1 may not countertransport protons (Dode et al. 2005, 2006). SPCA proteins are generally more compact and lack several regulatory elements that are present in SERCA (Fig. 3A,B). In addition, SPCA1 and SPCA2 contain longer amino and carboxyl termini, of which the regulatory roles are gradually emerging (Wei et al. 1999; Feng et al. 2010; Smaardijk et al. 2017; Chen et al. 2019). SPCA proteins are inhibited by high (micromolar) concentrations of the commonly used SERCA inhibitors thapsigargin, BHQ, and CPA. Specific and potent SPCA inhibitors are not yet reported, but the distinct structure–activity relationship of thapsigargin inhibition in SERCA versus SPCA1 indicates that SPCA-specific inhibitors may be developed based on the thapsigargin scaffold (Chen et al. 2017).

The higher apparent Ca^{2+} affinity renders the SPCA activity less sensitive to fluctuations in the cytosolic Ca^{2+} concentration than SERCA (Dode et al. 2005). SPCA ensures a constant filling of the Golgi with Ca^{2+} and also Mn^{2+} . Both ions are cofactors of many Golgi enzymes required for adequate protein processing by posttranslational modifications and trafficking. In particular, there is a Ca^{2+} gradient across the secretory pathway from the ER (0.5–1 mM), *cis*-Golgi (250 μM) to the *trans*-Golgi network (TGN) (100 μM) (Pizzo et al. 2011; Suzuki et al. 2016), which is generated by the combined activities of SERCA (in ER and early Golgi compartments) and SPCA (all Golgi compartments) (Fig. 1A). The luminal $\text{Ca}^{2+}/\text{Mn}^{2+}$ levels need to fall in a physiological window because either a diminished (Okunade et al. 2007) or excessive SPCA1 (Smaardijk et al. 2018) activity induces signs of Golgi stress such as Golgi swelling and fragmentation.

SPCA Isoforms

SPCA1 is ubiquitously expressed and displays a predominant distribution in the *trans*-Golgi (re-

viewed in Vangheluwe et al. 2009). In humans, alternative splicing of the *ATP2C1* gene generates four experimentally confirmed protein variants that differ at the carboxyl terminus (SPCA1a–d), among which the SPCA1c is an inactive form and SPCA1d is the longest (Table 1; Missiaen et al. 2004; Micaroni et al. 2016). However, their tissue-specific expression pattern and subcellular localization remain incompletely understood. SPCA1 is the main SPCA isoform in brain, in which it plays a crucial role in establishing neural polarity during development (Sepúlveda et al. 2009). Neurons are highly sensitive to Ca^{2+} dyshomeostasis in the Golgi (Sepúlveda et al. 2009) and to Mn^{2+} toxicity (Olanow 2004; Sepúlveda et al. 2012b).

SPCA2 expression is more restricted to the brain, testis, gastrointestinal and respiratory tracts, and to actively secreting cells like prostate, thyroid, salivary, and mammary glands (Table 1), suggesting more specialized functions than SPCA1 (Vanoevenen et al. 2005; Xiang et al. 2005). Compared to SPCA1, SPCA2 displays a broader subcellular localization in the secretory pathway such as the Golgi, ER, and secretory vesicles (Vanoevenen et al. 2005; Xiang et al. 2005; Feng et al. 2010; Pestov et al. 2012).

The SPCA1 and SPCA2 proteins have distinct functional properties. The SPCA2 maximal activity is 2.5-fold higher than SPCA1d, but presents a similar apparent Ca^{2+} affinity. This relates to an increased rate of E1~P to E2-P conversion, a reduced rate of the E2 to E1 transition, and an enhanced rate of the E2-P dephosphorylation (Dode et al. 2006). However, purified SPCA1a displays a lower apparent Ca^{2+} affinity than purified SPCA2, but a twofold higher ATPase activity in the presence of Ca^{2+} . In contrast, the maximal activity and apparent affinity for Mn^{2+} are comparable for both isoforms. The distinct Ca^{2+} -dependent properties of SPCA1a relate to the presence of an amino-terminal Ca^{2+} -binding EF-hand-like motif that is absent in SPCA2. Ca^{2+} binding to the amino-terminal EF-hand-like motif promotes the activity of SPCA1a by facilitating the autophosphorylation step. This may be important in cells with a high Ca^{2+} load, such as mammary gland cells during lactation, or in cells with a low ATP content,

such as keratinocytes (Chen et al. 2019). Also, the *S. cerevisiae* ortholog PMR1 contains a Ca²⁺-binding EF-hand-like motif in the amino terminus that is important for substrate affinity and ion selectivity (Wei et al. 1999). Based on a partial proteolytic digestion analysis, a model was proposed that the amino terminus of PMR1 interacts with the carboxy-terminal half of the protein to control its functional properties (Wei et al. 1999), but the precise molecular mechanism remains unclear.

Regulation of SPCA

The activity and expression of multiple Ca²⁺ transporters in the mammary gland is tightly coordinated during pregnancy, lactation after parturition, and the process of involution (reviewed in Cross et al. 2014). These remarkable changes are required to support the release of large amounts of Ca²⁺ in the milk (8–60 mM, depending on the species) (Neville 2005). SPCA1 and SPCA2 expression is highly up-regulated in mammary glands during lactation (McAndrew et al. 2011; Cross et al. 2013). Via its amino- and carboxy-terminal extensions, SPCA2 directly interacts with and activates the plasma membrane Ca²⁺ channel Orai1, which leads to an increased Ca²⁺ influx. This happens independent of a change in the intracellular Ca²⁺ store content or the relocation of STIM1, which typically activates Orai1 dependent on Ca²⁺ store depletion. The SPCA2-Orai1 coupling is therefore dubbed “store-independent Ca²⁺ entry” (SICE) (Feng et al. 2010). Once activated, SPCA2 transfers the incoming Ca²⁺ into the Golgi/secretory pathway (Smaardijk et al. 2017). Later, SPCA1 was also found to induce SICE through Orai1, which controls the luminal Ca²⁺ content of the Golgi/secretory pathway (Smaardijk et al. 2018). The incoming Ca²⁺ via Orai1 occurs at the basolateral side of the mammary gland epithelial cells (Cross et al. 2013). At the apical side, Ca²⁺ is delivered into the milk by direct Ca²⁺ transport by PMCA2 over the plasma membrane (60%) and by secretion of Ca²⁺ that was sequestered in the secretory pathway by SERCA and SPCA (40%) (Reinhardt et al. 2004; Faddy et al. 2008; Cross et al. 2013).

The TGN sorts proteins destined for endolysosomes, secretory storage granules, and the plasma membrane via several parallel sorting systems that package cargo into specific vesicles. Of interest, SPCA is activated by the actin filament severing protein cofilin-1, which determines protein sorting and secretion (Kienzle et al. 2014). The transient increase of the local luminal Ca²⁺ concentration in the TGN induces the polymerization of luminal 45-kDa, Ca²⁺-binding protein (Cab45) (Crevenna et al. 2016). In its polymerized state, Cab45 selectively interacts with specific cargo molecules for secretion (von Blume et al. 2012; Crevenna et al. 2016). More recently, it was shown that SPCA1 also influences cell contractility by disrupting actin dynamics and the localization of cofilin-1. This process is important during embryonic development to organize neuronal tube closure, which goes wrong in the *ATP2C1* knockout embryo (Brown and Garcia-Garcia 2018).

SPCA Isoforms in Disease

In humans, heterozygous mutations in *ATP2A2* result in Darier disease, whereas heterozygous *ATP2C1* mutations cause Hailey–Hailey disease (HHD), a related chronic skin disease with similar symptoms, showing blisters and itchy erosions mainly at the sites of sweating and friction such as the groin and the axillar regions (Hu et al. 2000). Although most studied SPCA1 mutations show a loss of transport function (Fairclough et al. 2003), some mutants are unable to couple with Orai1 to elicit a SICE response (Smaardijk et al. 2018). Irrespective of the mechanism, disease-causing mutations appear less potent to fill the Golgi/secretory pathway with Ca²⁺ (Smaardijk et al. 2018), which may cause haploinsufficiency.

Remarkably, both *Atp2a2*^{+/-} (Prasad et al. 2005) and *Atp2c1*^{+/-} (Okunade et al. 2007) heterozygous mice display an increased incidence of squamous tumors, which markedly differs from the skin disease phenotype in humans. Loss of *Atp2c1* is embryonically lethal as a result of the failure of neural tube closure (Okunade et al. 2007; Brown and Garcia-Garcia 2018).

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SPCA1 levels are increased in basal-type breast cancer, whereas SPCA2 is increased mainly in luminal types of breast cancer (Grice et al. 2010; Dang et al. 2017). Knockdown of SPCA1 in the basal-type breast cancer cell line MDA-MB-231 leads to impaired processing of the insulin-like growth factor 1 receptor (IGF1R) (Grice et al. 2010), suggesting that SPCA1 inhibition may be of interest for breast cancer therapy (Christopoulos et al. 2018). Furthermore, the up-regulation of SPCA2 leads to a constitutive activation of Orai1 and, consequently, a pathological elevated cytosolic Ca^{2+} concentration that increases proliferation and oncogenic activity (Feng et al. 2010). Preventing the pathological Ca^{2+} influx from the Orai1-SPCA2 complex, or inhibition of SPCA1 activity, may be considered for breast cancer therapy (Feng et al. 2010). Furthermore, breast calcification is a radiographic feature that is linked to poorer survival in breast cancer patients. In vitro microcalcifications in human breast cancer cells depend on SPCA Ca^{2+} transport activity (Dang et al. 2017).

SPCA1 is required as a host factor for virus maturation and spreading possibly by maintaining high luminal Ca^{2+} and Mn^{2+} levels for (viral) protein maturation. Thus, SPCA1 may be an interesting target for viral diseases, in particular, involving flaviviruses and togaviruses (Hoffmann et al. 2017). Furthermore, disrupting PMR1 in various model organisms counters the cytosolic Ca^{2+} toxicity that is elicited by the ectopic expression of α -synuclein, indicating that inhibition of PMR1/SPCA may be of interest to lower α -synuclein toxicity, the major constituent of the brain plaques present in the brain of Parkinson's disease patients (Büttner et al. 2013).

Finally, SPCA1 may be implicated in man-ganism, a Parkinson-like disease that affects miners, welders, and steel and battery workers because of Mn^{2+} intoxication. Toxic Mn^{2+} accumulation in brain areas correlates with a higher presence of SPCA1 (Sepúlveda et al. 2012a). In addition, Mn^{2+} overload affects survival of neurons and glia by inhibiting SPCA activity and inducing Golgi fragmentation (Sepúlveda et al. 2012b), a phenomenon also described in other

neurodegenerative diseases (Gonatas et al. 2006). Conversely, PMR1 in yeast is important for Mn^{2+} detoxification by uptake of Mn^{2+} in the secretory pathway for subsequent secretion. Likewise, SPCA1 may play a role in Mn^{2+} detoxification in the liver (Leitch et al. 2011).

PMCA

PMCA was identified (Schatzmann 1966) and purified (Niggli et al. 1979) in erythrocytes, in which it is the only type of Ca^{2+} pump. Over the years, the simple view that PMCA reduce cytosolic Ca^{2+} to avoid cellular overload shifted to a more complex picture in which PMCA isoforms fine-tune local Ca^{2+} events that originate close to the plasma membrane. Because it is impossible to include the vast literature on PMCA in this review, the reader is also referred to Brini and Carafoli (2011), Strehler (2015), and Stafford et al. (2017).

In mammals, four PMCA isoforms and more than 20 spliced variants were described with diverse kinetic properties (Strehler and Zacharias 2001), each comprising about 1200 amino acids (Table 1). PMCA displays a Ca^{2+} /ATP molar stoichiometry of 1/1 as does SPCA while it behaves like a $\text{Ca}^{2+}/\text{H}^{+}$ countertransporter akin to SERCA (Hao et al. 1994; Salvador et al. 1998). Like other Ca^{2+} -ATPases, PMCA isoforms contain three cytoplasmic domains and 10 TM helices (Fig. 3C), but they fundamentally differ in regulatory regions. PMCA are marked by the presence of an autoinhibitor domain at the carboxyl terminus, which constitutes the CaM-binding domain. Recently, a cryo-EM structure of PMCA1 in the E1- Mg^{2+} intermediate state has been resolved to about 4 Å in complex with the protein NPTN/basigin (BASI) (Gong et al. 2018), which recently identified auxiliary subunits that form a complex with PMCA to regulate Ca^{2+} clearance in different types of cells (Korthals et al. 2017; Schmidt et al. 2017). NPTN/BASI binds primarily with M10 of PMCA1 (Fig. 3C; Go and Soboloff 2018; Gong et al. 2018), apparently at the same site as the 2b-tail binds in the SERCA2b protein (Inoue et al. 2019). Heterotetramers of two PMCA1s and two regulatory subunits have

been proposed to improve the efficiency of Ca²⁺ transport by facilitating the transition from E2 to E1 (Gong et al. 2018).

PMCA Isoforms

The diverse set of PMCA protein variants is generated via alternative processing of the primary transcripts of the four PMCA genes (named *ATP2B1–4*) in two main sites (A and C) (Table 1). Splicing site A (rendering splice variants w, x, z) is located in the first cytosolic domain, between the interaction sites for the CaM-binding domain and a binding site of acidic phospholipids. Splicing at this site not only changes the length of the domain, but also alters the autoinhibition and sensitivity toward acidic phospholipid regulation (Brini et al. 2010). Splicing site C (variants a, b, c, d, e) is situated in the middle of the carboxy-terminal CaM-binding domain, and affects the regulatory and functional properties of PMCA, such as the affinity of the pumps for Ca²⁺-CaM and the kinetics of their activation (reviewed in Strehler and Zacharias 2001; Krebs 2015; Strehler 2015). In general, “a” variants display a shortened C-tail and present a lower CaM affinity, but increased basal activity and moderate CaM stimulation than “b” splice variants. However, there are also differences between “b” variants; for example, PMCA4b shows high basal activity and is highly stimulated by CaM, but PMCA2b is marked by high basal activity, but a modest stimulation by CaM (Elwess et al. 1997).

All tissues express at least one PMCA isoform, but their abundance and distribution appear to be isoform specific. However, not all variants have been confirmed at the protein level because of the lack of specific antibodies. PMCA1 is ubiquitous, although variant expression levels and distribution change especially during development. It is the earliest PMCA isoform to be expressed in the embryo and seems to exert an essential housekeeping or developmental function (Okunade et al. 2004). There is a switch from PMCA1b to PMCA1a during brain development (Brandt and Neve 1992) that reflects a differential expression of variants according to the developmental stage. PMCA2 displays a more restricted distribution, but is

abundant in cerebellar Purkinje neurons and hair cells in the inner ear (Furuta et al. 1998). PMCA2 is also highly expressed in the apical membrane of the epithelia of lactating mammary glands, in which it is important for Ca²⁺ extrusion into the milk (Reinhardt et al. 2004). PMCA3 is the least characterized isoform and is broadly expressed in the developing embryo, but, like PMCA2, shows a more restricted distribution in adults, in which it is mainly expressed in the brain, particularly in cerebellar synaptic terminals and choroid plexus (Eakin et al. 1995; Marcos et al. 2009). Finally, PMCA4 is present in most cells and tissues as PMCA1, and therefore fulfills a housekeeping role.

Regulation of PMCA

In resting conditions at about 100 nM cytosolic Ca²⁺, PMCA is trapped in an autoinhibited state because of blocking of the ATP-binding site by the carboxy-terminal CaM-binding domain that interacts with two cytosolic regions of PMCA. The first region is located within the A-domain, preceding an acidic phospholipids-binding site. The second region is located within the N-domain after the autophosphorylation site. At increased cytosolic Ca²⁺ concentrations, Ca²⁺-bound CaM interacts with the CaM-binding domain of PMCA, which relieves the autoinhibition. This leads to a significant increase in the Ca²⁺ affinity and turnover rate of PMCA (for review, see Brini and Carafoli 2009; Lopreiato et al. 2014). Ca²⁺-loaded CaM binds to two sites along the carboxy-terminal regulatory domain, which facilitates a two-step PMCA activation mechanism that allows a tight control of intracellular Ca²⁺ levels over a broad range of physiological conditions in eukaryotic cells (Tidow et al. 2012).

The C-tail of PMCA is subjected to phosphorylation by protein kinases A and C (Zylin-ska et al. 1998) and tyrosine kinases such as Src (Ghosh et al. 2011, 2016), regulates self-association (Kosk-Kosicka and Bzdega 1988), and undergoes cleavage by proteases such as caspases or calpain (Pászty et al. 2002; Guerini et al. 2003). Furthermore, “b” splice variants contain a PDZ domain for protein–protein interactions.

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Different PDZ proteins from the membrane-associated guanylate kinase (MAGUK) family of scaffold proteins are involved in the recruitment and retention of different PMCA splice variants in specific Ca^{2+} microdomains (Kim et al. 1998; DeMarco and Strehler 2001; Schuh et al. 2003; Strehler 2015). These interactions also allow the regulation of specific PMCA by affecting the affinity for Ca^{2+} or CaM. Interestingly, besides suppressing SERCA expression and stability (Dremina et al. 2004), Bcl-2 may also influence cell fate by suppressing the Ca^{2+} extrusion via PMCA (Ferdek et al. 2012).

Protein-lipid interactions also play a key role in PMCA function. There are two binding sites for acidic phospholipids that modulate PMCA activity (Niggli et al. 1981), one that precedes M3, and another one that is positioned in the carboxy-terminal region. The lipid composition of cellular membranes or subdomains like lipid rafts also affect PMCA activity and localization (Sepúlveda et al. 2006; Jiang et al. 2007; Marques-da-Silva and Gutiérrez-Merino 2014). Conversely, changes in the lipid environment, for example, in the aging brain or in the development of age-dependent neurodegenerative disorders, drastically affect activity and distribution of PMCA (Michaelis et al. 1996; Farooqui et al. 1997; Jiang et al. 2012).

PMCA in Disease

The relatively low number of diseases that are linked to PMCA mutations contrasts with the high diversity of PMCA variants and physiological roles, which may point to functional redundancy between isoforms. Still, all PMCA isoforms have been implicated in specific human disorders.

Single-nucleotide polymorphisms in the *ATP2B1* gene are associated with higher risk for hypertension and cardiovascular disease (Cho et al. 2009; Hong et al. 2010; Kobayashi et al. 2012; Wang et al. 2017). A reduced PMCA1 expression may lead to a raised blood pressure associated with elevated cytosolic Ca^{2+} and vascular remodeling (Kobayashi et al. 2012; Shin et al. 2013). PMCA1 defects are also associated with impaired bone mineralization and

dietary Ca^{2+} absorption in the small intestine (Kim et al. 2012; Liu et al. 2013; Ryan et al. 2015; Ehara et al. 2018). In addition, the key role of PMCA1 in the embryo becomes clear from the homozygous *Atp2b1* knockout, which caused embryo lethality, whereas heterozygous mutants presented no overt disease phenotype (Okunade et al. 2004).

PMCA2 deficiency mainly affects tissues in which PMCA2 is highly expressed, highlighting specific physiological functions of PMCA2. PMCA2 null mice display balance control and hearing problems (Kozel et al. 1998; Kurnellas et al. 2007). Moreover, spontaneous mutations in the *ATP2B2* gene are associated with hearing loss, deafness, or ataxia in humans and mice (Street et al. 1998; Takahashi and Kitamura 1999; Ueno et al. 2002; Ficarella et al. 2007; Spiden et al. 2008; Vicario et al. 2018), which is the result of impaired sensory transduction in hair cells of the inner ear. During lactation, PMCA2 contributes to Ca^{2+} uptake in the milk, whereas the reduction in PMCA2 expression during physiological mammary gland involution induces apoptosis. Conversely, PMCA2 overexpression in breast cancer cells is coupled to proliferation and resistance to apoptosis (Van-Houten et al. 2010; Peters et al. 2016). Genetic studies further linked PMCA2 mutations with autism in line with the abundant expression of PMCA2 in the brain (Hu et al. 2009; Carayol et al. 2011).

PMCA3 mutations were associated with congenital cerebellar ataxia in humans, which may relate to the high PMCA3 expression in cerebellum (Zanni et al. 2012; Cali et al. 2016; Vicario et al. 2017). However, other PMCA3 mutations were identified in adrenal aldosterone-producing adenomas. These PMCA3 mutants present a lower Ca^{2+} -ATPase activity and may trigger Ca^{2+} influx because of an increased Ca^{2+} leak (Beuschlein et al. 2013; Dutta et al. 2014; Tauber et al. 2016). Remarkably, the PMCA3 knockout mice did not present any of these phenotypes, but instead showed an interesting long-sleeper phenotype (Tatsuki et al. 2016).

PMCA4 is the major isoform in erythrocytes (Strehler et al. 1990) and several single-nucleotide polymorphisms in PMCA4 were reported

to confer resistance to malaria infection (Bedu-Addo et al. 2013; Lessard et al. 2017), which makes PMCA4 an interesting target for antimalaria therapy. Loss of *Atp2b4* in mice is not lethal, but induces sterility in males by impaired sperm motility (Okunade et al. 2004; Schuh et al. 2004). In addition, the *Atp2b4* knockout mice present vascular smooth muscle dysfunction in support of a specific role of PMCA4 in cardiovascular physiology (Oceandy et al. 2007; Mohamed et al. 2011; Prasad et al. 2014). Isoform PMCA4 is also abundant in nervous tissue (Krizaj et al. 2002; Burette et al. 2003; Marcos et al. 2009), in which Ca²⁺ dyshomeostasis contributes to aging and neurodegenerative diseases (Berridge 2011; Rivero-Rios et al. 2014). In fact, PMCA4 is inhibited by amyloid- β peptide and tau, two hallmarks in Alzheimer's disease (Berrocal et al. 2009, 2015), is affected by glutamate excitotoxicity (Pottorf et al. 2006), and its mutation may cause a familial spastic paraplegia (Akin et al. 2013; Li et al. 2014; Ho et al. 2015).

CONCLUDING REMARKS

Over the last few decades, a plethora of Ca²⁺ pump isoforms and splice variants have been discovered, which are mapped differently across cell types and subcellular compartments, in which they fine tune Ca²⁺ transport according to the local physiological needs. With detailed insights into the Ca²⁺ transport mechanism of primary active transporters at hand, the field now focuses on understanding the isoform-specific regulation of Ca²⁺ pumps at the molecular level. Isoform-specific elements alter the molecular dynamics and kinetic behavior, allow posttranslational control, and provide regulation by intramolecular domains or protein interaction. In addition, genetic screenings continue to provide new correlations between primary Ca²⁺ transport dysfunction and human diseases, which helps to establish Ca²⁺ pumps as therapeutic targets. Without any doubt, insights into the molecular aspects of Ca²⁺ pump regulation will provide new therapeutic opportunities to restore aberrant Ca²⁺ transport in disease.

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