World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v1.i6.201

World J Biol Chem 2010 June 26; 1(6): 201-208
ISSN 1949-8454 (online)

TOPIC HIGHLIGHT

Emanuel E Strehler, PhD, Professor, *Series Editor*

Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways

Mary Louisa Holton, Weiguang Wang, Michael Emerson, Ludwig Neyses, Angel L Armesilla

Mary Louisa Holton, Angel L Armesilla, Molecular Pharmacology Group, Department of Pharmacy, Research Institute in Healthcare Sciences, Room MA 228, School of Applied sciences, University of Wolverhampton, WV1 1SB, Wolverhampton, United Kingdom
Weiguang Wang, Oncology Group, Research Institute in Healthcare Sciences, School of Applied Sciences, University of Wolverhampton, Wulfruna Street, WV1 1SB, Wolverhampton, United Kingdom
Michael Emerson, National Heart and Lung Institute, Imperial College London, Exhibition Road, SW7 2AZ, London, United Kingdom
Ludwig Neyses, Cardiovascular Research Group, Manchester Academic Health Science Centre, Biomedical Research Centre and School of Biomedicine, University of Manchester, M13 9PT, Manchester, United Kingdom
Author contributions: Holton ML and Armesilla AL wrote the manuscript; Wang W, Emerson M and Neyses L corrected and critically read the manuscript.
Supported by The Breast Cancer Campaign and the Research Institute in Healthcare Sciences (Armesilla AL); The Wellcome Trust (Emerson M)
Correspondence to: Angel L Armesilla, PhD, Molecular Pharmacology Group, Department of Pharmacy, Research Institute in Healthcare Sciences, Room MA 228, School of Applied sciences, University of Wolverhampton, WV1 1SB, Wolverhampton, United Kingdom. a.armesilla@wlv.ac.uk
Telephone: +44-1902-322756 Fax: +44-1902-323465
Received: May 18, 2010 Revised: June 22, 2010
Accepted: June 24, 2010
Published online: June 26, 2010

Abstract

Emerging evidence suggests that plasma membrane calcium ATPases (PMCAs) play a key role as regulators of calcium-triggered signal transduction pathways *via* interaction with partner proteins. PMCAs regulate these pathways by targeting specific proteins to cellular sub-domains where the levels of intracellular free calcium are kept low by the calcium ejection properties of PMCAs. According to this model, PMCAs have been shown to interact functionally with the calcium-sensitive proteins neuronal nitric oxide synthase, calmodulin-dependent serine protein kinase, calcineurin and endothelial nitric oxidase synthase. Transgenic animals with altered expression of PMCAs are being used to evaluate the physiological significance of these interactions. To date, PMCA interactions with calcium-dependent partner proteins have been demonstrated to play a crucial role in the pathophysiology of the cardiovascular system *via* regulation of the nitric oxide and calcineurin/nuclear factor of activated T cells pathways. This new evidence suggests that PMCAs play a more sophisticated role than the mere ejection of calcium from the cells, by acting as modulators of signaling transduction pathways.

Key words: Plasma membrane calcium ATPase; Signal transduction; Regulation; Nitric oxide; Calcineurin; Nuclear factor of activated T cells

Peer reviewers: Osvaldo Rey, PhD, Associate Professor of Medicine, Department of Medicine, Division of Digestive Diseases, David Geffen School of Medicine at UCLA, 900 Veteran Place North, Warren Hall 11-115, Los Angeles, CA 90095-1768, United States; Carlos Bandeira Duarte, Professor, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal; Hong-Gang Wang, PhD, Professor, Lois High Berstler Professor of Pharmacology, Penn State College of Medicine, Penn State Hershey Cancer Institute, CH74, 500 University Drive, PO Box 850, Hershey, PA 17033-0850, United States

Holton ML, Wang W, Emerson M, Neyses L, Armesilla AL. Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways. *World J Biol Chem* 2010; 1(6): 201-208 Available from: URL: http://www.wjgnet.com/1949-8454/full/v1/i6/201.htm DOI: http://dx.doi.org/10.4331/wjbc.v1.i6.201

INTRODUCTION

Calcium is well known to play a pivotal role in the regulation of cellular physiology[1]. Tight control of intracellular calcium homeostasis is essential for normal cellular function. The plasma membrane calcium ATPases (PMCAs) contribute to the maintenance of appropriate cytoplasmic calcium levels by removing calcium from the cell to the extracellular environment[2]. There are four different PMCA isoforms (PMCA1-4) that are encoded by four independent genes[3-9]. PMCA1 and 4 are expressed ubiquitously, whereas the expression of PMCA2 and 3 is restricted to specific cells and tissues[4,9]. Additional isoform diversity is generated by alternative splicing of primary transcripts, which raises more than 20 different PMCA versions[10]. Structurally, PMCAs consist of 10 transmembrane domains, two major intracellular loops, and N- and C-cytoplasmic domains[11].

In addition to their traditional role as calcium transporters, a growing body of evidence suggests that PMCAs perform more specialized functions by establishing molecular interactions between their intracellular domains and cytoplasmic partner proteins. In this context, we and others have identified interactions between the cytoplasmic C-terminal end of PMCA and a number of PDZ [postsynaptic density 95 (PSD-95), Drosophila discs large protein and zona occludens-1] domain-containing proteins such as, members of the membrane-associated guanylate kinase (MAGUK) family[12,13], the cytoskeletal CLP36 protein[14], the Na^+/H^+ exchanger regulatory factor-2[15], the PMCA-interacting single-PDZ domain protein[16], neuronal nitric oxide synthase (nNOS, NOS-1)[17], the calcium/calmodulin-dependent serine protein kinase (CASK)[18], and the scaffold protein Ania-3/Homer[19]. Interactions with molecular partners are not limited to the C-terminal domain of PMCAs but also involve other intracellular domains. In fact, the big catalytic intracellular domain located between transmembrane regions 4 and 5 of PMCA has been reported to interact with the tumor suppressor protein Ras-associated factor 1 (Rassf1)[20], the calcium-dependent phosphatase calcineurin[21], the cytoskeletal scaffolding protein α-1 syntrophin[22], and endothelial NOS (eNOS; NOS-3)[23]. Finally, the N-terminal region of PMCA1, 3 and 4 has been shown to interact with the isoform ε of the 14-3-3 protein[24,25].

These interactions fulfill several purposes, from targeted localization of the pump or interaction partner to a particular subcellular domain[15], to the modification of the functional activity of PMCA[24,25] or the associated protein[17,18,20,21,23]. In this sense, interaction with PMCA has been reported to downregulate the enzymatic activity of signaling partner proteins that play key roles in the transduction of signals within the cell[17,18,21-23]. These findings suggest that PMCAs might participate in the regulation of signal transduction pathways. According to this idea, PMCAs have been found in caveolae and lipid-rafts in different cellular types[26-29]. It is well established that caveolae and lipid-rafts are specialized membrane sub-domains that are enriched in a variety of molecules implicated in the integration and regulation of cellular signaling events[30]. The sub-cellular localization of PMCAs in caveolae is, therefore, well in agreement with their putative role as signaling regulators.

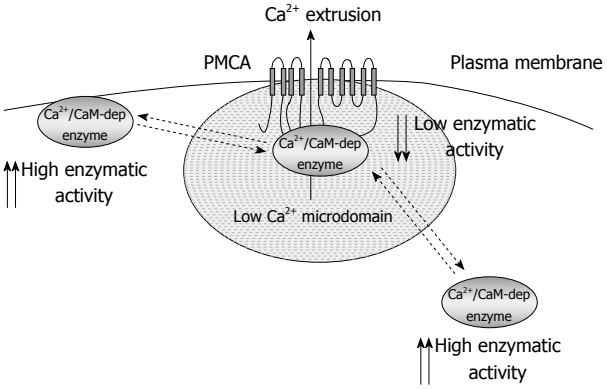

Figure 1 Model of plasma membrane calcium ATPase (PMCA) as a regulator of signal transduction pathways. PMCA pumps calcium out of the cell and generates a microenvironment where the intracellular calcium concentrations are very low. Interaction with the intracellular domains of PMCA tethers partner proteins to this low-calcium microenvironment, which results in downregulation of the enzymatic activity of calcium/calmodulin-dependent proteins. Ca^{2+}/CaM-dep, calcium/calmodulin-dependent enzyme.

This review outlines recent evidence that supports the novel role for PMCA as a regulator of signal transduction pathways, and highlights the first approaches to analyze the functional significance of the interactions between PMCA and partner proteins using transgenic animal models.

PMCAs AS REGULATORS OF CALCIUM-DEPENDENT SIGNAL TRANSDUCTION PATHWAYS

A number of the PMCA partner proteins identified so far are well-established calcium-regulated enzymes that participate in the transduction of calcium signals within the cell (Table 1)[17,18,21,23]. It is thought that PMCAs inhibit the activity of these molecules by tethering them to low calcium micro-domains that are created by the calcium extrusion function of the pump (Figure 1). Two different sets of data support this hypothesis. (1) PMCAs have been found to be concentrated up to 25-fold in caveolae[26,29]. Highly concentrated PMCA clusters might create small cellular microenvironments where intracellular calcium is maintained at a low level by the calcium-extrusion activity of PMCAs. In this location, the activity of calcium-dependent enzymes is downregulated; (2) The interaction between PMCAs and partner proteins is essential for downregulation of the activity of the associated protein. As described below, experiments in which the interaction between PMCA and its interaction partner was disrupted, or impaired, reversed the PMCA-mediated inhibition of the activity of the partner protein. Importantly, these experiments didnot alter the

Table 1 Functional interactions between PMCA and calcium-dependent partner proteins

Protein partner	Domain of PMCA implicated in the interaction	Domain of partner protein implicated in the interaction	Functional consequence of the interaction	Proposed mechanism of regulation
nNOS, NOS-1	C-terminal PDZ-binding domain	PDZ domain	Decrease nNOS activity, decrease NO production	PMCA interactions tethers partner protein to a low calcium micro-environment, this results in inhibition of the enzymatic activity of the partner proteins
CASK	C-terminal PDZ-binding domain	PDZ domain	Decrease in T-element-dependent transcriptional activit	PMCA interactions tethers partner protein to a low calcium micro-environment, the complex CASK/Tbr-1 cannot be established
eNOS, NOS-3	Proximal region of the big, catalytic intracellular loop located between transmembrane domains 4 and 5 (amino acids 462-684 and 428-651 of PMCA2 and 4, respectively)	Region 735-934 of eNOS	Decrease eNOS activity, decrease NO production	PMCA interactions tethers partner protein to a low calcium micro-environment, this results in inhibition of the enzymatic activity of the partner proteins
Calcineurin A	Proximal region of the big, catalytic intracellular loop located between transmembrane domains 4 and 5 (amino acids 462-684 and 428-651 of PMCA2 and 4, respectively)	Region 58-143 of calcineurin A	Decrease in calcineurin/NFAT-dependent transcriptional activity	PMCA interactions tethers partner protein to a low calcium micro-environment, this results in inhibition of the enzymatic activity of the partner proteins

PMCA: Plasma membrane calcium ATPase; NOS: Nitric oxide synthase; nNOS: Neuronal NOS; eNOS: Endothelial NOS; CASK: Calmodulin-dependent serine protein kinase.

calcium-extruding properties of PMCAs that were still fully active, which demonstrates that general clearance of calcium by PMCAs is not sufficient to modify the activity of the partner proteins, and that the localization of the partner proteins to a particular sub-domain with low calcium levels is a more plausible mechanism.

In concurrence with this hypothesis, PMCAs have been reported to regulate NO- and calcineurin-dependent signal transduction pathways *via* interaction with NOSs and calcineurin, respectively.

PMCA negatively modulates NO-dependent signaling

The first evidence to show the involvement of PMCAs in the regulation of NO signaling was reported by Schuh *et al*[17] almost 10 years ago. Ectopic expression of recombinant human PMCA4b and nNOS in HEK-293 cells demonstrated the interaction between the two proteins[17]. Binding of PMCA4b to nNOS resulted in significant inhibition of nNOS activity, which suggests that PMCA4b is implicated in the modulation of NO synthesis and, therefore, NO-dependent signaling[17].

Further to this first observation, immunoprecipitation experiments with cardiac proteins have demonstrated that endogenous PMCA4b and nNOS form a ternary complex together with α-1 syntrophin[22]. PMCA and α-1 syntrophin act synergistically to regulate negatively nNOS activity[22], which introduces a new level of regulation on the PMCA-mediated control of nNOS activity.

The relevant role of NO in the control of cardiovascular physiology[31] has prompted the groups of Neyses and Husein to investigate the physiological relevance of the PMCA4b/nNOS interaction in the cardiovascular system. Work by these groups has demonstrated the interaction between endogenous PMCA4b and nNOS in mouse cardiomyocytes and smooth muscle cells[22,32-35]. The generation of transgenic mice with altered expression of PMCA4b in cardiovascular cells has corroborated the functionality of the PMCA4b/nNOS interaction in a physiological system.

Transgenic mice that express human PMCA4b under the control of the arterial-smooth-muscle-specific SM22α promoter have shown depressed nNOS activity[33], in association with increased vasomotor responsiveness and blood pressure[32,33], which indicates that PMCA plays a significant role in the regulation of vascular tone.

Likewise, to investigate the physiological importance of the PMCA/nNOS interaction as a regulator of NO signaling in cardiac physiology, Oceandy *et al*[34] overexpressed human PMCA4b in the heart of transgenic mice under the control of the myosin light chain (MLC2v) promoter. β-adrenergic stimulation of cardiac contractility was significantly attenuated in the animals that overexpressed PMCA4b[34]. To ascertain that this effect was a consequence of PMCA4b-mediated inhibition of nNOS, Oceandy *et al*[34] also generated mice that overexpressed PMCA4 ct120 (a mutant form of human PMCA4b that lacks 120 amino acid residues at the C terminus, including the PDZ-binding motif[36]) in the heart of the transgenic animals. PMCA4 ct120 is very active as a calcium pump[36] but it is unable to downregulate nNOS activity due to a lack of interaction[17]. Animals that overexpressed this non-nNOS binding form of PMCA4 exhibited normal β-adrenergic stimulation of cardiac contractility[34], which suggests that the PMCA4b/nNOS interaction is indeed involved in the inotropic response of mouse cardiomyocytes to β-adrenergic stimuli. Moreover, when wild-type animals or transgenic mice that expressed the PMCA4 ct120 mutant were treated with the specific nNOS in-

hibitor N-propyl-L-arginine (L-nPA), the β-adrenergic-induced response in cardiac contractility was inhibited[34], however, L-nPA had no significant effect in the response of PMCA4b-overexpressing mice[34].

The molecular analysis of cardiomyocytes from PMCA4b-overexpressing transgenic animals has also revealed the PMCA/nNOS-downstream effectors that are implicated in the modulation of the β-adrenergic response in cardiac cells[35]. It seems that PMCA-mediated reduction of nNOS activity leads to a decrease in NO levels and a concomitant reduction in the levels of cGMP produced by the soluble guanylyl cyclase. This reduction in the cGMP levels translates into a decrease in phosphodiesterase activity that prevents degradation of cAMP and results in strong elevation of cAMP intracellular levels in cardiomyocytes. Increased cAMP levels activate the cAMP-dependent protein kinase, which leads to enhanced phosphorylation of its major substrates in cardiac cells, the proteins phospholamban and cardiac troponin I (cTn I)[35]. This cascade of molecular events, which ends with increased phosphorylation of phospholamban and cTn I, explains the reduced β-adrenergic response that is observed in the cardiac-specific transgenic mice that overexpress PMCA4b[34] (Figure 2).

A recent study by Beigi *et al*[37] has shown that cardiac PMCA4b and nNOS are implicated in the formation of a ternary complex with the nNOS adaptor protein CAPON (carboxy-terminal PDZ ligand of NOS1). The authors have demonstrated that PMCA interacts with CAPON in cardiac cells. The interaction between the two proteins is dependent on the presence of nNOS and increases following myocardial infarction. The complex CAPON/nNOS (initially located in the sarcoplasmic reticulum) redistributes to caveolae after myocardial infarction[37]. The presence of PMCA in cardiac caveolae[28] suggests that the pump can be involved in the redistribution of nNOS that occurs after myocardial infarction *via* its interaction with the complex CAPON/nNOS. Further investigations are necessary to elucidate if the interaction PMCA/CAPON/nNOS results in a decrease in nNOS activity in injured myocardium.

Our work in endothelial cells has recently shown that PMCAs interact with eNOS[23]. This interaction has been mapped to the catalytic, big intracellular loop located between transmembrane domains 4 and 5 of PMCAs, and the region 735-934 of eNOS. PMCA/eNOS association results in a significant decrease in eNOS activity, and subsequent NO production in resting and acetylcholine-stimulated endothelial cells. A first insight into the molecular mechanisms responsible for inhibition of eNOS activity has shown that interaction with PMCA leads to an increase in the phosphorylation status of the residue Thr-495 of eNOS[23]. Phosphorylation of Thr-495 is well known to inhibit eNOS activity[38], which suggests that PMCA negatively regulates eNOS activity by promoting Thr-495 phosphorylation. The *in vivo* analysis of the physiological relevance of this interaction must wait for the generation of genetically modified animals with altered expression of PMCAs in endothelial cells.

PMCA inhibits the calcineurin/nuclear factor of activated T cells signal transduction pathway

More evidence in support of a role for PMCA in the regulation of calcium-dependent signaling pathways has come from the identification of a functional interaction between PMCA and the catalytic subunit of the calcium-sensitive serine-threonine phosphatase calcineurin[21,23,39,40]. Calcineurin plays a crucial role in the coupling of calcium signals to cellular responses. Increments in the levels of cytoplasmic calcium result in activation of calcineurin, which in turn dephosphorylates specific target substrates[41]. The best-characterized substrate of calcineurin is the nuclear factor of activated T cells (NFAT) family of transcription factors[42]. NFATs are expressed as constitutively phosphorylated proteins in the cytoplasm of resting cells. Activated calcineurin mediates dephosphorylation of the NFAT transcription factors and their subsequent translocation from the cytoplasm to the nucleus. Once in the nucleus, NFATs bind to specific sequences in the regulatory regions of target genes and switch on their expression[42]. Activation of the calcineurin/NFAT pathway has been implicated in the progression of a large variety of processes including: T-cell activation and differentiation[43], osteoblast growth and differentiation[44], skeletal muscle growth and development[45], neural development and axon growth[46], beta-cell growth and function[47], heart valve morphogenesis[48], and cardiac hypertrophy[49]. The broad spectrum of biological processes that are orchestrated by calcineurin/NFAT-mediated signals underlines the importance of the proper regulation of this pathway. We and others have recently shown that the interaction between PMCA and calcineurin leads to inhibition of the calcineurin/NFAT activity[21,39,40], which indicates that PMCA plays a relevant role in the control of this pathway. To evaluate the *in vivo* significance of this interaction, Wu *et al*[40] have generated inducible, cardiac-specific, PMCA4b transgenic mice. Overexpression of the PMCA4b isoform in the heart antagonizes cardiac hypertrophy induced by transverse aortic constriction or phenylephrine/angiotensin Ⅱ infusion[40]. In contrast, Oceandy *et al*[34] have found that mice that overexpress PMCA4b in the heart display an increased hypertrophic response after chronic stimulation with isoproterenol. The reasons behind this discrepancy are not clear at present. Further investigations in cardiac, and other tissues where the calcineurin/NFAT pathway modulates biological responses, are required to gain a full understanding of the role of PMCA as a cellular regulator of calcineurin.

PMCA downregulates CASK/Tbr-1 signaling

Additional evidence for the role of PMCA as a regulator of calcium signaling has been provided by the characterization of a molecular interaction between PMCA4b and CASK in protein extracts isolated from rat brain and kidney[18]. CASK is a MAGUK family member and, like other members of the family mentioned before, contains a PDZ-domain that is responsible for binding to PMCA4b. CASK can form a molecular complex with the

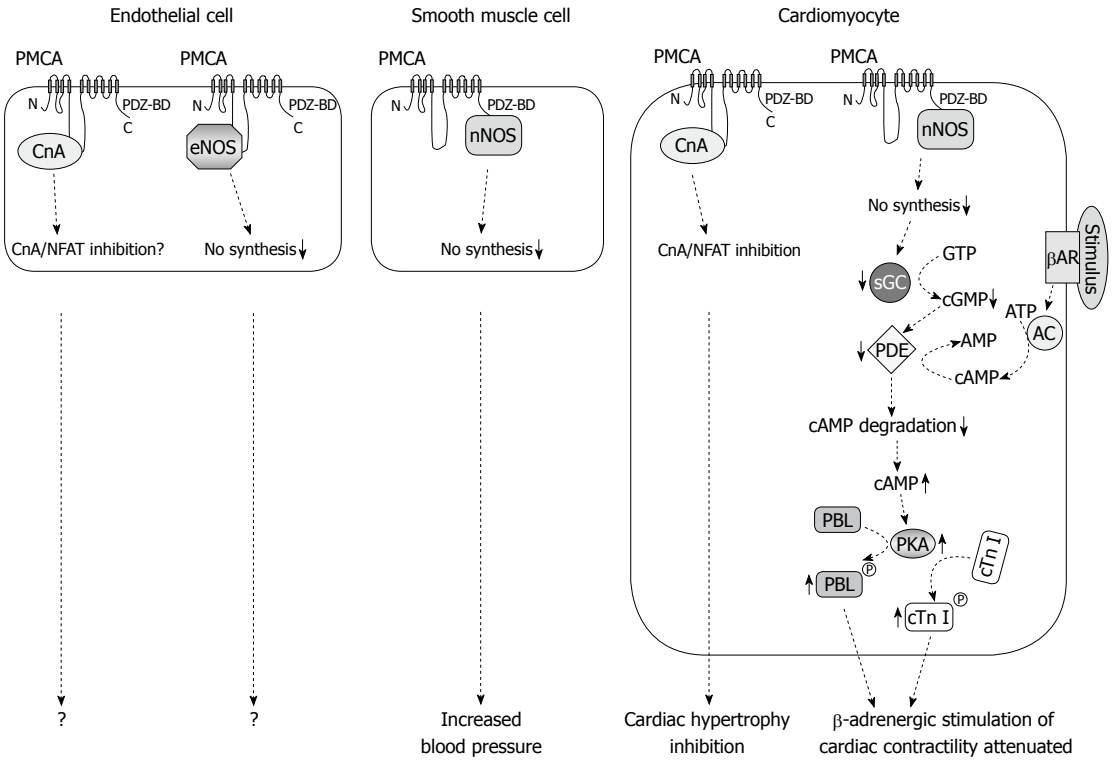

Figure 2 Physiological consequences of the interaction between PMCAs and signaling partner proteins in the cardiovascular system. The figure depicts regulatory interactions between PMCA and calcium-dependent signaling proteins in cardiovascular cells. These interactions play a pivotal role in the regulation of cardiovascular physiology *via* regulation of the NO and calcineurin/NFAT signal transduction pathways. CnA: Calcineurin A; sGC: Soluble guanylyl cyclase; PDE: Phosphodiesterase; PKA: Protein kinase A; PBL: Phospholamban; cTn I: Cardiac troponin I; βAR: β-adrenergic receptor; AC: Adenylyl cyclase; NFAT: Nuclear factor of activated T cells.

transcription factor Tbr-1[50]. Once formed, the complex CASK/Tbr-1 enters the nucleus and binds to T-element sequences (AATTTCACACCTAGGTGTGAAATT) that are located in the promoter regions of specific target genes[50]. PMCA interaction with CASK results in a dramatic reduction (80% decrease) in T-element-dependent transcriptional activity[18]. As described above for the PMCA-mediated regulation of other partner proteins, PMCA4b seems to modulate CASK/Tbr-1 functionality by depletion of calcium in the proximity of the pump. Supporting this idea, a mutated PMCA4b ($Asp^{672} \rightarrow Glu$) (with the calcium pumping activity severely compromised but still able to interact with CASK[51]) or the PMCA4 ct120 mutant (which retains full calcium extrusion capabilities but is unable to bind to CASK) had only a small influence on the transcriptional activity of a T-element-driven luciferase reporter vector[18]. The characterization of a functional interaction between PMCA4b and CASK/Tbr-1 reinforces the link between PMCAs and the regulation of calcium-dependent gene transcription that is suggested by the PMCA/calcineurin interaction.

The molecular events behind the PMCA-mediated downregulation of CASK/Tbr-1 activity are not understood at present. It has been shown that the calmodulin-binding site of CASK binds calmodulin in a calcium-dependent manner[52]. It is tempting to speculate that calcium/calmodulin binding to CASK might alter the conformation of the protein, which causes its release from PMCA and leads to its binding to Tbr-1. CASK/Tbr-1 would then travel together to the nucleus and activate the expression of target genes. In this case, PMCA-mediated targeting of CASK to a low calcium cellular sub-domain would not be directly involved in regulating the catalytic activity of the partner protein (as is the case for NOS or calcineurin), but would control calcium-dependent interactions between the partner protein CASK and its effector Tbr-1. The physiological relevance of the PMCA-mediated regulation of CASK/Tbr-1 signaling requires further investigation.

CONCLUSION AND FUTURE PERSPECTIVES

From the studies that we have discussed in this review, we can conclude that PMCAs play a significant role in the negative regulation of signal transduction pathways. In some instances, PMCAs seem to create local low calcium microenvironments that decrease the catalytic activity of calcium-sensitive signal transduction proteins. In other cases, it seems that low calcium levels might impair the interaction between key signaling proteins and their effectors. The use of transgenic animal models with modified expression of PMCA proteins in cardiovascular tissues is starting to reveal the physiological functionality of the PMCA/nNOS and PMCA/calcineurin interactions in the regulation of cardiovascular signal transduction (Figure 2),

although additional work must be conducted in the future in other organs and cellular types.

PMCA-mediated low-calcium microenvironments might be the result of localization of the pump to specific plasma membrane microdomains. In this sense, the interaction between the PSD-95 scaffolding protein and PMCA4b has been shown to induce the formation of high-density PMCA4b clusters in the plasma membrane[53]. Although the physiological consequences of the PSD-95/PMCA4b interaction remain to be determined, PSD-95 has also been reported to induce clustering of other proteins (such as potassium channels and neurotransmitter receptors) that play an important role in the regulation of synaptic signal transduction[54-56], which suggests that PMCA4b participates in the regulation of calcium signaling in the synaptic nerve terminals *via* PSD-95-induced clustering. In support of the function of PMCA as a regulator of calcium signaling at synapses, Garside *et al*[57] have analyzed PMCA interactions in synapse-enriched brain tissue from rats, and have found that PMCA2 interacts with the postsynaptic protein PSD-95, and the NMDA glutamate receptor subunits NR1 and NR2a. At the pre-synapse, PMCA2 interacts with the presynaptic protein syntaxin-1A. By establishing interactions with synaptic partner proteins, PMCAs might form part of signaling macromolecular complexes and participate in the regulation of synaptic calcium-dependent signal transduction pathways, *via* control of local calcium dynamics at specific sites of the synapse.

To date, no studies have evaluated the influence of calcium in the interaction between PMCA and partner proteins. It is well recognized that the intracellular levels of calcium play a dynamic role in the regulation of protein-protein interactions, for instance, increments in the intracellular levels of calcium/calmodulin abolish the interaction between eNOS and caveolin-1 and lead to activation of eNOS[58]. In a similar way, increments in intracellular calcium might disrupt the PMCA/eNOS interaction, which allows eNOS to escape from the low-calcium microdomain that is created by PMCA activity. This possibility introduces a new dimension to the regulation of partner proteins by PMCAs, and as such, requires further investigation.

PMCAs also seem to be involved in the regulation of other signaling pathways where the role of calcium is not so evident, for example, in the regulation of the Ras/Erk pathway *via* interaction with Rassf1A. In these cases, PMCA might act as a macromolecular protein organizer that recruits proteins to specific cellular domains. This function might be comparable to that of the scaffold protein CNK1 in Ras-mediated apoptosis. CNK1 interacts with the signaling protein Ras[59]. Ras-promoted apoptosis requires the participation of the Mst kinases. To place the Mst1/2 kinases in contact with Ras, CNK1 interacts with Rassf1 which, in turn, interacts with Mst1/2 kinases[59]. Disruption of the interaction CNK1/Rassf1A disassembles the complex and suppresses Ras-mediated apoptosis[59]. It seems entirely plausible that PMCAs might be playing a similar role as recruiters of macromolecular signaling complexes in caveolae, and therefore, might be placing Rassf1 in contact with other signaling proteins. This exciting possibility deserves further investigation.

In summary, data from several groups support a novel role for PMCA as a regulator of signal transduction pathways. It will be interesting to exploit this new functionality of PMCA with therapeutic purposes through the design of new drugs that regulate its activity. This strategy could be used to modulate essential biological processes in the cardiovascular system, and likely, other organs and tissues.

REFERENCES

1 **Carafoli E**. Calcium signaling: a tale for all seasons. *Proc Natl Acad Sci USA* 2002; **99**: 1115-1122
2 **Carafoli E**. Biogenesis: plasma membrane calcium ATPase: 15 years of work on the purified enzyme. *FASEB J* 1994; **8**: 993-1002
3 **Shull GE**, Greeb J. Molecular cloning of two isoforms of the plasma membrane Ca2+-transporting ATPase from rat brain. Structural and functional domains exhibit similarity to Na+,K+- and other cation transport ATPases. *J Biol Chem* 1988; **263**: 8646-8657
4 **Greeb J**, Shull GE. Molecular cloning of a third isoform of the calmodulin-sensitive plasma membrane Ca2+-transporting ATPase that is expressed predominantly in brain and skeletal muscle. *J Biol Chem* 1989; **264**: 18569-18576
5 **Verma AK**, Filoteo AG, Stanford DR, Wieben ED, Penniston JT, Strehler EE, Fischer R, Heim R, Vogel G, Mathews S. Complete primary structure of a human plasma membrane Ca2+ pump. *J Biol Chem* 1988; **263**: 14152-14159
6 **Strehler EE**, James P, Fischer R, Heim R, Vorherr T, Filoteo AG, Penniston JT, Carafoli E. Peptide sequence analysis and molecular cloning reveal two calcium pump isoforms in the human erythrocyte membrane. *J Biol Chem* 1990; **265**: 2835-2842
7 **Heim R**, Iwata T, Zvaritch E, Adamo HP, Rutishauser B, Strehler EE, Guerini D, Carafoli E. Expression, purification, and properties of the plasma membrane Ca2+ pump and of its N-terminally truncated 105-kDa fragment. *J Biol Chem* 1992; **267**: 24476-24484
8 **Brown BJ**, Hilfiker H, DeMarco SJ, Zacharias DA, Greenwood TM, Guerini D, Strehler EE. Primary structure of human plasma membrane Ca(2+)-ATPase isoform 3. *Biochim Biophys Acta* 1996; **1283**: 10-13
9 **Keeton TP**, Shull GE. Primary structure of rat plasma membrane Ca(2+)-ATPase isoform 4 and analysis of alternative splicing patterns at splice site A. *Biochem J* 1995; **306** (Pt 3): 779-785
10 **Strehler EE**, Zacharias DA. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol Rev* 2001; **81**: 21-50
11 **Di Leva F**, Domi T, Fedrizzi L, Lim D, Carafoli E. The plasma membrane Ca2+ ATPase of animal cells: structure, function and regulation. *Arch Biochem Biophys* 2008; **476**: 65-74
12 **Kim E**, DeMarco SJ, Marfatia SM, Chishti AH, Sheng M, Strehler EE. Plasma membrane Ca2+ ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. *J Biol Chem* 1998; **273**: 1591-1595
13 **DeMarco SJ**, Strehler EE. Plasma membrane Ca2+-atpase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PSD95/Dlg/ZO-1) domain-containing proteins. *J Biol Chem* 2001; **276**: 21594-21600

14 **Bozulic LD**, Malik MT, Powell DW, Nanez A, Link AJ, Ramos KS, Dean WL. Plasma membrane Ca(2+) -ATPase associates with CLP36, alpha-actinin and actin in human platelets. *Thromb Haemost* 2007; **97**: 587-597

15 **DeMarco SJ**, Chicka MC, Strehler EE. Plasma membrane Ca2+ ATPase isoform 2b interacts preferentially with Na+/H+ exchanger regulatory factor 2 in apical plasma membranes. *J Biol Chem* 2002; **277**: 10506-10511

16 **Goellner GM**, DeMarco SJ, Strehler EE. Characterization of PISP, a novel single-PDZ protein that binds to all plasma membrane Ca2+-ATPase b-splice variants. *Ann N Y Acad Sci* 2003; **986**: 461-471

17 **Schuh K**, Uldrijan S, Telkamp M, Rothlein N, Neyses L. The plasmamembrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase I. *J Cell Biol* 2001; **155**: 201-205

18 **Schuh K**, Uldrijan S, Gambaryan S, Roethlein N, Neyses L. Interaction of the plasma membrane Ca2+ pump 4b/CI with the Ca2+/calmodulin-dependent membrane-associated kinase CASK. *J Biol Chem* 2003; **278**: 9778-9783

19 **Sgambato-Faure V**, Xiong Y, Berke JD, Hyman SE, Strehler EE. The Homer-1 protein Ania-3 interacts with the plasma membrane calcium pump. *Biochem Biophys Res Commun* 2006; **343**: 630-637

20 **Armesilla AL**, Williams JC, Buch MH, Pickard A, Emerson M, Cartwright EJ, Oceandy D, Vos MD, Gillies S, Clark GJ, Neyses L. Novel functional interaction between the plasma membrane Ca2+ pump 4b and the proapoptotic tumor suppressor Ras-associated factor 1 (RASSF1). *J Biol Chem* 2004; **279**: 31318-31328

21 **Buch MH**, Pickard A, Rodriguez A, Gillies S, Maass AH, Emerson M, Cartwright EJ, Williams JC, Oceandy D, Redondo JM, Neyses L, Armesilla AL. The sarcolemmal calcium pump inhibits the calcineurin/nuclear factor of activated T-cell pathway via interaction with the calcineurin A catalytic subunit. *J Biol Chem* 2005; **280**: 29479-29487

22 **Williams JC**, Armesilla AL, Mohamed TM, Hagarty CL, McIntyre FH, Schomburg S, Zaki AO, Oceandy D, Cartwright EJ, Buch MH, Emerson M, Neyses L. The sarcolemmal calcium pump, alpha-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. *J Biol Chem* 2006; **281**: 23341-23348

23 **Holton M**, Mohamed TM, Oceandy D, Wang W, Lamas S, Emerson M, Neyses L, Armesilla AL. Endothelial nitric oxide synthase activity is inhibited by the plasma membrane calcium ATPase in human endothelial cells. *Cardiovasc Res* 2010; Epub ahead of print

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

25 **Linde CI**, Di Leva F, Domi T, Tosatto SC, Brini M, Carafoli E. Inhibitory interaction of the 14-3-3 proteins with ubiquitous (PMCA1) and tissue-specific (PMCA3) isoforms of the plasma membrane Ca2+ pump. *Cell Calcium* 2008; **43**: 550-561

26 **Fujimoto T**. Calcium pump of the plasma membrane is localized in caveolae. *J Cell Biol* 1993; **120**: 1147-1157

27 **Sepúlveda MR**, Berrocal-Carrillo M, Gasset M, Mata AM. The plasma membrane Ca2+-ATPase isoform 4 is localized in lipid rafts of cerebellum synaptic plasma membranes. *J Biol Chem* 2006; **281**: 447-453

28 **Hammes A**, Oberdorf-Maass S, Rother T, Nething K, Gollnick F, Linz KW, Meyer R, Hu K, Han H, Gaudron P, Ertl G, Hoffmann S, Ganten U, Vetter R, Schuh K, Benkwitz C, Zimmer HG, Neyses L. Overexpression of the sarcolemmal calcium pump in the myocardium of transgenic rats. *Circ Res* 1998; **83**: 877-888

29 **Schnitzer JE**, Oh P, Jacobson BS, Dvorak AM. Caveolae from luminal plasmalemma of rat lung endothelium: microdomains enriched in caveolin, Ca(2+)-ATPase, and inositol trisphosphate receptor. *Proc Natl Acad Sci USA* 1995; **92**: 1759-1763

30 **Anderson RG**. Caveolae: where incoming and outgoing messengers meet. *Proc Natl Acad Sci USA* 1993; **90**: 10909-10913

31 **Michel T**, Smith TW. Nitric oxide synthases and cardiovascular signaling. *Am J Cardiol* 1993; **72**: 33C-38C

32 **Schuh K**, Quaschning T, Knauer S, Hu K, Kocak S, Roethlein N, Neyses L. Regulation of vascular tone in animals overexpressing the sarcolemmal calcium pump. *J Biol Chem* 2003; **278**: 41246-41252

33 **Gros R**, Afroze T, You XM, Kabir G, Van Wert R, Kalair W, Hoque AE, Mungrue IN, Husain M. Plasma membrane calcium ATPase overexpression in arterial smooth muscle increases vasomotor responsiveness and blood pressure. *Circ Res* 2003; **93**: 614-621

34 **Oceandy D**, Cartwright EJ, Emerson M, Prehar S, Baudoin FM, Zi M, Alatwi N, Venetucci L, Schuh K, Williams JC, Armesilla AL, Neyses L. Neuronal nitric oxide synthase signaling in the heart is regulated by the sarcolemmal calcium pump 4b. *Circulation* 2007; **115**: 483-492

35 **Mohamed TM**, Oceandy D, Prehar S, Alatwi N, Hegab Z, Baudoin FM, Pickard A, Zaki AO, Nadif R, Cartwright EJ, Neyses L. Specific role of neuronal nitric-oxide synthase when tethered to the plasma membrane calcium pump in regulating the beta-adrenergic signal in the myocardium. *J Biol Chem* 2009; **284**: 12091-12098

36 **Enyedi A**, Verma AK, Filoteo AG, Penniston JT. A highly active 120-kDa truncated mutant of the plasma membrane Ca2+ pump. *J Biol Chem* 1993; **268**: 10621-10626

37 **Beigi F**, Oskouei BN, Zheng M, Cooke CA, Lamirault G, Hare JM. Cardiac nitric oxide synthase-1 localization within the cardiomyocyte is accompanied by the adaptor protein, CAPON. *Nitric Oxide* 2009; **21**: 226-233

38 **Dudzinski DM**, Michel T. Life history of eNOS: partners and pathways. *Cardiovasc Res* 2007; **75**: 247-260

39 **Holton M**, Yang D, Wang W, Mohamed TM, Neyses L, Armesilla AL. The interaction between endogenous calcineurin and the plasma membrane calcium-dependent ATPase is isoform specific in breast cancer cells. *FEBS Lett* 2007; **581**: 4115-4119

40 **Wu X**, Chang B, Blair NS, Sargent M, York AJ, Robbins J, Shull GE, Molkentin JD. Plasma membrane Ca2+-ATPase isoform 4 antagonizes cardiac hypertrophy in association with calcineurin inhibition in rodents. *J Clin Invest* 2009; **119**: 976-985

41 **Klee CB**, Ren H, Wang X. Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. *J Biol Chem* 1998; **273**: 13367-13370

42 **Rao A**, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 1997; **15**: 707-747

43 **Macian F**. NFAT proteins: key regulators of T-cell development and function. *Nat Rev Immunol* 2005; **5**: 472-484

44 **Zayzafoon M**. Calcium/calmodulin signaling controls osteoblast growth and differentiation. *J Cell Biochem* 2006; **97**: 56-70

45 **Al-Shanti N**, Stewart CE. Ca2+/calmodulin-dependent transcriptional pathways: potential mediators of skeletal muscle growth and development. *Biol Rev Camb Philos Soc* 2009; **84**: 637-652

46 **Nguyen T**, Di Giovanni S. NFAT signaling in neural development and axon growth. *Int J Dev Neurosci* 2008; **26**: 141-145

47 **Heit JJ**, Apelqvist AA, Gu X, Winslow MM, Neilson JR, Crabtree GR, Kim SK. Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature* 2006; **443**: 345-349

48 **Chang CP**, Neilson JR, Bayle JH, Gestwicki JE, Kuo A, Stankunas K, Graef IA, Crabtree GR. A field of myocardial-endocardial NFAT signaling underlies heart valve morpho-

genesis. *Cell* 2004; **118**: 649-663
49 **Wilkins BJ**, Molkentin JD. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem Biophys Res Commun* 2004; **322**: 1178-1191
50 **Hsueh YP**, Wang TF, Yang FC, Sheng M. Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2. *Nature* 2000; **404**: 298-302
51 **Adamo HP**, Filoteo AG, Enyedi A, Penniston JT. Mutants in the putative nucleotide-binding region of the plasma membrane Ca(2+)-pump. A reduction in activity due to slow dephosphorylation. *J Biol Chem* 1995; **270**: 30111-30114
52 **Hata Y**, Butz S, Südhof TC. CASK: a novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. *J Neurosci* 1996; **16**: 2488-2494
53 **Padányi R**, Pászty K, Strehler EE, Enyedi A. PSD-95 mediates membrane clustering of the human plasma membrane Ca2+ pump isoform 4b. *Biochim Biophys Acta* 2009; **1793**: 1023-1032
54 **Kim E**, Cho KO, Rothschild A, Sheng M. Heteromultimerization and NMDA receptor-clustering activity of Chapsyn-110, a member of the PSD-95 family of proteins. *Neuron* 1996; **17**: 103-113
55 **Imamura F**, Maeda S, Doi T, Fujiyoshi Y. Ligand binding of the second PDZ domain regulates clustering of PSD-95 with the Kv1.4 potassium channel. *J Biol Chem* 2002; **277**: 3640-3646
56 **Wong W**, Newell EW, Jugloff DG, Jones OT, Schlichter LC. Cell surface targeting and clustering interactions between heterologously expressed PSD-95 and the Shal voltage-gated potassium channel, Kv4.2. *J Biol Chem* 2002; **277**: 20423-20430
57 **Garside ML**, Turner PR, Austen B, Strehler EE, Beesley PW, Empson RM. Molecular interactions of the plasma membrane calcium ATPase 2 at pre- and post-synaptic sites in rat cerebellum. *Neuroscience* 2009; **162**: 383-395
58 **Feron O**, Michel JB, Sase K, Michel T. Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. *Biochemistry* 1998; **37**: 193-200
59 **Rabizadeh S**, Xavier RJ, Ishiguro K, Bernabeortiz J, Lopez-Ilasaca M, Khokhlatchev A, Mollahan P, Pfeifer GP, Avruch J, Seed B. The scaffold protein CNK1 interacts with the tumor suppressor RASSF1A and augments RASSF1A-induced cell death. *J Biol Chem* 2004; **279**: 29247-29254

S- Editor Cheng JX **L- Editor** Kerr C **E- Editor** Zheng XM