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Review

Local signals with global impacts and clinical implications: Lessons from the plasma membrane calcium pump (PMCA4)☆

Delvac Oceandy, Tamer M.A. Mohamed, Elizabeth J. Cartwright, Ludwig Neyses *

The Cardiovascular Medicine Research Group, School of Biomedicine, University of Manchester, Manchester Academic Health Science Centre, UK

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ABSTRACT

Calcium has been unequivocally regarded as a key signal messenger in almost every cell type. Calcium regulates a number of important cellular functions including cell growth, myofilament contraction, cell survival and apoptosis as well as gene transcription. A complex regulatory mechanism of cellular calcium is needed to fine tune the precise calcium concentration in each subcellular location and also to transmit the signals carried by the calcium pool to the correct end target. In this article we will review the recently emerging role of the plasma membrane calcium/calmodulin dependent ATPase isoform 4 (PMCA4) in regulating calcium signalling. We will then focus on the function of this molecule in cardiomyocytes, in which PMCA4 forms protein–protein interactions with several key signalling molecules. Recent evidence has shown in vivo physiological functionalities and possible clinical implications of the PMCA4 signalling complex. This article is part of a Special Issue entitled: 11th European Symposium on Calcium.

1. Introduction

Calcium is central in the regulation of a large number of fundamental cellular physiological functions. In most cell types intracellular calcium is highly dynamic, for example in cardiomyocytes calcium concentration fluctuates from a resting diastolic level of 100 nM to a peak systolic level of 1 μM [1,2]. Calcium travels throughout subcellullar compartments whilst transmitting signals that are key for cell functions, such as myofilament contraction, gene transcription, cell growth and even cell death or apoptosis. Variations in its concentration (amplitude), frequency of the oscillations and spatial locations determine the calcium signals [3]. These signals will be decoded by the effectors, usually these are calcium/calmodulin binding proteins, which will translate the signals to some specific actions, such as enzymatic activity, protein binding and conformational changes.

The complexity of calcium signal coding and decoding processes are accurately controlled by a set of proteins that not only regulate intracellular calcium flux, but also direct the signal to its precise target molecules. The calcium ATPases (also known as calcium pumps) are the chief players in this matter (reviewed in [2]). These pumps are membrane bound and therefore are responsible for transporting the ion across the membrane. Three types of this pump have been recognised according to their localization: i) plasma membrane calcium ATPase = PMCA; ii) sarcoplasmic reticulum ATPase (SERCA) and iii) secretory pathway ATPase (SPCA), which is located at the Golgi membrane. Our focus in this paper is isoform 4 of the PMCA (PMCA4), which is expressed in all cell types [4]. In particular we will review the role of PMCA4 in cardiomyocytes, where it precisely controls the calcium signals to ensure they reach the correct destination (target/effector) in the right strength (amplitude/frequency).

2. Protein binding domains are vital in PMCA4

In cardiomyocytes PMCA4 is localized in the caveolae [5,6], traditionally known as a hub of the signals that cross the plasma membrane. A large number of signalling molecules are situated in this sub-cellular compartment and understandably a considerable amount of molecular signal including calcium may reach caveolae [7]. In this regard PMCA4 displays its key role: selecting the target for the calcium signal. As a calcium ATPase, PMCA4 has a high affinity with calcium and in addition to that feature, PMCA4 interacts with a number of key signalling molecules [8], enabled by the fact that PMCA4 contains many protein binding domains.

Abbreviations: PMCA, plasma membrane calcium/calmodulin dependent ATPase; SERCA, sarco/endoplasmic reticulum calcium ATPase; SPCA, secretory pathway calcium ATPase; PDZ, PSD 95, Drosophila Discs large protein and Zona occludens-1; MAGUK, membrane-associated guanylate kinase; nNOS, neuronal nitric oxide synthase; CASK, calcium/calmodulin dependent serine protein kinase; PISP, PMCA-interacting single-PDZ domain; RASSF1A, Ras-association domain family protein 1A; CAPON, C-terminal PDZ domain ligand of neuronal nitric oxide synthase; Nav1.5, sodium channel, voltage-gated, type V; PDE, phosphodiesterase; NFAT, nuclear factor of activated T-cells

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* Corresponding author. The University of Manchester, Manchester Academic Health Science Centre, School of Biomedicine, Room 1.302 Stopford Building, Oxford Road, Manchester M13 9PT, UK. Tel.: +44 161 275 1628; fax: +44 161 275 5669.
E-mail address: Ludwig.neyses@cmft.nhs.uk (L. Neyses).

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We and others have identified several domains within PMCA4 which are responsible for key interactions with potential targets of calcium signalling including the PDZ binding domain, large intracellular loop and the N-terminal tail (Table 1).

2.1. PDZ binding domain

PDZ domains were first identified in the postsynaptic density protein (PSD95) [9], Drosophila homologue discs large tumour suppressor (DlgA) [10] and zonula occludens-1 (ZO1), a tight-junction protein [11]. These domains containing approximately 80–90 amino acids that act as scaffolds for protein–protein interactions [12,13]. The PDZ domain interaction is a specific type of interaction module bearing a structurally well-defined interaction "pocket" that can be filled by a PDZ motif 'ligand' [14]. These ligands are consensus sequences that are normally, but not always [15], located at the extreme intracellular carboxyl terminus [16]. Four types of PDZ domain motifs have been classified: type I (S/T-x-Φ), type II (Φ-x-Φ), type III (Ψ-x-Φ) and type IV (D-x-V), where x is any amino acid, Φ is a hydrophobic amino acid (V, I, L, A, G,W, C, M, F) and Ψ is a basic, hydrophilic amino acid (H, R, K) [16].

Most partners for PMCA have been found to interact with a PDZ ligand in the C-terminal region especially of isoforms 4*b* of the pump. The carboxy terminal tail of the human PMCA1b, -2b, and -3b indeed contain a PDZ-ligand motif -ETSL*, whereas hPMCA4b carries a more perfect motif –ETSV*. The first identified PDZ interaction partners of PMCA are the members of the MAGUK (membrane-associated guanylate kinase) family of kinases, a PDZ-domain containing protein family [17]. More interesting is the finding that PMCA4b interacts with neuronal nitric oxide synthase (nNOS) [18] (the only NOS isoform that structurally contain a PDZ domain) [19]. Another PDZ-mediated interaction partner of PMCA4 is the calcium/calmodulin-dependent serine protein kinase (CASK) which is another MAGUK protein and a co-activator of the transcription of T-element containing promoters. This interaction has been found in brain and kidney tissues. Functionally, overexpression of PMCA4b down-regulates the T-element-dependent reporter activity which is CASK dependent [20]. As was suggested for nNOS, the negative regulation of CASK by PMCA4 could be the result of Ca^{2+} depletion in close proximity to the enzyme.

Other structural PDZ interactions have been demonstrated for PMCA4b isoform. These include the PMCA-interacting single-PDZ domain (PISP), a ubiquitously expressed protein, which through its interaction with PMCA4 influences their localisation to the plasma membrane [21]. PMCA4b and Ania-3 also bind via PDZ interaction. Ania3 is a member of the Homer family of scaffolding proteins that couple NMDA (*N*-methyl-D-aspartate) receptors in the brain and link extracellular signals to Ca^{2+} release from intracellular stores [22]. The interaction between Ania-3 and PMCA4 may represent a novel mechanism by which local calcium signalling and hence synaptic function can be modulated in neurons, however further studies are needed to clarify the functionality of this interaction [22]. The most recently discovered PDZ-mediated interaction partner of PMCA4b is CLP36 (C-terminal LIM domain protein), a protein that interacts with the actin cytoskeleton in platelets. This interaction has been found to be involved in CLP36 translocation during platelet activation [23].

2.2. Calmodulin binding site

The calmodulin binding domain is located at the C-terminal loop of PMCA4. It plays a crucial role in regulating the pump activity. The binding of calmodulin to this site (located approximately 40 amino acid residues downstream of the last transmembrane domain) [24] releases the inhibitory effect caused by the binding of the C-terminal to the large cytosolic loop which contains the major catalytic unit between the phosphorylation and the ATP binding site [25].

2.3. Main intracellular loop

The largest intracellular loop of PMCA is located between transmembrane domains 4 and 5. This loop contains catalytic sites including the ATP binding site and the aspartate residue which is phosphorylated during the calcium transport cycle [26]. The main intracellular loop of PMCA4 has been found to have several functional interactions with signalling proteins as well as structural/adaptor proteins. These interaction partners include the tumour suppressor Ras-association domain family protein isoform 1A (RASSF1A) [27], α1 syntrophin [28] and calcineurin [29].

2.4. N-terminal region

The N-terminal region of PMCA has a very low degree of sequence homology amongst isoforms, and thus is the region commonly used to raise isoform specific antibodies. Isoform ε of 14-3-3 protein interacts with PMCA4 and functionally inhibits the ability of PMCA4 to eject calcium [30]. Isoform ε of 14-3-3 protein is the first identified inhibitory partner for PMCA4. This molecule also interacts with PMCA1 and PMCA3 and similar to the interaction with PMCA4, 14-3-3 protein also inhibits PMCA1 and PMCA3 activity [31].

Table 1
Interaction partners of PMCA4 and the possible functions.

Binding domain	Interaction partners	Cell types/tissues investigated	Function	References
PDZ-binding domain	DLG4/PSD95, a member of membrane-associated guanylate kinase (MAGUK) family	COS-7	Unknown	[17]
PDZ-binding domain	nNOS (neuronal nitric oxide synthase)	HEK293, neuro-2a and heart	Regulation of nNOS activity, cardiac β-adrenergic response and cardiac hypertrophy	[18,34,36]
PDZ-binding domain	CASK (calcium/calmodulin-dependent serine protein kinase)	Brain, kidney and HEK293 cells	Regulation of T-element-dependent reporter (Tbr-1) activity	[20]
PDZ-binding domain	PISP (PMCA-interacting single-PDZ domain)	MDCK cells	Sorting PMCA4 to or from the plasma membrane	[21]
PDZ-binding domain	Ania-3	MDCK cells and hippocampal neurons	Synaptic function	[22]
PDZ-binding domain	CLP36	Platelets	Translocation during platelet activation	[23]
Calmodulin binding site	Calmodulin	Purified protein	PMCA activation	[25]
Main intracellular loop	RASSF1A (Ras-association domain family protein member 1A)	HEK293 and primary rat cardiomyocytes	Regulation of Ras-MAPK signalling pathway	[27]
Main intracellular loop	alpha-1 syntrophin	HEK293 cells and heart	Regulation of nNOS activity	[28]
Main intracellular loop	Calcineurin A	HEK293 cells and heart	Regulation of calcineurin activity and cardiac hypertrophy	[29,35]
N-Terminal	14-3-3ϵ	HeLa cells	Regulation of PMCA activity	[30]