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Review

The plasma membrane Ca^{2+} ATPase of animal cells: Structure, function and regulation

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Abstract

Most important processes in cell life are regulated by calcium (Ca^{2+}). A number of mechanisms have thus been developed to maintain the concentration of free Ca^{2+} inside cells at the level (100–200 nM) necessary for the optimal operation of the targets of its regulatory function. The systems that move Ca^{2+} back and forth across membranes are important actors in its control. The plasma membrane calcium ATPase (PMCA pump) which ejects Ca^{2+} from all eukaryotic cell types will be the topic of this contribution.

The pump uses a molecule of ATP to transport one molecule of Ca^{2+} from the cytosol to the external environment. It is a P-type ATPase encoded by four genes (*ATP2B1–4*), the transcripts of which undergo different types of alternative splicing. Many pump variants thus exist. Their multiplicity is best explained by the specific Ca^{2+} demands in different cell types. In keeping with these demands, the isoforms are differently expressed in tissues and cell types and have differential Ca^{2+} extruding properties.

At very low Ca^{2+} concentrations the PMCAs are nearly inactive. They must be activated by calmodulin, by acid phospholipids, by protein kinases, and by other means, e.g., a dimerization process.

Other proteins interact with the PMCAs (i.e., MAGUK and NHERF at the PDZ domain and calcineurin A in the main intracellular domain) to sort them to specific regions of the cell membrane or to regulate their function. In some cases the interaction is isoform, or even splice variant specific. PMCAs knock out (KO) mice have been generated and have contributed information on the importance of PMCAs to cells and organisms. So far, only one human genetic disease, hearing loss, has been traced back to a PMCA defect.

Keywords: PMCA pump; Ca^{2+} homeostasis; Ca^{2+} signalling; PMCA KO; Hereditary deafness

General properties of PMCA pumps

The plasma membrane Ca^{2+} pump belongs to the P-type pump family, which is characterized by the formation of a high-energy phosphorylated intermediate during the reaction cycle. The pump ejects Ca^{2+} from all eukaryotic cells, but PMCA-like pumps have also been described in intracellular membranes in yeasts [1]. Na^+/Ca^{2+} exchangers, which are particularly important in excitable cells, also eject Ca^{2+} and do so with higher transport capacity. Two conformational states of the phosphorylated PMCA have been described, E1 and E2 [2]. In the E1 state the enzyme is assumed to expose a high affinity Ca^{2+} binding site to the cytoplasmic side of the plasma membrane. The phosphorylation of an invariant aspartate by ATP (see Fig. 1) promotes a conformational change of the enzyme and the E1∼P → E2-P transition. In the E2 conformation, the enzyme exposes the bound Ca^{2+} to the extracellular face and decreases the affinity of its binding site, liberating it outside the cell. After Ca^{2+} has been liberated, the E2-P intermediate is cleaved and the enzyme returns to the E1 conformation.

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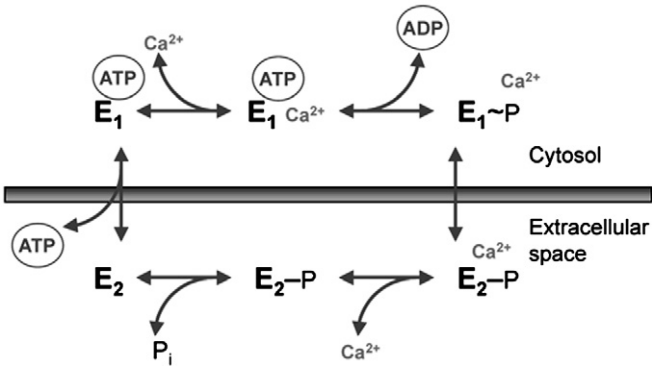

Fig. 1. A scheme of the reaction cycle of the PMCA pump. In the E1 conformation of the pump, Ca^{2+} is bound with high affinity at the cytoplasmic site of the plasma membrane. In the E2 configuration, the binding site exposes Ca^{2+} to the external site of the plasma membrane. In this configuration its lower affinity for Ca^{2+} favours its release.

At variance with the SERCA pump, which transport 2 Ca^{2+} ions per ATP hydrolyzed, the PMCA pump hydrolizes 1 ATP molecule per Ca^{2+} ion transported [3]. It thus only has one Ca^{2+} binding site, which is assumed to correspond to the site 2 now molecularly defined in this structure of the SERCA pump [4,5]. The PMCA exchanges 1 Ca^{2+} for 1 H^+, i.e., the transport operation is partially electrogenic [6].

As all other P-type pumps, PMCAs are inhibited by orthovanadate ($VO_3(OH)^{2-}$) and by La^{3+}. Interestingly, La^{3+} increases the steady-state level of the phosphorylated intermediate as it slows down the E1∼P → E2-P transition [7–9]. This is at variance with all other P-type pumps, where La^{3+} instead reduces the steady-state level of the phospho-intermediate. The property is useful in the identification of the phosphorylated PMCAs in preparations containing much higher amounts of other pumps, such as the SERCA pump: the amount of PMCA pumps usually does not exceed 0.1% of the total membrane proteins.

The structure

In mammals, the PMCAs are the products of four distinct genes (*ATP2B1–4*), located on human chromosomal loci 12q21-q23, 3p25-p26, Xq28 and 1q25-q32 [10–13]. They are single polypeptide chains organized in 10 transmembrane domains (TM)[1] and four main cytosolic domains: this topology scheme follows that experimentally established for the first time for the SERCA pump [4,5]. Fig. 2 shows a molecular modelling cartoon with the superposition of the PMCA (isoform 2) on structural template of the SERCA pump. Following the nomenclature introduced for the SERCA pump, the cytosolic domains of the PMCA pump are an actuator domain, A, a phosphorylation domain, P, and a nucleotide-binding domain, N (see Fig. 3). The N-terminal cytoplasmic domain, encompassing the first 80–90 amino acids, is the most variable pump portion in the four basic isoforms. Together with the intracellular loop between TM2 and TM3, it forms the A-domain, which contains the TGE sequence motif, shown to be important in the SERCA pump during the phosphorylation process of the aspartate residue in the P-domain. This loop is predicted to be mainly composed of β-sheets, and contains a stretch of basic amino acids which is one of the two binding sites for activatory acidic phospholipid (PL) (see below). The intracellular loop between TM4 and TM5 contains the catalytic core of the pump, the P-domain. This domain contains the phosphorylated aspartate residue (D), whereas the N-domain contains the conserved lysine (K) which is part of the binding of ATP. The C-terminal region of the pump is longer than in the other Ca^{2+} ATPases: it is of particular interest since it contains a calmodulin-binding domain (CaM-BD), which also acts as an autoinhibitory sequence by binding to two sites in the main body of the pump. The binding with CaM is assumed to remove the CaM-BD from the inhibitory binding sites in the pump, freeing it from autoinhibition.

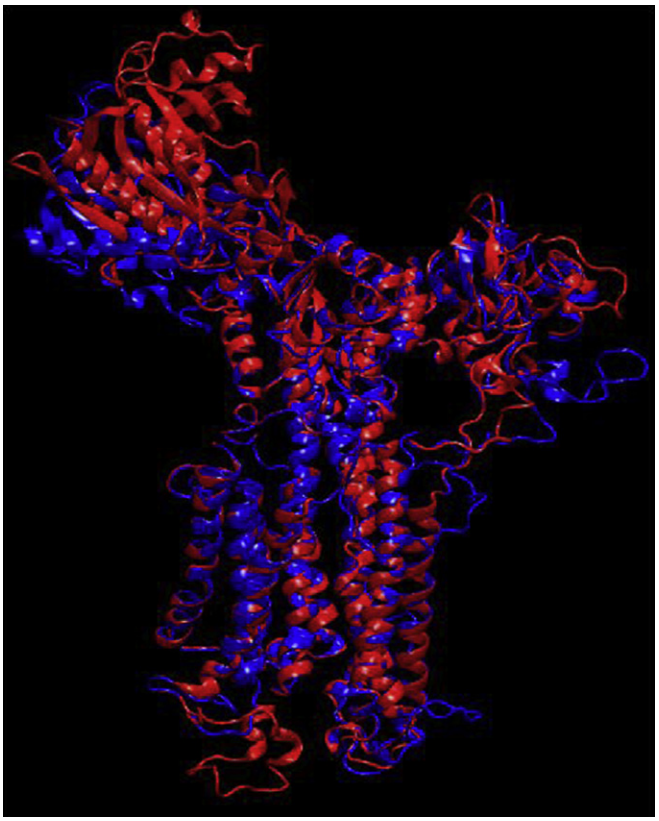

Fig. 2. Structural superposition of the PMCA structure on the SERCA structural template (E1). Blue ribbon, PMCA pump; red ribbon, SERCA pump. Courtesy of Sergio Pantano (Montevideo, Uruguay).

[1] *Abbreviations used:* TM, transmembrane domains; PL, phospholipid; CaM-BD, calmodulin-binding domain; PIP, phosphatidylinositol bisphosphate; InsP3, inositol triphosphate; DAG, diacylglycerol; PKA, protein kinase A; FAK, focal adhesion kinase; NOS-I, nitric oxide synthase I; CASK, calcium/calmodulin-dependent serine protein kinase; NMDA (*N*-methyl-D-aspartate); CaN, calcineurin; MET, mechano-electrical transduction; MECs, mammary epithelial cells.