Final Progress Report OTKA K101064

Title: Targeting of plasma membrane Ca^{2+}ATPases and their role in shaping the Ca^{2+} signal.

Project period: 2012 01.01 – 2016 06.30.

Principal Investigator: Ágnes Enyedi

Abstract

Cellular Ca^{2+} controls a variety of cell functions including growth, differentiation and apoptosis among others. The existence of a well-orchestrated Ca^{2+} signaling machinery ensures an appropriate response of each particular cell-type. This requires proper expression, localization and function of the participating proteins. Among the Ca^{2+} signaling proteins the plasma membrane Ca^{2+}ATPases (PMCAs) are responsible for removing excess Ca^{2+} from the cytosol not only for maintaining low cytosolic Ca^{2+} levels – which is their housekeeping function - but also for generating PMCA variant-specific Ca^{2+} signaling patterns for a variety of cell types. With the support of OTKA 1. / A specific localization motif was identified that affected cellular distribution of a particular PMCA variant; 2. / A mathematical model has been developed for predicting Ca^{2+} signaling patterns in the presence of different PMCA variants; 3. / It was found that PMCAs bound specific signaling inositol-lipid molecules by which they inhibited the release of Ca^{2+} from intracellular Ca^{2+} pools; 4. / Differentiation inducing agents (histone deacetylase inhibitors) greatly enhanced the expression of the PMCA4b variant in a breast cancer cell line. In line with these findings, immunohistochemistry analysis of normal breast tissue showed high PMCA4 expression level in the ductal epithelium.

Introduction

Intracellular calcium transients are shaped by complex mechanisms including Ca^{2+} influx through the plasma membrane, Ca^{2+} release from intracellular stores and also by different calcium removal systems such as the plasma membrane Ca^{2+}ATPases (PMCAs) or the sarco/endoplasmic Ca^{2+}ATPases (SERCAs). The main goal of the OTKA K101064 research grant were to find out how distinct PMCA isoforms influence intracellular Ca^{2+} signaling; *specifically (1) to identify localization signals in the C-terminal region of PMCA isoform 4; (2) to determine how PMCAs with different localization and kinetic characteristics alter cytosolic Ca^{2+} signaling; and (3) to develop tools for studies determining near plasma membrane Ca^{2+} spikes in the vicinity of the PMCA*. Therefore, they studied localization of the PMCAs in epithelial and endothelial cells, studied how these pumps changed the pattern of Ca^{2+} transients

induced by store operated Ca^{2+} entry (SOCE) and developed Ca^{2+} sensors to study near-membrane Ca^{2+} fluxes for future studies.

Major new findings supported by OTKA K101064

A di-leucine-like LLL motif in the C-terminal tail controls plasma membrane expression and internalization of PMCA4b. A novel finding of this project period was that localization of PMCA4b highly depended on cell density: the PI and coworkers found that in highly confluent epithelial or endothelial cell cultures PMCA4b localized in the plasma membrane as expected but in low-density cells it was found mostly in intracellular compartments. In correlation with these results they found a significantly more efficient Ca^{2+} clearance in confluent versus non-confluent PMCA4b expressing cell cultures. Further, they showed that three leucine residues at positions 1167-1169 were responsible for the internalization of the PMCA4b. Alteration of these leucine residues to alanines resulted in enhanced cell surface expression, suggesting that the LLL motif – which resembles an atypical di-leucine-like motif found also in other membrane proteins - was crucial in internalization of PMCA4b. Loss of cell contact resulted in the translocation of the wild type pump to the early endosomal compartment, which was diminished by the mutation of the LLL motif. These data suggest that a di-leucine-like internalization motif was responsible for the internalization-mediated loss of function of the pump at low degree of cell-cell contact. These results are described in BBA Mol Cell Research [1].

PMCA modulates phosphatidylinositol 4,5-bisphosphate (PIP_2) signaling by binding PIP_2. The PI's group in collaboration with John Penniston at Harvard Medical School described for the first time that apart from its known function – that is to remove Ca^{2+} from the cytosol - PMCA can regulate the IP_3-mediated Ca^{2+} signaling by controlling the accessibility of the signaling molecule phosphatidylinositol-4,5-bisphosphate (PIP_2). Their studies demonstrated that PMCAs can attract PIP_2 molecules in the plasma membrane and protect them from the depletion by phospholipase C (PLC). The protection operates by two mechanisms; one is the removal of the Ca^{2+} required for sustained PLC activity and the other is PIP_2 binding. Molecular dynamics simulation revealed a region of positively charged side-chains which formed four binding pockets for the phosphorylated inositol head group of PIP_2. The group suggests that binding of PIP_2 to the pockets can control the accessibility of PIP_2 for phospholipase C resulting in decreased inositol 1,4,5-triphosphate formation and hence release of Ca^{2+} from the intracellular pools. These data are described in the Journal of Cell Science [2].

Plasma membrane Ca^{2+}-ATPases shape the pattern of Ca^{2+} transients induced by store-operated Ca^{2+} entry. During the 2012 – 2016 OTKA period the PI's group found that each PMCA isoform could produce distinct pattern of the SOCE signal; 1/ the expression of PMCA4b induced periodic baseline oscillations; 2/ in PMCA4a expressing cells a quick initial rise in intracellular Ca^{2+} was followed by a rapid return to a new elevated level; and 3/ the expression of PMCA2b resulted in complete Ca^{2+} clearance. The distinct pattern produced by the different PMCAs in HeLa cells could also be generated in other cells such as the PMCA over-expressing human embryonic kidney 293 (HEK293) cells or the primary endothelial cells isolated from human umbilical veins (HUVECs) expressing PMCA4b endogenously. In these studies we successfully applied the genetically encoded calcium sensor, GCaMP2.

In collaboration with Emanuel Strehler at the Mayo Clinic they developed a mathematical model and showed that the distinct kinetic properties of the PMCAs was sufficient to generate the distinct Ca^{2+} signaling pattern. The experimental data were adequately fitted to the model using the kinetic parameters of the pumps previously determined in "*in vitro*" experiments. The data suggested that the unique shape of each Ca^{2+} signaling curve attributed to the different kinetic and regulatory characteristics of the PMCA isoforms and that delayed activation was critical to the sustained oscillation induced by the PMCA4b pump variant. These data were published in Science Signaling [3].

Developing Ca^{2+} sensors to detect Ca^{2+} fluctuations near the plasma membrane. The aim was to study how local Ca^{2+} efflux and specifically targeted PMCAs influenced the amplitude and duration of locally generated Ca^{2+} signals. To address these issues, we generated GCaMP2-based Ca^{2+} sensors, which can be targeted to specific cellular locales. These sensors now are being used in a variety of different cell models to study local Ca^{2+} signals in the vicinity of the PMCA. These tools will provide great advantage in determining spatial and temporal aspects of calcium signaling in these cells. Manuscript in preparation.

Histone deacetylase inhibitor enhances PMCA4b expression in MCF-7 breast cancer cells. Previous studies have suggested that expression of PMCA4b is altered in several types of cancer cells suggesting that they are involved in cancer progression. Therefore, the PI's group has initiated studies on the expression and function of PMCAs in different kinds of cancer cells, including breast and colon cancer cells. First, they studied how differentiation inducing agents such as the histone deacetylase inhibitors (HDACis) affected PMCA expression in MCF-7 breast cancer cell line. They found that differentiation of these cells led to strong upregulation

of PMCA4b protein expression while no change in the expression of PMCA1 was seen. In addition, combination of HDACis with phorbol-12-myristate 13-acetate (PMA) further augmented cell differentiation and PMCA4b expression both at the mRNA and protein levels. Furthermore, the increased PMCA4b expression led to enhanced Ca^{2+} clearance from stimulated MCF-7 cells suggesting that the newly expressed PMCA protein was functional. The expression of PMCA4 could also be detected in ductal epithelial cells indicating that PMCA4 is a physiologically relevant actor of the normal breast epithelium. These findings were published in Cell Calcium [4].

Selective upregulation of the expression of plasma membrane calcium ATPase isoforms upon differentiation and 1,25(OH)$_2$D$_3$-vitamin treatment of colon cancer cells. PMCA proteins are involved both in intestinal Ca^{2+} absorption and adenocarcinoma cell differentiation. Interestingly, 1,25(OH)$_2$D$_3$ stimulated PMCA1b, but not PMCA4b expression in enterocyte-like colon carcinoma cells. In contrast, PMCA4b but not PMCA1b was upregulated during post-confluency induced differentiation of enterocyte- and goblet cell-type colon cancer cells. These data suggest that while PMCA4b participates in the reorganization of the Ca^{2+} signaling machinery during cell differentiation, PMCA1b must have a specific role in 1,25(OH)$_2$D$_3$-stimulated intestinal Ca^{2+} absorption. Data were published in the Biochemical and Biophysical Research Communications [5].

Impact

Work during the period of 2012 – 2016 provided preliminary data for two new OTKA grant applications; one of which is granted by OTKA-FWF which is based on a newly developed international collaboration. The success of the present project stimulated the generation of a homozygous transgenic rat strain stably expressing the GCaMP2 calcium sensor protein that yielded two scientific papers in high impact journals in collaboration with Balázs Sarkadi, Ágota Apáti and Tamás Orbán [6-8]. Altogether, during the present OTKA period the research group published 10 scientific papers - cumulative IF= 52.25 - including a review article that also summarizes the basic findings of this project [9].

References

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