Regulation of plasma membrane calcium ATPase (PMCA) by actin cytoskeleton

Vigil MA[a], Picco ME[b], Rinaldi DE[a], Rossi RC[a], Rey O[b], Rossi JP[a], Ferreira-Gomes MS[a]
a - Cátedra de Fisicoquímica Biológica-Departamento de Química Biológica, Universidad de Buenos Aires, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB) UBA-CONICET.
b - Instituto de Inmunología, Genética y Metabolismo (INIGEM)

The Plasma Membrane Calcium ATPase (PMCA) is a calmodulin-modulated P-type ATPase responsible for the maintenance of low intracellular concentrations of Ca^{2+} in most eukaryotic cells. Our group have previously shown that purified actin can exert a dual modulation on the activity of Ca^{2+}-ATPase 4b isoform (hPMCA4b): F-actin inhibits it while short actin oligomers may contribute to its activation. These studies had to be performed with purified proteins given the nature of the biophysical and biochemical approaches used.

On the other hand, in HEK293 human cells that overexpressed PMCA2w/b isoform, the actin depolymerization upon Cytochalasin D (CytD) treatment significantly increased PMCA2-mediated Ca^{2+} extrusion and when F-actin was stabilized using jasplakinolide, PMCA2w/b activity was completely abolished.

In order to assess whether the functional interaction between the hPMCA4 isoform and the actin cytoskeleton may be of physiological relevance, we decided to further characterize it in the context of a living cell by monitoring in real-time the changes in the actin polymerization and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). For this, hPMCA4 isoform was transiently expressed in HEK293T cells. The dynamics of $[Ca^{2+}]_{cyt}$ was performed using the fluorescent probe Fluo-4 and studying the alterations in $[Ca^{2+}]_{cyt}$ generated by Ca^{2+} release from the endoplasmic reticulum, and by extracellular Ca^{2+} entry through store-operated Ca^{2+} channels. The dynamics of actin polymerization was performed transiently expressing LifeAct-Ruby.

Results show that the alteration of actin polymerization by CytD treatment significantly increased hPMCA4 activity (102%). On the other hand, in absent of CytD, actin polymerization dynamics did not change after TG stimulus, while after Ca^{2+} stimulus, an actin reorganization was observed. This reorganization takes place at the same times that the hPMCA4 increases its activity, suggesting that hPMCA4 may be activated by actin depolymerization in the cells.

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