TRPM7, Calcium and the cytoskeleton

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Summary

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Ions play an important role in many cellular process. They may act as regulators of enzymes or as second messenger: changes in intracellular ion concentration are induced by stimuli (like hormones) from the outside of the cell. Therefore cells maintain a tightly regulated gradient of ions over the plasma-membrane. Sodium and potassium are the main ions that control the membrane potential of cells whereas calcium (Ca^{2+}) and magnesium (Mg^{2+}) regulate many enzymes and cellular processess. The plasma-membrane consists of various lipids through which ions can not pass. As a result, cells have adopted specialized transporters and channels to control the distribution ions over the plasma-membrane.

TRPM7 channels are widely expressed and play a crucial role in the Mg^{2+} homeostasis of cells. This channel permeates divalent ions, such as Ca^{2+} and Mg^{2+}. Above all, TRPM7 channels have an unique feature at their C-terminus: an α-kinase domain.

It is well established that TRPM7 channels may be activated by depletion of internal Mg^{2+} or magnesium-nucleotides in "whole-cell" patch clamp experiments (a method for measuring ionic currents whereby the contents of the cell is dialyzed). Activation of TRPM7 currents by agonist-induced second messengers was still unknown. In **Chapter II** we describe a receptor-mediated signaling pathway that mediates TRPM7 channel activation in unperturbed cells. In wild-type N1E-115 cells, stimulation of G-Protein Coupled Receptors that couple to PLC cause a single transient intracellular Ca^{2+} elevation through Ca2+ release from the endoplasmatic reticulum, meaured by fluorescent Ca^{2+} dyes. In TRPM7 transduced N1E-115 cells, this single transient Ca^{2+} elevation is followed by a second, sustained Ca^{2+} increase as a result of Ca^{2+} influx through TRPM7 channels. The activation of TRPM7 by PLC activation was validated by perforated-patch experiments (a patch-clamp assay that leaves the contents of the cells unperturbed). Furthermore, in this chapter we show that opening of TRPM7 channels is restricted to PLC-coupled stimuli: using various fluorescent indicators ("FRET assays") we confirmed that TRPM7 opening correlates well with PLC activity, but not other GPCR-derived second messengers such as cyclic AMP and cyclic GMP.

The above described PLC regulation of TRPM7 currents contradicts with results of other groups: they show that activation of PLC leads to closure of TRPM7 cells in "whole-cell" patch-clamp configuration. In **Chapter III** we continued to examine this discrepancy. Here we show that this difference can be ascribed to the difference in the patch-clamp assays. We use minimal invasive techniques like Ca^{2+} experiments and the perforated-patch clamp technique, which both do not interfere with the functioning of the cell. We show that the inhibitory effect of PLC is only observed in "whole-cell" experiments where intracellular Mg^{2+} is artificially clamped at low concentrations. To convincingly proove this, we have developed a method to lower intracellular Mg^{2+} levels in perforated-patch: pretreatment of cells with a membrane-permeable Mg^{2+} buffer. De currents induced in this way are both biohysically and pharmacoligically identical to the TRPM7 currents measured activated by Mg^{2+} depletion measured in whole-cell configuration. Moreover, the data described in this chapter show that a gradual loss of PIP2 at the plasma-membrane probably underlies the artificial whole-cell results.

Mild overexpression of the TRPM7 channel in neuroblastoma cells induces cell spreading, cell adhesion and formation of focal adhesions. In **Chapter IV** we show that all these features are enhanced after PLC-mediated TRPM7 activation. The underlying mechanism is a Ca^{2+}- and kinase-dependent association with the actomyosin cytoskeleton. This association of the kinase domain and the cytoskeleton results in phosphorylation of myosin IIA and releases the tension of the actomyosin cytoskeleton. Furthermore, podosomes are formed *de novo* or out of existing focal adhesions. Thus, TRPM7 regulates cell adhesion by changing actomyosin contractility.

In **Chapter V** we describe a second Ca^{2+} dependent signaling pathway that regulates the cytoskeleton. In prostate carcinoma cells elevation of intracellular Ca^{2+}, either by receptor stimulation or pharmacologically, activates the small G-protein Rac. This activated protein causes lamellipodia formation: dynamic, flat structures of the plasma-membrane that contain large amounts of actin. The experiments described in this chapter show that the Ca^{2+} increase activates a kinase (a so-called conventional protein kinase C) that phosphorylates an activator of Rac (RhoGDIα) and causes translocation of Rac to the plasma-membrane.

We conclude that TRPM7, which combines a ion channel with a kinase, affects the cytoskeleton in several ways.