The channel-kinase TRPM7, revealing the untold story of Mg\textsuperscript{2+} in cellular signaling

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Abstract. Ion homeostasis dysregulations have severe effects on human health, impairing the effectiveness and appropriateness of major cellular events, including immune responses. The adverse effects of Mg\textsuperscript{2+} deficiency on cellular physiology are well known and documented, but mechanistic insights into Mg\textsuperscript{2+} sensitive signal transduction are still lacking. TRPM7 and its sister channel TRPM6 stand out as the only known fusions of an ion pore with a Ser/Thr kinase domain. Both channels are permeable to divalent cations and are central regulators of Mg\textsuperscript{2+} homeostasis. One crucial aspect of TRPM7 function we have extensively studied is the relationship between its ion channel portion and its C-terminal Ser/Thr kinase domain. The modulation of ion channels by phosphorylation through exogenous kinases is common, however the covalent bond between the TRPM7 channel and its kinase suggests a novel kind of link between ion-entry and signal transduction events. Current knowledge supports a reciprocal “two-way street” model where TRPM7-kinase modulates ion transport function through Ser/Thr phosphorylation, and in turn, channel gating and ionic conditions in close proximity to the pore regulate TRPM7-kinase mediated signaling. We have shown that TRPM7 acts as a sensor of Mg\textsuperscript{2+}-availability, adjusting key cellular functions such as the rate of cellular protein translation to the Mg\textsuperscript{2+} nutritional status. Since molecular mechanisms controlling rates of protein translation are critical for cell growth and division in response to nutrient availability, this could have relevance for example for therapies targeted at molecules shaping the cancerous translational apparatus. In our quest to understand the biology of Mg\textsuperscript{2+} in the context of immune responses, we found that TRPM7 associates with, and phosphorylates phospholipase C gamma 2 (PLC\textsubscript{\gamma}2), a pivotal molecule in the signaling pathway following B-cell receptor (BCR) activation. This contributes to the Mg\textsuperscript{2+}-dependent modulation of the Ca\textsuperscript{2+} response elicited by BCR ligation, and provides the first molecular pathway underlying the Mg\textsuperscript{2+}-sensitivity of immune responses. Expanding our knowledge about the modulation of immunoreceptor signaling in response to Mg\textsuperscript{2+} availability could allow for the development of unexplored strategies for therapeutic intervention in autoimmune diseases, immunodeficiencies, and lymphoma.

Key words: TRPM6, TRPM7, channel-kinase, nutritional signaling, phospholipase C, immunoreceptor signaling, eEF2 kinase, MagT1
Mg$^{2+}$, the maintenance ion taken for granted?

Because Mg$^{2+}$ is the most abundant divalent cation in living cells, and an essential cofactor for hundreds of enzymes involved in virtually all physiological processes, as well as a regulator of macromolecule conformation, phosphoinositide metabolism, and various transporters and ion channels, it is often seen as a "cellular maintenance ion". In contrast to the "signaling ion" Ca$^{2+}$, the gradient for free Mg$^{2+}$ across biological membranes is mild, perhaps reinforcing the perception that Mg$^{2+}$-homeostasis does not require the intricate and tight regulation associated with cellular control of Ca$^{2+}$-levels. Mg$^{2+}$ is so essential and omnipresent that it might have been taken for granted. However, in the past decade, the discovery of diverse components of the molecular machinery of Mg$^{2+}$-homeostasis regulation has revealed that Mg$^{2+}$ concentrations and its subcellular distribution are exquisitely surveyed and adjusted by the cell, and that information about Mg$^{2+}$-availability is integrated into classic signaling pathways of nutritional sensing and receptor activation [1-6].

Clinically, numerous studies have established the role of Mg$^{2+}$ as an essential nutrient contributing to the development of major risk factors leading to cardio-vascular diseases, such as diabetes mellitus, hyperlipidemia, atherosclerosis, and hypertension. Hypomagnesemia is a frequent corollary of other illnesses, often as a result of renal Mg$^{2+}$-wasting caused by medication, predisposing patients to serious, even life-threatening effects. Functionally defective Mg$^{2+}$ transporters have been documented in humans, and were shown to cause severe pathologies [7, 8]. Patients with deficiencies in TRPM6 develop Hypomagnesemia with Secondary Hypocalcemia (HSH), an autosomal recessive disorder leading to muscle spasms, tetany, and seizures, which can be treated by Mg$^{2+}$-supplementation [9, 10]. Mutations in the Mg$^{2+}$-transporter MagT1 are linked to a human immune disease named XMEN (X-linked immunodeficiency with Magnesium defect, chronic Epstein–Barr virus infections and Neoplasia) and an ATRX syndrome variant [11-14]. Hermosura and colleagues demonstrated a direct correlation between the TRPM7 T1482I polymorphism, impaired Ca$^{2+}$ and Mg$^{2+}$ intake, and the risk of colorectal carcinogenesis [16]. Although past studies have attributed the involvement of TRPM7 in tumor development mostly to changes in Ca$^{2+}$ flux, a growing number of studies tie Mg$^{2+}$ to cancer occurrence as well [17]. Future studies that will provide insights into the complex relationship between Ca$^{2+}$, Mg$^{2+}$, TRPM7 function, and carcinogenesis are needed, as exemplified by Dai et al. In addition, given TRPM6’s high expression levels in the colon, and its known association with TRPM7 [18, 19], it is possible that TRPM6 kinase activity might play a role in this context by modulating the biological function of TRPM7, and adjusting its Mg$^{2+}$ sensitivity via phosphorylation. Future elucidation of the underlying mechanisms involved in Mg$^{2+}$ dependent signal transduction could provide opportunities to develop novel therapeutic approaches to treat nutrient deficiencies and their associated diseases.

TRPM6 and TRPM7, at the heart of Mg$^{2+}$-homeostasis regulation

TRPM7 and its sister channel TRPM6 stand out as the only known fusions of an ion pore with a Ser/Thr kinase domain [1]. While all TRP channels are thought to form pores by tetramerizing, not all can build heteromers with other related TRP channels [20]. As previously mentioned, TRPM6 and TRPM7 associate, building functional TRPM6/M7 pores. Although these two channel kinases share many biophysical and biochemical properties [21-24], we found that their kinases do not have identical substrate specificities, since TRPM7 is a substrate of TRPM6-kinase, but we could find no evidence of the opposite [18]. We found that TRPM6 via its kinase modulates TRPM7, and has an inhibitory effect on TRPM7 dependent cell growth under hypomagnesia seen in patients with TRPM6 defects, and the considerable decrease in total intracellular Mg$^{2+}$ observed in TRPM7-deficient DT40 cells.
The Mg\(^{2+}\) sensor TRPM7 functions as a signaling module

[25], suggest that one crucial role of both channels is to act as a Mg\(^{2+}\)-sensing and uptake mechanism involved in the regulation of Ca\(^{2+}\) and Mg\(^{2+}\) homeostasis [25]. TRPM7-deficient chicken DT40 B-cells experience growth arrest within 24 hr, become Mg\(^{2+}\)-deficient and die. Both, the viability and the proliferation of TRPM7-deficient DT40 cells are rescued by supplementing the culture media with mM Mg\(^{2+}\) amounts (not with other ions tested, including Mn\(^{2+}\), Ca\(^{2+}\), Zn\(^{2+}\), Ni\(^{2+}\)), which is reminiscent of TRPM6-deficient humans who can live a normal life if provided with a Mg\(^{2+}\)-rich diet [9, 10, 25]. Both channels are permeable to Ca\(^{2+}\) and Mg\(^{2+}\), but TRPM7 also provides a novel entry mechanism for trace metal ions like Ni\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\) and others [26], particularly in suboptimal concentrations of Mg\(^{2+}\), which promote TRPM7 pore opening. The biological role of trace metal entry via TRPM7 (and potentially TRPM6) still remains to be defined.

Since cloning TRPM7 fifteen years ago, we have remained intrigued by the biological significance of the covalent link between its channel and kinase portions. Our main hypothesis and a major focus of our research is therefore how TRPM7-kinase adjusts cellular functions in accordance to Mg\(^{2+}\) availability. Known substrates of TRPM7 include eukaryotic elongation factor 2-kinase (eEF2-k) [27], a Ser/Thr kinase that regulates rates of protein translation and is a substrate of the master regulator of nutritional signaling mTOR. Moreover, the involvement of TRPM7 in regulating components of the actin cytoskeleton machinery (i.e. via myosin phosphorylation [28]) could impact cellular processes ranging from motility to receptor membrane distribution and signaling. TRPM7 appears to also directly adjust the signaling threshold of central molecular components, such as phospholipase C (PLC), a pivotal enzyme crucial to phosphoinositide metabolism and the generation of Ca\(^{2+}\) signals following receptor activation, which can be phosphorylated by TRPM7 kinase [29]. These data suggest TRPM7 might dually contribute to signaling, through its ion transport function, and via its Ser/Thr kinase activity.

The channel kinase TRPM7: a unique signaling concept?

Our working model is that Mg\(^{2+}\) availability is carefully monitored by the cell, and that cellular functions are actively, and not just passively, adjusted to it. We would like to propose that the covalent bound between the ionic pore and the kinase of TRPM7 is at the heart of this process, and represent a two way street allowing for the reciprocal regulation between channel and kinase (figure 1).

We have previously shown that mutations in the kinase domain of TRPM7 abrogating its phosphotransferase activity do not affect the capacity
of TRPM7-currents to be activated to a similar extent as WT hTRPM7 with nominal zero intracellular Mg\(^{2+}\) pipette solution [25]. However, the sensitivity of TRPM7 kinase-dead point mutant channels (K1648R, G1799D) towards Mg\(^{2+}\) is decreased, since significantly higher Mg\(^{2+}\) concentrations are required in the intracellular patch-clamp solutions in order to inhibit TRPM7-mediated currents as compared to WT TRPM7 [25]. These results lead to the conclusion that while phosphotransferase activity is not required for channel activation [25, 30], mutations that affect the enzymatic activity of the kinase domain do influence channel activity, supporting the concept of a functional coupling between TRPM7 channel and kinase domains [25]. In a subsequent study we have shown that TRPM6 and TRPM7 wildtype channels can cross-phosphorylate associated kinase-deleted versions of themselves, confirming that the channels are their own kinases’ substrates [18]. Moreover, using our TRPM7-deficient DT40 cell line complemented with kinase-dead point mutants (see figure 2, model), we have been able to show the TRPM7-kinase dependent increase in phosphorylation of the protein elongation factor eEF2 under hypomagnesic conditions [27]. This is to our knowledge the first example of a cellular adaptation (in this case a reduced rate of protein translation) to suboptimal Mg\(^{2+}\)-availability that is mediated by TRPM7-kinase.

Conversely, in order to investigate the impact of channel activity on kinase-function, it is necessary to dissociate TRPM7’s ion-permeation from its phosphotransferase activity by molecular manipulation(s) in regions expected to be crucial for ion transport. Our unpublished data indicate that the auto-phosphorylation of the channel appears substantially decreased in pore-mutants with an altered permeability profile, indicating that ionic permeation through the TRPM7-pore, and/or gating events associated with it, are central to kinase-function. Based on these observations, we propose that a direct coupling of the ion-conducting activity of the channel to the signaling function of the kinase is required for TRPM6/7 to fulfill their biological role. An attractive model is that their structural duality reflects the reciprocal relationship of these two domains: The kinase modulates channel activity, and ion

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**Figure 2.** Studying TRPM7 function and signaling through a complementation approach in TRPM7 deficient DT40 cells: Genetically engineered TRPM7\(^{-/-}\) cells are complemented with human TRPM7 WT and mutants (such as kinase dead), and biological functions such as growth rates or signaling events evaluated, particularly those involving known TRPM7 kinase substrates.
transport through the channel regulates kinase activity, which can in turn modulate cellular responses.

**Immunoreceptor signaling and protein translation, two examples of the broad impact of TRPM7-mediated signaling**

To illustrate the general significance of TRPM7-mediated signaling for cell physiology, we would like to highlight in more details the afore mentioned involvement of TRPM7 in adjusting protein translational rate and PLC-dependent immune signaling.

Phospholipase C (PLC) isoforms are multidomain signaling proteins that are widely used downstream of the activation of receptors, such as GPCRs or immune receptors. PLCs convert membrane-bound phosphatidylinositol 4,5-bisphosphate (PtdIns4,5P₂) to the second messengers Diacylglycerol (DAG), and the ER Ca²⁺-store mobilizing agent 1,4,5-trisphosphate (IP₃). IP₃-mediated store depletion followed by Ca²⁺ re-entry from the extracellular space via STIM/Orai is essential to immune responses [31], and Ca²⁺ transfer from the ER to the mitochondria drives apoptotic and autophagic pathways [32-34] and affects many other processes.

Multiple PLC isoforms have been shown to associate with the kinase domain of TRPM7 [35, 36], however subsequent studies have concentrated on the influence of phosphoinositide metabolism and PLC signaling on TRPM7 function [36-39]. Our recent report was the first to investigate the reverse possibility that PLCs can be substrates of TRPM7 kinase. We have shown that TRPM7 kinase phosphorylates a conserved serine residue at position 1164 in the human PLCγ2 C2 domain. Upon B-cell receptor stimulation, PLCγ2 deficient DT40 cells complemented with the PLCγ2 S1164A mutant exhibit a normal Ca²⁺ response under physiological 1mM Mg²⁺ conditions, but a reduced Ca²⁺ response following Mg²⁺ deprivation, providing the first molecular mechanism mediating Mg²⁺-sensitivity of an immune response [29].

In a different study we found that TRPM7 deficiency in DT40 B lymphocytes can partially be compensated by overexpressing the Mg²⁺ transporter MagT1 [40]. Mutations in the gene encoding MagT1 are associated to an X-linked human immunodeficiency showing reduced Mg²⁺ influx followed by impaired PLC activation and Ca²⁺ response following T cell receptor stimulation, further supporting the notion that Mg²⁺ plays a crucial role in PLC-dependent signaling in the immune context [11].

An important question for future investigations is the integration of TRPM7 into known nutritional signaling networks. The elongation step of protein translation is highly energy consuming, and its rate is tightly linked to the energy and nutrient status of the cell via phosphorylation of eukaryotic elongation factor 2 (eEF2) [41-43], which represents a branch of the mTOR kinase signaling network. The only described kinase of eEF2, the mTOR substrate eEF2-k, phosphorylates eEF2 at Thr56 leading to its failure to bind ribosomes, and therefore its inactivation. We found that TRPM7 increases native inhibitory Thr56 phosphorylation of eEF2 upon Mg²⁺ withdrawal from the cell culture media, which correlates with reduced cell growth in a TRPM7-kinase dependent manner [27]. TRPM7 kinase does not appear to directly phosphorylate eEF2, but rather to influence the activity of eEF2-k via phosphorylation on Ser77. Our results indicate that TRPM7 acts as a master regulator of Mg²⁺ homeostasis coordinating cell growth in response to Mg²⁺ availability, providing a potential mechanistic explanation for the documented effect of Mg²⁺ on protein synthesis and cell proliferation [44].

**Conclusions**

Current knowledge suggests that TRPM7’s structural duality ensures ideal positioning of its kinase to sense Mg²⁺-mediated gating of the channel, and channel-mediated Mg²⁺ uptake. This allows for the adjustment of essential cellular functions through Ser/Thr phosphorylation of relevant substrates by TRPM7-kinase in accordance to the availability of environmental Mg²⁺.

Recent findings establish Mg²⁺ as a crucial parameter of nutritional sensing networks, and have uncovered unsuspected aspects of Mg²⁺-mediated signaling. The identification of new elements of Mg²⁺-sensitive signal transduction could be of public health relevance particularly in the context of environmental and nutritional paucity in Ca²⁺ and Mg²⁺, and could help in...
developing improved strategies to prevent or treat conditions ranging from immunodeficiencies to cancer.

Disclosure

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