Editorials

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TRPC4 Knockout Mice
The Coming of Age of TRP Channels as Gates of Calcium Entry
Responsible for Cellular Responses

Lutz Birnbaumer

Agents that lead to IP$_3$ formation are those that act through receptors that are coupled to intracellular responses by either PLC$_{\beta}$ activated by G proteins of the Gq and Gi/o classes, or PLC$_{\gamma}$ activated by tyrosine kinases, thus reflecting the distinct responsiveness of these two types of PLC. PLC$_{\beta}$s respond to Gq-derived GTP-Gq and to Gi/o-derived G$p$$\gamma$; PLC$_{\gamma}$s are activated by tyrosine phosphorylation. Stimulation of dedifferentiated fibroblast cell lines that lack voltage- and ligand-gated ion channels, in the presence of extracellular Ca$^{2+}$, with an agonist that acts through a Gq-coupled receptor, typically shows an initial, rapid (within seconds) rise of [Ca$^{2+}$], to peak levels that can be as high as 100- to a 1000-fold basal, followed by a decline to a plateau level that averages between 2- and 4-fold the prestimulation level and persists until the stimulating agent is removed. The same experiment without extracellular Ca$^{2+}$ shows the initial peak to be little affected but the plateau to be abolished. Readmission of Ca$^{2+}$ to the medium after [Ca$^{2+}$], has returned to baseline—agonist continuously present—now shows an immediate influx of Ca$^{2+}$ and establishment of the plateau phase of elevated [Ca$^{2+}$], that persists until the causative agonist is removed. With small variations, this type of experiment has been repeated with the same outcome by researchers across the world with almost all cells that have been isolated and tested for their [Ca$^{2+}$], changes in response to agonists, chemokines, cytokines, neurotrophic peptides, etc. Cells with this type of [Ca$^{2+}$], response pattern are not only of the nonexcitable type but also include excitable cells such as neurons and myocytes, visualized after blocking their voltage- and/or ligand-gated channels. This underscores the ubiquitous nature of the [Ca$^{2+}$], response to maneuvers that elevate IP$_3$ levels in cells, and by extension, the ubiquitous nature of the Ca$^{2+}$ entry pathway activated in conjunction with increases in cytosolic IP$_3$.

What are the molecular components of this Ca$^{2+}$ entry pathway? The hunt for channels through which the Ca$^{2+}$ enters began about 10 years ago with clues coming from studies with mammalian cells and insect photoreceptor cells. Mammalian cells were found to activate a similar Ca$^{2+}$ entry pathway without prior stimulation of IP$_3$ formation by simply doing what IP$_3$ does, ie, by depleting the internal stores with the drug thapsigargin (TG). IP$_3$ does this by binding to its intracellular receptor, the IP$_3$R, which is a Ca$^{2+}$ release channel located in the endoplasmic reticulum membrane that delimits the internal store. TG instead acts by inhibiting sarco(endo)plasmic reticulum ATPases (SERCAs), the Ca$^{2+}$ pumps responsible for transporting Ca$^{2+}$ from cytosol to the store. Ca$^{2+}$ entry promoted by mere store depletion without overt activation of a PLC system was referred to as
capacitative Ca\(^{2+}\) entry, for it eventually restored the capacity of the store to release Ca\(^{2+}\). Experimental maneuvers could now differentiate between agonist and IP\(_3\)-mediated Ca\(^{2+}\) entry from mere store depletion-induced Ca\(^{2+}\) entry.

Although susceptible to blockade by nonspecific agents that block all Ca\(^{2+}\)-permeable ion channels, such as the lanthanides, curiously—or strangely—this universal form of Ca\(^{2+}\) influx knows of no specific inhibitors. Electrophysiological characterization of the channel(s) through which Ca\(^{2+}\) enters cells after store depletion was elusive, until in 1992, Hoth and Penner\(^6\) characterized a store depletion–activated current in mast cells. Noise analysis indicated that single-channel currents had to be in the sub–picosiemen (pS) range. Ion permeation studies showed the channel to be exquisitely selective for Ca\(^{2+}\) over other divergent cations, including Mg\(^{2+}\).

The term CRAC for Ca\(^{2+}\) release–activated current was coined. In 1993, Penner’s group\(^6\) reported the existence in the same cells of receptor-activated nonspecific (Ca\(^{2+}\)-permeable) cation channels with much larger single-channel conductances than those responsible for CRAC, 50 pS, and thus laid the foundation for the fact that Ca\(^{2+}\) entry activated by the agonist-PLC-IP\(_3\) pathway, which includes store-activated capacitative Ca\(^{2+}\) entry channels, were likely to be heterogeneous. Numerous other examples confirmed this assumption.

TRP channels entered the scene at about the same time, when in 1992 Hardie and Minke\(^7\) reported that a light-activated Ca\(^{2+}\) current responsible for the sustained phase of the fly’s compound eye electroretinogram was absent in a Drosophila mutant termed transient receptor potential, TRP. In invertebrates, unlike in vertebrates, light activation of rhodopsin, instead of hyperpolarizing the photoreceptor cell, causes its depolarization through a pathway formed of rhodopsin, a Gq-like G protein, and PLC, akin to the one that elicits in mammalian cells the IP\(_3\) and store depletion–activated Ca\(^{2+}\) entry. Interestingly, the \(trp\) gene product predicted a structure with some characteristics of an ion channel with limited sequence homology to voltage-gated Na\(^+\) and Ca\(^{2+}\) channels.\(^8\) Could it be that mammalian homologues that existed for receptor store depletion- and/or agonist-activated Ca\(^{2+}\) entries? By 1996, six mammalian TRP homologues (now referred to as TRPC channels) had been identified.\(^9\) Full-length cDNAs were available for several of them by 1997, including TRPC4, originally referred to as Trp4 and CCE1.\(^10\)

Absence of cells lacking capacitative and/or PLC-IP\(_3\)-activated Ca\(^{2+}\) entry made it difficult to establish physiological role(s) for the proteins encoded in TRPC cDNAs. Seven TRPC genes were eventually identified. Importantly, expression of none recapitulated CRAC in terms of ion selectivity. Although TRPC1, TRPC2, and TRPC4, when expressed in model cells, were susceptible to activation by mere store depletion (TG treatment), channels appearing in TRPC3- and TRPC6-transfected cells seem insensitive to store depletion and require a receptor-PLC-IP\(_3\)-IP\(_3\)R pathway for their activation.

Formation of hybrid TRPC channels composed of more than one TRPC is at present the best explanation for the elusive nature of CRAC properties of expressed TRPC cDNAs and the relative paucity of data from normal cells that predict the electrophysiological characteristics of the channels that appear upon transfection of TRPC cDNAs. In their quest for unequivocal roles, matters turned out still worse for TRPCs, the close homologues to Drosophila TRP, for they have been found to form part of a superfamily of TRP-related proteins. TRP-related proteins include the vanilloid and vanilloid-related receptors (VR1 and VRL1) and the cold and menthol receptor (CMR1), responsible for heat and cold sensing, the presumed anti–oncogene melastatin-1 (MLSN1), the poly cystic kidney disease 2 gene product (PKD2), the epithelial calcium channels ECaC1 and ECaC2 (also CaT2 and CaT1), and channels with unrelated protein folds in their C-terminal domains such as an atypical kinase that interacts with PLC (TRP-PLIK) or a NuDix domain able to sense reactive oxygen species and ADP-ribose (see recent reviews\(^11,12\); see nomenclature rules\(^13\)).

Although not addressing specifically the question of subunit complexity of TRP channels—this may have to be solved by applying proteomics approaches—targeted inactivation studies are beginning to shed light on the functional significance of individual TRP channels. So, for example, an antibody approach showed an essential role of TRPC2 in sperm’s acrosome reaction triggered by oocyte zona pellucida protein,\(^14\) and ablation studies showed that TRPM7 (TRP-PLIK/LTRPC7) is essential for cell viability\(^15\) and that TRPC4 (Trp4/CCE1) contributes to endothelium-mediated vascular smooth muscle relaxation.\(^2\)

Tiruppathi et al expand the studies of Freichel and collaborators with TRPC4\(^{-/}\) mice\(^2\) by studying properties of lung endothelial cells both in isolation and in situ.1 Thrombin, or a thrombin receptor–derived peptide with agonist activity, was used as a trigger of Gq activation to regulate endothelial cell functions such as Ca\(^{2+}\) entry, stress fiber formation, and cellular retraction. Further, in a model that assesses endothelial cell–dependent microvessel liquid permeability, they measured thrombin-induced permeability increases. In all instances, loss of TRPC4 correlates with a loss of endothelial cell responses, showing participation of TRPC4 in the response of endothelial cells to thrombin or its surrogate PAR1 receptor–derived agonist. Reduction of Ca\(^{2+}\) influx was expected from previous results with TRPC4–/– aortic endothelial cells,\(^2\) but the strict dependence on agonist-activated Ca\(^{2+}\) entry for stress fiber formation, and its function in retraction and relaxation of cell-cell interaction as seen in the microvascular permeability studies, was not.

It is noteworthy that impairment of thrombin’s responses was not always total. In aortic strips, endothelium-dependent Gq-activated relaxation was lost by only 50%.\(^2\) Likewise, endothelial cell retraction and microvascular permeability increases were only reduced by \(\sim 50\%\) in TRPC4–/– mice. On the other hand, stress fiber formation was fully eliminated, and Ca\(^{2+}\) influx assessed by the Ca\(^{2+}\) readdition assay was reduced by 80% to 90%. These results very likely indicate that other TRPCs or one or more of the TRP-related molecules—some known, others just suspected of having the ability to form ion channels—may cover for the loss of TRPC4 in endothelial cell function. It is for future research to answer these questions. In the meantime, it is clear that TRPC4, originally called CCE1, is indeed a critical compo-
nent of capacitative Ca\(^{2+}\) influx, thus validating the efforts of many in the field who, like me, betted on TRPCs being important in agonist and capacitative Ca\(^{2+}\) entry.

**References**


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