

Commentary

Sorting out MIC, TRP, and CRAC Ion Channels

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A horde of cation-nonspecific ion channel clans populate cell membranes. Their number is further augmented by removing extracellular calcium ($[Ca^{2+}]_o$), turning well-behaved Ca^{2+} -selective channels into less discriminating sieves (Kostyuk and Krishtal, 1977; Almers and McCleskey, 1984). The Ca^{2+} ion stems the flood of ions moving through many cation channels by lodging in their throats, obstructing the path for monovalent ions by clinging to acidic groups in the narrow pore (Tsien et al., 1987; Hille, 2001). The trick of reducing $[Ca^{2+}]_o$ uncovers channels whose conductances are inconveniently low in physiological solution. One of the most frustratingly low conductance Ca^{2+} channels has been the Ca^{2+} release-activated channel (CRAC) channel (Parekh and Penner, 1997; Lewis, 1999). The CRAC current is typically induced by the "store depletion" protocol in which thapsigargin, inositol trisphosphate (IP_3), and/or dialysis using a Ca^{2+} chelator slowly evokes an anemic 10–50 pA of whole-cell inward current. Analysis of CRAC current variance estimates CRAC single channel conductance (even in 110 mM $[Ca^{2+}]_o$) of <30 fS, outside the reach of patch clamp measurements of reasonable bandwidth (Zweifach and Lewis, 1993).

Hoth and Penner (1993) and Lepple-Wienhues and Cahalan (1996) recorded a transient monovalent CRAC current upon external divalent removal. Subsequently, Kerschbaum and Cahalan (1998) recorded a much larger and noninactivating monovalent current when internal Mg^{2+} was also omitted. Thus, it was logical to look for the highly Ca^{2+} -selective CRAC channel single channel conductance by lowering $[Ca^{2+}]_o$. Cahalan and colleagues reasonably assumed they had uncovered the CRAC single channel conductance by removing divalents from both sides of the CRAC-loaded Jurkat T lymphocyte in whole-cell recordings (Kerschbaum and Cahalan, 1999; see also Lepple-Wienhues and Cahalan, 1996; Kerschbaum and Cahalan, 1998; Fomina et al., 2000). The presumed CRAC single channels in monovalent solution were 35–40 pS in size, leading to estimates of only 15–150 channels in resting and activated T cells, respectively (Fomina et al., 2000). Identification of the presumed ~40 pS CRAC monovalent single channel conductance was a hopeful development as a useful new tool for identification of CRAC

channels. The monovalent whole-cell conductance was 40 times higher than in $[Ca^{2+}]_o$ -containing solutions, blocked in a voltage-dependent manner by 10 μM $[Ca^{2+}]_o$, and was inactivated by intracellular Mg^{2+} ions. But inactivation of the presumed monovalent CRAC current by $[Mg^{2+}]_i$, turns out to be very important, and ultimately distinguishes I_{CRAC} from the newly identified monovalent current they had unwittingly revealed.

Now, three recently published papers clearly show that the monovalent current, proposed by Cahalan and colleagues to be I_{CRAC} , differs significantly from bona fide I_{CRAC} (Hermosura et al., 2002; Prakriya and Lewis, 2002; Kozak and Cahalan, 2002, this issue). Hermosura et al. (2002) showed that the monovalent current (which they called MagNum, for magnesium- and nucleotide-regulated metal current) could be separated from I_{CRAC} by its kinetics and internal Mg^{2+} sensitivity. In a very detailed study in the May issue of the *Journal of General Physiology*, Prakriya and Lewis (2002) clearly separate CRAC and from the Mg^{2+} -inhibited current (which they called MIC). Finally, and again in the *Journal of General Physiology*, Kozak and Cahalan (2002) also show that the monovalent current is composed primarily of MIC channels that have intrinsic selectivity properties and can be selectively blocked or induced to run down.

It is apparent from the careful work of Prakriya and Lewis (2002) that I_{CRAC} 's single channel conductance, even in monovalent conditions, is <1 pS. This is an important finding for several reasons. First, Yue et al. (2001) proposed that the Ca^{2+} -selective TRPV6 is part of the CRAC channel, based partly on the similar monovalent single channel conductance measured in Cahalan and colleagues' previous work (e.g., Kerschbaum and Cahalan, 1999) and on TRPV6's unique inwardly rectifying (CRAC-like) whole-cell current-voltage relation. The small conductance of CRAC, even under monovalent conditions, mitigates against the conclusion that TRPV6 (CaT1) encodes the CRAC pore itself. It does not exclude the possibility that TRPV6 is part of a heteromeric protein comprising CRAC (but see also Voets et al., 2001). Second, the low conductance in monovalent conditions lacking Ca^{2+} brings up an interesting point about the highly Ca^{2+} -selective CRAC channel. Most Ca^{2+} channels (in partic-

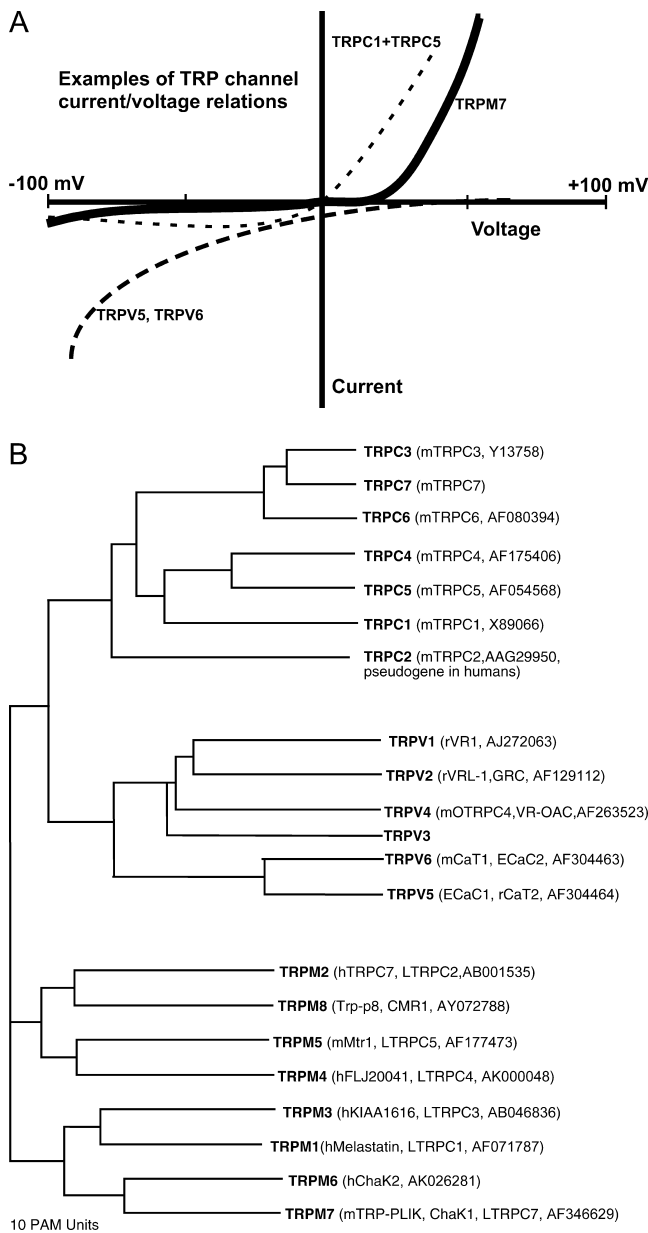


FIGURE 1. (A) Representative current-voltage relations of TRP currents. TRPM7 (TRP-PLIK, ChaK1) probably encodes MIC. Note that the TRPV5/6 I-V closely resembles that for I_{CRAC} . Also, TRPs may heteromultimerize to yield unique I-V relations (TRPC1+TRPC5). (B) TRP ion channel family.

ular the large class of voltage-dependent Ca_v s) are selective for Ca^{2+} because they have one or more high affinity sites for Ca^{2+} in the mouth of the pore, usually implemented by a ring of negatively charged (glutamates or aspartate) amino acids (Ellinor et al., 1995). CRAC somehow accomplishes its selectivity for Ca^{2+} without the need to bind external Ca^{2+} ions in its presumed pore. It is tantalizing to speculate that this feature implies a unique channel or transporter-like structure for CRAC. But further speculation about how

this is accomplished must await unequivocal identification of the molecule encoding CRAC.

If MIC is not CRAC, what is it? Jaded electrophysiologists are usually more annoyed than pleased by the appearance of small inward whole-cell currents, because they are difficult to distinguish from artifactual leak currents. With the wealth of K^+ channels that can produce large currents at positive membrane potentials, they are also inured to the gradual appearance of yet another outward current. But it is this combination of features that typifies many of the transient receptor potential TRP channels (Fig. 1 A). When expressed in heterologous systems, many of the known TRP channels produce relatively nonselective currents, small at potentials <0 mV, but steeply rectifying and large at positive potentials (Clapham et al., 2001). This current-voltage relationship is also grossly similar to that recorded from TRPV1–4, TRPM7, and TRPM8-expressing cells (Fig. 1, A and B). The short answer to the question posed at the beginning of this paragraph is that MIC is most likely encoded by TRPM7 (TRP-PLIK, ChaK1, LTRPC7) or its close relative TRPM6 (ChaK2). The argument that MIC is encoded by TRPM7 is best made by comparing the detailed work of the three original MIC papers and three papers on TRPM7 (Runnels et al., 2001, 2002; Nadler et al., 2001), but the relevant features that separate MIC from CRAC and align it with TRPM7 are summarized in Table I.

The first member of the TRPM group to be functionally expressed as an ion channel, TRPM7, has the unique feature of also being itself a protein kinase. TRPM7 is an 1,863 amino acid-containing protein identified in a yeast two-hybrid screen as a protein interacting with PLC- β_1 (Runnels et al., 2001, 2002). TRPM7 is a relatively nonselective, ~ 80 -pS (chord conductance at 100 mV) channel that exhibits a steeply outwardly rectifying conductance when expressed in mammalian cells (Nadler et al. 2001; Runnels et al., 2001). The mechanism of its activation is still unclear, but does not require the kinase domain for activity (Runnels et al., 2002; unpublished data). Receptor-mediated activation of PLC by hormones or growth factors inhibits channel activity by hydrolyzing and reducing local PIP_2 concentrations (Runnels et al., 2002). Most important for this story, TRPM7 is inhibited by $[Mg^{2+}]_i$ ($IC_{50} \sim 0.5$ mM; Nadler et al., 2001) and appears to be expressed in every cell examined, including blood cells and cell lines commonly used for expression. Like MIC, TRPM7's Mg^{2+} inhibition is not a simple blockade and may involve another diffusible molecule.

In hindsight, I_{CRAC} and I_{MIC} are not very similar. But, the apparently wide distribution of TRPM7 (presumably encoding I_{MIC}) and its presence in cell lines used for expressing novel channel genes, will continue to entrap unwary investigators. Given the varied state of MIC

T A B L E I
Comparison of I_{CRAC} , I_{MIC} , and I_{TRPM7}

Property	I_{CRAC}	I_{MIC}	I_{TRPM7}
Permeation			
Inhibition by free $[Mg^{2+}]_i$	No	$IC_{50} \sim 0.6$ mM	$IC_{50} < 0.5$ mM
Mg^{2+} permeability	Not measurable	Yes	Yes
P_{Ca}/P_{Na}	~ 0.1	~ 1	~ 1
$P_{Ca^{2+}}/P_{Na^+}$	$\sim 1,000$	ND	1-3
DVF chord γ to Na^+ at -110 (pS)	~ 0.2 pS	$\sim 40-45$ pS	ND (~ 80 pS in Ca^{2+} at 100 mV)
I-V			
With divalents in bath	Strong inward rectification	Strong outward rectification	Strong outward rectification
In DVF	Strong inward rectification	Near linear	ND
Gating			
Store depletion protocol activates	Yes	No	No
PIP_2 control	ND	ND	Yes
Kinetics			
Onset of current	Variable, 1-2 min in store depletion cond.	Variable $\sim 3-10$ min	Variable
Offset of current	Transient in DVF	Sustained in DVF	Sustained in DVF
Pharmacology			
Inhibition by SKF96365	90% at 20 μ M	10% at 20 μ M	ND
Inhibition by 2-APB	$IC_{50} = 10$ μ M irreversible (3 μ M increases)	$IC_{50} \sim 50$ μ M, reversible	ND

DVF, divalent free.

activation at the initiation of whole-cell recordings and the small size of inward currents, leak subtraction is particularly hazardous. Until the discovery of toxins or other agents that specifically block I_{MIC} , one can eliminate MIC by including high $[Mg^{2+}]_i$ in the pipette (e.g., 10 mM). However, this alone will not solve the problem of separating MIC from other ion channels that are potentially Mg^{2+} -sensitive, and confounds the use of Mg^{2+} binding moieties such as ATP (and thus the regulation of PIP_2). But the most difficult issue for future recordings will be separating MIC from other TRPs, particularly TRPV1-4 and TRPM8.

What can we learn from these MIC, CRAC, and TRPM7 studies? Almost all molecularly identified TRPs, as well as CRAC, dribble Ca^{2+} into cells at potentials < 0 mV. They are activated by as yet unclear mechanisms, many involving the complex phospholipase C and phosphatidylinositol pathways. Evidence is steadily emerging that these channels, eerily reminiscent of glutamatergic channels, are localized in signal transduction complexes by scaffolding proteins. My guess is that CRAC and TRP channels are tethered sources that provide localized Ca^{2+} increases for spatially defined signal transduction pathways. It seems doubtful that their main purpose is the repletion of intracellular Ca^{2+} stores. But for CRAC addicts the question remains, what gene or genes encode CRAC?

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