## TRPC channels: on-going discovery of molecular physiological function in relation to store operated $Ca^{2+}$ entry and Orai proteins

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The year 1988 saw the molecular cloning of the gene that, when mutated, was responsible for changing the receptor potential of *Drosophila*'s compound eye to what was termed a transient receptor potential (trp). The TRP protein was predicted to span the membrane six times with cytosolic N- and C-termini, no voltage sensor, but limited sequence similarity to voltage-gated $Ca^{2+}$ channels. The trp phenotype was shown in 1992 to be due to absence of a sustained $Ca^{2+}$ influx current. Visual signal transduction studies showed invertebrate light signal transduction to be akin to mammalian Gq-PLC-$IP_3$ signaling. These findings offered an hypothesis for the origin of capacitative $Ca^{2+}$ entry (CCE – now referred to as store operated $Ca^{2+}$ entry or SOCE) into cells. In 1992, an electrophysiological correlate to SOCE was discovered and called $Ca^{2+}$ release activated $Ca^{2+}$ current ($I_{crac}$). 1995 saw the molecular cloning of the first mammalian trp homologue (now referred to as TRPC, the classical or canonical TRP, of which there are seven). At present the broad TRP family of cation channels counts 31 members grouped into C, V, M, P, ML and A subfamilies. All TRP channels are of interest as they transduce sensory signals. Most are non-selective cation channels with variable selectivity for $Ca^{2+}$.

The roles of TRPC channels remain a matter of some speculation, having been replaced in the minds of many, if not most, researchers in the field by Orai channels (Orai1, 2 and/or 3). In contrast, as deduced from RNA and genetic suppression studies, TRPCs are being identified as critical in complex physiological and pathophysiological situations related to their non-selective cation channel properties which causes their activation to mediate: 1. membrane depolarization; 2. $Ca^{2+}$entry; 3. $Na^+$ entry; and 4. $K^+$ efflux. Membrane depolarization in turn activates voltage-gated ion channels that vary with the cell type studied contributing to the complexity of the cellular functions affected by TRPCs. Yet, involvement of TRPCs as essential components in store operated $Ca^{2+}$ entry and $I_{crac}$ has not been conclusively proven or disproven. There are competing hypotheses. One states that the CRAC channel is formed of Orai proteins (which span membranes four times), activated by the single transmembrane STIM proteins, that reside in the endoplasmic reticulium (ER) membrane, when ER $Ca^{2+}$ levels fall. This activation process involves clustering of STIM proteins, and promotion of assembly of the Orai channel at ER-PM junctional complexes to allow for $Ca^{2+}$entry. In support: a. Orai and STIM proteins are ubiquitously expressed; b. genetic ablation or suppressor RNA- induced down regulation of Orai1 and STIM1 leads to loss of SOCE and $I_{crac}$; c. co-expression of Orai1 and STIM1 leads to appearance of very large CRAC currents (*giant*Icrac) and very much enhanced SOCE (*monster*SOCE); and d. point mutations in Orai alter the cation selectivity profile of *giant*$I_{crac}$. The second hypothesis states that TRPCs are part of the molecular CRAC channel makeup and ascribe to Orai the role of a regulatory subunit of the TRPC channel. In support of TRPCs being part of CRAC channels: a. SOCE can be enhanced in a TRPC-dependent manner by Orai expression; b. neither SOCE nor $I_{crac}$ have ever been analyzed in TRPC-null cells; and c. TRPC-mediated $Ba^{2+}$ and [$Gd^{3+}$-resistant] $Ca^{2+}$ entry is inhibited by dominant negative forms of Orai (Orai1[R91W; Orai1[G98A]). STIM is viewed as interacting with both Orai and TRPC. A third hypothesis states that Orai CRAC channels co-exist with store activated TRPC channels, so-called SOCs. Here STIM activates Orai and TRPC in parallel. In sum, TRPCs are non-selective $Ca^{2+}$-permeable cation channels that participate in a large and varied number of physiological and pathophysiological processes. They may do so as free-standing STIM-activated channels or in complex with Orai molecules. It remains to be seen to what extent the TRPC functions depend on Orai molecules, and whether TRPCs and Orais form channels independently of each other other remains to be determined.

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