Capacitative calcium entry: sensing the calcium stores

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A long-standing mystery in the cell biology of calcium channel regulation is the nature of the signal linking intracellular calcium stores to plasma membrane capacitative calcium entry channels. An RNAi-based screen of selected *Drosophila* genes has revealed that a calcium-binding protein, stromal interaction molecule (STIM), plays an essential role in the activation of these channels and may be the long sought sensor of calcium store content.

Virtually all cell types depend in some manner upon the generation of cytoplasmic Ca^{2+} signals to regulate cell function, or to trigger specific responses. Usually, these signals involve some combination of release of Ca^{2+} from intracellular stores and influx of Ca^{2+} across the plasma membrane. The release of Ca^{2+} from intracellular stores is often signaled by the messenger inositol 1,4,5-trisphosphate (IP_3), and additionally by a process of calcium-induced calcium release (Berridge, 1997). The influx of Ca^{2+} across the plasma membrane can be signaled by a variety of mechanisms (Barritt, 1999). In most cell types, depletion of intracellular Ca^{2+} stores signals the activation of capacitative calcium entry, occurring through store-operated calcium channels (Putney, 1997). However, the nature of the store-operated channels as well as the mechanism linking their activation to the Ca^{2+} content of intracellular stores has remained a mystery. Now, Roos et al. (2005) provide exciting new information on a key player in this elusive mechanism (on page 435 of this issue).

The authors used an RNAi screen in *Drosophila* S2 cells using thapsigargin-activated Ca^{2+} entry as a marker for store-operated channels. They screened 170 genes, including a number of transient receptor potential genes, other known calcium permeable channel genes, and a number of genes for potentially interacting signaling molecules. One gene gave a substantially reduced Ca^{2+} entry, coding for the protein stromal interaction molecule (STIM). Direct measurement of the store-operated current in S2 cells confirmed that the Ca^{2+}-release–activated Ca^{2+} current (I_{crac}) was essentially null in STIM knockdown S2 cells. There are two homologues of STIM in mammalian cells, STIM1 and STIM2, both of which appear to be distributed ubiquitously (Williams et al., 2001). Knockdown of STIM1 by RNAi substantially reduced I_{crac} in Jurkat T cells, and store-operated Ca^{2+} entry in HEK293 epithelial cells and SH-SY5Y neuroblastoma cells. However, knockdown of the closely related STIM2 had no effect.

These results make a strong case for an essential role of STIM (*Drosophila*) and STIM1 (mammals) in the mechanism of activation of store-operated channels. It is unlikely that STIM1 is the store-operated channel itself. It has no channel-like sequence, and overexpression of the protein only modestly enhanced Ca^{2+} entry. The obvious next question, then, is: what role does STIM1 play? Clues to the action of STIM1 may come from its domain structure and cellular localization. Apparently, the protein is located both on the plasma and intracellular membranes (Manji et al., 2000), presumably the ER. The protein sequence suggests that it spans the membrane once, with its NH_2 terminus oriented toward the lumen of the ER or the extracellular space (Fig. 1). The NH_2 terminus contains an EF-hand domain, and thus, as Roos et al. (2005) point out, the protein could function as the long-sought Ca^{2+} sensor in the ER. The protein also contains protein–protein interaction domains, notably coiled-coiled domains in the cytoplasm and a sterile α motif (SAM) in the ER (or extracellular space), both near the predicted transmembrane domain (Fig. 1). STIM1 can oligomerize and Roos et al. (2005) speculate that the protein in the ER and plasma membrane could interact bridging the two. This idea is reminiscent of the conformational coupling hypothesis (Irvine, 1990; Berridge, 1995), according to which ER stores communicate with the plasma membrane by means of protein–protein interactions. Finally, STIM1 has an extended COOH terminus that contains a proline/serine-rich domain and a lysine-rich domain. However, most of the sequence downstream of the coiled-coil domain is missing in *Drosophila* STIM, indicating that it is not of prime importance in store-operated Ca^{2+} entry.

A proposed mechanism considered as an alternative to the conformational coupling hypothesis involves the action of a diffusible signal, a calcium influx factor (CIF; Randriamampita and Tsien, 1993; Kim et al., 1995). CIF is believed to act through activation of a Ca^{2+}-independent phospholipase A_2 (Smani et al., 2004); however, how the formation of CIF is triggered in response to depletion of ER Ca^{2+} stores is not known, and STIM1 could conceivably play a role in activating its formation.

The details of the precise action of STIM1 in capacitative calcium entry are far from clear at present. But it is the early

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Abbreviations used in this paper: CIF, calcium influx factor; IP_3, inositol 1,4,5-trisphosphate; SAM, sterile α motif; STIM, stromal interaction molecule.

The Journal of Cell Biology, Vol. 169, No. 3, May 9, 2005 381–382
http://www.jcb.org/cgi/doi/10.1083/jcb.200503161

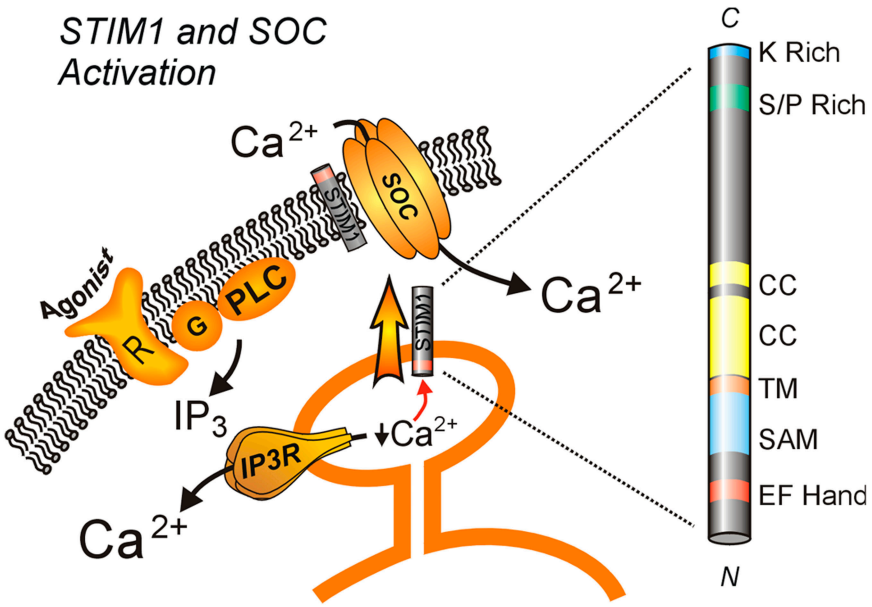

Figure 1. **STIM1 and SOC activation.** In this example, cell activation begins with an agonist binding to a surface membrane receptor (R), coupled to PLC through a G-protein (G) mechanism. PLC activation leads to the production of IP_3, which in turn activates the IP_3 receptor (IP_3R) causing release of Ca^{2+} from a critical compartment of the ER. The fall in ER Ca^{2+} then signals to plasma membrane store-operated channels (SOC) through a mechanism that involves STIM1 in the ER, plasma membrane, or both. The structure of STIM1 includes an EF hand and SAM domain NH_2-terminal to a single transmembrane (TM) domain; these domains would face the lumen of the ER and extracellular space, and the EF hand in particular may be involved in sensing ER Ca^{2+} levels, or in Ca^{2+} regulation at the plasma membrane. COOH-terminal to the TM domain are two coiled-coil (CC) domains, and a serine/proline-rich (S/P Rich) and lysine (K Rich) domain. The EF hand domain is shown in red on the signaling diagram as well as the domain map to indicate the presumed orientation of the protein across ER and plasma membranes.

days. The discovery of a key player in this ubiquitous signaling pathway no doubt opens the way for intriguing new disclosures in the very near future.

Submitted: 29 March 2005
Accepted: 4 April 2005

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