CALCIUM

# An effective get-together

> …a new mechanism for ion channel activation…

The release of calcium ($Ca^{2+}$) from the endoplasmic reticulum (ER) is followed by the influx of $Ca^{2+}$ across the plasma membrane through $Ca^{2+}$-release-activated $Ca^{2+}$ (CRAC) channels, which are crucial for sustained $Ca^{2+}$ signalling in many cell types. Three studies now provide new insights into how $Ca^{2+}$ depletion is sensed in the ER and discover a unique mechanism for ion channel assembly and activation.

The activity of CRAC channels relies on the ER transmembrane protein stromal interaction molecule-1 (STIM1), which senses the depletion of $Ca^{2+}$ in the ER and physically relays this information to the plasma membrane protein ORAI1, which forms the pore of the $Ca^{2+}$ channel.

Using nuclear magnetic resonance (NMR), Stathopulos *et al.* determined the three-dimensional atomic structure of the $Ca^{2+}$-sensing region of STIM1, which consists of the EF-hand and sterile α-motif (SAM) domains (EF–SAM domains). They found a 'hidden' EF-hand domain that stabilizes the EF–SAM intramolecular interaction. Mutations that alter the structure of the SAM, EF-hand or hidden EF-hand domains unfold the EF–SAM domain and disrupt $Ca^{2+}$ sensitivity. Furthermore, they induce $Ca^{2+}$ flux independently of the ER $Ca^{2+}$ store. So, the conformation of the STIM1 $Ca^{2+}$-sensing region is crucial for store-operated $Ca^{2+}$ entry.

Penna *et al.* transfected haemagglutinin (HA)-tagged *Drosophila melanogaster* Orai into S2 cells and performed chemical crosslinking assays on intact cells and total cell lysates. They found that Orai forms homodimers in the plasma membrane of resting cells. Similar results were obtained in a human kidney cell line using gel electrophoresis under non-dissociating conditions.

Expression of the cytosolic C-terminal region of Stim (C-Stim), together with green fluorescent protein (GFP)-tagged Orai, showed colocalization of the two proteins and induced constitutive $Ca^{2+}$ influx in S2 cells without causing $Ca^{2+}$ store depletion. Furthermore, C-Stim induced homomultimeric association of Orai in biochemical assays. So, the oligomeric state of Orai depends on C-Stim binding and the C-Stim–Orai interaction is independent of the ER $Ca^{2+}$ store content.

In an independent study, Ji *et al.* used single-molecule imaging and fluorescence resonance energy transfer (FRET) to study the assembly of functional CRAC channels, and found that four human ORAI1 subunits and two STIM1 molecules assemble to form the active channel. However, Ji *et al.* also found that ORAI1 formed tetramers in resting cells.

Penna *et al.* then investigated the mechanism that underlies the assembly and activation of CRAC channel. Imaging of single molecules of GFP–Orai that were expressed in *Xenopus laevis* oocytes by total internal reflection microscopy (TIRFM) and photobleaching confirmed the dimeric basal state of Orai that was initially observed in S2 cells. Furthermore, coexpression of C-Stim induced the assembly of GFP–Orai into tetramers that constitute the active CRAC channel.

Together, these data illustrate a new mechanism for ion channel activation, which requires an activator protein (STIM1) to assemble and open the ORAI1 tetrameric channel in the plasma membrane.

*Francesca Cesari*

**ORIGINAL RESEARCH PAPERS** Penna, A. *et al.* The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 28 Sept 2008 (doi:10.1038/nature07338) | Ji, W. *et al.* Functional stoichiometry of the unitary calcium-release-activated calcium channel. *Proc. Natl Acad. Sci. USA* **105**, 13668–13673 (2008) | Stathopulos, P. B. *et al.* Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell* **135**, 110–122 (2008)