

## Unlocking SOAR releases STIM

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**A crucial component of the receptor-evoked Ca<sup>2+</sup> signal is Ca<sup>2+</sup> influx mediated by the store-operated Ca<sup>2+</sup> channels (SOCs). The molecular makeup of one SOC is the endoplasmic reticulum (ER) Ca<sup>2+</sup> sensor STIM1 and the pore-forming Orai1. Ca<sup>2+</sup> release from the ER leads to co-clustering of STIM1 and Orai1 to activate Orai1. The short STIM1 SOAR/CAD domain (STIM1 Orai1-activating region/CRAC-activating domain), which has two coiled-coil (C–C) domains, interacts with the Orai1 C terminus C–C domain to activate the channel. How the function of SOAR is regulated is not known. Korzeniewski *et al* (2010) and Muik *et al* (2011; this issue) now identified an auto-inhibitory domain in STIM1 that occludes SOAR. Release of SOAR involves a conformational transition that is aided by the Orai1 C–C domain.**

The receptor-evoked Ca<sup>2+</sup> signal initiates with Ca<sup>2+</sup> release from internal stores and is followed by activation of Ca<sup>2+</sup> influx by the store-operated channels (SOCs), which is required for sustaining the Ca<sup>2+</sup> signal and thus all Ca<sup>2+</sup>-dependent functions (Parekh and Putney, 2005). The molecular makeup of the SOCs was revealed with the discovery of the endoplasmic reticulum (ER) Ca<sup>2+</sup> sensor STIM1 (Liou *et al*, 2005; Roos *et al*, 2005) and the Ora1s (Hogan *et al*, 2010). Since then, rapid progress was made in defining the domains and function of STIM1 and Orai1. STIM1 has a single transmembrane span with an N-terminus EF hand and SAM domain that reside in the ER lumen. The STIM1 cytoplasmic C terminus contains three coiled-coil (C–C) domains (CC1, CC2 and CC3), with CC2 and CC3 residing within SOAR/CAD that fully activate the Ora1s (Park *et al*, 2009; Yuan *et al*, 2009), an S/P domain and a polybasic domain (Figure 1A). Orai1 is a four transmembrane-span protein that assembles into a tetramer to form the channel pore (Hogan *et al*, 2010).

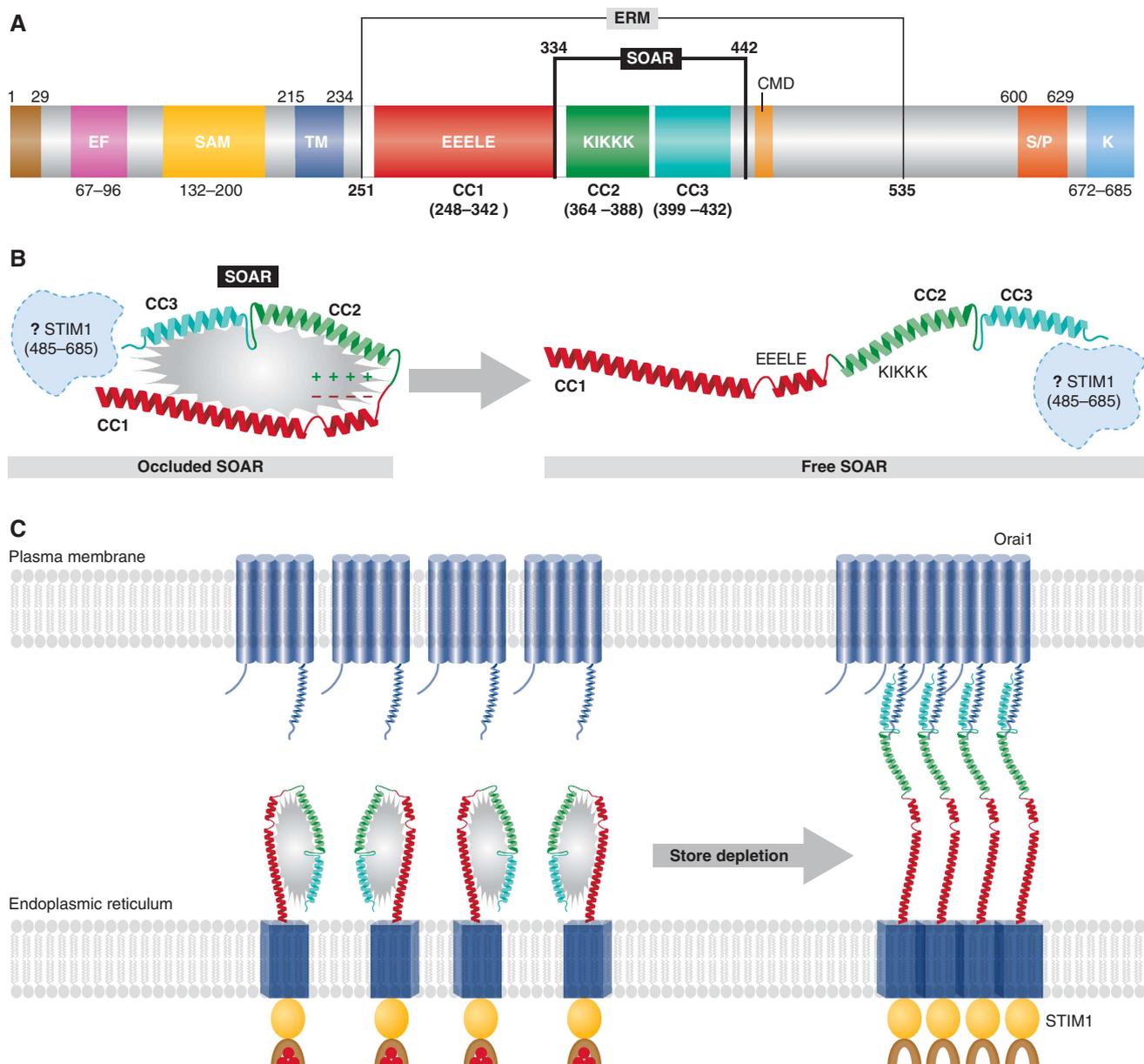
STIM1 is obligatory for Orai1 functioning. Ca<sup>2+</sup> store depletion and dissociation from the EF hand leads to assembly of STIM1 into multimers that cluster with Orai1 to form Ca<sup>2+</sup> influx units. Domain analysis revealed that the Orai1 C terminus likely folds into a C–C and disruption of the C–C prevents activation of Orai1 by STIM1 (Muik *et al*, 2008). Although STIM1 C terminus can activate Orai1 (Huang *et al*, 2006), the 100-residue SOAR/CAD is all that is needed to fully activate Ora1s (Park *et al*, 2009; Yuan *et al*, 2009). Moreover, SOAR/CAD is more effective than STIM1 C terminus in activating Orai1 (Korzeniewski *et al*, 2010). The SOAR/CAD domain encompasses CC2 and CC3 of STIM1 (Figure 1A) and

interacts with the C (Park *et al*, 2009; Yuan *et al*, 2009) and N terminus (Park *et al*, 2009) of Orai1 to activate the channel. Disruption of Orai1 C–C domain prevents interaction with and activation of Orai1 by SOAR (Yuan *et al*, 2009).

The finding that SOAR activates Orai1 better than STIM1 C terminus raised the possibility that SOAR is occluded in STIM1, and it has to be released by a conformational transition to activate Orai1. Two recent independent studies by Korzeniewski *et al* (2010) and Muik *et al* (2011) addressed this possibility in two different approaches and arrived at a similar conclusion.

The study by Korzeniewski *et al* (2010) reported that STIM1 fragments that include SOAR and STIM1 CC1 were less active than SOAR in activating Orai1. They noticed that the STIM1 CC1 contains an acidic patch while SOAR has a basic patch (Figure 1B), and the CC1 acidic patch has spacing similar to an acidic patch in the C terminus of Orai1. This led to the hypothesis of occlusion of SOAR by the STIM1 CC1 and competition between CC1 and Orai1 acidic patches for interaction with SOAR. Dissociation of the CC1/SOAR is needed for interaction of SOAR with Orai1 C terminus and activation of the channel. Accordingly, neutralization of the CC1 acidic patch resulted in constitutively active STIM1 and STIM1 fragments that included CC1, while neutralization of the SOAR basic patch resulted in inactive STIM1 and fragments. Significantly, targeting STIM1(238–343) containing only the CC1 acidic patch to the plasma membrane inhibited activation of Orai1 by STIM1 fragments (Korzeniewski *et al*, 2010). Korzeniewski *et al* concluded that, in the resting state, SOAR is occluded by electrostatic interaction between the CC1 acidic patch and the SOAR basic patch, and activation of Orai1 by STIM1 requires release of SOAR (Figure 1B).

How the occluded SOAR might be released and whether a conformational transition is involved was directly addressed by Muik *et al* (2011) using the STIM1 conformational sensor YFP-OASF-CFP. Intramolecular interaction resulted in compact folding of YFP-OASF-CFP and strong FRET. As interaction of C–C domains is stabilized by hydrophobic interactions, Muik *et al* inserted mutations that disrupt the C–C domains and/or hydrophobic interactions in YFP-OASF-CFP. The mutants reduced YFP-OASF-CFP FRET, altered the CD spectra and increased the mobility of OASF, indicating extended conformation. Most notably, plasma membrane Orai1 with intact C–C domain facilitated transition of YFP-OASF-CFP to the extended conformation and bound better to YFP-OASF-CFP mutants with extended conformation. They conclude that



**Figure 1** The STIM1 domains (A) and potential activation mechanism of Orai1 by SOAR (B, C). (A) C-C, coiled-coil; CMD, CRAC modulatory domain; EF, EF hand; ERM, Ezrin/radixin/moesin; K, lysine-rich domain; SAM, sterile  $\alpha$ -motif; SOAR, STIM1 Orai1-activating region; S/P, proline-, serine-rich segment; TM, transmembrane. Also shown are the acidic glutamate (residues 318–322) and basic lysine (residues 382–386) patches. (B) CC2 and CC3 are folded with CC1, and the interaction is stabilized by hydrophobic (grey star) and electrostatic interactions. STIM1(485–685) may (?) also participate in stabilizing the occluded SOAR state (blue domain). (C) The interaction of CC2 and CC3 with CC1 occludes SOAR.  $\text{Ca}^{2+}$  release from the ER clusters STIM1 in a microdomain with Orai1 to facilitate interaction of the folded STIM1 C-C domains with Orai1 C terminus. This releases SOAR that activates Orai1 to initiate  $\text{Ca}^{2+}$  influx.

hydrophobic interactions between CC1 and SOAR occlude SOAR, and release of SOAR is facilitated by interaction of CC1 with Orai1 C-C domain (Figure 1C).

A twist in the theme may be provided by a recent study examining the expression and function of STIM1(1–485) (Yu *et al*, 2011). Cytoplasmic STIM1 constructs that terminate at residue 485 are constitutively active (Yuan *et al*, 2009) and STIM1(1–463) retains some constitutive activity (Korzeniowski *et al*, 2010). The activity of STIM1(1–485) could not be assayed directly as it remains in the ER, where it traps Orai1, suggesting that the SOAR domain of STIM1 (1–485) is in the unoccluded state. Remarkably, fusing mCherry or GFP to the C terminus of STIM1(1–485) rescued

its trafficking and the occluded SOAR state (Yu *et al*, 2011), suggesting that the C terminus end of STIM1 participates in SOAR occlusion (blue domain in Figure 1B).

The combined findings lead to the model in Figure 1C. In the resting state, the STIM1 EF hand is bound with  $\text{Ca}^{2+}$  to maintain STIM1 in unclustered state. The STIM1 C-C domains are in compact fold, with CC1 interacting with CC2 and CC3 hydrophobically and electrostatically.  $\text{Ca}^{2+}$  release from the ER leads to clustering of STIM1 and co-clustering with Orai1 to allow access of the Orai1 C-C domain to the folded CC1–SOAR. The Orai1 C-C domain can now effectively compete with STIM1 CC1 to unwind the STIM1 C-C domain fold, release and bind SOAR and activate Orai1

and  $\text{Ca}^{2+}$  influx. Such an arrangement may serve to ensure tight coupling of  $\text{Ca}^{2+}$  influx to  $\text{Ca}^{2+}$  release so that only when STIM1 and Orai1 are co-clustered will SOAR be in the unoccluded state. This can also reduce  $\text{Ca}^{2+}$  leak in the face of the large inward  $\text{Ca}^{2+}$  gradient.

The autoinhibitory mechanism of STIM1 by CC1, discovered by Korzeniewski *et al* (2010) and Muik *et al* (2011; this issue), adds considerably to the mechanism by which STIM1 activity and  $\text{Ca}^{2+}$  influx are regulated. We now need to understand the molecular mechanism by which  $\text{Ca}^{2+}$  store

depletion and receptor stimulation releases SOAR and the exact role of Orai1 in SOAR release. Other open questions are the role of STIM1 C terminus end in this regulation and whether other STIM1 domains participate in the regulation, as may be suggested by the reduced FRET of N and C terminally extended YFP-OASF-CFP.

## Conflict of interest

The authors declare that they have no conflict of interest.

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