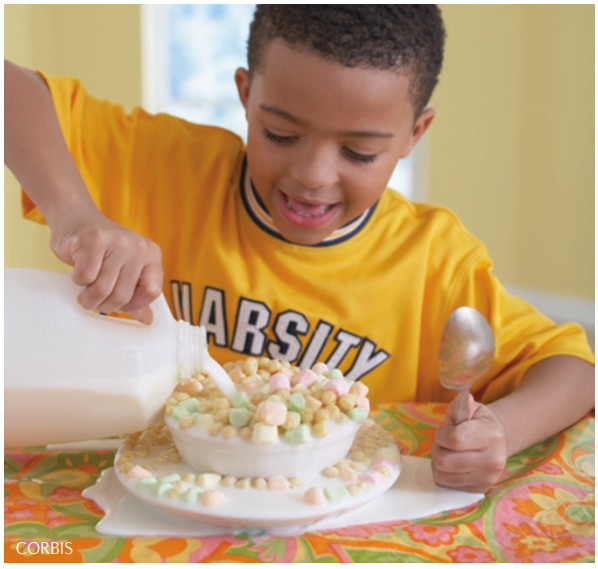
SIGNALLING

Stop refilling (Ca^{2+} stores)

Store-operated Ca^{2+} entry (SOCE) is the cellular response to loss of Ca^{2+} from the endoplasmic reticulum (ER), which allows ER Ca^{2+} refilling following Ca^{2+} influx through the plasma membrane. SOCE is regulated to determine the magnitude and duration of Ca^{2+} influx. The mechanisms that inactivate SOCE are not entirely understood, and Palty *et al.* now identify SARAF (SOCE-associated regulatory factor) as a factor that promotes slow inactivation of SOCE and prevents excess Ca^{2+} refill.

Intracellular Ca^{2+} functions as a signal that regulates a wide range of cellular functions. Following stimulation, cells can rapidly release Ca^{2+} from the ER, the main intracellular Ca^{2+} storage site, and this is followed by a slower Ca^{2+} entry from outside the cell. Ca^{2+} entry through the plasma membrane is coupled to Ca^{2+} release from the ER by Ca^{2+} release-activated Ca^{2+} (CRAC) channels. STIM and ORAI proteins are essential components of CRAC channels, with ORAI forming a channel at the plasma membrane, and STIM residing at the ER membrane. STIM proteins detect changes in ER Ca^{2+} levels and, following Ca^{2+} depletion in the ER, they oligomerize and relocalize at ER–plasma membrane junctions, where they bind to ORAI and cause the opening of the plasma membrane channels, thus promoting SOCE.

> SARAF function seems to prevent cell overloading with excessive Ca^{2+}



CORBIS

Palty *et al.* performed a functional expression screen by transfecting a cDNA library into human embryonic kidney (HEK) cells, and identified a clone that alone could lower basal mitochondrial Ca^{2+} levels and greatly decrease ER and cytosolic Ca^{2+} levels. This clone was found to code for SARAF. Interestingly, fluorescently labelled SARAF colocalized with ER markers, indicating that the ER is the primary subcellular localization of SARAF.

To test whether SARAF regulates SOCE, the authors heterologously expressed STIM1 and ORAI1 in HEK cells and measured Ca^{2+} influx through CRAC channels in response to Ca^{2+} depletion from the ER in the presence or absence of SARAF. Although Ca^{2+} influx was detected in both cases, SOCE was more rapidly inactivated in the presence of SARAF. Importantly, the addition of Ca^{2+} chelators showed that CRAC channel inactivation required increased free cytosolic Ca^{2+} levels, whereas the use of inhibitors of ER Ca^{2+} refilling indicated that store refilling contributes, in part, to SOCE inactivation. Thus, SARAF regulates Ca^{2+} entry through CRAC channels, and this depends on both cytosolic Ca^{2+} levels and ER Ca^{2+} levels.

So how does SARAF regulate SOCE? Following ER Ca^{2+} depletion, SARAF translocated, in a STIM1-dependent manner, to the sites of STIM1–ORAI1 aggregation at the specialized ER–plasma membrane junctions, which suggests that SARAF directly inhibits SOCE. Human SARAF is predicted to have a single membrane-spanning domain, a cytosol-facing domain and an ER lumen-facing domain. Truncation experiments showed that the cytosolic domain is responsible for SOCE inactivation, whereas the luminal domain has an autoregulatory function, as its deletion led to strong inhibition of SOCE regardless of ER Ca^{2+} levels.

Using a FRET (fluorescence resonance energy transfer)-based approach, Palty *et al.* found that SARAF and STIM1 interacted during resting conditions, and that SOCE activation triggered molecular rearrangements between SARAF and STIM1. These rearrangements were ORAI-dependent and reduced the numbers of active STIM1 oligomers that underlie SOCE activation following Ca^{2+} store refilling. Moreover, when Ca^{2+} ER stores were refilled, the rearranged interaction between SARAF and STIM1 was reversed. Together, these results suggest that the association of SARAF with STIM1 facilitates the disaggregation of STIM1 oligomers to efficiently turn off ORAI1 when the ER lumen has been filled with Ca^{2+}. However, further studies are required to elucidate the molecular mechanisms underlying the capacity of SARAF to sense Ca^{2+} levels and to directly inactivate SOCE.

SARAF function seems to prevent cell overloading with excessive Ca^{2+}, and this may be therapeutically relevant for conditions that are accompanied by abnormal responses to intracellular Ca^{2+} levels such as Alzheimer's disease and prostate cancer.

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ORIGINAL RESEARCH PAPER Palty, R. *et al.* SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling. *Cell* 29 Mar 2012 (doi:10.1016/j.cell.2012.01.055)