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Calcium Signals: STIM Dynamics Mediate Spatially Unique Oscillations

Receptor-induced Ca^{2+} oscillations provide 'digitized' signals that confer precise activation of downstream targets. New studies reveal that STIM proteins — sensors of endoplasmic reticulum Ca^{2+} levels — cyclically translocate during oscillations, transiently coupling to activate cell-surface Ca^{2+} entry channels, resulting in a spatially unique signal that selectively triggers immediate-early gene expression.

Salvatore Mancarella, Youjun Wang, and Donald L. Gill

Calcium signals, crucial to the control of a plethora of cellular functions, involve extraordinary spatial and temporal precision within cells [1]. In most cells, physiological receptor activation induces repetitive oscillations of cytosolic Ca^{2+} mediated by cyclic release of Ca^{2+} from endoplasmic reticulum (ER) stores [2,3]. These 'digital' Ca^{2+} signals confer unique specificity, sensitivity and accuracy in the activation of downstream target functions [3]. As ER Ca^{2+} stores empty, Ca^{2+} enters through highly specific store-operated channels (SOCs) in the plasma membrane that are controlled by STIM proteins, sensors of ER luminal Ca^{2+} levels [4]. Rather than merely replenishing depleted stores, it has recently been revealed that Ca^{2+} entry through SOCs contributes crucially to the spatial signature of Ca^{2+} oscillations [5]. Indeed, in a study by Bird *et al.* [6] in this issue of *Current Biology*, the STIM1 protein is now shown to translocate cyclically in and out of ER–plasma membrane junctions during each Ca^{2+} oscillatory spike. This STIM-mediated Ca^{2+} entry component of the digitized Ca^{2+} signals appears to be crucial for the Ca^{2+}-mediated control of gene expression [5,6].

In all cells, Ca^{2+} release from ER stores and Ca^{2+} entry through SOCs in the plasma membrane are highly coordinated events [2,4]. Indeed, the dynamic ER Ca^{2+}-sensing STIM proteins (STIM1 and STIM2) are remarkable integrators of the two processes [4,7,8]. STIM proteins detect subtle changes in ER luminal Ca^{2+} levels and undergo profound migration within the ER membrane to enter ER–plasma membrane junctions, where they trap and activate members of the Orai family of highly Ca^{2+}-selective SOCs [4,7–10]. The activation and function of STIM1 and STIM2 proteins is significantly different [11,12]. STIM1 requires quite substantial Ca^{2+} release from the ER to undergo translocation, but activates opening of Orai channels very efficiently [4]. In contrast, STIM2 appears sensitive to small changes in ER luminal Ca^{2+} and rapidly translocates into ER–plasma membrane junctions with minimal store depletion [6,12]; however, STIM2 is poor at activating Orai channels [6,13] and, when overexpressed, has a dominant-inhibitory effect on channel activation [11].

The function of STIM proteins and their translocation and coupling to Orai channels has mainly been studied in response to substantial emptying of ER Ca^{2+} stores activated, for example, by high levels of agonists for phospholipase C (PLC)-coupled receptors that produce large global increases in inositol (1,4,5) trisphosphate ($InsP_3$) levels, or by applying the powerful ER Ca^{2+} pump blocker thapsigargin [4]. However, these are non-physiological conditions. Instead, cells *in vivo* are exposed to much lower levels of receptor agonist, resulting in lower $InsP_3$ production, which may remain local to the cell periphery and penetrate less deeply into the cell interior. The new study of Bird *et al.* [6] and the recent work by Di Capite *et al.* [5] examined the function of SOCs under physiological agonist activation conditions in which continuous oscillations of Ca^{2+} are observed, driven by fluctuating $InsP_3$-induced Ca^{2+} release through $InsP_3$ receptors ($InsP_3Rs$) in the ER membrane. The repetitive Ca^{2+} spikes are regenerative ER Ca^{2+} release events thought to involve complex negative and positive feedback of Ca^{2+} on the $InsP_3R$ [2,3].

Clearly, external Ca^{2+} entry is required for the oscillations to continue: without such entry, stores cannot refill and the oscillations run down and cease. Although the Ca^{2+} entry route had been debated, it is now clear that SOCs mediate the entry to maintain oscillations [6,14,15]. The elimination of STIM1 or Orai1 channels suppresses the oscillations [6,15]. Since each spike induced by low levels of agonist represents a rather small total release of ER Ca^{2+}, it might have been expected that the more 'sensitive' Ca^{2+} sensor, STIM2, would be the major instigator of the replenishing Ca^{2+} entry process. Surprisingly, only knockdown of STIM1, and not STIM2, inhibited Ca^{2+} oscillations [6]. An interesting inference can be drawn from this observation. Given that STIM1 needs a more substantial discharge of luminal Ca^{2+} to be activated [6,12], the rather small release events detected by STIM1 during Ca^{2+} spikes likely represent large decreases of Ca^{2+} in just a relatively few discrete Ca^{2+} stores (Figure 1), as opposed to a more global ER Ca^{2+} release event involving a relatively small overall change in luminal Ca^{2+}. The results could also suggest that these local stores are predominantly localized at the cell periphery, likely in close proximity to pre-existing ER–plasma membrane junctions into which STIM1 molecules could easily and rapidly move to activate Orai channels. Indeed, these might also be the stores closest to the source of $InsP_3$ production, as depicted in Figure 1.

The studies of Bird *et al.* [6] provide remarkable evidence for the movement of STIM proteins during Ca^{2+} oscillations and also reveal the proximity of discrete plasma-membrane-associated subsets of ER. The authors used total internal reflection microscopy to view STIM1 (tagged with enhanced yellow fluorescent protein, EYFP), in the ER undergoing translocation within close proximity (<100 nm) of the plasma membrane, while simultaneously recording low-agonist-induced Ca^{2+} oscillations in the cytosol. A small amount of the EYFP–STIM1 was clearly observed to

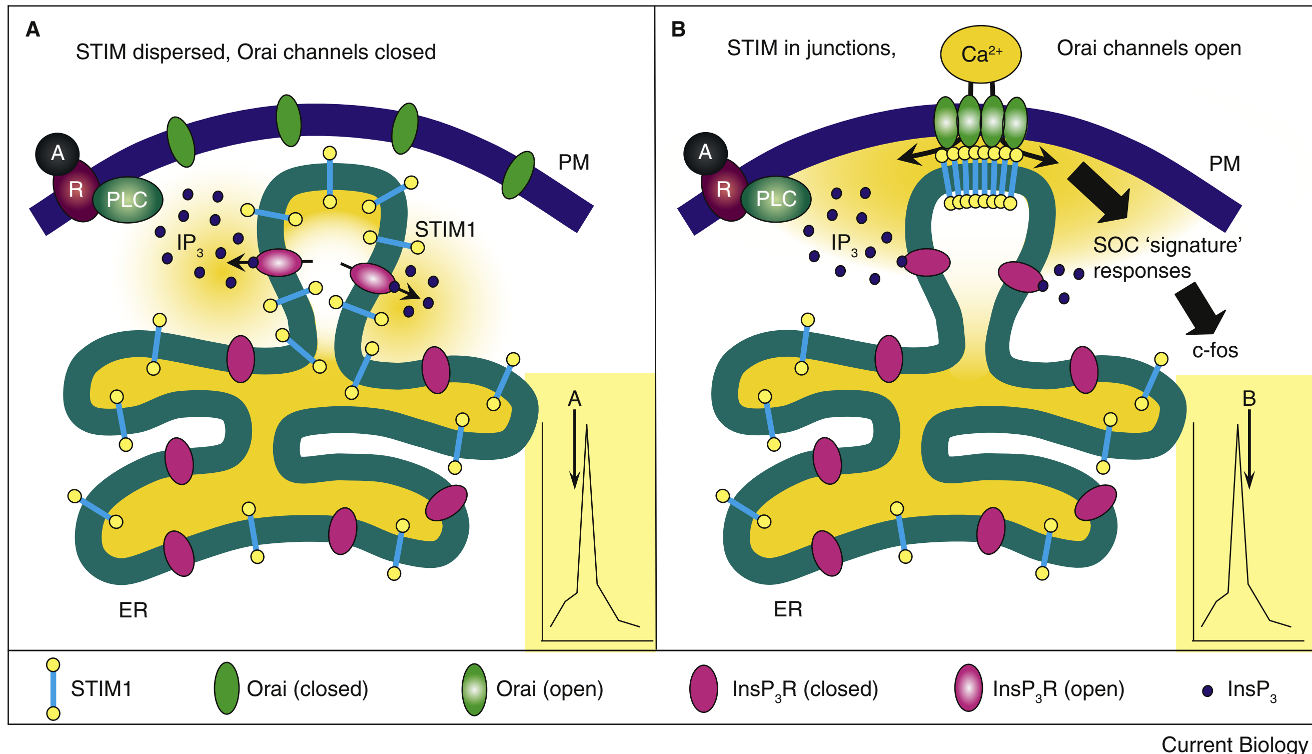

Figure 1. Coordination of spatially distinct Ca^{2+} signals during oscillations.

The figure shows a hypothetical model depicting the coordinated opening of $InsP_3R$ Ca^{2+} release channels in the ER and store-operated Orai Ca^{2+} entry channels in the plasma membrane (PM) during different phases of oscillations induced by submaximal stimulation of PLC-coupled receptors. (A) During the initial rising phase of spikes (see inset), activation of PLC-coupled receptors (denoted by R) by agonist (denoted by A) results in locally increased $InsP_3$ levels opening $InsP_3Rs$ predominantly in peripheral ER. The rise in cytoplasmic Ca^{2+} represents rapid Ca^{2+} release and depletion of stores near the plasma membrane, while the bulk of the ER remains Ca^{2+} replete without compromising crucial ER functions. (B) During the falling phase, Ca^{2+} has been exhausted and/or $InsP_3Rs$ are deactivated. The substantial luminal Ca^{2+} decrease surpasses the Ca^{2+}-sensing threshold required for STIM1 activation, causing STIM1 molecules to aggregate and translocate into ER–plasma membrane junctions. There, the STIM1 carboxyl termini lie within 10–20 nm of the plasma membrane where they bind, trap and activate Orai channels within the plasma membrane. Ca^{2+} enters through the Orai channels where it provides a spatially unique local Ca^{2+} signaling 'signature' required for triggering a number of crucial processes, including immediate early gene expression. The entering Ca^{2+} also bathes the region of the depleted stores and is pumped back to effect their refilling. The STIM1 Ca^{2+} sensor is saturated causing a rapid reversal of Orai activation, disaggregation of STIM1 and diffusion of STIM1 away from junctions. The cyclical movement of STIM1 is hence an important part of controlling the generation of Ca^{2+} oscillations. The specific spatial 'signature' of SOC-mediated Ca^{2+} entry provides a unique signal controlling gene expression.

translocate into ER–plasma membrane junctions then move away again during each oscillation. This is consistent with studies indicating that STIM1 molecules act locally, moving on average less than 2 μm to reach ER–plasma membrane junctions [16]. The oscillation-driven STIM1 translocation was found to be even more pronounced if Ca^{2+} were excluded from the cytosol [6]. In this case, STIM1 appeared to continue to accumulate to even higher levels during the course of Ca^{2+} oscillations. Obviously, with no Ca^{2+} outside, the movement of STIM1 molecules to activate Orai channels results in no entry of Ca^{2+} and hence no replenishment of stores. Presumably, in this case, the flight of STIM1 molecules into junctional ER is essentially irreversible and they continue to accumulate. The lack of Ca^{2+} entry prevents the stores from refilling and the oscillations wind down.

Interestingly, EYFP-tagged STIM2 molecules expressed in cells also accumulated into junctions close to the plasma membrane during oscillations [6]. The STIM2 translocation initiated a little faster than that of STIM1 during the onset of oscillations and was not as enhanced as that of STIM1 following Ca^{2+} removal. This would be consistent with the more constitutive ability of STIM2 to enter junctions, with greater store depletion having little further effect. However, as stated above, STIM2 does not effectively couple with endogenous Orai channels to mediate Ca^{2+} entry. Whereas Bird *et al.* [6] suggest that this is because the STIM2 molecule is intrinsically less able to couple with Orai channels, our own recent studies militate against a simple coupling difference between STIM1 and STIM2 [17]: the cytoplasmic carboxy-terminal Orai-interacting domains of STIM1 and STIM2 can each bind to and activate Orai1 channels [17]. The functional differences between STIM1 and STIM2 lie more within their amino-terminal ER luminal regions. Although reportedly more sensitive to ER Ca^{2+} changes [12], the difference in affinity between the Ca^{2+}-binding EF-hands of STIM1 and STIM2 is not substantial [18]. Instead, a small amino-terminal

domain that differs between STIM1 and STIM2 may confer substantial differences in the ability of STIM1 and STIM2 to aggregate and conformationally couple to Orai channels [13,19]. Hence, STIM2 can have a dominant-inhibitory effect by virtue of it being a poor partial agonist in the activation of Orai channels. Certainly, STIM2 is more constitutively 'available' at ER–plasma membrane junctions, but this may be a result of poor clearance from junctions rather than increased sensitization to enter junctions. Regardless, it is likely important that STIM2's constitutive presence in junctions is attenuated by its poor ability to activate channels, having a distinct role in controlling Ca^{2+} homeostasis [12] and/or functioning as a negative regulator of STIM1-mediated Ca^{2+} entry [13].

Perhaps the most significant conclusion from the new work linking SOCs with the Ca^{2+} oscillatory response is the functional role of Ca^{2+} entering through SOCs during Ca^{2+} oscillations. Particularly in the work of Di Capite *et al.* [5] we learn that the SOC-mediated Ca^{2+} entry provides a 'spatial signature' that specifically controls Ca^{2+}-dependent gene expression. Thus, the prevailing theory on the significance of store-operated Ca^{2+} entry during oscillations is that it is necessary to prevent run-down and to allow replenishment of Ca^{2+} stores to facilitate the continuation of oscillations. While this function is clearly important, Di Capite *et al.* [5] used a trick which allowed Ca^{2+} oscillations to continue normally without any Ca^{2+} entry component. Thus, they applied high levels of La^{3+} to block movement of Ca^{2+} across the plasma membrane — both entry through SOCs and exit via plasma membrane Ca^{2+} pumps — essentially isolating the cytoplasm from the cell exterior, allowing $InsP_3$-mediated oscillations in response to low-agonist-induced activation to continue normally without any net Ca^{2+} entry. In mast cells, the proinflammatory leukotriene LTC_4 induces expression of the immediate early gene *c-fos* through PLC-driven $InsP_3$-mediated oscillatory Ca^{2+} signals. Using the La^{3+} block to prevent SOC-mediated Ca^{2+} entry, submaximal LTC_4 levels led to activation of Ca^{2+} oscillations identical to those observed without the block, but there was no *c-fos* expression. Thus, it is concluded that an important function of Ca^{2+} oscillations is to activate SOCs, which mediate spatially defined entry of Ca^{2+} that is essential for the activation of gene expression. Indeed, both recent papers [5,6] refer to a number of other examples in which the SOC-mediated Ca^{2+} entry component is important for coupling to Ca^{2+}-dependent downstream effectors. Such a specific role for Ca^{2+} entry is highly consistent with studies in neuronal cells which revealed that the signature of Ca^{2+} entry signals through L-type channels or NMDA receptors is crucial for defining gene induction responses [20].

The function of a specific subset of ER Ca^{2+} stores tightly coupled to the activation of SOCs makes much sense. Thus, the maintenance of Ca^{2+} within the bulk ER is essential in order to preserve protein synthesis and trafficking and to prevent protein misfolding and stress responses. Considering that STIM1 is the major mediator of coupling to activate SOCs and requires substantial luminal Ca^{2+} decreases to become activated, it is logical that such release be restricted to a small fraction of stores. As shown in Figure 1, it is also logical that these restricted stores are near the plasma membrane, where they are not only exposed to the highest levels of $InsP_3$, but also proximal to ER–plasma membrane junctions to optimize STIM1-mediated coupling to activate Orai channels.

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Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140, USA.
E-mail: dgill@temple.edu

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