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SPOTLIGHT

EB1 traps STIM1 and regulates local store-operated Ca^{2+} entry

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STIM1 activates store-operated Ca^{2+} entry when Ca^{2+} in the endoplasmic reticulum (ER) is depleted. In this issue, Chang et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201711151) demonstrate that EB1 traps STIM1 at dynamic contacts between the ER and microtubule plus ends, delaying STIM1 translocation to ER–plasma membrane junctions and preventing Ca^{2+} overload.

The ER is the major intracellular Ca^{2+} store and plays a central role in regulating cytosolic Ca^{2+} signaling through store-operated Ca^{2+} entry (SOCE). Physiologically, SOCE is initiated by activation of plasma membrane (PM) receptors that stimulate phospholipase C to hydrolyze phosphatidylinositol-4,5-bisphosphate (PIP_2). Breakdown of PIP_2 produces inositol-1,4,5-trisphosphate, a second messenger that rapidly releases Ca^{2+} from the ER. The transient decrease in ER Ca^{2+} is sensed by the stromal interaction molecule 1 (STIM1), an ER transmembrane (TM) protein with an EF hand motif localized in the ER lumen. STIM1 is normally distributed throughout the ER membrane when Ca^{2+} is replete. However, Ca^{2+} loss leads to the dissociation of Ca^{2+} from the EF hand motif, and STIM1 undergoes a conformational change, oligomerizes, and exposes its C terminus, which interacts with phospholipids on the PM. The STIM1–phospholipid interaction retains STIM1 in a junction structure formed by a close contact of <30 nm between the PM and cortical ER. In the ER–PM junction, STIM1 activates Orai1, the critical Ca^{2+} channel in SOCE that allows extracellular Ca^{2+} flux into the cytosol. The elevation of cytosolic Ca^{2+} by SOCE controls a variety of cellular processes and pathological events.

Endogenous STIM1 diffuses passively at slow speeds in cells with plentiful ER Ca^{2+}. However, overexpression of STIM1 leads to its interaction with microtubule (MT) end-binding proteins through an EB1 binding motif in the C terminus of STIM1, and overexpressed STIM1 exhibits a fast comet-like movement (Grigoriev et al., 2008). This traveling wave of STIM1 represents a transient contact between ER and polymerizing MT ends that is significant in three ways. First, STIM1 is concentrated on the ER that is attached to the tips of growing MTs. Second, this ER extension through a tip attachment complex mechanism facilitates rearrangement of ER tubules to form cortical ER, providing platforms for ER–PM junction formation (Shen et al., 2011). Third, the interaction of STIM1 and EB1 places STIM1 under the regulation of MTs in addition to the ER. Although the STIM1–EB1 interaction is not essential for SOCE (Grigoriev et al., 2008), it has been proposed that this interaction may underlie the functional delay of ∼35 s between STIM1 oligomerization and its translocation to ER–PM junctions (Liou et al., 2007). However, the exact roles of the STIM1–EB1 interaction in controlling STIM1 translocation and SOCE remain obscure.

In this issue, Chang et al. created a synthetic protein, inducible membrane-attached peripheral ER (iMAPPER-633), to probe the functional significance of the STIM1–EB1 interaction. iMAPPER-633 included the STIM1 TM domain, signal peptide for ER targeting, and its C-terminal (CT) region for EB1 and phospholipid binding (PB). The optimal MAPPER linker was included to separate the cytosolic and luminal regions of STIM1 by a distance equivalent to the ER–PM junction. A fluorescent protein and a tandem FK506-binding protein (FKBP) motif (2×FKBP) were inserted into the luminal region to permit imaging and drug-induced oligomerization. As expected, iMAPPER-633 exhibited ER localization but tracked MT-like structures and, in live cells, moved toward the cell periphery in a comet-like manner. Drug-induced oligomerization of the FKBP motifs led to the formation of iMAPPER-633 puncta that localized with three ER–PM markers, MAPPER, Syt2, and Syt3, indicating that iMAPPER-633 preferentially translocates to ER–PM junctions and binds phospholipids after oligomerization. Therefore, Chang et al. (2018) confirmed that iMAPPER-633 possesses key features of endogenous STIM1 including ER localization, EB1 binding, oligomerization, and translocation to ER–PM junction but lacks the domains involved in the complex regulation of cytosolic and ER Ca^{2+} levels via other STIM1 regulators. For the first time, this powerful tool

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allowed Chang et al. (2018) to dissect the function of EB1 binding in regulating STIM1 puncta at ER–PM junctions.

Chang et al. (2018) first asked whether EB1 formed an obstruction preventing STIM1 from reaching ER–PM junctions. iMAP PER-633 was dissociated from MTs by collapsing them with nocodazole, which led to the rapid translocation of iMAPPER-633 to ER–PM junctions in 2 min. Other approaches to disturb the interaction between STIM1 and EB1 such as EB1 knockdown and mutation of the EB1 binding residues of the TRIP motif in STIM1 resulted in iMAPPER-633 preoccupying the ER–PM junctions. Drug-induced oligomerization of iMAPPER-633 did not produce more puncta of ER–PM junctions, suggesting that the interaction of STIM1 with EB1 is a major mechanism that retards its translocation. It is surprising that elimination of MT binding alone could cause translocation of iMAPPER-633 to ER–PM junctions, which means that PB is sufficient to trigger this event when there is no restriction of STIM1 by EB1. Together, these data confirm that MT binding prevents iMAPPER-633 localization to ER–PM junctions.

Chang et al. (2018) next tested whether endogenous STIM1 was under similar restriction by EB1 as the synthetic iMAPPER-633. They coexpressed STIM1-TRNN with iMAPPER-633–TRNN (both have mutations of the EB1 binding motif) and found that STIM-TRNN was diffusely distributed rather than forming puncta like the iMAPPER-633–TRNN. However, depletion of ER Ca^{2+} stores with thapsigargin (TG) led to STIM1-TRNN colocalizing with iMAPPER-633–TRNN, consistent with the CT domain of STIM1 not being fully exposed for EB1 and PB until Ca^{2+} becomes dissociated from the EF hand motif (Zhou et al., 2013). This also indicates that ER–PM junctions are predefined rather than determined by ER Ca^{2+} depletion (Smyth et al., 2008). Moreover, when the PB motif was duplicated in the C terminus of STIM1 to generate STIM1-2K with enhanced PB activity, an increase in puncta formation was observed that could not be further boosted by TG. In contrast with STIM1-D76A, a constitutively active mutant that readily forms puncta and elevates resting cytosolic Ca^{2+}, STIM1-2K could not increase resting cytosolic Ca^{2+}. These data suggest that enhanced phospholipid interaction can overcome the limitation on STIM1 imposed by MT binding and promote ER–PM junction formation; however, STIM1 translocation to the PM is not sufficient to open SOCE channels (Park et al., 2009).

What is the role of EB1 binding in the regulation of STIM1 translocation to ER–PM junctions? To address this question, Chang et al. (2018) first performed a FRAP experiment and found that STIM1-TRNN recovered faster than STIM1 in the photobleached region. Furthermore, ∼25% of overexpressed STIM1 translocated to ER–PM junctions after MTs were dissembled by nocodazole, but no increase was noticed in STIM1-TRNN–expressing cells. Critically, 17% of the endogenous STIM1 was trapped by MTs. In addition, in a stable cell line expressing STIM1-YFP at low level, a comet-like structure of STIM1 was seen, which was eliminated by subsequent nocodazole treatment. Chang et al. (2018) conclude that EB1 binding to STIM1 is not an artificial effect caused by overexpression and that this interaction restricts the movement of STIM1. The trapping of STIM1 at ER–MT contact sites likely explains why STIM1 recovered more slowly than STIM1-TRNN in the FRAP assay, suggesting that EB1 binding is the rate-limiting step for STIM1 translocation. It will be important to know the quantitative distribution of STIM1 in EB1-binding and EB1-free conditions as the interaction between STIM1 and EB1 likely plays a critical housekeeping role in SOCE.

Chang et al. (2018) next examined whether the interaction between EB1 and STIM1 was altered after ER Ca^{2+} is depleted. Imaging and coimmunoprecipitation experiments support interaction occurring between STIM1 and EB1 before and after Ca^{2+} depletion. Dissociation of STIM1 from TG-induced puncta with ML-9, a potent inhibitor of myosin light chain kinase, eliminated STIM1 from puncta, but STIM1 remained distributed with and tracking EB1, and the ER–PM junctions were retained. More interestingly, puncta formed by coexpression of STIM1 and Orai1 were not affected by ML-9 treatment, which is consistent with previous research (Smyth et al., 2008). These data support the notion that STIM1 puncta may be stabilized by both ML-9–insensitive STIM1–Orai1 interactions and ML-9–sensitive STIM1–phospholipid interactions, with ER depletion as a trigger. This idea was further supported by the fact that STIM1-D76A puncta disappeared in response to ML-9 treatment and, simultaneously, STIM1 colocalization with EB1 appeared. In contrast, STIM1-D76A–TRNN puncta also disappeared but did not colocalize with EB1. Overall, these results indicate that EB1 retains STIM1- and MT-binding activity regardless of whether ER Ca^{2+} was depleted. It is worth mentioning that the mechanism of how ML-9 affects STIM1 function has not been fully revealed (Smyth et al., 2008), and it seems that the explanation proffered by Chang et al. (2018) that ML-9 inhibits STIM1 and phospholipid interaction is the most straightforward one. It would be very interesting to determine how ML-9 disrupts STIM1–phospholipid interaction with the strategies used in previous work by Chen et al. (2017).

Given that EB1–STIM1 interaction exists both before and after ER depletion, a major question is how EB1 binding influences the translocation of STIM1 during ER depletion. By analyzing STIM1 and STIM1–TRNN translocation after ionomycin or TG treatment, Chang et al. (2018) observed that STIM1-TRNN has faster kinetics of ER–PM translocation and that knockdown of EB1 had a similar effect. They determined that STIM1-TRNN also accelerated Orai1 translocation to the ER–PM junctions after TG treatment. Collectively, these data suggest that EB1 binding impedes SOCE initiation time by restricting STIM1 translocation to the PM to activate Orai1. EB1 may trap STIM1 to down-regulate SOCE, which may also partially explain the delay observed in cells between STIM1 oligomerization and ER–PM junction formation. Intriguingly, puncta formed by STIM1-TRNN and Orai1 seem to disassemble more quickly than those formed by STIM1 and Orai1, possibly indicating a role of EB1 in SOCE inactivation.

What are the functional consequences of STIM1 restriction by EB1 in regulating SOCE? By imaging Ca^{2+} with Fura2, Chang et al. (2018) observed that SOCE was increased when STIM1 and EB1 interaction was disrupted via EB1 knockdown or by introducing TRNN mutations into STIM1 and STIM1-D76A. Therefore, the interaction of STIM1 with EB1 limits the amplitude of SOCE. STIM1-TRNN–transfected cells consistently display elevated resting ER Ca^{2+} and enhanced Ca^{2+} refilling after BHQ-induced ER depletion. The ability of EB1 to limit the kinetics and efficiency of STIM1 and Orai1 translocation to ER–PM junctions, possibly

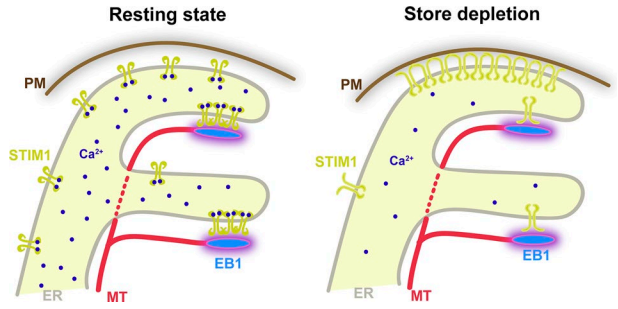

Figure 1. **Schematic showing role of STIM1–EB1 interaction in STIM1 translocation to ER–PM junctions during ER Ca^{2+} depletion.** The ER is a dynamic organelle that continuously rearranges its structure through the MT to maintain its network and contacts with other organelles including the PM. The binding of STIM1 to EB1 enables ER movement along MT. In the resting state (left), the ER is replete with Ca^{2+}, and STIM1 is distributed throughout the ER. EB1 dynamically binds a few STIM1 molecules and traps them from translocating to the ER–PM junction. After ER Ca^{2+} depletion (right), free STIM1 without EB1 restriction is readily translocated to ER–PM junctions once Ca^{2+} is dissociated from its EF hand localized in the ER lumen. EB1 still binds some STIM1 to prevent enhanced SOCE from inducing overload of cytosolic and ER Ca^{2+}. This restrictive mechanism is critical for migrating cells in which EB1 restricts STIM1 translocation to activate SOCE at the leading edge of the cells. Enhanced SOCE induces elevation of cytosolic and ER Ca^{2+}, which leads to termination of cell migration.

together with other negative regulators of SOCE, may form a fine-tuning mechanism that cells use to control the dynamics and amplitude of SOCE and protect the ER from Ca^{2+} overload. Sarcoplasmic reticulum, a specialized ER in excitable cells, can undergo Ca^{2+} overload physiologically (Garaschuk et al., 1997). In nonexcitable cells, ER Ca^{2+} overload occurs because of malfunction in some "leaky channels" (Camello et al., 2002). Recently, both Presenilins and TMCO1 have been shown to form ER Ca^{2+} channels that prevent ER Ca^{2+} from becoming overloaded (Tu et al., 2006; Wang et al., 2016). In their study, Chang et al. (2018) have revealed a novel mechanism of how the cells may prevent ER Ca^{2+} overload through the trapping STIM1 in the ER–PM junctions by EB1, highlighting the significance of the cytoskeleton in regulating ER Ca^{2+} homeostasis. It is understandable that cells appear to integrate multiple approaches that work together to prevent ER Ca^{2+} overfilling and thus maintain intracellular Ca^{2+} homeostasis and signaling.

Overall, this excellent study has addressed an important question regarding whether the EB1–STIM1 interaction plays a role in SOCE. Chang et al. (2018) wisely designed iMAPPER-633 rather than using full-length STIM1 at the beginning of their study to investigate the possibility that EB1 binding may impede ER–PM targeting of STIM1. This clever approach avoided the potentially confounding influence of the complex regulatory mechanisms of STIM1 and clearly demonstrated that EB1 binding with STIM1 occurs both before and after ER depletion. EB1 binding reduces the rate and efficiency of STIM1 translocation to ER–PM junctions and down-regulates SOCE to prevent ER Ca^{2+} overload (Fig. 1). Therefore, Chang et al. (2018) identified a novel ER Ca^{2+} regulation mechanism and provide new insight into how the cytoskeleton controls Ca^{2+} homeostasis.

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