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Regulation of Store-operated Calcium Entry during Cell Division

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Abstract

Store-operate calcium channels gate calcium entry into the cytoplasm in response to the depletion of calcium from endoplasmic reticulum calcium stores. The major molecular components of store-operated calcium entry are STIM1 (and in some instances STIM2) that serves as the endoplasmic reticulum calcium sensor, and Orai (Orai1, 2 and 3) which function as pore-forming subunits of the store-operated channel. It has been known for some time that store-operated calcium entry is shut down during cell division. Recent work has revealed complex mechanisms regulating the functions and locations of both STIM1 and Orai1 in dividing cells.

Keywords

calcium entry; store-operated channels; STIM proteins; Orai; cell division; protein phosphorylation

Store-operated Calcium Entry

Store-operated calcium entry is a process whereby a decrease in endoplasmic reticulum Ca^{2+} content activates calcium entry into the cytoplasm across the plasma membrane [22]. Despite intensive research since the initial concept was proposed [26], the molecular basis for this pervasive signaling pathway was not solved until large scale genomic screens revealed the two fundamental players: STIM proteins that sense Ca^{2+} levels in the endoplasmic reticulum [15;27], and Orai proteins that constitute the basic pore-forming subunits of the store-operated channels [7;35;46]. Mammalian cells express two STIM proteins, STIM1 and STIM2 [38]. The major Ca^{2+} sensing protein involved in store-operated Ca^{2+} entry appears to be STIM1, since its ablation results in almost complete loss of store-operated entry in most cell types investigated so far [1;2;5;24;37]. Depletion of Orai1 similarly results in substantial loss of Ca^{2+} entry in numerous cell types, whereas knockdown of Orai2 or Orai3 has little to no effect, leading to the conclusion that Orai1 is the predominant Orai isoform. Mice lacking STIM1 die perinatally, while mice lacking STIM2 may live for 4 weeks or so [19]. Knockout of Orai1 in mice produces a severe phenotype [9;34], similar to that seen with knockout of STIM1, and mouse knockout models of the other mammalian Orais, Orai2 and Orai3, have not yet been reported.

Orai1 is primarily expressed in the plasma membrane, although a vesicular pool of variable size has been reported as will be discussed below. STIM1 was initially identified as a plasma membrane protein [21], but is also expressed in the endoplasmic reticulum where it functions as a Ca^{2+} sensor to signal store-operated Ca^{2+} entry. The function, if any, of plasma membrane STIM1 in store-operated Ca^{2+} entry is unclear but it has been suggested to play a role in another class of Ca^{2+} channels activated by arachidonic acid [17]. Within the endoplasmic reticulum, STIM1 does not distribute randomly but appears to align

partially with the microtubular network, at least when overexpressed with a fluorescent tag [30]. As discussed below, this association is not a direct one but involves STIM1 binding to a microtubule accessory protein, EB1 [8].

The domain structure of STIM1 is shown in Figure 1. The protein traverses the limiting membrane of the endoplasmic reticulum once. The lumenally directed N-terminus contains a calcium binding EF-hand motif that is apparently bound to Ca^{2+} under resting conditions. There is also a second somewhat degenerated EF-hand motif downstream of the first (not shown in the Figure) which does not likely bind Ca^{2+} but is probably involved in the conformational change that occurs when Ca^{2+} dissociates as is the case for other Ca^{2+} sensing EF-hand proteins [32]. When Ca^{2+} decreases in the endoplasmic reticulum, for example in response to the activated formation of inositol 1,4,5-trisphosphate, Ca^{2+} dissociates from the N-terminus resulting in a conformational change that permits self-association of STIM1 initially through interactions between sterile α -motif (SAM) domains [31]. STIM1 rapidly forms dimers and subsequently higher order oligomeric complexes [14]. These oligomeric structures interact with moieties in the plasma membrane at close ER-plasma membrane junctions resulting in the accumulation of STIM1 in these junctions. Here, STIM1 oligomers induce the co-clustering of Orai subunits and interact with them, probably directly, to induce channel opening and Ca^{2+} influx [12;23;40;45]. The domain within STIM1 that is responsible for activation of Orai channels was termed SOAR (STIM-Orai Activating Region) by Yuan *et al.* [45] and essentially similar data were published by other groups shortly thereafter [11;18;23]. These clusters of STIM1 and Orai appear as distinct sites when constructs with fluorescent protein tags are expressed in cells, and the sites of concentrated STIM1 and Orai are generally referred to as puncta. The coalescence of full-length STIM1 into puncta can be readily observed with confocal microscopes. Additionally, since the formation of puncta involves migration of STIM1 to near plasma membrane junctions, store depletion results in a substantial increase in the fluorescence signal measured by total internal reflection (TIRF) microscopy from fluorescent protein-tagged STIM1. Full-length STIM1 will form puncta in the absence of stoichiometric Orai, but Orai will not move to puncta unless there is a comparable quantity of STIM1. Experimentally, this means that overexpressed STIM1 will form observable puncta without the need for overexpressing Orai, but overexpressed Orai will not form observable puncta unless STIM1 is also overexpressed. The ability of STIM1 to form puncta independently of Orai depends upon a polybasic region at the far C-terminus of the protein [14], leading to speculation that this region may interact with plasma membrane polyphosphoinositides. However, strategies that deplete plasma membrane phosphatidylinositol 4,5-bisphosphate do not affect STIM1 movements nor do they alter store-operated Ca^{2+} entry [13]. In the absence of the polybasic tail, STIM1 can still form puncta that depend entirely upon Orai presumably through direct interaction with Orai; this interaction results in seemingly undiminished store-operated Ca^{2+} entry. Herein two major questions remain: with what does the polybasic region of STIM1 interact, and what is the role of this interaction? It is possible that STIM1 interactions with plasma membrane components help stabilize the ER-PM junctions. Consistent with this idea, overexpression of full-length STIM1 increases the size of these junctions [20]. It is noteworthy, however, that STIM in *Drosophila* has a significantly shorter C-terminus, lacking a polybasic region. Yet *Drosophila* cells exhibit store-operated currents essentially identical to those in mammalian cells [41].

The vast majority of studies on STIM1 cell biology have been carried out at room temperature, a condition in which STIM1 clearly acts as a depletion-dependent activator of Orai channels by relocating to ER-PM junctions. Interestingly, a recent report demonstrated that at physiological temperature, 37°C, STIM1 forms puncta spontaneously in the absence of store depletion, yet does not activate Orai [39]. Apparently these spontaneous puncta form because higher temperatures favor the interaction between the polybasic tails and its

cognate ligand, but inhibit interactions between the SOAR domain of STIM1 and Orai1. Further work is needed to determine if endogenous STIM1 also exists largely in these junctional areas providing highly localized communication between Ca^{2+} stores and plasma membrane channels.

Calcium entry is lost in dividing cells

Volpi and Berlin [36] first reported that the sustained elevation of $[\text{Ca}^{2+}]_i$ in histamine-activated HeLa cells was lost during mitosis. Histamine stimulation of $^{45}\text{Ca}^{2+}$ uptake was lost as well. The initial peak $[\text{Ca}^{2+}]_i$ signal remained, however, leading to the conclusion that Ca^{2+} influx was specifically impaired. Interestingly, in their discussion, they consider as possible mechanisms inactivation by phosphorylation or channel internalization, both of which have turned out to be true (although it is not the channel, but STIM1 that is phosphorylated, see below). The first implication of store-operated entry came a few years thereafter when Preston *et al.* [25] demonstrated that thapsigargin-induced sustained Ca^{2+} signaling, as well as $^{45}\text{Ca}^{2+}$ uptake, were lost during mitosis. More recently, the regulation of the current underlying store-operated Ca^{2+} entry, I_{crac} , was followed through the cell cycle in the mast cell line, RBL-2H3 [33]. Confirming the previous findings, I_{crac} was substantially suppressed in mitosis, and, interestingly, was significantly increased during G1/S and S phases.

Store operated Ca^{2+} entry is also diminished during meiosis, suggesting that it is a general occurrence during cell division. However, as discussed below, it is possible that the mechanisms involved in meiosis are somewhat different than in mitosis. Further the significance of Ca^{2+} influx suppression during cell division, whether meiosis or mitosis, is completely unknown; therefore, it will be important to elucidate the underlying mechanisms in order to begin to address the impact of this regulatory process.

Mechanisms for loss of SOCE during cell division

The two most obvious molecular candidates for sites of disruption of SOCE are STIM1 and Orai1. Perhaps not surprisingly, therefore, there is evidence for modification of both molecules during cell division. Yu *et al.* [42] in studies of SOCE during meiosis in *Xenopus* noted that during meiosis (1) STIM1 fails to cluster into puncta, (2) STIM1 becomes extensively phosphorylated, and (3) Orai1 is internalized. The latter phenomenon depends upon constant cycling of Orai1 in and out of the plasma membrane [44], a process similar to that which has been previously reported for certain TRP channels [4;28].

The inactivation of SOCE apparently results from the activation of maturation-promoting factor (MPF, Cdk1 and cyclin B) [16]. Presumably this underlies the hyperphosphorylation of STIM1 that occurs during both meiosis and mitosis [29;42]. However, there are conflicting findings with regard to the role played by phosphorylation in the inhibition of STIM1 clustering. Yu *et al.* [42] reported that mutation of multiple potential MPF phosphorylation sites to alanine did not rescue STIM1 clustering during meiosis. On the other hand, Smyth *et al.* [29] made large deletions in the far C-terminal region of STIM1 where all potential MPF sites are located, thereby preventing phosphorylation (detected by a phosphopeptide antibody, MPM-2 [6]). When co-expressed with Orai1, in mitotic cells the truncated STIM1 relocated to near plasma membrane sites upon store depletion, and SOCE was substantially restored. In this same study, two of the sites phosphorylated in mitosis were identified, serines at positions 486 and 668; interestingly, serine 668 appears to be a direct target of MPF, at least *in vitro*. Clearly, however, additional sites were still phosphorylated as revealed by binding of the MPM-2 antibody after mutation of these two sites to alanine. Nonetheless, this 2-site mutant produced a partial rescue of peripheral localization and SOCE when co-expressed with Orai1 [29], further bolstering support for the

role of STIM1 phosphorylation in SOCE suppression. Note that in the study by Smyth *et al.* [29], it was not possible to rescue SOCE unless the mutant STIM1 constructs were co-expressed with Orai1, both driven by strong viral promoters. This may indicate that in the mitotic cells, a substantial fraction of Orai1 is internalized, as was the case for the meiotic cells.

Why then did these two studies come to such different conclusions regarding the consequences of STIM1 phosphorylation? It is possible that the mechanism underlying down regulation of STIM1 function in meiosis is fundamentally different than that in mitosis. In a recent publication, Yu *et al.* [43] substituted the C-terminal sequence containing all known MPM-2 phosphorylation site with a fluorescent protein followed by the STIM1 polybasic region. This construct was fully capable of forming puncta and signaling SOCE in interphase cells, yet despite the absence of any predicted phosphorylation sites, no puncta formed in cells in meiosis (eggs). However, this construct, unlike the unmodified STIM1 protein, was now capable of translocating to near membrane areas and interacting with plasma membrane components [43]. Thus, it seems that removal of phosphorylation sites did at least partially rescue STIM1 function in meiosis; rescue of Ca^{2+} entry would not likely occur, since, as these investigators showed previously, during meiosis virtually all Orai1 is internalized [42]. The failure to form distinct and visible puncta is perhaps not a particularly reliable indicator of STIM clustering, since it likely reflects the morphological arrangement of endoplasmic reticulum – plasma membrane junctions [20], which may themselves be altered during meiosis.

Consequences of STIM/Orai Regulation in Dividing Cells

Studies on the consequences of STIM and Orai regulation may have to await strategies to reverse or rescue both STIM1 function and Orai1 retention in the plasma membrane. Speculation has focused on two general ideas: the need to inactivate Ca^{2+} entry, and the need to prevent STIM1 association with microtubules. Note that these two possibilities may not be mutually exclusive. In order to examine the consequences of rescued Ca^{2+} entry on cell division, Smyth *et al.* [29] overexpressed both the C-terminal truncation and Orai1 in HEK293 cells and determined the growth rate of the cells in culture. The control for this experiment was the overexpression of wild-type STIM1 and Orai1 (which does not rescue SOCE in mitosis); unfortunately, this condition resulted in substantial slowing of cell division, possibly owing to the rechanneling of cell resources by the strong viral promoters on the transfected constructs. Nonetheless, cells transfected with the truncated STIM1 grew significantly more slowly than those transfected with the wild-type protein [29]. A clearer assessment may come when the specific sites of phosphorylation have been identified and a more subtle mutation can be tested.

Regarding STIM1 association with microtubules, it is possible that phosphorylation may play a role in regulating this interaction although this idea has not yet been tested. The interaction of STIM1 with microtubules is not direct, but occurs through a microtubule plus-end tracking protein known as EB1 [8]. This interaction with the growing ends of microtubules results in the comet-like movements of STIM1 seen with TIRF video imaging [2;8]. The STIM1 sequence through which STIM1 binds to EB1 is a TRIP in the C-terminus, and interestingly this site falls within the domain containing multiple MPF sites [8]. Mutation of this TRIP sequence prevents STIM1 interaction with EB1 and microtubules [10], and also diminishes the ability of growing ends of microtubules to associate with and extend endoplasmic reticulum tubules [8]. During mitosis, microtubules rearrange to form the mitotic spindle in the pro-nucleus, while endoplasmic reticulum is largely excluded from this region and distributes to the cell periphery. Thus it is possible that phosphorylation-dependent dissociation of STIM1 from EB1 is required during mitosis to properly segregate

the endoplasmic reticulum from spindle microtubules. It is known, however, that regulation of STIM1-microtubule interaction is unlikely to contribute to the loss of SOCE since previous studies have shown that disruption of this interaction has little impact on SOCE [3;8;30].

Conclusions

Store-operated calcium entry shuts down during cell division by a complex mechanism involving redistribution and internalization of Orai channels as well as phosphorylation and inactivation of the calcium sensor, STIM1. Some remaining questions pertain to the signals leading to redistribution of Orai, the kinase or kinases regulating STIM1, and the relationship to STIM1 microtubule interactions. Indeed, it is still not altogether clear what is the physiological significance of this profound functional down regulation of this widely encountered pathway of cell signaling during the critical period of cell division.

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Abbreviations

STIM	Stromal Interacting Molecule
SOAR	STIM-Orai Activating Region
SAM	Sterile α -Motif
MPF	Maturation-Promoting Factor

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Domain Structure of STIM1

Figure 1. Domain structure of STIM1

EF Hand, EF hand Ca^{2+} binding motif; SAM, sterile α motif; TM, transmembrane domain; SOAR, stim-orai activating region; FI, fast inactivation; Regul., regulatory (phosphorylation sites) domain; EB1 binding, domain for interaction with microtubule +end protein, EB1; K Rich, polybasic C-terminus.