Regulation of store-operated Ca\textsuperscript{2+} entry during the cell cycle

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Summary
Cytoplasmic Ca\textsuperscript{2+} signals are central to numerous cell physiological processes, including cellular proliferation. Historically, much of the research effort in this area has focused on the role of Ca\textsuperscript{2+} signals in cell-cycle progression. It is becoming clear, however, that the relationship between Ca\textsuperscript{2+} signaling and the cell cycle is a ‘two-way street’. Specifically, Ca\textsuperscript{2+}-signaling pathways are remodeled during M phase, leading to altered Ca\textsuperscript{2+} dynamics. Such remodeling probably better serves the large variety of functions that cells must perform during cell division compared with during interphase. This is clearly the case during oocyte meiosis, because remodeling of Ca\textsuperscript{2+} signals partially defines the competence of the egg to activate at fertilization. Store-operated Ca\textsuperscript{2+} entry (SOCE) is a ubiquitous Ca\textsuperscript{2+}-signaling pathway that is regulated during M phase. In this Commentary, we discuss the latest advances in our understanding of how SOCE is regulated during cell division.

Key words: Calcium signaling, Cell division, Store-operated Ca\textsuperscript{2+} entry

Introduction
Intracellular Ca\textsuperscript{2+} signals are central to practically all aspects of cellular physiology, including secretion, contraction, fertilization, synaptic transmission, cell division and gene expression. The fact that Ca\textsuperscript{2+} signals mediate disparate cellular responses, often in the same cell, necessitates a high level of specificity and versatility. Specificity is encoded by the spatial and temporal features of the Ca\textsuperscript{2+} signal itself, and by the sensitivity, availability and localization of downstream Ca\textsuperscript{2+}-dependent effectors (Berridge et al., 2000; Clapham, 1995). In addition, the astounding temporal (microseconds to hours) and concentration (nanomolar to millimolar) ranges across which Ca\textsuperscript{2+} acts contribute to its versatility and specificity as an effective signaling module (Berridge et al., 2003; Clapham, 2007).

Ca\textsuperscript{2+} signaling is found on cells providing a low background by maintaining cytoplasmic Ca\textsuperscript{2+} levels in the nanomolar range (~100 nM). Regulation of cytoplasmic Ca\textsuperscript{2+} in resting conditions is a homeostatic mechanism mediated by the balance of Ca\textsuperscript{2+} influx and extrusion at the cell membrane, as well as by Ca\textsuperscript{2+} uptake and release from intracellular Ca\textsuperscript{2+} stores. Similar mechanisms are involved in defining the spatial, temporal and amplitude features of Ca\textsuperscript{2+} signals. Extracellular Ca\textsuperscript{2+} concentration is typically 1-2 mM, whereas Ca\textsuperscript{2+} concentration in the endoplasmic reticulum (ER) – the primary intracellular Ca\textsuperscript{2+}-storage organelle – is in the 250-600 µM range (Demaurex and Frieden, 2003). Given the existence of these Ca\textsuperscript{2+} gradients across the cell and ER membranes, Ca\textsuperscript{2+} flows down its concentration gradient into the cytoplasm whenever a Ca\textsuperscript{2+}-permeable pathway is open. The active interplay between Ca\textsuperscript{2+} release from intracellular stores and influx from the extracellular space defines Ca\textsuperscript{2+}-signaling dynamics and hence the ensuing cellular response. In fact, both pathways (influx and release from stores) can be functionally linked through the store-operated Ca\textsuperscript{2+} entry (SOCE) pathway, which is activated in response to Ca\textsuperscript{2+}-store depletion. In addition, cells possess a myriad of Ca\textsuperscript{2+}-influx pathways, including voltage-gated, ligand-gated, receptor-operated, stretch-activated and second-messenger-gated channels (Berridge et al., 2000; Clapham, 2007).

As Ca\textsuperscript{2+} is vital for all cells, tight regulation of the mechanisms involved in Ca\textsuperscript{2+} homeostasis is of paramount importance for preventing dysfunctions that lead to pathological conditions (Missiaen et al., 2000). This regulation is highly complex and varies not only with cell type but also with the developmental stage of the cell (Lipskaia and Lompré, 2004). Given the central role of Ca\textsuperscript{2+} signaling in cellular physiology, it is not surprising that Ca\textsuperscript{2+} signals have been shown to play important roles during cell-cycle progression (Whitaker, 2006). Ca\textsuperscript{2+} signals are required for nuclear-envelope breakdown, and for chromosome condensation and disjunction during mitosis (Ciapa et al., 1994; Groigno and Whitaker, 1998; Kao et al., 1990; Steinhardt and Alderton, 1988; Tombes et al., 1992; Twigg et al., 1988; Wilding et al., 1996). By contrast, Ca\textsuperscript{2+} signals are dispensable for the breakdown of the germinal vesicle during vertebrate meiosis, but are required for the completion of meiosis I (Sun et al., 2008; Sun and Machaca, 2004; Tombes et al., 1992). The idea that Ca\textsuperscript{2+} is involved in cell-cycle progression is strengthened by genetic and biochemical evidence supporting a role for calmodulin (CaM) and Ca\textsuperscript{2+}-calmodulin-dependent protein kinase II (CaMKII) in mitosis and G1 (Means, 1994; Rasmussen and Means, 1989; Takuwa et al., 1993; Whitaker, 1995; Whitaker and Larmar, 2001). Furthermore, the Ca\textsuperscript{2+}-CaM-CaMKII module is required for centrosome duplication, which is necessary for spindle formation and chromosome segregation: when this module is defective, genomic instability results (Matsumoto and Maller, 2002). Moreover, the Ca\textsuperscript{2+}-dependent phosphatase calcineurin has also been implicated in G1-S progression (Kahl and Means, 2003).

Therefore, there is significant evidence supporting a crucial role for Ca\textsuperscript{2+} signals in regulating the cell cycle. Probably the best-defined example is at fertilization, which leads to the completion of the second meiotic division (Perry and Verlhac, 2008). The fertilization-induced Ca\textsuperscript{2+} transient takes the form of a single
sweeping Ca\textsuperscript{2+} wave, or multiple Ca\textsuperscript{2+} oscillations, depending on the species (Stricker, 1999). In vertebrates, the Ca\textsuperscript{2+} transient that occurs at fertilization activates CaMKII (Lorca et al., 1993), which mediates resumption of meiosis by inactivating cytostatic factor (CSF), a protein whose activity maintains metaphase II arrest (Knott et al., 2006; Lorca et al., 1993; Morin et al., 1994; Tunquist and Maller, 2003). CaMKII phosphorylates the anaphase-promoting complex (APC) inhibitor Emi2 (Hansen et al., 2006; Liu and Maller, 2005; Rauh et al., 2005), which primes it for additional phosphorylation by polo-like kinase. The dually phosphorylated Emi2 is targeted for degradation, thereby activating APC and releasing CSF-mediated arrest. This provides an elegant example of how a Ca\textsuperscript{2+} signal triggers a cascade of events that controls cell-cycle progression – in this case, completion of meiosis II.

Given the well-established role of Ca\textsuperscript{2+} signals in cell-cycle progression, an emerging area of interest is to understand how Ca\textsuperscript{2+}-signaling pathways themselves are regulated during the cell cycle, particularly during the cell-division phase. It is becoming evident that Ca\textsuperscript{2+} signaling is remodeled during M phase of the cell cycle. For example, inositol (1,4,5)-triphosphate [Ins(1,4,5)P\textsubscript{3}]-dependent Ca\textsuperscript{2+} release is sensitized during both meiosis (Fujiwara and Rios, 2007; Stiber et al., 2008). SOCE is an intricate physiological phenomenon whereby Ca\textsuperscript{2+} release from intracellular stores is increased by cytosolic calcium. SOCE is a mechanism that facilitates Ca\textsuperscript{2+} influx in essentially all non-excitable cells in response to agonist stimulation (Parekh and Putney, 2005). SOCE signals are thought to serve as an essential means of refilling Ca\textsuperscript{2+} stores, particularly during the cell-division phase. It is becoming clear that SOCE represents a primary Ca\textsuperscript{2+}-entry route in the cell cycle, particularly during the cell-division phase. It is becoming evident that Ca\textsuperscript{2+} signaling is remodeled during M phase of the cell cycle. For example, inositol (1,4,5)-triphosphate [Ins(1,4,5)P\textsubscript{3}]-dependent Ca\textsuperscript{2+} release is sensitized during both meiosis (Fujiwara et al., 1993; Machaca, 2004) and mitosis (Malathi et al., 2003), and this sensitization depends on the cell-cycle kinase cascade (Lee et al., 2006; Lim et al., 2003; Sun et al., 2009). In addition, during the maturation of Xenopus oocytes, the number of functional Ins(1,4,5)P\textsubscript{3} receptors increases following their release from annulate lamellae, vesicular compartments in oocytes in which Ins(1,4,5)P\textsubscript{3} receptor function is suppressed (Boulware and Marchant, 2005; Boulware and Marchant, 2008). Furthermore, SOCE is inhibited both during Xenopus oocyte meiosis and during mammalian cell mitosis (Machaca and Haun, 2000; Preston et al., 1991). In this Commentary, we discuss current knowledge of the mechanisms that mediate SOCE inactivation during M phase and their physiological significance.

### Store-operated Ca\textsuperscript{2+} entry

The idea that Ca\textsuperscript{2+}-store content regulates Ca\textsuperscript{2+} influx at the cell membrane was first formalized by Putney in 1986 in the context of the capacitative Ca\textsuperscript{2+}-entry model (Putney, 1986). In the ensuing two decades, it became clear that SOCE represents a primary Ca\textsuperscript{2+}-influx route in essentially all non-excitable cells in response to agonist stimulation (Parekh and Putney, 2005). SOCE signals are also operative, albeit in a secondary role, in excitable cells such as neurons (Empgate et al., 2001) and in skeletal muscle (Launikonis and Rios, 2007; Stiber et al., 2008). SOCE is an intricate physiological phenomenon whereby Ca\textsuperscript{2+} release from intracellular stores [typically induced by Ins(1,4,5)P\textsubscript{3}-coupled agonists] is followed by slow and sustained entry of extracellular Ca\textsuperscript{2+} (Putney et al., 2001). However, Ca\textsuperscript{2+} release per se is not a prerequisite for SOCE activation; rather, it is the coupling between the filling state of ER Ca\textsuperscript{2+} stores and store-operated Ca\textsuperscript{2+} channels that regulates SOCE (Parekh and Putney, 2005). In addition to store refilling, SOCE is involved in a myriad of cellular functions, including exocytosis (Fornia and Nowycky, 1999), sperm capacitation (O’Toole et al., 2000) and T-cell activation (Serafini et al., 1995). The best-characterized store-operated current is the highly Ca\textsuperscript{2+}-selective Ca\textsuperscript{2+}-release-activated current (I\textsubscript{CRAC}) (Hoth and Penner, 1992; Zweifach and Lewis, 1993). For example, antigen stimulation of T cells crosslinks T-cell receptors, thereby activating phospholipase C\textsubscript{γ} (PLC\textsubscript{γ}), leading to Ins(1,4,5)P\textsubscript{3}-dependent Ca\textsuperscript{2+} release from stores, followed by Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+}-release-activated Ca\textsuperscript{2+} (CRAC) channels. I\textsubscript{CRAC} produces a sustained Ca\textsuperscript{2+} transient that is required for calcineurin activation. Calcineurin dephosphorylates the transcriptional regulator nuclear factor of activated T cells (NFAT), resulting in its nuclear translocation and the subsequent expression of NFAT-regulated cytokines (Lewis, 2001; Oh-hora and Rao, 2008). In addition, several non-immune cell types possess a cation non-selective, Ca\textsuperscript{2+}-permeable SOCE pathway that is mediated by classical transient receptor potential channels (TRPCs) (Bailly et al., 1991).

Despite the intense interest in SOCE, both the identity of the SOCE channel and the nature of the coupling mechanism remained a matter of intense controversy for several years (Parekh and Putney, 2005). It is only in the past few years that the molecular players underlying SOCE were elucidated. The breakthrough came with the use of RNAi screens together with high-throughput functional assays to identify genes essential for SOCE. These screens initially identified stromal interaction molecule 1 (STIM1), an ER transmembrane protein with luminal EF-hands, as the sensor of luminal ER Ca\textsuperscript{2+} that links ER depletion to SOCE activation (Liu et al., 2005; Roos et al., 2005). This discovery was followed by the identification of Orai1 (also known as CRACM1) as the CRAC channel (Feske et al., 2006; Vig et al., 2006b; Zhang et al., 2006). The Orai1 protein spans the membrane four times and has no sequence homology to other known channels. Several lines of evidence support the conclusion that Orai1 contributes to and possibly defines the CRAC channel pore. Most importantly, mutations of key glutamates in the first and second membrane-spanning domains of Orai1 alter the ionic selectivity and permeation properties of SOCE (Prakriya et al., 2006; Vig et al., 2006a; Yeromin et al., 2006). In addition, a mutation in the Orai1 gene (R91W) abrogates Ca\textsuperscript{2+} influx in T cells and causes severe combined immunodeficiency disorder (SCID), a lethal immune disorder in humans (Feske et al., 2006). The establishment of STIM1 and Orai1 as the bona fide molecular mediators of CRAC is further strengthened by studies showing that coexpression of STIM1 and Orai1 produces large I\textsubscript{CRAC}-like currents with high Ca\textsuperscript{2+} selectivity, inward rectification and dependence on store depletion (Peinelt et al., 2006; Soboloff et al., 2006; Zhang et al., 2006). In addition, in some cell types, the ER Ca\textsuperscript{2+}-sensor STIM1 also couples to and gates TRPCs to induce SOCE (Huang et al., 2006; Yuan et al., 2007; Zeng et al., 2008). Nonetheless, the identification of STIM1 and Orai1 opened the door for studies targeted at obtaining a mechanistic understanding of SOCE inactivation during cell division, as discussed below.

### STIM1 and Orai1 coupling

Much has already been learned about how the elegant complexity of STIM1 and Orai1 coupling leads to SOCE (see Fig. 1, interphase). Depletion of Ca\textsuperscript{2+} stores leads to clustering of STIM1 into large puncta that are readily visible by light microscopy (Liou et al., 2007; Stathopoulos et al., 2006). These puncta are stabilized in a sub-plasma-membrane cortical ER domain that localizes within 10-20 nm of the cell membrane, where they physically recruit Orai1 into coincident puncta at the cell membrane, leading to Orai1 gating and Ca\textsuperscript{2+} influx (Luik et al., 2006; Prakriya et al., 2006; Vig et al., 2006b; Wu et al., 2006; Yeromin et al., 2006) (Fig. 1, interphase). Interestingly, a recent study argued that STIM1 is directly involved in the formation of these specialized cortical ER domains: expression of a constitutively active STIM1 mutant was shown to lead to the formation of multilayered cortical ER structures that are enriched in so-called pre-cortical ER domains.
that have similar morphology to the cortical ER, but are distant from the plasma membrane (Orci et al., 2009).

Mammalian genomes contain two STIM homologues, STIM1 and STIM2 (Cahalan, 2009). STIM1 has been shown to be essential for SOCE, whereas STIM2 appears to be primarily involved in maintaining Ca²⁺ homeostasis (Brandman et al., 2007). STIM1 is a single-pass integral membrane protein that localizes mainly to the ER membrane (Liou et al., 2005; Roos et al., 2005). STIM1 has a modular construction, with lumenal EF-hands and a sterile-α-motif (SAM) (Fahrner et al., 2009), the structure of which has been elucidated (Stathopulos et al., 2008). The STIM1 cytosolic domain contains two predicted coiled-coil domains followed by a Ser/Pro-rich region and a Lys-rich region at the C-terminal end of the molecule (Fahrner et al., 2009).

The STIM1 cytosolic domain is rich in functional domains that have been identified through structure-function studies, which have provided crucial insights into the coupling function of STIM1 (Fig. 1). Several groups have identified a minimal domain within the STIM1 cytoplasmic region that is necessary and sufficient for coupling to and activating Orai1 (Kawasaki et al., 2009; Muik et al., 2009). This so-called SOAR/CAD domain is a potent activator of SOCE, binds directly to Orai1, is sufficient to induce SOCE independently of Ca²⁺-store depletion and is required for STIM1-Orai1 coupling. In a region that is located C terminal to the SOAR/CAD and overlaps with it (residues 400-474) is a sequence referred to as the STIM-homomerization domain (SHD), which is required for STIM1 oligomerization (Muik et al., 2009). C terminal to the SHD is a short sequence of ~12 residues that mediates the Ca²⁺-dependent inactivation of I⁡CRAC (Lee et al., 2009; Mullins et al., 2009). Ca²⁺-dependent inactivation is a negative-feedback mechanism whereby Ca²⁺ that enters through the CRAC channels inactivates I⁡CRAC (Zweifach and Lewis, 1995).

Interestingly, expression of the STIM1 cytoplasmic domain alone produces constitutively active SOCE (Huang et al., 2006). Although smaller fragments within the STIM1 cytoplasmic region, such as the SOAR/CAD domain, are more potent activators of SOCE, this result shows that the dissociation of the cytoplasmic domain from the ER membrane changes its conformation in a way that allows this fragment to gate Orai1. This is consistent with the fact that the STIM1 cytoplasmic domain localizes below the cell membrane and can interact with Orai1, as indicated by fluorescence resonance energy transfer (FRET) analysis (Muik et al., 2009). Given that STIM1 oligomerization is initiated by depletion of Ca²⁺ stores and the association of SAM domains in the ER lumen, these results collectively suggest a ‘zippering’ mechanism for STIM1 oligomerization: STIM1 oligomerization initiates in the ER lumen, leading to structural changes in the STIM1 cytoplasmic domain that allow it to form stable large oligomers that bind to and gate Orai1. This is consistent with data showing that STIM1 oligomerization occurs independent of its ER lumenal domain is sufficient to activate SOCE (Luik et al., 2008). However, STIM1 oligomerization per se does not seem to be required for gating Orai1; rather, current data suggest that structural changes in STIM1 oligomerization.
following oligomerization expose the SOAR/CAD domain, allowing it to gate Orai1. This is supported by the finding that STIM1 mutants within the SOAR/CAD domain do not affect the ability of STIM1 to cluster and recruit Orai1, but do abrogate its ability to gate Orai1 (Yuan et al., 2009). In addition, the expression of STIM1 from worms in mammalian cells leads to the formation of STIM1 puncta even when Ca^{2+} stores are full (Gao et al., 2009). Pre-clustered worm STIM1 can recruit Orai1, leading to Ca^{2+} influx, only after store depletion. Nonetheless, given the essential role of STIM1 in gating Orai1 channels and its role in modulating the Ca^{2+} dependency of SOCE (Lee et al., 2009; Mullins et al., 2009), it can be considered an integral component of the SOCE channel.

**SOCE inactivates during mitosis**

The cell cycle is a sophisticated and tightly controlled physiological process that involves multiple finely coordinated signaling pathways that ensure correct cell division and transmission of genetic information. The cell cycle transitions through four phases: G1 growth phase, DNA synthesis (S phase), the G2 phase and cell division (M phase) (Murray and Hunt, 1993). This cycle is unidirectional and sequential; this is crucial for ascertaining proper chromosomal duplication and equal segregation to daughter cells (Murray and Hunt, 1993). The unidirectionality of the cycle is guaranteed by several checkpoints that prevent progression to the next phase unless key events have been satisfied (Murray and Hunt, 1993). Transitions between different phases of the cell cycle are driven by cyclin-dependent kinases (CDKs) and their cyclin partners (Murray and Hunt, 1993). Deregulation of the tight control of this cycle leads to cellular transformation, with devastating pathological consequences (Vermeulen et al., 2003).

The division phase of the cycle has unique cellular requirements, as illustrated by the dramatic morphological alterations observed during M phase compared with during interphase, including nuclear-envelope breakdown, chromosome condensation, and remodeling of the cytoskeleton and intracellular organelles. These changes ensure equal segregation to daughter cells not only of genetic material but also of intracellular organelles such as the Golgi and ER. The Golgi, for example, is structured as a series of stacks in mammalian cells and these stacks fragment during mitosis to ensure equal partitioning into daughter cells (for a review, see Wei and Seemann, 2009). As discussed above, Ca^{2+}-signaling pathways also undergo significant remodeling during M phase, which involves inhibition of SOCE.

The first suggestion that Ca^{2+} influx is inhibited during cell division was reported in 1988 in a study of HeLa cells (Volpi and Berlin, 1988). In this study, Volpi and Berlin showed that histamine division was reported in 1988 in a study of HeLa cells (Volpi and Berlin, 1988). In this study, Volpi and Berlin showed that histamine stimulation during interphase produced an initial Ca^{2+} rise, owing to Ca^{2+} release from stores, followed by an elevated plateau, owing to Ca^{2+} influx from the extracellular space. By contrast, in mitotic cells, only the Ca^{2+}-release phase was observed, arguing that Ca^{2+} influx is inhibited during mitosis. This observation was made around the same time that ideas regarding SOCE were being formulated. The same group later argued that SOCE inhibition during mitosis occurs through uncoupling of store depletion from SOCE, as thapsigargin (an agent that causes store depletion) activated SOCE in interphase but not mitotic cells (Preston et al., 1991). The incentive to investigate Ca^{2+} dynamics during mitosis was to determine whether changes in intracellular signaling might underlie the observed inhibition of vesicular trafficking during cell division (Volpi and Berlin, 1988). In an interesting twist of events, recent data indicate that vesicular trafficking is involved in inhibiting SOCE during M phase, as discussed in more detail below (Yu et al., 2009).

More recent studies confirmed that SOCE is inactivated during mitosis in HeLa, RBL-2H3, HEK293 and Cos-7 cells (Russa et al., 2008; Smyth et al., 2009; Tani et al., 2007). Investigating SOCE levels throughout the cell cycle showed that there is a slight enhancement of SOCE during the G1 and S phases, and dramatic downregulation during M phase (Tani et al., 2007). It has also been argued that SOCE inhibition during mitosis in Cos-7 cells is the result of the microtubule-network remodeling that accompanies mitosis (Russa et al., 2008).

What is the physiological significance of SOCE inactivation during mitosis? The most likely answer to this question is that tight regulation of Ca^{2+} signaling is required during mitosis because of its important functional role at multiple steps throughout the process. Ca^{2+} signals are implicated in nuclear-envelope breakdown (Baitinger et al., 1990; Wilding et al., 1996), anaphase onset (Groggino and Whitaker, 1998; Keith et al., 1985; Morin et al., 1994; Poenie et al., 1986) and cell cleavage (Poenie et al., 1985). The Ca^{2+} transients involved in these processes must be temporally and spatially controlled to mediate their intended cellular functions. Hence, SOCE inactivation might represent a safety mechanism that prevents sporadic Ca^{2+} signals from occurring during cell division that could derail its sequential progression. Erratic Ca^{2+} influx through SOCE might occur during mitosis due to ER remodeling that results in localized store depletion.

**SOCE inactivation during oocyte maturation (meiosis)**

It was almost a decade after the initial studies by Berlin and colleagues that the issue of Ca^{2+} influx during M phase was revisited in a different physiological context – that of gamete maturation in preparation for fertilization. Indeed, SOCE inactivates completely during Xenopus oocyte meiosis (Machaca and Haun, 2000; Machaca and Haun, 2002). This inhibition is important in the context of the overall Ca^{2+}-signaling remodeling that endows the egg (the fully mature Xenopus oocyte arrested at metaphase of meiosis II) with the capacity to produce the specialized Ca^{2+} transient required at fertilization (Machaca, 2007; Ullah et al., 2007).

Before acquiring the ability to activate at fertilization, fully grown oocytes in vertebrates undergo a cellular differentiation pathway known as oocyte maturation, which consists of coordinated morphological, biochemical and physiological changes (Masui, 2001), including remodeling of Ca^{2+}-signaling pathways (Machaca, 2007). Egg activation encompasses crucial events at fertilization that are essential for the egg-to-embryo transition, such as prevention of polyspermy and completion of meiosis. In all sexually reproducing species investigated to date, egg activation is mediated by a cytoplasmic Ca^{2+} rise that has specialized spatial and temporal dynamics (Stricker, 1999). The remodeling of Ca^{2+} signaling during oocyte maturation has been best defined for Xenopus oocyte maturation, in which it encompasses Ca^{2+} release, influx and extrusion. Ins(1,4,5)P_3-dependent Ca^{2+} release is sensitized through spatial and functional remodeling during oocyte maturation (Boulware and Marchant, 2005; Machaca, 2004; Sun et al., 2009; Terasaki et al., 2001). The plasma membrane Ca^{2+}-ATPase (PMCA) is internalized, thereby inhibiting Ca^{2+} extrusion in the Xenopus egg (El Jouni et al., 2005; El Jouni et al., 2008), and SOCE inactivates during oocyte maturation (Machaca and Haun, 2000;
Machaca and Haun, 2002). Combined, these alterations to primary Ca\(^{2+}\)-signaling pathways shape the fertilization-specific Ca\(^{2+}\) transient. Because a localized Ca\(^{2+}\) rise is sufficient to activate the mature egg in the absence of fertilization, SOCE inactivation might represent a safety mechanism that prevents premature sporadic egg activation in the absence of sperm.

Immature *Xenopus* oocytes possess a robust SOCE current that has similar biophysical properties to the CRAC current observed in mammalian cells (Hartzell, 1996; Machaca and Haun, 2000; Yao and Tsien, 1997). By contrast, in mature eggs that are arrested at metaphase II of meiosis, SOCE can no longer be activated by store depletion (Machaca and Haun, 2000). Single-oocyte analysis of the changes in SOCE current that occur following manipulation of the different kinases that drive *Xenopus* oocyte maturation showed that maturation-promoting factor (MPF, composed of Cdk1 and cyclin B) is necessary and sufficient for SOCE inactivation (Machaca and Haun, 2002). Therefore, the fertilization-specific Ca\(^{2+}\) transient in *Xenopus* eggs is generated without the contribution of Ca\(^{2+}\) influx through the SOCE pathway. *Xenopus* eggs respond to sperm entry with a single sweeping Ca\(^{2+}\) transient that lasts for several minutes (Busa and Nuccitelli, 1985; Fontanilla and Nuccitelli, 1998). This Ca\(^{2+}\) signal encodes all of the subsequent events associated with egg activation, including, in the following order: (1) the fast block to polyspermy due to Ca\(^{2+}\)-activated Cl\(^{-}\) channels that depolarize the cell membrane; (2) the slow block to polyspermy due to cortical granule fusion; and (3) completion of meiosis (for a review, see Machaca et al., 2001; Machaca, 2007). The ability of the Ca\(^{2+}\) signal to encode these crucial physiological responses in a sequential manner depends on its duration and spatial propagation. SOCE inactivation probably contributes to shaping the dynamics of the fertilization-specific Ca\(^{2+}\) signal in eggs. In contrast to immature oocytes, in which it is relatively straightforward to induce Ca\(^{2+}\) oscillations (Lechleiter et al., 1991), mature *Xenopus* eggs typically respond to Ca\(^{2+}\) mobilization with a single sweeping Ca\(^{2+}\) wave and are resistant to the generation of Ca\(^{2+}\) oscillations (El Jouni et al., 2005; Machaca, 2004). SOCE inhibition could contribute to this switch in the modality of Ca\(^{2+}\) signaling during maturation, as Ca\(^{2+}\) influx in the oocyte has been shown to increase the speed and frequency of Ca\(^{2+}\) oscillations (Girard and Clapham, 1993).

Interestingly, complete inhibition of SOCE does not appear to be a universal phenomenon during M phase, as store depletion in mammalian oocytes that are arrested at metaphase II induces Ca\(^{2+}\) entry (Igusa and Miyazaki, 1983; Kline and Kline, 1992; Machaty et al., 2002; Martin-Romero et al., 2008; Mohri et al., 2001). In contrast to what has been observed in *Xenopus* eggs, mammalian oocytes respond at fertilization with multiple Ca\(^{2+}\) oscillations that last for several hours (Kline and Kline, 1992). Maintenance of these Ca\(^{2+}\) oscillations depends on Ca\(^{2+}\) influx through SOCE, presumably to refill Ca\(^{2+}\) stores (Igusa and Miyazaki, 1983; Kline and Kline, 1992; Mohri et al., 2001). The data from *Xenopus* and mammalian oocytes indicate that there is a correlation between the occurrence of SOCE and the ability of the oocyte to entrain Ca\(^{2+}\) oscillations at fertilization. The Ca\(^{2+}\) transient at fertilization takes the form of single or multiple transients in a species-specific manner (Stricker, 1999). Unfortunately, as SOCE has not been carefully analyzed in oocytes of species other than frogs and mammals, it is unclear whether the correlation between SOCE and Ca\(^{2+}\) oscillations is conserved across all species. Nonetheless, it is tempting to speculate that the oocytes of species that have a single Ca\(^{2+}\) transient at fertilization are unable to entrain Ca\(^{2+}\) oscillations because of the absence of SOCE, which translates to inadequate refilling of Ca\(^{2+}\) stores following the dramatic first Ca\(^{2+}\) transient. By contrast, oocytes from species that generate an oscillatory Ca\(^{2+}\) signal at fertilization would rely on SOCE to refill stores between Ca\(^{2+}\) spikes.

Although SOCE is detectable in mature mammalian oocytes, it is not clear whether SOCE is downregulated compared with that in immature oocytes. It is possible that SOCE is downregulated but not completely inactivated in mammalian oocytes during maturation, as it serves a Ca\(^{2+}\)-replenishing function that entrains prolonged Ca\(^{2+}\) oscillations. This is an attractive possibility given the dependence of SOCE inactivation on MPF and the conserved nature of the kinase cascade that drives oocyte maturation in both mammals and frogs.

**Mechanisms regulating SOCE inactivation during M phase**

Aside from the role of MPF in SOCE inhibition, very little was known regarding the mechanistic regulation of SOCE inactivation during M phase. However, recent studies investigating the behavior of STIM1 and Orai1 during M phase have provided important insights (Smyth et al., 2009; Yu et al., 2009). During *Xenopus* oocyte meiosis, SOCE inactivation is mediated by removal of Orai1 from the cell membrane into an endosomal compartment and by inhibition of STIM1 clustering (Yu et al., 2009). In immature oocytes arrested in an interphase-like state with an intact germinal vesicle, Orai1 is enriched at the cell membrane, and continuously recycles between the cell membrane and an endosomal compartment (Yu et al., 2009) (Fig. 1). During meiosis resumption, however, Orai1 is removed from the cell membrane and becomes enriched intracellularly in endosomes, which contributes to SOCE inactivation. This was the first report that addressed Orai1 trafficking; hence, it is not clear whether this mechanism is involved in SOCE inactivation during mitosis. However, internalization of membrane channels, receptors and transporters has been documented during meiosis of both *Xenopus* and mouse oocytes (El Jouni et al., 2008; Muller et al., 1993; Schmalzing et al., 1990; Zhou et al., 2009). These internalized membrane proteins incorporate into newly formed blastomeres during early embryogenesis and, as such, contribute to the formation of the polarized epithelium during the blastocoele stage of *Xenopus* embryogenesis (Angres et al., 1991; Gawantka et al., 1992; Muller, 2001).

In addition to Orai1 internalization, another factor that contributes to SOCE inactivation is the inability of STIM1 to reorganize into puncta in response to store depletion in *Xenopus* eggs (Yu et al., 2009) (Fig. 1, M phase). Inhibition of STIM1 clustering correlates with the activation state of MPF (Yu et al., 2009), consistent with the earlier finding that MPF is required for SOCE inactivation (Machaca and Haun, 2002). Importantly, however, inhibition of STIM1 clustering does not affect the ability of STIM1 to interact with Orai1, as revealed by analysis of a constitutively active STIM1 mutant. The cytoplasmic domain of STIM1, which constitutively activates the SOCE current, co-segregates with Orai1 into the endosomal compartment during meiosis, showing that this mutant can still interact with Orai1 (Yu et al., 2009).

The inability of STIM1 to form large puncta following store depletion is associated with its hyperphosphorylation during meiosis (Yu et al., 2009) (Fig. 1). Coupled with the finding that MPF is involved in SOCE inactivation, this argued that the phosphorylation
of STIM1 is responsible for inhibition of its clustering. However, this is not the case, because mutations of STIM1 that affect its phosphorylation state (either alanine substitutions or phosphomimetic mutations at consensus MPF sites between residues 485 and 685) did not affect the inhibition of STIM1 clustering (Yu et al., 2009). This argues that the inability of STIM1 to cluster during meiosis is not mediated by its phosphorylation at consensus MPF sites.

As is the case during meiosis, STIM1 is also hyperphosphorylated and fails to form puncta in response to store depletion in mitotic HeLa and HEK293 cells (Smyth et al., 2009) (Fig. 1). Interestingly, a deletion mutant of STIM1 missing residues C terminal to residue 482 – a region that contains all of the consensus MPF phosphorylation sites – partially rescues both the SOCE current and puncta formation in mitotic cells (Smyth et al., 2009). In addition, alanine substitution at two residues (Ser486 and Ser668) was sufficient to partially rescue SOCE, although to a lesser extent than the 482 deletion mutant (Smyth et al., 2009). These data argue that, in contrast to what occurs during meiosis, STIM1 phosphorylation underlies the inhibition of its clustering during mitosis. However, another potential contributing factor to the discrepancy is the use of nocodazole in the study of mitotic cells. Nocodazole is a microtubule-disrupting drug that does not replicate all aspects of mitosis; this could influence the robustness of SOCE inactivation in cells arrested in M phase using this pharmacological agent. By contrast, in the meiosis studies,egis are physiologically arrested at metaphase of meiosis II until fertilization, thereby providing a better model to study the functional behavior of SOCE during metaphase.

Another factor that might modulate the ability of STIM1 to form large puncta in response to Ca2+-store depletion during M phase is the remodeling of the ER. During both mitosis and meiosis, the ER undergoes dramatic restructuring. This has been well documented during oocyte meiosis in several species (Campanella et al., 1984; Charbonneau and Grey, 1984; Jaffé and Terasaki, 1994; Mehlmann et al., 1996; Shiraiishi et al., 1995; Terasaki et al., 2001). Additional studies have described ER remodeling in a similar manner during mitosis (Lu et al., 2009; McCullough and Luocoq, 2005; Poteryaev et al., 2005). Overall, the evidence suggests that the tubular reticular structure of the ER that is typical of cells in interphase is re-structured into a more patchy cisternal organization during M phase. The function of STIM1 is tightly correlated with ER structure, as STIM1 puncta formation depends on STIM1 lateral diffusion within the ER membrane. In addition, store depletion leads not only to STIM1 clustering but also to enrichment of cortical ER, which puts large STIM1 puncta to form or to stabilize. Therefore, SOCE inactivation during M phase appears to be mediated by both Orai1 internalization and inhibition of STIM1 clustering. Further comparative studies that address the inhibition of STIM1 clustering during mitosis and meiosis will be instrumental in defining the molecular mechanisms underlying this process.

**Perspectives**

With the discovery of STIM and Orai, much is being learned about the molecular mechanisms that control SOCE activation in response to Ca2+-store depletion, and about the functional domains in STIM1 and Orai1 that mediate their coupling. Cell division provides an interesting physiological example in which cells have devised mechanisms to reverse the coupling between Ca2+-store depletion and the activation of Ca2+ influx at the cell membrane. Such inhibition of SOCE during M phase is liable to be important for cell-cycle progression and oocyte activation at fertilization. Furthermore, understanding SOCE inhibition during M phase will undoubtedly provide important clues regarding the basic mechanisms controlling SOCE activation and regulation. More than two decades after the appearance of Ca2+ influx during cell division was initially described, we are now beginning to understand the mechanisms involved. However, several fundamental questions remain unanswered. What are the mechanisms controlling Orai1 internalization during M phase? How do mammalian oocytes maintain functional SOCE during meiosis? How is STIM1 clustering inhibited during meiosis? Are homotypic STIM1-STIM1 interactions inhibited during M phase or is it the ability of STIM1 to form large puncta that is repressed? This last issue will be important to elucidate, as STIM1 is thought to form lower oligomers (possibly dimers) in resting conditions when Ca2+ stores are full (Baba et al., 2006; Penna et al., 2008) and to reorganize into the large puncta below the cell membrane after store depletion. Therefore, the regulation of SOCE during cell division promises to be an exciting area of research, with broad physiological implications ranging from cell-cycle regulation to the activation of development at fertilization.

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**References**


