Published in final edited form as:

Cell Calcium. 2010 November ; 48(5): 243–250. doi:10.1016/j.ceca.2010.10.003.

Ca²⁺ signaling, genes and the cell cycle

Khaled Machaca

Department of Physiology and Biophysics, Weill Cornell Medical College in Qatar (WCMC-Q), Education City – Qatar Foundation, Doha, Qatar

Abstract

Changes in the concentration and spatial distribution of Ca²⁺ ions in the cytoplasm constitute a ubiquitous intracellular signaling module in cellular physiology. With the advent of Ca²⁺ dyes that allow direct visualization of Ca²⁺ transients, combined with powerful experimental tools such as electrophysiological recordings, intracellular Ca²⁺ transients have been implicated in practically every aspect of cellular physiology, including cellular proliferation. Ca²⁺ signals are associated with different phases of the cell cycle and interfering with Ca²⁺ signaling or downstream pathways often disrupts progression of the cell cycle. Although there exists a dependence between Ca²⁺ signals and the cell cycle the mechanisms involved are not well defined and given the cross-talk between Ca²⁺ and other signaling modules, it is difficult to assess the exact role of Ca²⁺ signals in cell cycle progression. Two exceptions however, include fertilization and T-cell activation, where well-defined roles for Ca²⁺ signals in mediating progression through specific stages of the cell cycle have been clearly established. In the case of T-cell activation Ca²⁺ regulates entry into the cell cycle through the induction of gene transcription.

Introduction

Ca²⁺ signals have been implicated in various aspects of the cell cycle using different approaches and experimental systems. This has led to a consensus in the field for a requirement for Ca²⁺ in cellular proliferation. The cell cycle is essential for organismal development and its deregulation leads to disastrous results such as tumor growth. The cell cycle consists of four phases, two growth phases G1 and G2 interspersed by the DNA synthesis phase (S) and the cell division phase (M) [1]. To ensure proper cellular proliferation and survival the cell cycle is unidirectional and sequential. This guarantees that DNA synthesis precedes cell division to ensure diploidy in the two daughter cells [1].

The idea that Ca²⁺ signals may be involved in cell cycle progression is quite attractive given the versatile nature of Ca²⁺ signaling. Intracellular Ca²⁺ signals tend to be complex in terms of their spatial localization, temporal features and amplitude [2;3]. Ca²⁺ signals can be very localized, limited to small region surrounding the mouth of a channel for example [4], or can take the form of Ca²⁺ release waves that sweep through the entire cell or even across multiple cells. They can range in duration from μ seconds during vesicular exocytosis for example, to hours after fertilization in mammals [5]. In fact specificity in Ca²⁺ signaling is dependent on this versatility. Ca²⁺ transients activate different Ca²⁺ binding proteins with

© 2010 Elsevier Ltd. All rights reserved.

Address correspondence to: Khaled Machaca, PhD, Weill Cornell Medical College in Qatar, PO Box 24144, Doha, Qatar, Voice: 646-291-6345, Fax: +974-4492-8422, Khm2002@qatar-med.cornell.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

varying enzymatic activities such as kinases or phosphatases, or signal transducers such as calmodulin that then branch out and modulate a multitude of signaling pathways to affect cell function. Based on the association and dissociation constants of the different Ca^{2+} binding proteins, Ca^{2+} signals with disparate dynamics induce distinct cellular responses.

Intracellular Ca^{2+} signaling is possible because cells maintain a low Ca^{2+} background in the cytoplasm with concentrations of ~ 100 nM. Ca^{2+} signals are generated due to Ca^{2+} influx from the extracellular space with concentration around 1-2 mM, or Ca^{2+} release from intracellular Ca^{2+} stores, primarily the endoplasmic reticulum (ER) with concentrations of 250-600 μM [6]. Channels and pumps on the ER and plasma membranes coordinately regulate Ca^{2+} homeostasis in the ER and cytoplasm.

Ca^{2+} signals have been detected at various stages of the cell cycle and manipulation of Ca^{2+} signaling often affects cellular proliferation. This generated the consensus that Ca^{2+} signals are essential for cellular proliferation. However, given the ubiquitous and versatile nature of Ca^{2+} signaling and the integration of Ca^{2+} signals with other signaling modules regulating cellular physiology, it is quite difficult to identify the specific signaling pathway downstream of the Ca^{2+} signals. However, in at least two cases, following fertilization and during T-cell activation, Ca^{2+} signals have been shown to be essential for cell cycle progression, and the mechanisms through which Ca^{2+} signals mediate these cell cycle transitions are well defined.

I. Ca^{2+} signals are required at fertilization to induce completion of meiosis

As is often the case in biology the first studies to observe a correlation between the cell cycle and Ca^{2+} signals were performed to address a different problem, that of the role of Ca^{2+} ions in 'protoplasm gelation' following cellular stimulation [7]. Cellular stimulation was thought to be associated with an increased viscosity in the cytoplasm and Ca^{2+} was implicated in the process. One model of cell stimulation that was used to test the involvement of Ca^{2+} ions in colloid chemical changes in the cell was following fertilization of sea urchin eggs. Hence the earliest study to observe changes in Ca^{2+} dynamics associated with the cell cycle was performed in the mid 1930s in the context of fertilization, where a Ca^{2+} rise was observed following fertilization in of the sea urchin *Arabica* eggs [7]. Later studies confirmed this observation using $^{45}\text{Ca}^{2+}$ fluxes also in sea urchin [8-10]. Interestingly, initial hypotheses regarding the role of Ca^{2+} in inducing cell cycle progression at fertilization argued that Ca^{2+} has a physiochemical role in the formation of the mitotic spindle through increasing cytoplasmic viscosity [9;11]. The Ca^{2+} rise at fertilization was also linked to the observed increase in cellular respiration [9;12]. These studies sparked an interest in the potential role and regulation of Ca^{2+} signals in egg activation at fertilization and their role in cell cycle progression.

Other investigators became interested in the role of Ca^{2+} in cellular motility, wound healing and cellular cleavage. The common thread among these different cellular processes is that each involves contractions of the cell cortex. These interests were instigated by the known role at the time of Ca^{2+} in muscle contraction [13;14], it was therefore attractive to investigate the role of Ca^{2+} signals in other forms of cellular contractions in non-excitabile cells. In this case the *Xenopus laevis* oocyte and embryo were attractive systems for these studies given their unusually large size ($\sim 1.3\text{mm}$ for the fully grown stage VI oocyte). Indeed Ca^{2+} was shown to play important roles in wound healing in the oocyte and cell cleavage during early embryonic divisions [13;14]. These studies also stimulated research in the area of Ca^{2+} signaling during fertilization, egg activation and cell cycle progression.

Direct visualization of a Ca^{2+} rise at fertilization was first accomplished using aequorin in the medaka fish [15], and later in sea urchin [16]. These fertilization-induced Ca^{2+} increases

took the form of a propagating wave that swept through the entire egg [17]. However the fact that Ca^{2+} transients, although quite dramatic, were observed at fertilization did not necessarily establish a functional role for Ca^{2+} signals in egg activation. The correlation between Ca^{2+} and fertilization was extended to a causal relationship when an ionophore-mediated Ca^{2+} rise was shown to be sufficient to parthenogenetically activate sea urchin eggs [10;18]. Because ionophore treatment was also effective at activating eggs from other species independently of sperm, this argued that Ca^{2+} is the universal egg activator [19], a prediction that was validated by future studies in several other species [20].

Subsequent studies characterized the fertilization-dependent Ca^{2+} release in mammals and other vertebrates and showed specificity in the spatial and temporal pattern of these signals, where in mammals an oscillatory Ca^{2+} signal that lasts several hours is observed, whereas in the frog *Xenopus laevis* for example only a single Ca^{2+} transient is induced at fertilization and takes the form of a sweeping Ca^{2+} wave [20-22]. These differing Ca^{2+} dynamics among species are vital in mediating the complex events that need to occur at fertilization to induce the transition from gametogenesis to embryogenesis [20;22;23]. Central among these is the block to polyspermy and the release of the CSF arrest leading to the completion of meiosis and transitioning into the mitotic embryonic divisions.

Although not discussed in details here, eggs acquire the ability to produce the specialized Ca^{2+} transient at fertilization following a dramatic remodeling of the Ca^{2+} signaling machinery during oocyte maturation, which encompasses entry and progression through meiosis [22]. Hence not only are Ca^{2+} signals important for cell cycle progression, but also the Ca^{2+} signaling machinery remodels during the cell cycle to service the specific needs of the cell during its development [22;23].

Block to polyspermy—The polyspermy block is discussed here as an example of how Ca^{2+} signals during the cell cycle, in this case the egg arrested in meiosis II, are important to regulate physiological processes outside the central cell cycle machinery that are nonetheless essential for proper progression through the cell cycle. The block to polyspermy is mediated by the Ca^{2+} transient at fertilization and is critical to prevent polyploidy in the zygote, which will lead to its demise. Different species have devised distinct mechanisms to prevent polyploidy. The urodelas allow multiple sperms to penetrate the egg at fertilization, but only one sperm pronucleus fuses with the egg pronucleus and additional sperm nuclei disintegrate [24]. In contrast, in the more common monospermic fertilization only a single sperm is allowed to fuse with the egg [25]. This represents a challenge for species with large oocytes such *Xenopus laevis* given the large surface area of the egg and the number of sperm it is exposed to at fertilization. Hence such species have developed an electrical fast block to polyspermy to rapidly prevent additional sperm from fusing with the egg. This fast block is mediated by membrane depolarization due to the opening of Ca^{2+} -activated Cl^- channels following the initial Ca^{2+} transient at fertilization [26]. The Ca^{2+} rise at fertilization gates open Ca^{2+} -activated Cl^- channels leading to an inward current (Cl^- leaving the cell) and hence membrane depolarization. This change in membrane potential instantaneously blocks further sperm fusion because *Xenopus* sperm fusion is voltage-dependent [27]. Sea urchin eggs similarly exhibit a fast block to polyspermy [28]. A more sustained block to polyspermy, which is also observed in other species, occurs following the modification of the extracellular matrix surrounding the egg, making it impenetrable to sperm [29]. This is again a Ca^{2+} -dependent event due to the fusion of cortical granules as a Ca^{2+} wave sweeps through the egg [30]. Cortical granules release proteolytic and glycolytic enzymes that modify the egg extracellular matrix [29].

Release of the CSF-dependent meiosis II block—Following oocyte maturation vertebrate eggs arrest at metaphase of meiosis II until fertilization. In a landmark study

Masui and Market defined two activities from frog eggs at different stages of maturation that turn out to be essential drivers of M-phase. Microinjection experiments showed that cytoplasm from frog eggs arrested at metaphase of meiosis II when injected into blastomeres was capable of arresting their division due to a cell cycle arrest at metaphase. This activity was termed cytostatic factor (CSF) [31].

The second activity from those experiments was identified as an activity able to induce oocyte entry into and progression through meiosis (i.e. oocyte maturation), when cytoplasm from mature frog eggs was injected into immature oocytes arrest at the G2-M transition. This activity was termed maturation promoting factor (MPF) [31]. Biochemical purification and characterization of MPF showed that it is composed of the catalytic Cdk1 Ser/Thr kinase subunit and the regulatory cyclin B subunit [32-34]. The central role of MPF as the universal inducer of the G2-M transition was cemented when its catalytic Cdk1 subunit was shown to be equivalent to p34^{Cdc2} the product of the Cdc2 gene in fission yeast, which is required for cell cycle progression [35].

Entry into mitosis requires the activation of MPF leading to nuclear envelope breakdown, chromosome condensation, and formation of the mitotic spindle in metaphase. In a similar vein MPF inactivation, which occurs during anaphase and telophase, is essential for mitotic exit. MPF inactivation reverses the early mitotic events leading to chromosome decondensation, cell division and reformation of the nuclear envelope [36]. Cyclin B is targeted for degradation due to poly-ubiquitination and degradation by the 26S proteasome. Ubiquitination of cyclin B requires the anaphase-promoting complex (APC), which is a large molecular complex composed of 11 subunits and functions as an E3 ubiquitin ligase [36]. CSF maintains metaphase II meiotic arrest by inhibiting the APC and hence MPF degradation [37]. This is accomplished through a complex series of phosphorylation regulatory loops involving multiple cell cycle kinases, including Mos and the MAPK cascade, which are required to establish CSF arrest [37]. Ultimately however CSF arrest is regulated by the APC inhibitor Emi2 [38;39]. Emi2 binds to and inhibits the APC during metaphase II an activity that is stabilized through Emi2 phosphorylation by the Mos-MAPK-Rsk cascade, and dephosphorylation by protein phosphatase 2A. The fertilization-induced Ca²⁺ transient leads to the activation of CaMKII, which phosphorylates Emi2 [40-42]. CaMKII phosphorylated Emi2 becomes a target for further phosphorylation by polo-like kinase. The dually phosphorylated Emi2 is targeted for proteasomal degradation [38]. This activates APC and releases the CSF-mediated arrest. This complex signal transduction cascade provides an elegant example of the versatility of Ca²⁺ signaling and its essential role in releasing the CSF-mediated metaphase II arrest.

In addition to the role CaMKII in releasing the CSF-mediated meiotic arrest, calcineurin a Ca²⁺-dependent phosphatase is also important in this transition, arguing that the Ca²⁺-dependent regulation affects both kinases and phosphatase to coordinately regulate the release of CSF arrest [43;44]

From the brief description of the history of Ca²⁺ signaling in fertilization one can define a clear role for Ca²⁺ in mediating the meiotic metaphase-to-anaphase transition of the cell cycle. Importantly, in this case the downstream effectors linking Ca²⁺ signals to the cell cycle are well delineated. Given the diverse and promiscuous role of Ca²⁺ signals in various aspects of cellular function, it is often quite difficult to clearly define downstream targets. In the case of fertilization a conversion of multiple fields over a long period of time allowed this to happen. This required a good understanding of the biochemistry of the cell cycle with the complex kinase cascades involved, and tools to directly visualize the spatial-temporal features of the Ca²⁺ signal at fertilization. In addition, the biology of the egg is well adapted

for such studies as it provides a synchronous population of cells arrested at specific stages of meiosis.

II. Ca^{2+} signals during various stages of the cell cycle

The large size of oocytes allows easier experimental access to the cytoplasm in terms of the ability to insert ion sensitive electrodes or microinject aequorin to measure rapid transient Ca^{2+} changes. Such experiments in oocytes discussed in the previous section, coupled to the realization that the mitotic spindle is associated with intracellular membranes, lead to proposals for a role of Ca^{2+} signals in regulating the dynamics of mitosis. Initial attempts to test this idea were performed using the large endosperm cells of *Haemanthus* with various probes, and showed a correlation between anaphase onset and Ca^{2+} signals [45;46]. Similar studies aimed at identifying Ca^{2+} transients during the mitotic cell cycle of animal cells became possible with the development of membrane permeant Ca^{2+} -sensitive fluorescent probes such as Fura-2 [47;48]. Ca^{2+} transients associated with nuclear envelope breakdown, metaphase to anaphase transition and cytokinesis were observed [47-49]. Initial descriptive studies were followed by mechanistic experiments aimed at defining a causal relationship between the observed Ca^{2+} transients during mitosis and subsequent cellular events. It was shown using chelators and Ca^{2+} injection that a Ca^{2+} transient is necessary and sufficient to induce nuclear envelope breakdown (NEBD) and chromatin condensation in sea urchin embryos, and IP_3 -dependent Ca^{2+} release was implicated [50;51]. Interestingly, the Ca^{2+} dependency of these events required protein translation [51]. These observations were extended to mammalian cells, where it was shown in Swiss 3T3 fibroblasts using Ca^{2+} uncaging and mild chelation conditions that NEBD is dependent on Ca^{2+} transients, whereas the metaphase to anaphase transition was not as sensitive to alterations in Ca^{2+} signaling [52]. The fact that an inhibitory peptide to CaMKII was able to interfere with NEBD in sea urchin embryos argued that the Ca^{2+} -dependency of NEBD was mediated by CaMKII [53].

Surprisingly significant differences were observed in terms of the Ca^{2+} dependence of nuclear envelope breakdown during meiosis as compared to mitosis. In both mouse and *Xenopus* oocytes, germinal vesicle breakdown occurred independently of Ca^{2+} transients [54;55]. The differential Ca^{2+} -dependency of nuclear envelope breakdown in mitosis and meiosis is intriguing especially that in both cases MPF is required [56]. It was argued that GVBD may also be Ca^{2+} -dependent through early events that occur before the initiation of oocyte maturation [54;57], this seems unlikely though since long term Ca^{2+} -deprivation of *Xenopus* oocytes (up to 48hrs) does not affect their ability to undergo GVBD [55]. Therefore, the differential Ca^{2+} -dependency of GVBD and NEBD may reflect distinct mechanisms controlling both processes.

Oscillations in the levels of IP_3 were also observed to coincide with the mitotic cell cycle in sea urchin embryos, and blocking the IP_3 receptor using heparin inhibited mitosis arguing for a potential functional role for these IP_3 spikes in mitosis progression [58]. In contrast inhibiting Ca^{2+} signals in starfish, mouse and *Xenopus* oocytes did not affect their ability to enter meiosis and complete GVBD [54;55;59].

Downstream of Ca^{2+} both calmodulin (CaM) and Ca^{2+} -CaM-dependent protein kinase II (CaMKII) have been shown to play a role in cell cycle progression. Expression levels of CaM have been linked to cell cycle progression both experimentally and physiologically. CaM levels change during the cell cycle with a pronounced increase during the G1-S transition [60;61], and inducible expression of CaM in mouse cells affect progress through both the G1 and M-phases of the cell cycle [62]. Similarly, CaM in the fungus *Aspergillus nidulans* is required for the G2-M transition of the cell cycle [63;64], and genetic analyses in the yeast *S. cerevisiae* show that CaM is required for progression through mitosis [65-68]. Consistent with the idea that CaM effects on the cell cycle may be mediated by CaMKII,

knockout of the CaMKII gene in *Aspergillus* result in a G2-M arrest, showing that CaMKII is required for entry into M-phase [69]. Pharmacological inhibition of CaMKII in mammalian cells shows similar results to the knockout in *Aspergillus*, with an additional apparent requirement for CaMKII during the G1-phase of the cell cycle [70]. Furthermore, expression of a constitutively active CaMKII arrests the cell cycle in G2 [71].

In addition, increased CaM expression levels have been associated with cellular transformation [70]. A decrease in functional CaM levels in DT40 cells results in slower growth [72], and CaM inhibition in mammalian cells interferes with DNA replication and slows cellular proliferation [73;74]. Consistently, serum-dependent induction of DNA synthesis was shown to be dependent on extracellular Ca^{2+} and CaM in normal cells but not in transformed cells [75]. Phosphorylation of the retinoblastoma protein (Rb) also exhibited the same dependence on extracellular Ca^{2+} and CaM [75]. Rb phosphorylation by cyclin D/cdk4 and cyclin E/cdk2 relieves its inhibition of E2F transcription factors leading to the expression of genes required for entry into S-phase [76].

In addition to its well defined role in T-cell activation, calcineurin is also apparently involved in other aspects of cell cycle. During the metaphase to anaphase transition at fertilization in *Xenopus* eggs calcineurin has been shown to play a role although the details remain to be elucidated [43;44]. In pancreatic acinar cells cyclosporine (CsA) treatment inhibits CREB leading to a reduction in cyclin D expression and arrest in the G1-phase of the cell cycle [77]. However, CsA has also been reported to exhibit cell autonomous tumor promoting effects [78], arguing that inhibition of calcineurin activity depending on the cellular context can either lead to cell cycle arrest or promotion of cellular proliferation.

It is clear from these studies that Ca^{2+} signals play important roles during cell cycle progression. Interestingly however, it seems that the defects tend to be cell type specific arguing that Ca^{2+} signals may be used differentially in distinct cell types to modulate the cell cycle. In addition, the details of the mechanisms of action of Ca^{2+} signaling at different stages of the cell cycle are not well defined.

III. Role of Ca^{2+} signals in the induction of oocyte meiosis

In the addition to the CSF-mediated metaphase II arrest discussed above, vertebrate oocytes also arrest at an earlier stage during the G2-M transition during their growth and maturation in preparation for fertilization. As oocytes develop in the ovary they remain arrested at prophase of meiosis I in a G2-like state of the cell cycle with an intact germinal vesicle (nucleus) and active transcription [79-81]. In contrast, to the CSF-mediated arrest in meiosis II, this arrest is quite stable and can last for several decades in human oocytes as they grow and mature in a sequential fashion [82]. During this long arrest oocytes accumulate macromolecular components to allow them to undergo meiosis/oocyte maturation and then egg activation before transitioning to embryogenesis [79;83]. The signal inducing release from this meiotic arrest varies between species, but ultimately commits the oocyte to oocyte maturation in preparation for fertilization. In mammals meiotic arrest is relieved following release of the oocyte from the follicle at ovulation, whereas in *Xenopus* meiotic arrest is released in response to progesterone [84;85]. Independent of the signal however, oocyte maturation induces a complex kinase cascade that ultimately activates MPF and commits the oocyte to maturation. The details of this cascade are best defined in *Xenopus* oocytes with close parallels in mammals. Progesterone treatment results in a decrease in cAMP levels in *Xenopus* oocytes through the activation of a progesterone cell surface serpentine G-protein coupled receptor [86;87]. This induces poly-adenylation of oocyte mRNAs leading to accumulation of the oocyte specific MAP kinase kinase, Mos [88]. Induction of the MAPK cascade culminates in activation of p90Rsk (Rsk), which inhibits Myt1, a kinase that phosphorylates and inhibits MPF [88;89]. A parallel pathway to the MAPK cascade is also

activated leading to the induction of Cdc25C, which dephosphorylates and activates MPF. Combined Cdc25C activation and inhibition of Myt1 lead to a dramatic rapid rise in MPF activity. MPF is composed of cyclin B and Cdk1 the catalytic subunit which is maintained in an inactive state due to phosphorylation on Thr14 and Tyr15 mediated by Myt1 kinase [90;91]. Both residues localize to the ATP-binding loop of cdk, and are dephosphorylated by the dual-specificity phosphatase Cdc25 [92]. Dephosphorylation of these residues constitutes the rate limiting step in MPF activation [93].

Given the specific arrest stage of these oocytes at the G2-M transition, there has been a long standing interest in determining whether Ca^{2+} signals play a role in releasing their meiotic. The preponderance of the evidence argue that this is not the case and that release from meiotic arrest occurs independently from a Ca^{2+} signal. However there are reports arguing for a potential role of Ca^{2+} signals in the initiation of oocyte maturation. Incubation of oocytes in high Ca^{2+} and Mg^{2+} concentrations in the presence of ionophore was reported to induce oocyte maturation [94]. Similarly, Ca^{2+} electroporation was also effective at inducing oocyte maturation [95]. If indeed Ca^{2+} is important for the release from meiotic arrest, then one would expect Ca^{2+} transient to be associated with oocyte maturation. Such Ca^{2+} transients were reported following the induction of oocyte maturation in *Xenopus* by some groups [96-98], but could not be confirmed by others [99;100]. In addition, injection of IP_3 into the oocyte, which releases Ca^{2+} from intracellular stores, did not release meiotic arrest [101]. Furthermore, injection of Ca^{2+} chelators at high concentrations effectively blocked oocyte maturation [95;102]. However, this inhibition is apparently not due to Ca^{2+} chelation, but rather to chelation of transition metals, specifically Zn^{2+} [103]. This is plausible since Ca^{2+} chelators such as BAPTA are also powerful chelators of transition metals [104;105]. Indeed it was shown that these chelators when injected into the oocyte inhibit the activation of Cdc25C, and hence MPF and commitment to meiosis [103]. Cdc25C directly binds Zn^{2+} , and this is important for Cdc25C substrate recognition and dephosphorylation and activation of MPF [103]. Additional support against a role for Ca^{2+} signals in releasing meiotic arrest comes from a study where oocytes were completely deprived of Ca^{2+} signals by emptying Ca^{2+} stores and incubating the cells in nominally Ca^{2+} -free media. Under these conditions oocytes entered meiosis in response to progesterone and underwent GVBD, with the associated activation of the kinase cascade that drives oocyte maturation [55]. However, in the absence of Ca^{2+} signals such oocytes were not able to complete meiosis I because formation of the bipolar spindle and hence polar body extrusion was defective [55]. Similar results were obtained in mouse oocytes [54]. Together these data argue that Ca^{2+} signals are not required for the release of meiotic arrest. This is especially the case when one evaluates the consistent effects of interfering with Ca^{2+} signaling on fertilization in different species as compared to release of oocyte meiotic arrest.

IV. Ca^{2+} signals induce cell cycle reentry of quiescent T-cells following antigen stimulation

Another well defined example for a role for Ca^{2+} signals in cell cycle resumption is during T-cell activation in response to antigen stimulation. Naïve antigen-specific T-cells undergo clonal expansion following antigen stimulation leading to the formation of effector T-cells that are crucial for the adaptive immune response [106]. Antigen stimulation results in cross-linking of the T-cell receptor (TCR)-CD3 complex, which leads to activation of several signaling cascades including $\text{PLC}\gamma$. $\text{PLC}\gamma$ cleaves its substrate phosphatidylinositol 4,5-bisphosphate (PIP_2), generating IP_3 and diacylglycerol (DAG). DAG activates both PKC and RasGRP, leading to activation of NF- κ B and the MAPK cascade respectively [106;107]. IP_3 binds and gates the IP_3 receptor Ca^{2+} channel on the ER membrane hence releasing intracellular Ca^{2+} stores and leading to a transient Ca^{2+} rise. Store depletion activates the Ca^{2+} -release activated Ca^{2+} (CRAC) channel on the cell membrane resulting in a sustained low amplitude Ca^{2+} influx that is essential for NFAT activation and IL-2 transcription [108].

Together these signaling pathways converge on maintaining NFAT, NF- κ B and AP1 transcription factors activity leading to the transcription of genes required for cytokine production and T-cell activation [106;108]. Activation of T-cells results in their transition from the G0 quiescent phase to entry into the cell cycle leading to their proliferation and clonal expansion.

Ca^{2+} influx through SOCE activates calcineurin, a Ca^{2+} -dependent phosphatase that dephosphorylates and activates NFAT. The role of calcineurin in T-cell activation was initially revealed in attempts to elucidate the mechanism of action of the immunosuppressive drugs cyclosporine A (CsA) and FK506. Each drug binds to a distinct immunophilin, cyclophilin and FKBP respectively. The immunophilin-immunosuppressant complexes directly bind to and competitively inhibit calcineurin [109;110]. The introduction of these immunosuppressants to the clinic had a dramatic impact on improving patient outcome following organ transplantation, arguing that the major target for these agents are cells of the immune system. Calcineurin is a Ser/Thr phosphatase composed of two tightly associated subunits A and B. Calcineurin A is the catalytic subunit and contains a CaM binding site and an auto-inhibitory domain that maintains the enzyme inactive in the absence of Ca^{2+} [111]. Calcineurin B contains four EF-hands Ca^{2+} binding domains and has a similar dumbbell structure to CaM. At resting cytoplasmic Ca^{2+} concentrations ($\sim 100\text{nM}$) the high affinity Ca^{2+} binding site on calcineurin B are occupied but the phosphatase is inactive. Binding of Ca^{2+} -CaM induces a dramatic increase in the V_{max} of the enzyme leading to its activation [112]. Ca^{2+} and CaM exhibit a high degree of cooperativity in terms of activating calcineurin with a Hill coefficient of ~ 3 [112]. This allows the enzyme to respond to a threshold Ca^{2+} signal effectively and hence translate different Ca^{2+} dynamics into gene transcription [113].

NFAT at rest resides in the cytoplasm in a phosphorylated state that possesses a low DNA-affinity [114]. Calcineurin dephosphorylates NFAT, which unmask its nuclear localization sequence leading to its translocation to the nucleus where it can induce gene transcription [115]. However, NFAT is rapidly phosphorylated by several kinases leading to its export back out of the nucleus. Hence, maintenance of NFAT dependent transcription requires a sustained Ca^{2+} signal to keep NFAT in the nucleus, explaining the dependence of T-cell activation on Ca^{2+} influx through SOCE. SOCE provides a low amplitude sustained Ca^{2+} influx following the initial Ca^{2+} release transient downstream of TCR engagement.

The activation of T-cells is critically dependent on Ca^{2+} influx through the store-operated Ca^{2+} entry pathway which provides the sustained Ca^{2+} influx signal. This is illustrated by the fact that patients with defective SOCE present with severe combined immunodeficiency (SCID) [116-118]. This examples highlights the critical role of Ca^{2+} signals, in this case Ca^{2+} influx from the extracellular space through the store-operated Ca^{2+} entry pathway (SOCE) in inducing re-entry into the cell cycle. Furthermore, it highlights the integration of Ca^{2+} signaling pathways with other signaling modules to induce gene transcription, cell cycle progression and cell activation. Defects in T-cell activation or inactivation can result in serious clinical defects such as autoimmunity or immunodeficiencies.

Store-operated Ca^{2+} entry (SOCE)—The critical role that SOCE plays in the activation of cells in the immune system generated significant interest in identifying it molecularly. Biophysical characterization of the SOCE current in immune cells was the first step in that endeavor. The SOCE current in immune cells was referred to as the Ca^{2+} release-activated current (I_{CRAC}) and is the best characterized SOCE current biophysically. It is highly Ca^{2+} -selective and exhibits strong inward rectification [119;120]. The initial idea that Ca^{2+} store content regulates Ca^{2+} influx at the cell membrane was proposed in the capacitative model, which suggested that Ca^{2+} flows directly into the ER lumen [121]. Although we now know

that Ca^{2+} influx through SOCE channels flows into the cytosol, this model generated significant interest in this ubiquitous Ca^{2+} entry mechanism. SOCE is not only critical in the context of cell cycle re-entry of T-cells but is also involved in various physiological processes ranging from secretion to muscle development [122;123]. Despite the intense interest in SOCE it took two decades to identify the molecular players involved through the use of large scale RNAi screens. These experiments identified stromal interaction molecule 1 (STIM1) as the ER Ca^{2+} sensor that senses Ca^{2+} store depletion and transmits the signal to the cell membrane. STIM1 is an ER membrane protein with luminal EF hands that sense Ca^{2+} store content [124;125]. The CRAC channel at the cell membrane, Orai1, was identified as a mutation in two siblings with severe combined immunodeficiency (SCID) coupled to the use of genome wide RNAi screens in *Drosophila* [116;126;127]. Orai1 is an integral membrane protein with 4 transmembrane domains and N- and C-terminal cytoplasmic domains. Overexpression of STIM1 and Orai1 produces large I_{CRAC} -like currents that activated only after store depletion, are highly Ca^{2+} selectivity, and inward rectifying, which mirrors the properties of the endogenous I_{CRAC} [127-129].

STIM1 and Orai1 combine to couple Ca^{2+} store depletion to activation of Ca^{2+} influx at the cell membrane. Store depletion releases Ca^{2+} from the low affinity STIM1 EF-hand Ca^{2+} binding domain leading to clustering of STIM1 into large puncta that are stabilized in an ER domain that localizes 10-20nm below the cell membrane. These STIM1 puncta recruit Orai1 and gate it open thus stimulating Ca^{2+} entry at the cell membrane [130]. With the identification of STIM1 and Orai1, it became possible to directly test the requirement for SOCE in T-cell activation. Knockout studies of STIM1 and Orai1 coupled to identification of additional mutations in human patients with immunodeficiencies in both proteins nicely confirmed the essential role SOCE in T-cell activation, because the major phenotype in these patients and knockout animals was defects in the activation potential of cells of immune system leading to immunodeficiencies [131]. The absence of either STIM1 or Orai1 was also associated with congenital myopathy and ectodermal dysplasia consistent with the role of SOCE in myoblast differentiation [123;132].

Interestingly, in addition to the role of SOCE in mediating cell cycle progression in T-cells, the SOCE current itself is regulated during cell division. SOCE inactivates during both mitosis and meiosis arguing that this is a conserved mechanism of regulating Ca^{2+} influx during cellular division [133;134]. SOCE inactivation requires MPF activity [135]. Although speculative at this point, SOCE inactivation may prevent maverick Ca^{2+} signals during cellular division that may interfere with normal cell division especially given the potential role of Ca^{2+} signals during M-phase as discussed in details above [136]. SOCE inactivation was recently shown to be due to both internalization of the Orai1 channel and the inability of STIM1 to form clusters in response to store depletion in oocytes [137;138].

SOCE is not the only Ca^{2+} signaling pathway that is regulated during the cell cycle. In fact Ca^{2+} signaling are dramatically remodeled during oocyte meiosis. IP_3 -dependent Ca^{2+} release is sensitized [139-142] and the plasma membrane Ca^{2+} -ATPase (PMCA) is internalized [143;144]. This remodeling allow the egg to produce the fertilization-specific Ca^{2+} transient that is essential for egg activation [22].

Perspective

It is clear from this brief review that Ca^{2+} signals are associated with progression of the cell cycle. However, because Ca^{2+} signals are involved in so many aspects of cellular physiology it is tricky to define the relationship between Ca^{2+} signaling and the cell cycle machinery. That is interfering with Ca^{2+} signaling could eventually leads to cell cycle arrest through indirect mechanisms. However in at least two cases, at vertebrate fertilization and

during T-cell activation, there is a well defined link between Ca^{2+} signals, with specialized dynamics, and cell cycle progression. In both cases cells are arrested at a specific stage of the cell cycle, for the egg it is in metaphase of meiosis II by CSF, and for T-cell it is in the quiescent G0-phase of the cell cycle. In fact Ca^{2+} is the universal signal for egg activation at fertilization in all species studied to date, despite the fact that eggs from different species arrest at different stages of meiosis [20]. Furthermore the source of the Ca^{2+} transients differs. At fertilization it is primarily due to Ca^{2+} release from intracellular stores, whereas for cells of the immune system it is through Ca^{2+} influx through the store-operated Ca^{2+} entry pathway. The combination of different Ca^{2+} signaling pathways in distinct cell types generates disparate Ca^{2+} dynamics: a sustained Ca^{2+} rise in T-cells, versus prolonged Ca^{2+} oscillations following fertilization in mammals. Moreover, interfering with Ca^{2+} signaling in different cell types results in varied effects on the cell cycle. Together these observations argue that Ca^{2+} signals have been adapted through evolution to fit specific needs in cell cycle progression in a cell type specific fashion based on the cell's developmental stage. Ca^{2+} is a fitting second messenger for such a function because resumption of the cell cycle requires activation of a multitude of signaling cascades in a specific temporal sequence. Calcium's versatility as a second messenger and ability to branch out and activate different signaling modules given the diversity of Ca^{2+} -dependent enzymes and processes make it a well-adapted messenger for this function.

Acknowledgments

This work is funded by a grant from the NIH (GM61829) and grants from the Qatar National Research Fund (QNRF), NPRP08-395-3-088 and NPRP08-138-3-050.

References

1. Murray, A.; Hunt, T. *The Cell Cycle*. New York: Oxford University Press; 1993.
2. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol*. 2000; 1:11–21. [PubMed: 11413485]
3. Clapham DE. Calcium signaling. *Cell*. 2007; 131:1047–1058. [PubMed: 18083096]
4. Parekh AB. Ca^{2+} microdomains near plasma membrane Ca^{2+} channels: impact on cell function. *J Physiol*. 2008; 586:3043–3054. [PubMed: 18467365]
5. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*. 2003; 4:517–529. [PubMed: 12838335]
6. Demaurex N, Frieden M. Measurements of the free luminal ER Ca^{2+} concentration with targeted “cameleon” fluorescent proteins. *Cell Calcium*. 2003; 34:109–119. [PubMed: 12810053]
7. Mazia, d. The release of calcium in Arbacia eggs on fertilization. *J Comp Cell Phys*. 1937; 10:291–304.
8. Nigon V, Do F. Les mouvements du calcium dans l'oeuf d'Arbacia lixula au moment de la fécondation. *C R H Acad Sci*. 1963; 257:2178–2180.
9. Clothier G, Timourian H. Calcium uptake and release by dividing sea urchin eggs. *Exp Cell Res*. 1972; 75:105–110. [PubMed: 4635871]
10. Steinhardt RA, Epel D. Activation of sea-urchin eggs by a calcium ionophore. *Proc Natl Acad Sci U S A*. 1974; 71:1915–1919. [PubMed: 4525301]
11. Hutlin T. On the acid formation, breakdown of cytoplasmic inclusions, and increased viscosity in papcentrotus egg homogenates after the addition of calcium. *Exp Cell Res*. 1950; 1:272–283.
12. Nakazawa T, Asami K, Shoger R, Fujiwara A, Yasumasu I. Ca^{2+} uptake H^{+} ejection and respiration in sea urchin eggs on fertilization. *Exp Cell Res*. 1970; 63:143–146. [PubMed: 5531477]
13. Gingell D. Contractile responses at the surface of an amphibian egg. *J Embryol Exp Morphol*. 1970; 23:583–609. [PubMed: 4990699]

14. Baker PF, Warner AE. Intracellular calcium and cell cleavage in early embryos of *Xenopus laevis*. *J Cell Biol.* 1972; 53:579–581. [PubMed: 5063471]
15. Ridgway EB, Gilkey JC, Jaffe LF. Free calcium increases explosively in activating medaka eggs. *Proc Natl Acad Sci U S A.* 1977; 74:623–627. [PubMed: 322135]
16. Steinhardt RA, Zucker R, Schatten G. Intracellular calcium release at fertilization in the sea urchin egg. *Dev Biol.* 1977; 58:185–196. [PubMed: 326602]
17. Gilkey JC, Jaffe LF, Ridgway EB, Reynolds GT. A free calcium wave traverses the activating egg of the Medaka, *Oryzias latipes*. *J Cell Biol.* 1978; 76:448–466. [PubMed: 10605450]
18. Chambers EL, Pressman BC, Rose B. The activation of sea urchin eggs by the divalent ionophores A23187 and X-537A. *Biochem Biophys Res Commun.* 1974; 60:126–132. [PubMed: 4472499]
19. Steinhardt RA, Epel D, Carroll EJ Jr, Yanagimachi R. Is calcium ionophore a universal activator for unfertilised eggs? *Nature.* 1974; 252:41–43. [PubMed: 4473722]
20. Stricker SA. Comparative biology of calcium signaling during fertilization and egg activation in animals. *Dev Biol.* 1999; 211:157–176. [PubMed: 10395780]
21. Miyazaki S. Thirty years of calcium signals at fertilization. *Semin Cell Dev Biol.* 2006; 17:233–243. [PubMed: 16549376]
22. Machaca K. Ca²⁺ signaling differentiation during oocyte maturation. *Journal of Cellular Physiology.* 2007; 213:331–340. [PubMed: 17620315]
23. Machaca, K. Mechanisms controlling the remodeling of Ca²⁺ signaling pathways during *Xenopus* oocyte maturation in preparation for fertilization. In: Kubiak, J.; Ciemerych, MA.; Richard-Parpaillon, L., editors. *Cell Cycle and Development in Vertebrates*. Kerala, India: Research Signpost; 2009.
24. Fankhauser G, Moore C. Cytological and experimental studies of polyspermy in the newt, *Triturus viridescens*. I. Normal fertilization *Journal of Morphology.* 1941; 68:347–385.
25. Herlant M. Recherches sur les oeufs di-et-trispermiques de grenouille. *Arch Biol.* 1911; 26:103–336.
26. Machaca, K.; Qu, Z.; Kuruma, A.; Hartzell, HC.; McCarty, N. The Endogenous Calcium-Activated Cl Channel *Xenopus* Oocytes: A Physiologically and Biophysically Rich Model System. In: Fuller, CM., editor. *Calcium activates chloride channels*. Academic Press; San Diego: 2001. p. 3-39.
27. Jaffe LA, Cross NL, Picheral B. Studies of the Voltage-Dependent Polyspermy Block Using Cross-Species Fertilization of Amphibians. *Dev Biol.* 1983; 98:319–326. [PubMed: 6683683]
28. Jaffe LA. Fast block to polyspermy in sea urchin eggs is electrically mediated. *Nature.* 1976; 261:68–71. [PubMed: 944858]
29. Hedrick JL. A comparative analysis of molecular mechanisms for blocking polyspermy: identification of a lectin-ligand binding reaction in mammalian eggs. *Soc Reprod Fertil Suppl.* 2007; 63:409–419. [PubMed: 17566287]
30. Ducibella T. The cortical reaction and development of activation competence in mammalian oocytes. *Human Reproduction Update.* 1996; 2:29–42. [PubMed: 9079401]
31. Masui Y, Markert CL. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool.* 1971; 177:129–145. [PubMed: 5106340]
32. Dunphy WG, Brizuela L, Beach D, Newport J. The *Xenopus* cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell.* 1988; 54:423–431. [PubMed: 3293802]
33. Gautier J, Minshull J, Lohka M, Glotzer M, Hunt T, Maller JL. Cyclin is a component of maturation-promoting factor from *Xenopus*. *Cell.* 1990; 60:487–494. [PubMed: 1967981]
34. Lohka MJ, Hayes MK, Maller JL. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc Natl Acad Sci USA.* 1988; 85:3009–3013. [PubMed: 3283736]
35. Nurse P. Universal control mechanism regulating onset of M-phase. *Nature.* 1990; 344:503–508. [PubMed: 2138713]
36. Peters JM. The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol Cell.* 2002; 9:931–943. [PubMed: 12049731]

37. Tunquist BJ, Maller JL. Under arrest: cytotstatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev.* 2003; 17:683–710. [PubMed: 12651887]
38. Schmidt A, Duncan PI, Rauh NR, et al. *Xenopus* polo-like kinase Plx1 regulates XErp1, a novel inhibitor of APC/C activity. *Genes Dev.* 2005; 19:502–513. [PubMed: 15713843]
39. Tung JJ, Hansen DV, Ban KH, et al. A role for the anaphase-promoting complex inhibitor Emi2/XErp1, a homolog of early mitotic inhibitor 1, in cytotstatic factor arrest of *Xenopus* eggs. *Proc Natl Acad Sci U S A.* 2005; 102:4318–4323. [PubMed: 15753281]
40. Liu, j; Maller, JL. Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytotstatic factor. *Curr Biol.* 2005; 15:1458–1468. [PubMed: 16040245]
41. Hansen DV, Tung JJ, Jackson PK. CaMKII and polo-like kinase 1 sequentially phosphorylate the cytotstatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. *Proc Natl Acad Sci U S A.* 2006; 103:608–613. [PubMed: 16407128]
42. Rauh NR, Schmidt A, Bormann J, Nigg EA, Mayer TU. Calcium triggers exit from meiosis II by targeting the APC/C inhibitor XErp1 for degradation. *Nature.* 2005; 437:1048–1052. [PubMed: 16127448]
43. Mochida S, Hunt T. Calcineurin is required to release *Xenopus* egg extracts from meiotic M phase. *Nature.* 2007; 449:336–340. [PubMed: 17882219]
44. Nishiyama T, Yoshizaki N, Kishimoto T, Ohsumi K. Transient activation of calcineurin is essential to initiate embryonic development in *Xenopus laevis*. *Nature.* 2007; 449:341–345. [PubMed: 17882220]
45. Wolniak SM, Hepler PK, Jackson WT. Ionic changes in the mitotic apparatus at the metaphase/anaphase transition. *J Cell Biol.* 1983; 96:598–605. [PubMed: 6833373]
46. Keith CH, Ratan RR, Maxfield FR, Bajer A, Shelanski ML. Local cytoplasmic calcium gradients in living mitotic cells. *Nature.* 1985; 316:848–850. [PubMed: 4033782]
47. Poenie M, Alderton JM, Tsien RY, Steinhardt RA. Changes of free calcium levels with stages of the cell division cycle. *Nature.* 1985; 315:147–149. [PubMed: 3838803]
48. Poenie M, Alderton J, Steinhardt RA, Tsien RY. Calcium rises abruptly and briefly throughout the cell at the onset of anaphase. *Science.* 1986; 233:886–889. [PubMed: 3755550]
49. Ratan RR, Maxfield FR, Shelanski ML. Long-lasting and rapid calcium changes during mitosis. *J Cell Biol.* 1988; 107:993–999. [PubMed: 3417787]
50. Steinhardt RA, Alderton JM. Intracellular free calcium rise triggers nuclear envelope breakdown in the sea urchin embryo. *Nature.* 1988; 332:364–366. [PubMed: 3127727]
51. Twigg J, Patel R, Whitaker M. Translational control of InsP3-induced chromatin condensation during the early cell cycles of sea urchin embryos. *Nature.* 1988; 332:366–369. [PubMed: 3127728]
52. Kao JP, Alderton JM, Tsien RY, Steinhardt RA. Active involvement of Ca²⁺ in mitotic progression of Swiss 3T3 fibroblasts. *J Cell Biol.* 1990; 111:183–196. [PubMed: 2114410]
53. Baitinger C, Alderton J, Poenie M, Schulman H, Steinhardt RA. Multifunctional Ca²⁺/calmodulin-dependent protein kinase is necessary for nuclear envelope breakdown. *J Cell Biol.* 1990; 111:1763–1773. [PubMed: 2229172]
54. Tombes RM, Simerly C, Borisy GG, Schatten G. Meiosis, egg activation, and nuclear envelope breakdown are differentially reliant on Ca²⁺, whereas germinal vesicle breakdown is Ca²⁺ independent in the mouse oocyte. *J Cell Biol.* 1992; 117:799–811. [PubMed: 1577859]
55. Sun L, Machaca K. Ca²⁺cyt negatively regulates the initiation of oocyte maturation. *J Cell Biol.* 2004; 165:63–75. [PubMed: 15067021]
56. Lenart P, Ellenberg J. Nuclear envelope dynamics in oocytes: from germinal vesicle breakdown to mitosis. *Curr Opin Cell Biol.* 2003; 15:88–95. [PubMed: 12517709]
57. Homa ST, Carroll J, Swann K. The role of calcium in mammalian oocyte maturation and egg activation. *Human Reproduction.* 1993; 8:1274–1281. [PubMed: 8408526]
58. Ciapa B, Pesando D, Wilding M, Whitaker M. Cell-cycle calcium transients driven by cyclic changes in inositol trisphosphate levels. *Nature.* 1994; 368:875–878. [PubMed: 8159248]

59. Witchel HJ, Steinhardt RA. 1-Methyladenine can consistently induce a fura-detectable transient calcium increase which is neither necessary nor sufficient for maturation in oocytes of the starfish *Asterina miniata*. *Dev Biol*. 1990; 141:393–398. [PubMed: 2210042]
60. Chafouleas JG, Bolton WE, Hidaka H, Boyd AE III, Means AR. Calmodulin and the cell cycle: involvement in regulation of cell-cycle progression. *Cell*. 1982; 28:41–50. [PubMed: 7066986]
61. Chafouleas JG, Lagace L, Bolton WE, Boyd AE III, Means AR. Changes in calmodulin and its mRNA accompany reentry of quiescent (G0) cells into the cell cycle. *Cell*. 1984; 36:73–81. [PubMed: 6692470]
62. Rasmussen CD, Means AR. Calmodulin is required for cell-cycle progression during G1 and mitosis. *EMBO J*. 1989; 8:73–82. [PubMed: 2469574]
63. Lu KP, Rasmussen CD, May GS, Means AR. Cooperative regulation of cell proliferation by calcium and calmodulin in *Aspergillus nidulans*. *Mol Endocrinol*. 1992; 6:365–374. [PubMed: 1584213]
64. Lu KP, Osmani SA, Osmani AH, Means AR. Essential roles for calcium and calmodulin in G2/M progression in *Aspergillus nidulans*. *J Cell Biol*. 1993; 121:621–630. [PubMed: 8486741]
65. Davis TN. A temperature-sensitive calmodulin mutant loses viability during mitosis. *J Cell Biol*. 1992; 118:607–617. [PubMed: 1639846]
66. Moser MJ, Flory MR, Davis TN. Calmodulin localizes to the spindle pole body of *Schizosaccharomyces pombe* and performs an essential function in chromosome segregation. *J Cell Sci*. 1997; 110(Pt 15):1805–1812. [PubMed: 9264467]
67. Ohya Y, Anraku Y. A galactose-dependent *cmd1* mutant of *Saccharomyces cerevisiae*: involvement of calmodulin in nuclear division. *Curr Genet*. 1989; 15:113–120. [PubMed: 2663189]
68. Ohya Y, Botstein D. Diverse essential functions revealed by complementing yeast calmodulin mutants. *Science*. 1994; 263:963–966. [PubMed: 8310294]
69. Dayton JS, Means AR. Ca(2+)/calmodulin-dependent kinase is essential for both growth and nuclear division in *Aspergillus nidulans*. *Mol Biol Cell*. 1996; 7:1511–1519. [PubMed: 8898358]
70. Kahl CR, Means A. Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. *Endocrine Reviews*. 2003; 24:719–736. [PubMed: 14671000]
71. Planas-Silva MD, Means AR. Expression of a constitutive form of calcium/calmodulin dependent protein kinase II leads to arrest of the cell cycle in G2. *EMBO J*. 1992; 11:507–517. [PubMed: 1371461]
72. Schmalzigaug R, Ye Q, Berchtold MW. Calmodulin protects cells from death under normal growth conditions and mitogenic starvation but plays a mediating role in cell death upon B-cell receptor stimulation. *Immunology*. 2001; 103:332–342. [PubMed: 11454062]
73. Reddy GP, Reed WC, Sheehan E, Sacks DB. Calmodulin-specific monoclonal antibodies inhibit DNA replication in mammalian cells. *Biochem*. 1992; 31:10426–10430. [PubMed: 1420160]
74. Hidaka H, Sasaki Y, Tanaka T, et al. N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, a calmodulin antagonist, inhibits cell proliferation. *Proc Natl Acad Sci U S A*. 1981; 78:4354–4357. [PubMed: 6945588]
75. Takuwa N, Zhou W, Kumada M, Takuwa Y. Ca(2+)-dependent stimulation of retinoblastoma gene product phosphorylation and p34cdc2 kinase activation in serum-stimulated human fibroblasts. *J Biol Chem*. 1993; 268:138–145. [PubMed: 8416921]
76. Sherr CJ. The ins and outs of Rb: coupling gene expression to the cell cycle clock. *Trends Cell Biol*. 1994; 4:15–18. [PubMed: 14731824]
77. Schneider G, Oswald F, Wahl C, Greten FR, Adler G, Schmid RM. Cyclosporine inhibits growth through the activating transcription factor/cAMP-responsive element-binding protein binding site in the cyclin D1 promoter. *J Biol Chem*. 2002; 277:43599–43607. [PubMed: 12215435]
78. Hojo M, Morimoto T, Maluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature*. 1999; 397:530–534. [PubMed: 10028970]
79. Conti M, Andersen CB, Richard F, et al. Role of cyclic nucleotide signaling in oocyte maturation. *Mol Cell Endocrinol*. 2002; 187:153–159. [PubMed: 11988323]
80. Miyazaki S. Calcium signalling during mammalian fertilization. *Ciba Foundation Symposium*. 1995; 188:235–251. [PubMed: 7587620]

81. Smith LD. The induction of oocyte maturation: transmembrane signaling events and regulation of the cell cycle. *Development*. 1989; 107:685–699. [PubMed: 2698799]
82. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet*. 2001; 2:280–291. [PubMed: 11283700]
83. Voronina E, Wessel GM. The regulation of oocyte maturation. *Curr Top Dev Biol*. 2003; 58:53–110. [PubMed: 14711013]
84. Maller JL, Krebs EG. Regulation of oocyte maturation. *Curr Top Cell Regul*. 1980; 16:271–311. [PubMed: 6249544]
85. Eppig, JJ.; Viveiros, MM.; Marin-Bivens, C.; De La Fuente, R. Regulation of mammalian oocyte maturation. In: Adashi, EY.; Leung, PCK., editors. *The Ovary*. San Diego: Elsevier Press; 2004. p. 113-129.
86. Maller JL, Butcher FR, Krebs EG. Early effect of progesterone on levels of cyclic adenosine 3':5'-monophosphate in *Xenopus* oocytes. *J Biol Chem*. 1979; 254:579–582. [PubMed: 216678]
87. Josefsberg Ben-Yehoshua L, Lewellyn AL, Thomas P, Maller JL. The role of *Xenopus* membrane progesterone receptor beta in mediating the effect of progesterone on oocyte maturation. *Mol Endocrinol*. 2007; 21:664–673. [PubMed: 17185392]
88. Nebreda AR, Ferby I. Regulation of the meiotic cell cycle in oocytes. *Curr Opin Cell Biol*. 2000; 12:666–675. [PubMed: 11063930]
89. Perdiguero E, Nebreda AR. Regulation of Cdc25C activity during the meiotic G2/M transition. *Cell Cycle*. 2004; 3:733–737. [PubMed: 15136768]
90. Mueller PR, Coleman TR, Kumagai A, Dunphy WG. Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15. *Science*. 1995; 270:86–90. [PubMed: 7569953]
91. Kumagai A, Dunphy WG. The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system. *Cell*. 1991; 64:903–914. [PubMed: 1825803]
92. Nurse P. Checkpoint pathways come of age. *Cell*. 1997; 91:865–867. [PubMed: 9428508]
93. Kristjansdottir K, Rudolph J. Cdc25 phosphatases and cancer. *Chem Biol*. 2004; 11:1043–1051. [PubMed: 15324805]
94. Wasserman WJ, Masui Y. Initiation of meiotic maturation in *Xenopus laevis* oocytes by the combination of divalent cations and ionophore A23187. *J Exp Zool*. 1975; 193:369–375. [PubMed: 1176910]
95. Moreau M, Doree M, Guerrier P. Electrophoretic introduction of calcium into the cortex of *Xenopus laevis* oocytes triggers meiosis reinitiation. *J Exp Zool*. 1976; 197:443–449. [PubMed: 965921]
96. O'Connor CM, Robinson KR, Smith LD. Calcium, potassium, and sodium exchange by full-grown and maturing *Xenopus laevis* oocytes. *Dev Biol*. 1977; 61:28–40. [PubMed: 562807]
97. Wasserman WJ, Pinto LH, O'Connor CM, Smith LD. Progesterone induces a rapid increase in $[Ca^{2+}]_i$ in *Xenopus laevis* oocytes. *Proc Natl Acad Sci U S A*. 1980; 77:1534–1536. [PubMed: 6929506]
98. Moreau M, Vilain JP, Guerrier P. Free calcium changes associated with hormone action in amphibian oocytes. *Dev Biol*. 1980; 78:201–214. [PubMed: 6249687]
99. Robinson KR. Maturation of *Xenopus* oocytes is not accompanied by electrode-detectable calcium changes. *Dev Biol*. 1985; 109:504–508. [PubMed: 3996760]
100. Cork RJ, Cicirelli MF, Robinson KR. A rise in cytosolic calcium is not necessary for maturation of *Xenopus laevis* oocytes. *Dev Biol*. 1987; 121:41–47. [PubMed: 3032713]
101. Picard A, Giraud F, Le Bouffant F, Sladeczek F, Le Peuch C, Doree M. Inositol 1,4,5-trisphosphate microinjection triggers activation, but not meiotic maturation in amphibian and starfish oocytes. *FEBS Letters*. 1985; 182:446–450. [PubMed: 3920074]
102. Duesbery NS, Masui Y. The role of Ca^{2+} in progesterone-induced germinal vesicle breakdown of *Xenopus laevis* oocytes: the synergic effects of microtubule depolymerization and Ca^{2+} . *Dev Genes Evol*. 1996; 206:110–124.
103. Sun L, Chai Y, Hannigan R, Bhogaraju VK, Machaca K. Zinc regulates the ability of Cdc25C to activate MPF/cdk1. *Journal of Cellular Physiology*. 2007; 213:98–104. [PubMed: 17443687]

104. Tsien RY, Pozzan T, Rink TJ. Calcium Homeostasis in Intact Lymphocytes: Cytoplasmic Free Calcium Monitored With a New, Intracellularly Trapped Fluorescent Indicator. *J Cell Biol.* 1982; 94:325–334. [PubMed: 6980885]
105. Tsien RY. New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. *Biochem.* 1980; 19:2396–2404. [PubMed: 6770893]
106. Manicassamy S, Gupta S, Sun Z. Selective function of PKC-theta in T cells. *Cell Mol Immunol.* 2006; 3:263–270. [PubMed: 16978534]
107. Lorenzo PS, Kung JW, Bottorff DA, Garfield SH, Stone JC, Blumberg PM. Phorbol esters modulate the Ras exchange factor RasGRP3. *Cancer Res.* 2001; 61:943–949. [PubMed: 11221888]
108. Lewis RS. Calcium signaling mechanisms in T lymphocytes. *Annu Rev Immunol.* 2001; 19:497–521. [PubMed: 11244045]
109. Liu, j; Farmer, JD., Jr; Lane, WS.; Friedman, J.; Weissman, I.; Schreiber, SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell.* 1991; 66:807–815. [PubMed: 1715244]
110. Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature.* 1992; 357:695–697. [PubMed: 1377362]
111. Klee CB, Ren H, Wang X. Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. *J Biol Chem.* 1998; 273:13367–13370. [PubMed: 9593662]
112. Stemmer PM, Klee CB. Dual calcium ion regulation of calcineurin by calmodulin and calcineurin B. *Biochem.* 1994; 33:6859–6866. [PubMed: 8204620]
113. Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature.* 1998; 392:933–936. [PubMed: 9582075]
114. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol.* 1997; 15:707–747. [PubMed: 9143705]
115. Oh-hora M, Rao A. Calcium signaling in lymphocytes. *Curr Opin Immunol.* 2008; 20:250–258. [PubMed: 18515054]
116. Feske S, Gwack Y, Prakriya M, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature.* 2006; 441:179–185. [PubMed: 16582901]
117. Feske S, Prakriya M, Rao A, Lewis RS. A severe defect in CRAC Ca²⁺ channel activation and altered K⁺ channel gating in T cells from immunodeficient patients. *J Exp Med.* 2005; 202:651–662. [PubMed: 16147976]
118. Picard C, McCarl CA, Papolos A, et al. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med.* 2009; 360:1971–1980. [PubMed: 19420366]
119. Zweifach A, Lewis RS. Mitogen-regulated Ca²⁺ current of T lymphocytes is activated by depletion of intracellular Ca²⁺ stores. *Proc Natl Acad Sci U S A.* 1993; 90:6295–6299. [PubMed: 8392195]
120. Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature.* 1992; 355:353–356. [PubMed: 1309940]
121. Putney JW. A model for receptor-regulated calcium entry. *Cell Calcium.* 1986; 7:1–12. [PubMed: 2420465]
122. Parekh AB, Putney JW. Store-Operated Calcium Channels. *Physiol Rev.* 2005; 85:757–810. [PubMed: 15788710]
123. Dirksen RT. Checking your SOCCs and feet: the molecular mechanisms of Ca²⁺ entry in skeletal muscle. *J Physiol.* 2009; 587:3139–3147. [PubMed: 19406875]
124. Liou J, Kim ML, Heo WD, et al. STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol.* 2005; 15:1235–1241. [PubMed: 16005298]
125. Roos J, DiGregorio PJ, Yeromin AV, et al. STIM1, an essential and conserved component of store-operated Ca²⁺ channel function. *J Cell Biol.* 2005; 169:435–445. [PubMed: 15866891]
126. Vig M, Peinelt C, Beck A, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science.* 2006; 312:1220–1223. [PubMed: 16645049]

127. Zhang SL, Yeromin AV, Zhang XH, et al. Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc Natl Acad Sci U S A*. 2006; 103:9357–9362. [PubMed: 16751269]
128. Peinelt C, Vig M, Koomoa DL, et al. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol*. 2006; 8:771–773. [PubMed: 16733527]
129. Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem*. 2006; 281:20661–20665. [PubMed: 16766533]
130. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol*. 2010; 28:491–533. [PubMed: 20307213]
131. Feske S. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca²⁺ entry in the immune system and beyond. *Immunol Rev*. 2009; 231:189–209. [PubMed: 19754898]
132. Stiber J, Hawkins A, Zhang ZS, et al. STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. *Nat Cell Biol*. 2008; 10:688–697. [PubMed: 18488020]
133. Tani D, Monteilh-Zoller MK, Fleig A, Penner R. Cell cycle-dependent regulation of store-operated I(CRAC) and Mg²⁺-nucleotide-regulated MagNum (TRPM7) currents. *Cell Calcium*. 2007; 41:249–260. [PubMed: 17064762]
134. Machaca K, Haun S. Store-operated Calcium Entry Inactivates at the Germinal Vesicle Breakdown Stage of *Xenopus* Meiosis. *J Biol Chem*. 2000; 275:38710–38715. [PubMed: 10991950]
135. Machaca K, Haun S. Induction of maturation-promoting factor during *Xenopus* oocyte maturation uncouples Ca²⁺ store depletion from store-operated Ca²⁺ entry. *J Cell Biol*. 2002; 156:75–85. [PubMed: 11781335]
136. Arredouani A, Yu F, Sun L, Machaca K. Regulation of store-operated Ca²⁺ entry during the cell cycle. *J Cell Sci*. 2010; 123:2155–2162. [PubMed: 20554894]
137. Yu F, Sun L, Machaca K. Orai1 internalization and STIM1 clustering inhibition modulate SOCE inactivation during meiosis. *Proc Natl Acad Sci U S A*. 2009; 106:17401–17406. [PubMed: 19805124]
138. Yu F, Sun L, Machaca K. Constitutive recycling of the store-operated Ca²⁺ channel Orai1 and its internalization during meiosis. *Cell Biol*. 2010 In Press.
139. Machaca K. Increased sensitivity and clustering of elementary Ca²⁺ release events during oocyte maturation. *Dev Biol*. 2004; 275:170–182. [PubMed: 15464580]
140. Terasaki M, Runft LL, Hand AR. Changes in organization of the endoplasmic reticulum during *Xenopus* oocyte maturation and activation. *Mol Biol Cell*. 2001; 12:1103–1116. [PubMed: 11294910]
141. Boulware MJ, Marchant JS. IP₃ receptor activity is differentially regulated in endoplasmic reticulum subdomains during oocyte maturation. *Curr Biol*. 2005; 15:765–770. [PubMed: 15854911]
142. Sun L, Haun S, Jones RC, Edmondson RD, Machaca K. Kinase-dependent regulation of IP₃-dependent Ca²⁺ release during oocyte maturation. *J Biol Chem*. 2009; 284:20184–20196. [PubMed: 19473987]
143. El Jouni W, Jang B, Haun S, Machaca K. Calcium signaling differentiation during *Xenopus* oocyte maturation. *Dev Biol*. 2005; 288:514–525. [PubMed: 16330019]
144. El Jouni W, Haun S, Machaca K. Internalization of plasma membrane Ca²⁺-ATPase during *Xenopus* oocyte maturation. *Dev Biol*. 2008; 324:99–107. [PubMed: 18823969]