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Review

The cell cycle: a new entry in the field of Ca^{2+} signaling

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Abstract. Ca^{2+} signaling plays a crucial role in virtually all cellular processes, from the origin of new life at fertilization to the end of life when cells die. Both the influx of external Ca^{2+} through Ca^{2+}-permeable channels and its release from intracellular stores are essential to the signaling function. Intracellular Ca^{2+} is influenced by mitogenic factors which control the entry and progression of the cell cycle; this is a strong indication for a role of Ca^{2+} in the control of the cycle, but surprisingly, the possibility of such a role has only been paid scant attention in the literature. Substantial progress has nevertheless been made in recent years in relating Ca^{2+} and the principal decoder of its information, calmodulin, to the modulation of various cycle steps. The aim of this review is to critically discuss the evidence for a role of Ca^{2+} in the cell cycle and to discuss Ca^{2+}-dependent pathways regulating cell growth and differentiation.

Key words. Calcium signaling; mitosis; meiosis; cyclin-dependent kinase; maturation-promoting factor; $InsP_3$ fertilization.

The eukaryotic cell cycle: a brief survey

The control of the cell division cycle, which is critical for the normal development of multicellular organisms, is regulated by extracellular signals and coordinated by internal checkpoints [1–2]. The eukaryotic cell cycle comprises two main events, interphase and mitosis, which are regulated by cyclin-dependent kinases (CDKs) at the G_1, S and mitotic phases. Interphase includes the G_1, S and G_2 phases. G_1 is the time interval between mitosis and DNA synthesis (S) and is the time when CDK complexes are first expressed to prepare the cell for DNA replication in the S phase [3]. In the G_2 phase proteins are synthesized for the process of mitosis. At the end of G_2 comes nuclear division (M phase), which in turn is divided into prophase, metaphase, anaphase and telophase (fig. 1). CDKs activated in the M phase induce breakdown of the nuclear envelope, condensation of chromosomes, their movement to the center of the cell (prophase), assembly of the mitotic spindle and attachment of sister chromatids to the spindle at the kinetochores. The latter is a complex of proteins associated with centromeric DNA [4].

An important process that ensures the proper progression through the cell division cycle is that mediated by the ubiquitin-proteasome system [5]. Proteolysis of the cycle components is controlled by two classes of ubiquitin ligases: the SCF (Skp1/Cullin/F-box) and the anaphase promoting complex, or cyclosome (APC/C) [6]. APC is kept inactive during S and G_2 phases by binding to early mitotic inhibitor 1 (Emi1), allowing the accumulation of mitotic cyclins. The level of Emi1 oscillates: it accumulates in the S phase and is rapidly degraded in prometaphase where SCF mediates its degradation, thus contributing to activation of APC/C and to subsequent progression to anaphase [7]. Later, in anaphase, activated APC/C enables the decondensation of the separated chromosomes and reformation of the nuclear envelope around the nuclei of daughter cells (telophase), together with division of the cytoplasm at cytokinesis [8, 9].

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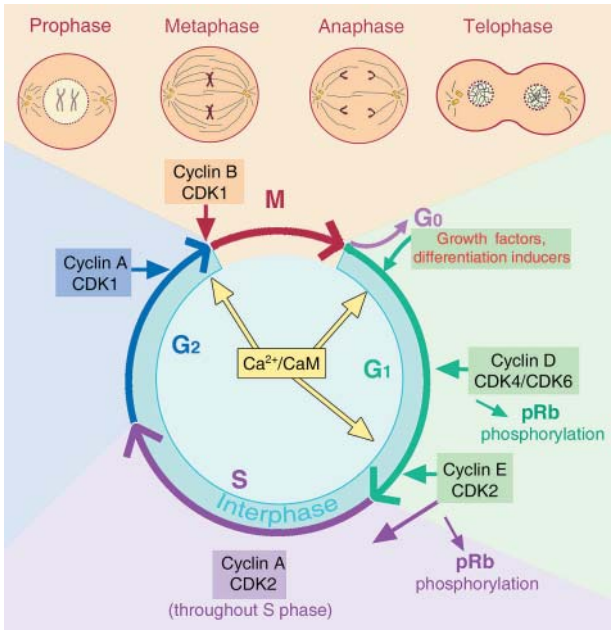

Figure 1. Phases of the mammalian cell cycle. The cell grows through interphase, which comprises G_1, S and G_2. The M phase (mitosis) includes: (i) Prophase, in which the chromosomes condense and the centrosomes initiate formation of the mitotic spindles by migrating to opposite sides of the nucleus. (ii) Metaphase, in which the chromosomes align in the center of the spindles. (iii) Anaphase, in which sister chromatids separate and move to the opposite poles. (iv) Telophase, during which the nuclear envelope reassembles and chromosomes decondense. Cell cycle transitions are regulated by a series of CDKs. As cells progress through G_1, cyclin D/CDK4 and CDK6 complexes and cyclin E/CDK2 are activated and phosphorylate pRb. As cells enter the S phase, cyclin A/CDK2 becomes activated and remains activated throughout the G_2 phase. The transition from G_2 to M is regulated by cyclin B/CDK1. Ca^{2+}/CaM are required early after mitogenic stimulation, and later near the G_1/S boundary. Furthermore, Ca^{2+}/CaM is involved in G_2/M transition, M-phase progression and exit from mitosis.

Growth factors control the cell cycle at a point in late G_1 called the restriction point, which represents a point of no return after which cells determine whether nutrients are sufficient to traverse the cell cycle. When proliferating cells are deprived of growth factors, they enter a resting stage called G_0, failing to enter the S phase (fig. 1) [3]. Progression through the cycle is also arrested at the G_1 and G_2 checkpoints in response to DNA damage. At the G_1 checkpoint, DNA damage leads to rapid increase of p53, a transcriptional activator, which arrests cells in G_1 and attempts to repair the DNA. Mutations in the *p53* gene are a common alteration in human cancer cells: p53 becomes inactivated, so that cells survive inappropriately replicating damaged DNA [10].

Considering that Ca^{2+} is now acknowledged to control all the most important events in the life of cells, it would be logical to assume that a process as important as the cell cycle would also have Ca^{2+} as an important regulator. Surprisingly, however, only marginal attention to the role of Ca^{2+} in the cell cycle has been paid in the literature. This is all the more unexpected in the face of the considerable evidence that has accumulated in recent years in favor of such a role. This article will try to put Ca^{2+} back in the cycle. It will consider all steps and regulation points where the evidence is clear for an intervention of Ca^{2+} as one of their important modulators.

Identification of the CDKs as regulators of the cell cycle

Biochemical and genetic experiments have led to identification of a conserved set of CDKs responsible for mitotic and meiotic cycle transitions. Fully grown *Xenopus* oocytes are arrested in the G_2 phase and are induced to enter meiosis I by progesterone. Stimulated oocytes progress through meiosis I and the subsequent interphase and become arrested at the metaphase of meiosis II [11]. In 1971 it was found that when the cytoplasm of unfertilized eggs arrested at the metaphase of meiosis II was injected into G_2-arrested oocytes, the oocytes resumed the maturation process in the absence of progesterone stimulation [12, 13]. The maturing factor which was evidently present in the cytoplasm was called maturation promoting factor (MPF), as the entry of oocytes into meiosis is commonly called maturation [14]. Later studies have shown that MPF plays a universal role as regulator of the transition from G_2 to M in the mitotic cell as well. Genetic analysis on yeasts has indicated that a collection of temperature-sensitive mutants arrest growth at specific points in the cell cycle [15]. Molecular cloning revealed that the yeast mutant *cdc2* gene encoded a protein which was the Cdc2 kinase [1]. Furthermore, studies in sea urchin eggs showed a cyclic accumulation of proteins throughout interphase and their degradation at the end of each mitosis [16]. MPF entered the picture when it was found that it was composed of the regulatory subunit cyclin B and the catalytic subunit of the Cdc2 protein kinase [17–20]. Cdc2 became CDK1, the first cyclin-dependent protein kinase, when it was confirmed that vertebrates and mammalian Cdc2 were able to bind cyclin [21]. While CDK protein levels remain stable during the cycle, cyclin levels rise and fall during the cell cycle, and in this way they periodically activate CDKs [16]. In mammalian cells cyclin/CDK complexes require phosphorylation on conserved threonine and tyrosine residues to become fully active and transmit their signals to multiple substrates by phosphorylating them at specific sites [22–24]. As one might expect, dephosphorylation on serine and threonine residues in cyclin/CDKs also plays an important role. Activation of MPF (cyclin B/CDK1) into mitosis requires dephosphorylation of inhibitory sites on the kinase by the Cdc25 phosphatase, which in vertebrates

and *Xenopus* early embryos is inhibited by phosphorylation at a single serine (S287) by the checkpoint kinases [25]. CDK activity can also be controlled by cell cycle inhibitory proteins called cyclin dependent kinase inhibitors (CKIs) that bind to CDK alone or to cyclin/CDK complexes [26]. Mitogenic stimulation promotes G_1 progression via cyclin D and E synthesis [3]. The primary target of the cyclinD/CDK4 complex is the retinoblastoma tumor suppressor gene product pRb, whose underphosphorylated form represses the transcription of the E2F-regulated genes, whose products (cyclin A, cyclin E and Cdc25) are required for S phase and DNA synthesis [2, 3]. In addition to phosphorylating pRb in mid-late G_1, D-type cyclins/CDK4 complexes also act to sequester p21 and p27 proteins (two classes of CKIs) away from cyclin E/CDK2, thereby activating this complex, which is responsible for the initiation of DNA replication [3]. Cyclin A binds to CDK2, and this complex is required throughout the S phase. In late G_2 and early M phase, cyclin A complexes CDK1 to promote entry into mitosis. Mitosis is further regulated by cyclin B and CDK1 (MPF) (fig. 1). Cyclins A and B contain a destruction box and cyclins D and E contain a PEST sequence. Both the box and the sequence promote efficient ubiquitin-mediated cyclin proteolysis before the metaphase-anaphase transition [27].

Ca^{2+} and the mitotic cycle

In the last few years abundant information has accumulated on the role of Ca^{2+} in controlling cell proliferation. Ca^{2+} increase induced by extracellular signals such as hormones, growth factors and cytokines is a key step in the regulation of processes that trigger normal and pathological cell proliferation [28–30]. Proliferation of several cell types is associated with major changes in intracellular Ca^{2+}-handling mechanisms. The increase of cell Ca^{2+} is controlled by proteins located in the plasma membrane [31–34] and in the membranes of cytoplasmic organelles [35–39]. During stimulation by growth factors, the temporally and spatially coordinated Ca^{2+} puffs and sparks that operate in quiescent cells are responsible for the generation of repetitive Ca^{2+} waves and oscillations [40]. Recent data point to a major role of Ca^{2+} influx in the control of cell growth and division, even if the contribution of Ca^{2+} released from intracellular stores cannot be excluded (see below). That Ca^{2+} entry plays a specific role in the cell cycle interphase at the G_0/G_1 or at a later stage (fig. 1) is indicated by the inhibitory effect of either EGTA or SKF 96365, a specific blocker of store-operated Ca^{2+} entry (SOCE) during the first 2–4 h following mitogenic stimulation [41]. Patch-clamp experiments and microfluorimetry work have shown that the reduction of SOCE correlates with cell arrest at the G_0/G_1 boundary [42, 43]. The regulated expression of plasma membrane Ca^{2+}-ATPase isoforms (PMCAs) plays a critical role in vascular smooth muscle cell proliferation. In these cells the G_1/S phase transition is triggered by an intracellular Ca^{2+} elevation which is associated with the downregulation of the PMCA1 isoform by the transcription factor c-Myb [44]. L-type Ca^{2+} channels also play a significant role in activation and proliferation processes. They mediate the Ca^{2+} influx pathways leading to T lymphocyte activation and proliferation in vitro and in vivo [45]. The importance of Ca^{2+} influx is underlined by other findings as well: stimulation of rat cardiac microvascular endothelial cells by epidermal growth factor induces Ca^{2+} oscillations that are prevented by removal of external Ca^{2+} or by inhibitors of capacitative Ca^{2+} entry (CCE) [46]. Thus, plasma membrane channels with different biophysical properties, and responsible for mitogen-activated Ca^{2+} entry, play an important role in control of proliferation in several cell types [29]. However, Ca^{2+} movements initiated by internal stores are also important. The induction of proliferation is associated with a decrease in the frequency of spontaneous sparks, probably associated with inhibition of ryanodine receptors (RyRs) [47]. In human prostate cancer cells, treatment with thapsigargin, the inhibitor of sarco-endoplasmic reticulum Ca^{2+} pump ATPase (SERCA), inhibits cell growth and prevents stimulation of cell proliferation by growth factors [48]. Upregulation of the SERCA2a pump isoform in smooth-muscle cells after 24-h stimulation with platelet-derived growth factor (PDGF) is associated with their entry into S phase [49]. Results from heterozygous mice with a null mutation in the SERCA2 gene also indicate the importance of the SERCA 2 isoform in growth and differentiation [50]. Furthermore, in the absence of growth factors, 3T3 fibroblasts enter the G_0 quiescent state. Cell cycle progression through and past the G_1 phase by serum stimulation is linked to an intracellular calcium increase that activates a MAPK-NF-kB (mitogen-activated protein kinase-nuclear factor kappa B) pathway through transcriptional regulation of D-type cyclins [51].

Direct measurements of intracellular Ca^{2+} with sensitive dyes have revealed both single transients and sustained increases during pronuclear migration, nuclear envelope breakdown, the metaphase-anaphase transition of mitosis and cytokinesis [52]. Large cells such as eggs, and embryos of various species, have been a useful tool to study cell division. Thus, cyclic increases in $InsP_3$ are responsible for the intracellular Ca^{2+} signals that mark the cell cycle events in sea urchin embryos that undergo a series of very rapid cell divisions after fertilization [53]. During the early cell division cycles of zebrafish embryos, Ca^{2+} transients, generated by release from intracellular stores, are associated with cytokinesis, and Ca^{2+} released via $InsP_3$ receptors has been shown to be essential for generating the furrow deepening [54]. Local calcium transients have

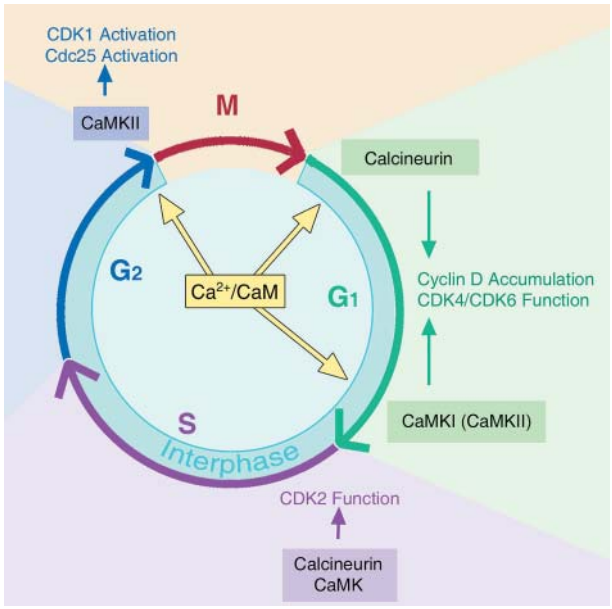

Figure 2. Calcineurin and CaMK regulation of mammalian cell transitions. In early/mid-G_1, inhibitors of both calcineurin and CaMKs arrest cells before cyclinD/CDK activation. In late G_1 or S phase, inhibition of calcineurin or CaMK leads to CDK2 inactivation through accumulation of p21 and p27, respectively.

been observed in the perinuclear region before mitosis entry [55]. This is in line with the finding that application of the Ca^{2+} chelator BAPTA/AM blocks, or severely delays, the metaphase/anaphase transition by a failure to activate APC/C [56]. A transient increase in intracellular Ca^{2+} precedes the separation of chromosomes in sea urchin embryos entering anaphase of the first cell cycle [57]. A strong correlation between cytosolic Ca^{2+} and cell cycle phase has also been found in the early stages of differentiation of *Dictyostelium discoideum* [58].

Ca^{2+}-binding proteins in mitosis

Calmodulin (CaM) is a small protein that contains four EF-hand motifs and is highly conserved among eukaryotes. Transduction of the Ca^{2+} signal by CaM consists of a series of conformational changes that occur upon Ca^{2+} binding and, after Ca^{2+} has become bound when CaM interacts with targets [33]. These targets are very numerous and include a family of multifunctional protein kinases (CaMKs) and the serine/threonine phosphatase calcineurin [59]. It is mostly (but not exclusively) through activating protein phosphorylation and dephosphorylation that Ca^{2+} and CaM act on the cell cycle, influencing many of its stages. They are required during re-entry from quiescence after extracellular stimulation, to traverse the G_1/S, G_2/M and metaphase/anaphase transition boundaries [60, 61]. Changes in CaM RNA accompany the re-entry of quiescent cells (G_0) into the cell cycle, and stabilization of the CaM messenger RNA (mRNA) transcript by a CaM inhibitor during the S phase inhibits cell growth and DNA replication [62]. CaM also activates DNA polymerases delta and alpha, and regulates the expression of the proliferating nuclear antigen (PCNA) [63, 64]. Ca^{2+} and CaM also play a role in nuclear envelope breakdown and cytokinesis, as indicated by data from different laboratories, and in regulation of the actomyosin contractile ring as well [65, 66]. The parallel distribution of CaM and the central spindle between separating chromatin masses regulates the cleavage plane and initiates furrowing [54, 66]. A number of studies have focused on the effect of the three CaM-activated protein kinases, exploring them mostly with the help of inhibitors. The results have shown cycle arrest in different phases (G_1 or G_2) depending on the cell type [61]. In addition, overexpression of constitutively active CaMKII leads to arrest of the cell cycle in

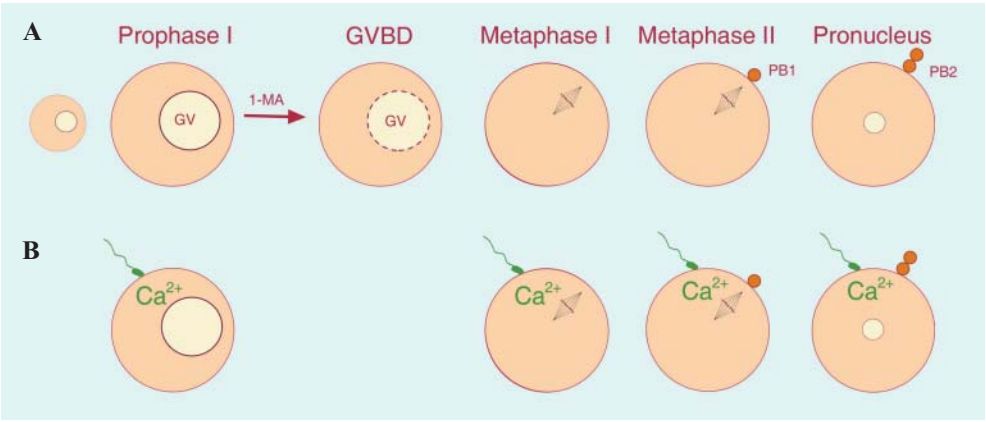

Figure 3. Phases of the meiotic cycle. (*A*) Reinitiation of the meiotic process (maturation) of a starfish oocyte. Fully grown oocytes arrested at the prophase of the first meiotic division are characterized by a large nucleus (GV). The addition of the maturing hormone 1-MA induces the breakdown of the nuclear envelope. As a result of the two meiotic divisions, the first and second polar bodies (PB1 and PB2) are extruded at telophase I and II. (*B*) State of oocyte maturation at fertilization. At fertilization, the sperm triggers a Ca^{2+} signal that releases the oocyte from meiotic arrest, the stage of which may vary with the species.

G_2, elevates cyclin B1 expression and delays mitosis in HeLa cells [67, 68]. Other effects have also been described, e.g., on the binding of PCNA with replicating factor C [64] or on the onset of centrosome duplication [69]. As is to be expected, calcineurin is also important in the cell cycle and in processes related to it, e.g., translocation of the nuclear factor of activated T cells (NFAT) to the nucleus [30, 70, 71]. Disruption of the calcineurin A gene in *Aspergillus nidulans* leads to early cell cycle arrest [72], and downregulation of calcineurin A expression arrests the majority of cells in G_1 (some cells are arrested in G_2 and M) [61]. Calcineurin A-deficient mutants display anomalies in cell and nuclear shape, microtubule arrays and spindle pole body positioning in interphase cells [73]. Recent studies have implicated calcineurin in regulation and transcription, and therefore expression, of both cyclin D and cyclinD/CDK4 (fig. 2) [61]. It has been shown in T lymphocytes that calcineurin dephosphorylates and inactivates CDK4 directly, suggesting a role in the inactivation of CDK4 in mitosis [74]. Calcineurin inhibition inactivates CDK2 in late G_1 or S phase, through the accumulation of p27 [61]. Studies on the function of calcineurin have been greatly facilitated by use of the inhibitory immunosuppressive drugs cyclosporin A (CsA) and FK506 [75]. Diploid human fibroblasts are arrested in G_1 by CsA via reduction in the amount of newly synthesized cyclin D1 protein. The same effect was obtained with the expression of constitutively active calcineurin, which induced cyclin D1 synthesis during mid-G_1 phase [76]. Thus, calcineurin regulates the transcription and translation of cyclin D1 as well the transcription and phosphorylation status of CDK4. Finally, interesting experiments using the non-competitive inhibitor of calcineurin CAIN [77] have shown attenuated agonist-induced cardiomyocyte hypertrophy [78].

Ca^{2+} and the meiotic cycle (maturation)

Understanding the regulation of the meiotic cycle phases comes essentially from studies on oocytes and eggs, which can be arrested at different stages of the first or second meiotic division. They resume meiosis by hormonal stimulation or by the addition of sperm (fig. 3). A hallmark of the meiotic cycle, the so-called maturation process, is the absence of DNA replication between the cell division phases. This is achieved by the MAPK pathway, which prevents a second round of DNA duplication during the two meiotic divisions [79]. During progesterone-induced maturation in *Xenopus* oocytes (meiosis I), activation of MPF correlates with dephosphorylation on Tyr15 and on Thr14 of the kinase by the phosphatase Cdc25, or by inhibition of the Myt1 protein kinase, which keeps MPF inactive by phosphorylating it on Thr14 and Tyr15 [11]. The main function of MPF is to assemble a meiotic bipolar spindle; MPF is then inactivated between meiosis I (during which a reductional division segregates homologous chromosomes) and meiosis II (in which an equational division segregates sister chromatids. Finally, MPF is reactivated at metaphase of the II division and kept active by the cytostatic factor (CSF) [12, 13].

At variance with the mitotic cycle, there has been a long-standing debate as to whether Ca^{2+} signals are required for reinitiation of the meiotic cycle (maturation). Recent results have shown a Ca^{2+}-dependent negative regulation of the MPF cascade during progesterone-induced *Xenopus* oocyte maturation upstream of Mos (a MAPK kinase) [80]. After the meiosis I and meiosis II transition, *Xenopus* eggs are blocked at the II metaphase by the activity of CSF, which prevents cyclin B degradation, thus maintaining high activity of MPF. CSF is arrested during oocyte maturation by the Mos, MAP kinase/ERK kinase (MEK), MAPKs and p90(Rsk) pathway [81]. The shape of the Ca^{2+} signal during maturation shows variability: in *Xenopus*, fertilization triggers a Ca^{2+} increase in the form of a single transient, which activates both CSF and MPF, restarting the meiotic cycle [82]. Oocytes that display sperm-triggered Ca^{2+} oscillations are also released from meiotic metaphase arrest by sperm [83]. These include those of some molluscs, nemerteans, annelid arthropods, ascidians and mammals [84] (fig. 3). Ca^{2+}-induced APC/C activation at metaphase targets destruction box-containing substrates, such as cyclin B1 and securin, targeted for degradation by 26S proteasome activity and MPF inactivation. Another model proposes that Ca^{2+} oscillations induced by sperm do not regulate 26S proteasome activity, but promote cyclin B degradation by directly stimulating destruction of the CSF [85]. In ascidian oocytes two series of sperm-induced Ca^{2+} waves triggered from two distinct Ca^{2+} wave pacemakers (PM1 and PM2) at the first metaphase characterize the two meiotic divisions preceding entry into the first interphase [86]. By manipulating MPF activity, it has been shown that Ca^{2+} oscillations, and not the first Ca^{2+} signal, are correlated with elevated MPF, and not with MAP kinase activity [87]. In mouse oocytes, instead, two series of $InsP_3$-dependent Ca^{2+} oscillations from metaphase II to interphase of the first cell cycle stimulate exit from metaphase arrest. The Ca^{2+}signals terminate following the formation of nuclei in the two-cell-stage embryo, and inhibition of pronuclei formation leads to persistent Ca^{2+} oscillations, indicating that the cessation of Ca^{2+} transients is linked to the sequestration of the newly forming pronuclei [88].

Starfish oocytes have also contributed to our understanding of oocyte maturation [89]. Fully grown oocytes dissected from the gonads are synchronously arrested at the prophase of the first meiotic division. At this stage they are characterized by a large nucleus (germinal vesicle, GV) that breaks down following stimulation by the matur-

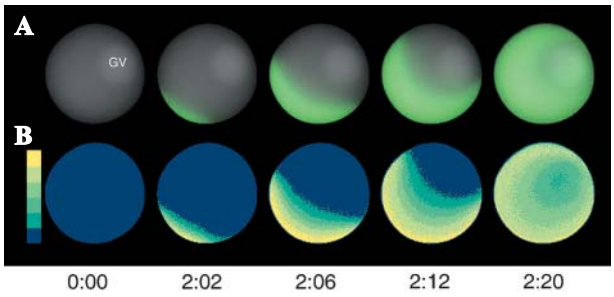

Figure 4. Ca^{2+} signaling during maturation of a starfish oocyte. The Ca^{2+} dye Oregon Green 488 BAPTA-1 is injected in a prophase I-arrested oocyte, which is then exposed to 1-MA. The Ca^{2+} increase starts at the vegetal hemisphere ~2 min after hormonal stimulation. The Ca^{2+} wave then propagates to the animal hemisphere in about 20 s. The figure shows an overlay of the fluorescence and the relative fluorescence (*A*), and pseudocolored relative fluorescence images (*B*) of the Ca^{2+} increase induced by 1-MA. Blue corresponds to low Ca^{2+} levels, green and yellow to higher Ca^{2+} levels.

ing hormone 1-methyldenine (1-MA). MPF activity is very low in immature oocytes not treated with the hormone. 1-MA activates the kinase Akt (or protein kinase B), which phosphorylates and downregulates the MPF inhibitory kinase Myt1, causing the initial activation of MPF at the meiotic G_2/M-phase transition [90]. The translocation of MPF to the GV along with Cdc25 has been shown to be important for final activation of the kinase [91, 92]. Results on Ca^{2+}-linked meiosis reinitiation have been conflicting. As early as 1978 starfish oocytes injected with the Ca^{2+}-sensitive photoprotein aequorin were found to emit light several minutes after the addition of 1-MA [93]. Earlier work in our laboratory has confirmed that 1-MA induces an increase in cytoplasmic calcium [94] which always starts at the vegetal hemisphere of the oocyte (fig. 4). The cytosolic Ca^{2+} increase is

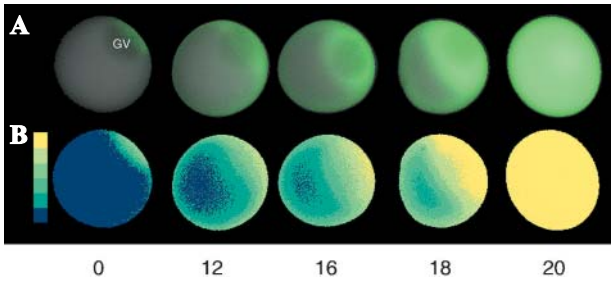

Figure 5. Spatiotemporal pattern of the $InsP_3$-induced Ca^{2+} release during starfish oocyte maturation. Prophase I-arrested oocytes are co-injected with the Ca^{2+} dye Oregon Green 488 BAPTA-1 and caged $InsP_3$. inositol-1,4,5-trisphosphate ($InsP_3$) is liberated by ultraviolet irradiation at different times after 1-MA stimulation. After global photoactivation, the increased sensitivity to $InsP_3$ starts at the animal hemisphere and in the perinuclear area. The increased sensitity then spreads along the animal/vegetal axis. Spatiotemporal changes after photoactivation of $InsP_3$ are shown as an overlay of the fluorescence and the relative fluorescence (*A*), and pseudocolored relative fluorescence images (*B*)

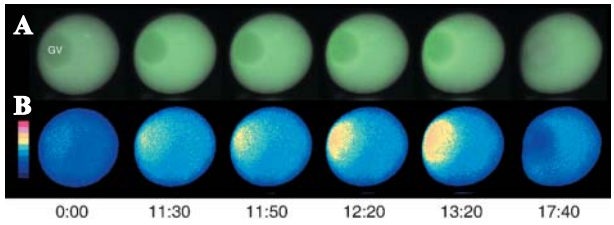

Figure 6. Spatiotemporal changes in Ca^{2+} concentration before GVBD. An increase of Ca^{2+} is measured in the perinuclear area of a maturing starfish oocyte injected with the Ca^{2+} dye Oregon Green 488 BAPTA-1 and treated with 1-MA for approximately 12 min. The Ca^{2+} signal is a consequence of the increased sensitivity of the Ca^{2+} stores to $InsP_3$, as shown in figure 5. The figure shows an overlay of the fluorescence and relative fluorescence images (*A*), and pseudocolored relative fluorescence images (*B*).

followed by calcium elevation in the GV, which could promote the phosphorylation of Cdc25, and thus activation of MPF: indeed, the nuclear injection of BAPTA blocks the continuation of meiosis [94].

The treatment of starfish oocytes with 1-MA increases the $InsP_3$ sensitivity of the Ca^{2+} stores, preparing the oocytes to generate a normal Ca^{2+} response at fertilization [95]. The observation is not limited to starfish, as similar results have been recently obtained in the *Xenopus* system [96]. Our laboratory has shown that the sensitivity of starfish oocytes to $InsP_3$ increases in the perinuclear area at the animal pole where the GV is located, then propagating to the entire oocyte along the animal/vegetal axis (fig. 5). [97]. The higher Ca^{2+} response to $InsP_3$ elevates Ca^{2+} around the GV just before meiosis entry (fig. 6). These results are clearly at variance with the idea that Ca^{2+} signals do not play a role in the maturation process of starfish oocytes, and they underscore the idea that Ca^{2+} is a universal activator of both the mitotic and the meiotic cycles.

Ca^{2+}-binding proteins and the meiotic cycle

Based on the discussion above showing that Ca^{2+}/CaM-dependent signaling pathways are also essential for the progression of oocyte through meiotic maturation, the role of CaM can now be discussed. CaM antagonists, antibodies and an inhibitory peptide corresponding to the CaM-binding domain of myosin-light-chain kinase injected into the GV of prophase I-arrested starfish oocytes completely inhibited the resumption of the meiotic cycle induced by 1-MA [98, 99]. Release from the metaphase I arrest in mollusc oocytes involves the activation of Ca^{2+}/CaM-dependent kinase III [100]. CaMKII, however, appears to have a more comprehensive role. It may participate in the MI to anaphase I transition (chromosome segregation) in mouse oocytes, as suggested by inhibition of the polar body 1 (PB1) extrusion by the inhibitors

KN-93 and AIP [101]. CaMKII also plays a role in the MII to anaphase II transition (sister chromatid segregation) [102]. Confocal microscopy has revealed CaM and CaMKII in the nucleus and in the periphery of GV-stage oocytes and in the condensed chromosomes after GVBD. Furthermore, during metaphase I and II, CaM is distributed to the whole spindle and CaMKII to the spindle poles. After transition into anaphase, both proteins translocate to the area between separating chromosomes [103]. CaMKII might be involved in the process of cyclin B degradation via ubiquitin proteasome. This is in line with the finding that proteasome activation in ascidians is linked to intracellular Ca^{2+} mobilization [104]. Mouse eggs generate Ca^{2+} oscillations that correlate with transient increases of activity of CaMKII for the first hour of fertilization. The continuing pulse of CaMKII activity is required to decrease the level of cyclin B, and for MPF activity and meiotic completion [105].

Conclusions

In surveying the literature on the cell cycle one is struck by the scant attention specialists have paid to Ca^{2+}. This is certainly surprising: considering the universal role of Ca^{2+} as a controller of cell function, it would be very odd if Ca^{2+} were irrelevant in a process as important as the cell cycle. Ample evidence has indeed accumulated that Ca^{2+} has a role in the control of the cycle, beginning with the finding that cells are induced to divide and proliferate by different mitogens that bind to membrane receptors to induce Ca^{2+} increase in the cell. As is usual for the signaling functions of Ca^{2+}, the Ca^{2+} message to the cell cycle is mostly decoded through CaM. And as one might expect, Ca^{2+}/CaM-dependent pathways are involved in several cell cycle steps and influence a number of important targets of the cycle.

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