Chapter 1

ARPP19 Phosphorylations by PKA and Greatwall: The Yin and the Yang of the Cell Decision to Divide

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Additional information is available at the end of the chapter

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Abstract

Entry into mitosis and meiosis is orchestrated by the phosphorylation of thousands of mitotic substrates under the control of active Cdk1-cyclin B complexes. To avoid futile cycles of phosphorylation/dephosphorylation, the specific Cdk1-antagonizing phosphatase, PP2A-B55δ, must be simultaneously inactivated. This process is achieved by the activation of the kinase Greatwall (Gwl), which phosphorylates ARPP19. Gwl-phosphorylated ARPP19 then inactivates PP2A-B55δ to allow Cdk1 activation as well as to secure the phosphorylation state of mitotic substrates. This chapter discusses a series of recent works showing that ARPP19 is also phosphorylated by another kinase, PKA. Phosphorylated by PKA, ARPP19 arrests *Xenopus* oocytes in G2 before the first meiotic division. Therefore, depending on its phosphorylation state by either PKA or Gwl, ARPP19 either restrains or activates Cdk1 in *Xenopus* oocytes. Beyond the understanding of the mechanisms of meiotic and mitotic cell division, the control of ARPP19 by its dual phosphorylation enlightens the cAMP-regulated signalization pathways that control vital functions in numerous eukaryotic cell types.

Keywords: cell division, meiosis, ARPP19, Cdk1, PKA, Greatwall, phosphatases, PP2A, kinases

1. Introduction

Cell division is fundamental for life. This process allows the development, the growth and the renewal of any organisms on earth, from bacteria to metazoan. Since its first description by Walther Flemming in 1882, this process has fascinated biologists. M-phase entry and exit are switch-like transitions that are predominantly orchestrated by phosphorylation and dephosphorylation of hundreds of proteins. These "mitotic substrates" govern the structural events that underlie the mechanics of cell division. These phosphorylations are controlled by a cohort

of mitotic kinases and phosphatases themselves regulated by two master enzymes: the universal mitotic kinase, Cdk1-cyclin B (or MPF for M-phase promoting factor) and its recently identified antagonizing phosphatase, PP2A, associated to its regulatory subunit B55δ. While we now have a detailed description of cell division mechanics and a long list, still expanding, of mitotic proteins orchestrating these structural cellular events, our basic knowledge of the molecular cascades converging to Cdk1 activation and entry into cell division remains incomplete. In particular, despite the well-documented role of protein kinases in mitosis, little is known regarding the control of Ser/Thr phosphatases that remove the mitotic phosphates and counterbalance the activation and the activity of Cdk1. In 2010, it was shown that the small protein ARPP19 is a substrate of the Greatwall kinase (Gwl) and plays a central role in M-phase through PP2A inhibition. Interestingly, ARPP19 stands for "cAMP-regulated phosphoprotein" and is phosphorylated by PKA in mammalian tissues and cell cultures. In 2014, we showed that ARPP19 phosphorylation by PKA in oocytes maintains these germ cells arrested in G2 by preventing the molecular cascade activating Cdk1. Therefore, ARPP19 is at a crossroad in the meiotic M-phase control network, integrating PKA signaling to halt cell cycle in G2 and switched by Gwl to an M-phase inducer by linking Cdk1 activation to PP2A-B55δ. In this review, we describe how the small ARPP19 protein is able to act as a switcher to guide the cell decision to divide or not. Beyond the understanding of the mechanisms of meiotic and mitotic cell division, deciphering the control of ARPP19 by phosphorylation should also enlighten the cAMP-regulated signalization pathway that controls vital functions in numerous eukaryotic cell types.

2. Cell division: good guys-bad guys, protein kinases-protein phosphatases

2.1. A prominent role of protein kinases ensuring the phosphorylation of mitotic proteins

During cell division, the structure of the cell is dramatically reorganized to properly segregate chromosomes. Since transcription is repressed during cell division, these structural rearrangements are mainly driven by posttranslational modifications of mitotic proteins. Of these, protein phosphorylation is the most abundant event that plays a key role in regulating cell cycle engines. During mitosis entry, approximately 7000 proteins are phosphorylated at multiple sites. This extensive network of phosphorylations is governed by multiple kinases (**Figure 1**), which are organized in cascade, hence amplifying a small initial signal to ensure irreversible transitions during the cell cycle. Of these, the kinase Cdk1 (cyclin-dependent kinase 1) is the most prominent one and is the universal inducer of M-phase entry in all eukaryotes [1–8]. To be active, Cdk1 must be associated to its regulatory subunit, cyclin B (Cdk1-cyclins B complexes, also called MPF for M-phase promoting factor), phosphorylated at the activatory T161 residue and further dephosphorylated at T14 and Y15, two residues that impair its activity when phosphorylated. During M-phase entry, Cdk1 is activated and acts in concert with other kinases to phosphorylate mitotic proteins responsible for the structural changes associated with cell division (**Figure 1**). Hence, entry and progression in mitosis have long been viewed as depending on a giant burst of protein phosphorylation under the control of a master kinase, Cdk1.

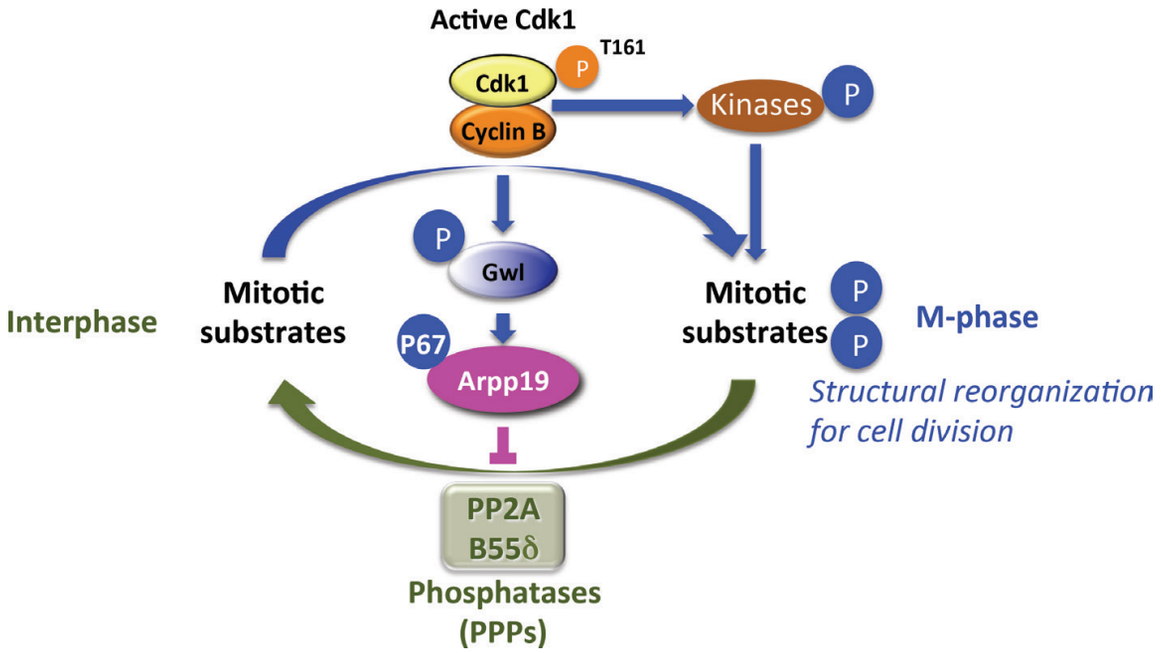

Figure 1. Cell division is controlled by kinases and phosphatases.

2.2. Cell division is not only a matter of kinases: requirement for a fine-tuning of protein phosphatases

Importantly, the return in interphase requires not only Cdk1 inactivation but also the mitotic proteins to be dephosphorylated. This process was at first thought to be controlled by the cellular machinery responsible for protein degradation, as it irreversibly inactivates Cdk1 through cyclin B degradation and consequently leads to the disappearance of phosphorylated mitotic proteins [9, 10]. Nevertheless, mitotic substrates need the action of phosphatases to be dephosphorylated and it is now admitted that the regulation of phosphatase activities is as essential as kinases, not only to exit from M-phase but also for the accurate progression in M-phase (**Figure 1**). Phosphatases are classified into three distinct groups based on their specificity toward residues that are dephosphorylated: the tyrosine phosphatases (cTPs), the dual specificity phosphatases (DSPs) and the serine/threonine phosphatases (STPs) [11]. From the cTPs and DSPs family, only Cdc14, Cdc25 and Ssu72 play an essential role in mitosis [12, 13], and most of the dephosphorylations during M-phase rely on STPs activities. Of these, the phosphoprotein phosphatase (PPPs) family, which includes the protein phosphatases 1 (PP1) and 2A (PP2A), is the largest group necessary for the proper regulation of mitosis [12, 14, 15]. The regulation and the diversity of these phosphatases result from the combinatorial association between conserved catalytic subunits and regulatory subunits that drive their substrate specificity, their cellular localization and their activity. In particular, PP2A is a trimeric complex composed of one catalytic C subunit ($PP2A_C$), one scaffold A subunit ($PP2A_A$) and one regulatory B subunit ($PP2A_B$) that provides substrate specificity. Each subunit is expressed as multiple cellular isoforms, resulting in approximately 100 heterotrimeric PP2A holoenzymes broad-acting *in vivo*. This variety also applies to other PPPs such as PP1, which associates to one or two variable regulatory subunits, thereby creating more than 650 distinct complexes

[14]. Hence, investigating the specific function of these PPPs *in vivo* has been challenging as most of their pharmacological inhibitors, such as okadaic acid or microcystin, do not distinguish among holocomplexes with identical catalytic subunits, yet harboring very different functions due to the variety of their regulatory subunits. Moreover, the effects of these inhibitors have been mostly characterized on purified phosphatases. These phosphatases have been long viewed as housekeeping enzymes, being constitutively active during cell cycle progression. However, failure to regulate their activities has severe consequences on cell division. Inhibiting their activities allows cells to enter mitosis without Cdk1 activation. Then cells proceed through aberrant mitosis with multiple defects on chromosome condensation and segregation [16, 17]. Moreover, their inhibition at metaphase prevents cells to exit from mitosis despite cyclin B degradation and Cdk1 inactivation [18]. More recently, it appeared that it is not just one but a network of PPP activities that temporally orchestrates the dephosphorylation of specific substrates during M-phase. These sequential dephosphorylations order the structural events necessary to exit from cell division, starting with chromosomes segregation and ending with cytokinesis. Hence, by antagonizing kinase activities, the precise regulation of PPP activities is required for the timely execution of mitosis in higher eukaryotes.

In 2010, a breakthrough in the field of cell cycle research came with the discovery that the specific PP2A holoenzyme associated to the B55δ regulatory subunit (PP2A-B55δ) counteracts the Cdk1-dependent phosphorylations in mitosis (**Figure 1**). The activity of PP2A-B55δ is high in interphase and low during mitosis [19]. Like many regulators of the cell cycle, the role and the biochemical mechanism controlling PP2A-B55δ activity were deciphered in CSF and cycling egg extracts from *Xenopus*, a powerful model system that reproduces *in vitro* all the biochemical events of mitosis [20, 21]. Depleting PP2A-B55δ from *Xenopus* egg extracts enhances Cdk1 substrate phosphorylations during mitotic onset and further prevents exit from mitosis due to a failure in dephosphorylating Cdk1 substrates [19]. This was the first evidence that the regulation of a specific phosphatase, PP2A-B55δ, is as important as the Cdk1 kinase for the proper progression in M-phase.

3. Under Gwl control, ARPP19 shuts down PP2A-B55δ to let Cdk1 acting

3.1. ARPP19, a specific inhibitor of PP2A-B55δ

The molecular mechanism underlying the negative control of PP2A-B55δ was further elucidated in *Xenopus* extracts. It revealed that two proteins, the kinase Greatwall (Gwl) and the small protein ARPP19 (cAMP-regulated phosphoprotein of 19 kDa), are the key regulators of PP2A-B55δ. Gwl is an evolutionary conserved protein that was identified in *Drosophila* by two independent genetic screens aimed at identifying new kinases involved in cell cycle progression [22–24]. As cells enter mitosis, Gwl is phosphorylated at multiple sites, probably by various kinases, among them Cdk1 that is critical as it activates Gwl [25–28]. Gwl then phosphorylates two proteins from the endosulfine (ENSA) family: α-endosulfine and its very close paralog ARPP19 at an S residue present in a highly conserved KYFDSGDY motif, corresponding to S67 in *Xenopus* ARPP19 [20, 21, 28]. These small proteins are expressed

ubiquitously in neurons as well as in nonneuronal cells and regulate a plethora of biological processes including insulin secretion, quiescence upon nutrient deprivation in yeast and neurite outgrowth [28–34]. Interestingly, these proteins preset the biochemical characteristics of phosphatase inhibitors, being basic-, heat- and acid-stable. When phosphorylated by Gwl at S67, ENSA/ARPP19 is converted into a potent and highly specific inhibitor of PP2A-B55δ that acts by direct binding and does not associate to other PP2A holoenzymes [20, 21] (**Figure 1**). ARPP19 is also a substrate of PP2A-B55δ that binds extremely tightly to the phosphatase and is dephosphorylated at a very low rate [35]. From these results, an "inhibition by unfair competition" model has been proposed wherein phosphorylated ARPP19 would inhibit PP2A-B55δ by blocking access of other substrates [35].

3.2. ARPP19-Gwl, a critical module for the phosphorylation of mitotic substrates, M-phase progression and exit

Because PP2A-B55δ inhibition is required to secure Cdk1 substrate phosphorylation, ENSA/ARPP19 phosphorylation by Gwl is both necessary and sufficient for M-phase entry, progression and exit. Indeed, preventing Gwl inactivation and ARPP19 dephosphorylation abolishes dephosphorylation of mitotic substrates, and consequently M-phase exit despite Cdk1 inactivation [20, 21]. The role of Gwl and ENSA/ARPP19 as key actors governing M-phase progression is largely conserved in somatic eukaryotic cells. In yeast, the Gwl homolog, Rim15p, is required for cellular nutrient responses, such as the arrest of cell proliferation and the induction of meiosis [29, 32, 36]. In human cells, the homolog of Gwl, MASTL, is surprisingly dispensable for the nuclear envelope breakdown (NEBD), a morphological event that signals M-phase entry. However, it remains necessary for important structural events beyond NEBD such as chromosome condensation, proper segregation of chromosomes and cytokinesis [37–43].

The exit from M-phase requires PP2A-B55δ reactivation that depends on Gwl inactivation and ENSA/ARPP19 dephosphorylation. Phosphorylated ARPP19 is present in molar excess over PP2A-B55δ [35], implying that other phosphatases must act during mitosis exit to break the loop established between Gwl, ENSA/ARPP19 and PP2A-B55δ (**Figure 2**). One of these other phosphatases could correspond to PP1. In mitosis, PP1 is inhibited by its Cdk1-dependent phosphorylation at T320. Upon mitosis exit, Cdk1 is inactivated and allows PP1 to dephosphorylate itself at T320 (**Figure 2**). As a result, PP1 is activated and dephosphorylates Gwl, resulting in its inactivation [44, 45]. Consequently, ENSA/ARPP19 is dephosphorylated and no longer inhibits PP2A-B55δ. PP2A-B55δ then dephosphorylates mitotic substrates and the cell shifts from a mitotic to an interphasic structural organization [44, 45]. PP1 also contributes to M-phase exit by dephosphorylating mitotic phosphoproteins [46]. Hence, PP1 acts in concert with PP2A-B55δ for the proper exit from mitosis. In conclusion, ARPP19 is at the center of the antagonistic regulation of an intricate network of kinases and phosphatases, which irreversibly drives M-phase progression by avoiding futile cycles of phosphorylation/dephosphorylation. During the G2/M transition, Cdk1 is activated, whereas PP2A-B55δ is inactivated by ARPP19, thus securing Cdk substrate phosphorylation and progression in M-phase. Upon Cdk1 inactivation by cyclin degradation, both PP1 and PP2A-B55δ are reactivated and consequently dephosphorylate Cdk substrates to promote M-phase exit and the return to interphase.

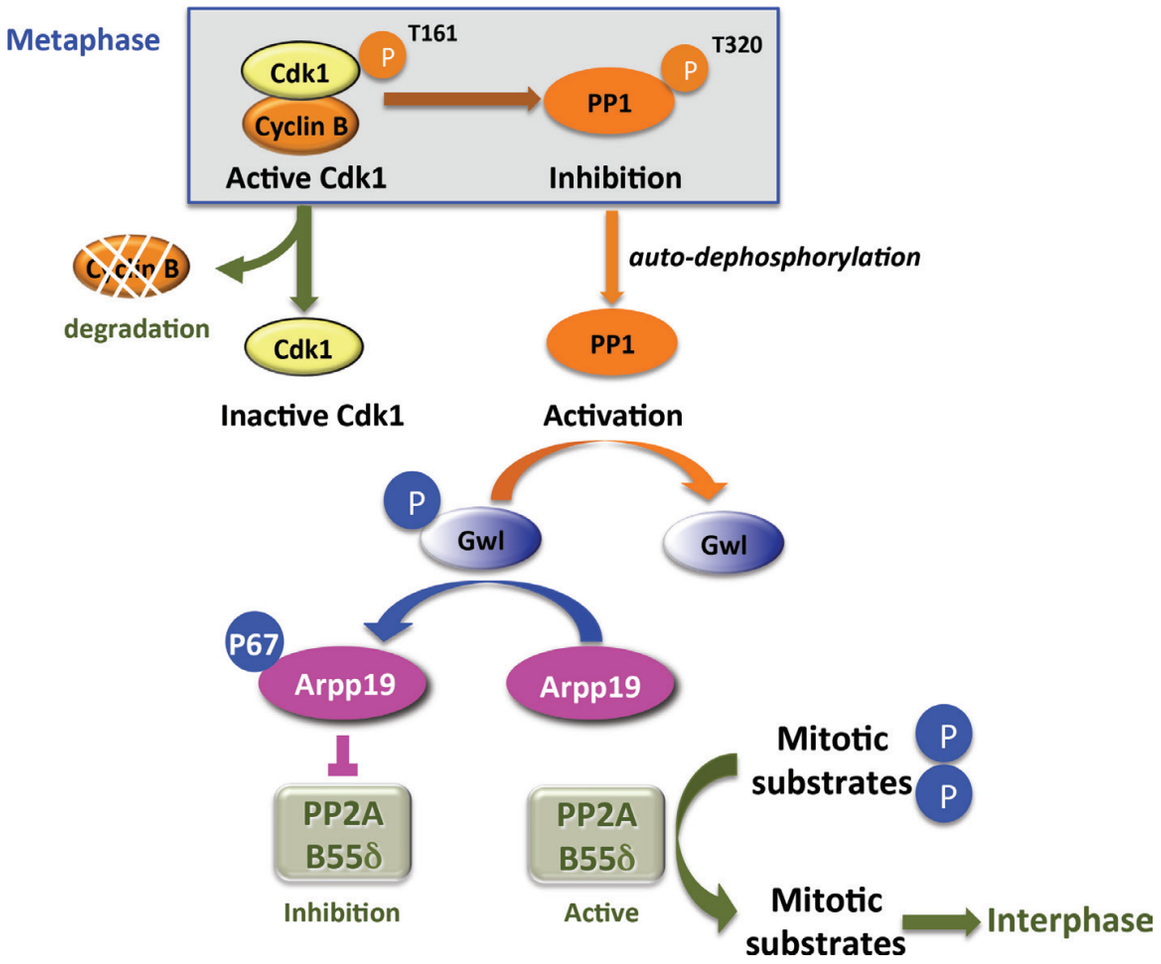

Figure 2. M-phase exit requires PP1 and PP2A-B55δ activation.

3.3. ARPP19-Gwl, a critical module for Cdk1 activation and M-phase entry

Besides acting on Cdk substrate phosphorylation, PPPs are also directly involved in the molecular mechanism responsible for Cdk1 activation. At the end of the G2 phase, Cdk1-cyclin B complexes have accumulated due to cyclin synthesis and are inhibited by Cdk1 phosphorylation at T14 and Y15 (**Figure 3**). These inhibitory phosphorylations depend on the balance of two antagonistic activities: the phosphatase Cdc25 on one side and the Wee1 and/or Myt1 kinases, two redundant members of the same family, on the other side [47–53]. This triad, Wee1/Myt1, Cdc25, and their substrate Cdk1, controls M-phase in all eukaryotes. The activities of Wee1/Myt1 and Cdc25 are regulated by phosphorylation, the first one being inactivated by phosphorylation, whereas the second one is activated. The expression level of Cdk1 being constant during the cell cycle, the formation of Cdk1-cyclin B complexes begins in interphase with the progressive accumulation of cyclin B. As cyclin B accumulates throughout G2, it associates with free Cdk1 molecules that get phosphorylated at T161. These Cdk1-cyclin B complexes are then held inactive by the Wee1/Myt1-dependent phosphorylation of Cdk1 at T14 and Y15 [3, 53]. This prevents precocious entry in M-phase (**Figure 3**). Entry into M-phase is triggered by the activation of Cdc25 that dephosphorylates Cdk1-cyclin B complexes at T14

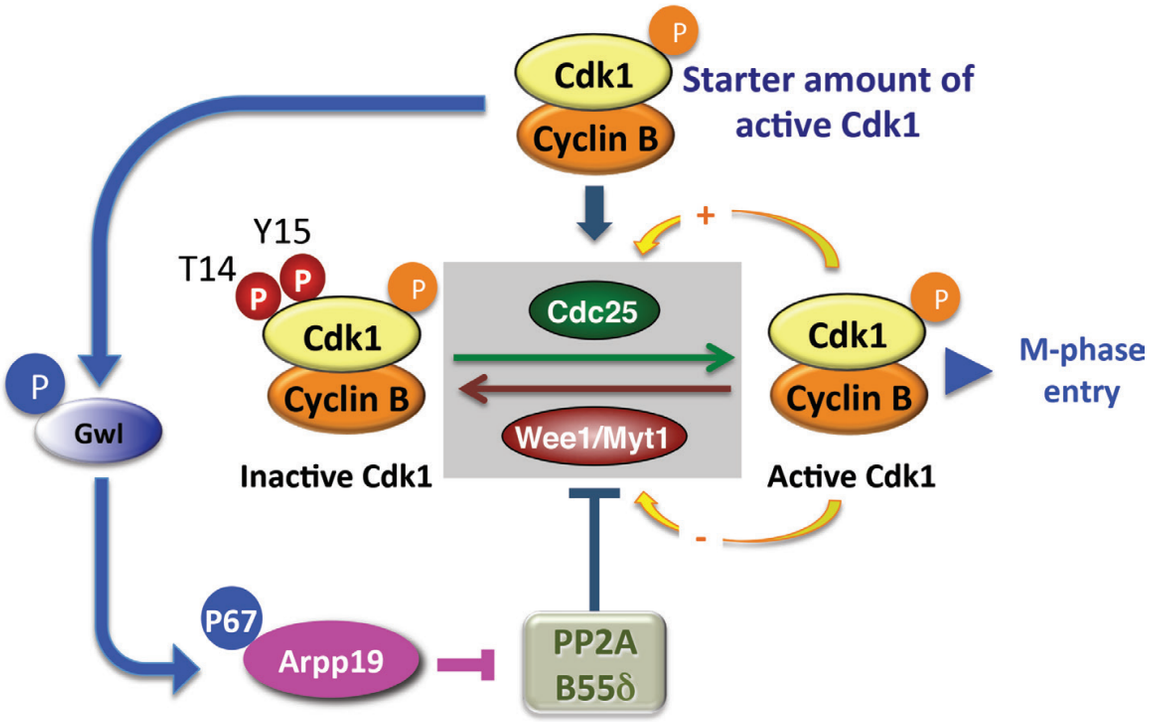

Figure 3. Cdk1 activation and M-phase entry require PP2A-B55δ inactivation.

and Y15. As a result, these complexes are activated and phosphorylate their own regulators, thus enhancing Wee1/Myt1 inhibition and Cdc25 activation [3, 53]: more Cdk1 is activated, more it activates its own activator, Cdc25, and inhibits its own inhibitor, Wee1/Myt1, and more it is activated itself. This mechanism constitutes the core part of a process called the Cdk1 autoamplification loop, which ensures the dephosphorylation of all Cdk1-cyclin B complexes. Furthermore, Cdk1 activates other kinases including Plk1 or Aurora-A to reinforce the loop [3, 54]. Importantly, this switch-like transition is counterbalanced by PP2A-B55δ activity. Indeed, this phosphatase opposes Cdk1 activity by dephosphorylating Cdc25 and Wee1/Myt1, thus compromising the initial activation of Cdk1 by Cdc25 and then the autoamplification process. Importantly, by phosphorylating and activating Gwl, Cdk1 enables ARPP19 phosphorylation at S67 and the consequent inactivation of its counteracting phosphatase. This generates a feedforward regulatory loop to ensure M-phase entry and progression [20–22, 25, 39, 55, 56] (**Figure 3**). Hence, ARPP19, under its Gwl-phosphorylated state, occupies a central position to activate the irreversible switch that engages the cell to divide.

4. PKA, a break of cell division

Kinases are commonly viewed as positive engines driving cell division. Only few kinases, as Wee1/Myt1, restrain the G2/M transition. In G2, some kinases can however inhibit mitosis entry and are usually activated by surveillance mechanisms, termed checkpoints [57]. These kinases, such as ATM, ATR and their substrates Chk1 and Chk2, are activated when DNA

integrity is at threat or when DNA replication is not properly completed. By indirectly regulating Wee1/Myt1 and Cdc25 activities, these kinases block M-phase entry and extend the G2 period, allowing the establishment of the DNA quality control and repair processes [57]. However, other kinases have the potential to block M-phase entry without being involved in checkpoint mechanisms. Of these is the well-conserved cAMP-dependent protein kinase A (PKA). PKA is the major mediator of the pleiotropic cellular regulator, cyclic AMP (cAMP). This kinase is one of the most prominent actors of signal transduction pathways and plays pivotal functions in various cellular processes including metabolism, cell survival, proliferation, differentiation and cell cycle regulation.

PKA is a heterotetradimer composed of two catalytic subunits (PKA_C) and two regulatory subunits (PKA_R). The PKA_C subunits contain the active site, an ATP-binding site and a binding domain for PKA_R. The PKA_R subunits bind one another in an antiparallel orientation to form a homodimer and further encompass a cAMP-binding domain also interacting with PKA_C and an "autoinhibitory" domain that serves as a pseudosubstrate for PKA_C. When the intracellular concentration of cAMP is low, PKA_R dimer binds and inactivates PKA_C. As the concentration of cAMP increases, cAMP binds to PKA_R and induces an allosteric conformational change, which unleashes PKA_C subunits. Free PKA_C subunits are then active and phosphorylate their intracellular targets. This process further involves scaffold proteins, termed AKAPs (A-kinase anchoring proteins), which tether the kinase to defined cellular sites in order to confer spatial and temporal specificity to PKA [58, 59]. While most of its contribution involves the activation of gene transcription, nongenomic effects of PKA have been implicated in M-phase progression. Remarkably, high PKA activity is responsible for arresting vertebrate oocytes in prophase of the first meiotic division by indirectly inhibiting Cdk1 activation. This negative effect of PKA exerted on M-phase entry is also conserved in somatic cells, in which keeping high levels of cAMP or PKA activity was found to prevent Cdk1 activation and block the G2/M transition [60–63]. While the role of PKA as a potent inhibitor of the mitotic and the meiotic cell division is well established since almost 40 years, its substrate in both somatic cells and oocytes had remained unidentified all over these years. Using meiotic division of *Xenopus* oocytes as a model system, this critical question has received a first answer in 2014 [64].

4.1. The oocyte meiotic division, a powerful system model to study the G2/M transition

The arrest in prophase is specific to female germ cells and is universal in the animal kingdom. This arrest allows oocytes to accumulate the components necessary to support embryo development. On the contrary to somatic cells, this physiological arrest is independent of checkpoint mechanisms. At the time of ovulation, the prophase arrest is released by external stimuli. In vertebrates, the luteinizing hormone (LH) promotes the synthesis, in ovarian follicular cells, of a second hormonal inducer that acts on the oocyte to induce meiosis resumption. In lower vertebrates as amphibians, this signal corresponds to a steroid hormone, progesterone [65–69]. Upon the hormonal stimulation, oocytes complete the first meiotic division and proceed through the second meiotic division with no intervening S-phase, which reduces the parental genome by half (**Figure 4**). These cells then halt their

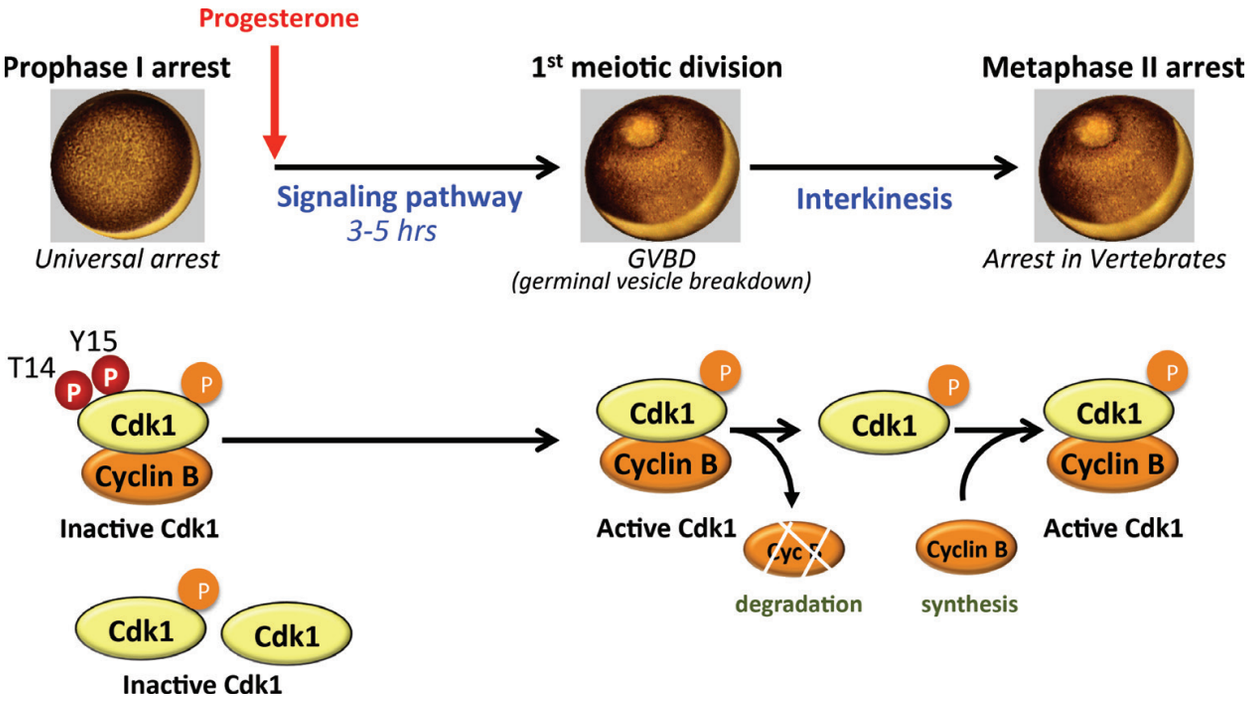

Figure 4. Meiotic maturation of *Xenopus* oocytes.

divisions in metaphase of the second meiotic division (metaphase II) in all vertebrates. This process, called meiotic maturation, generates haploid gametes ready to be fertilized. Similarly to mitosis, the progression through the meiotic maturation depends on Cdk1 that sustains two successive waves of activation to coordinate the two consecutive cell divisions (**Figure 4**). The first activation of Cdk1 promotes the reentry in the first meiotic division, a process marked by the nuclear envelope breakdown, or germinal vesicle breakdown (GVBD). Thereafter, Cdk1 is inactivated in metaphase I by the ubiquitin-dependent degradation of cyclin B to induce exit from the first meiotic division and the extrusion of the first polar body [70]. Concomitantly to cyclin B degradation, newly synthesized cyclin B molecules reaccumulate and quickly reactivate Cdk1 to enable entry into the second meiotic division (**Figure 4**). In metaphase II, an oocyte-specific kinase network, the Mos/MAPK/Rsk module, and its substrate Erp1/Emi2 inhibit cyclin B degradation [71–74]. This process stabilizes Cdk1 activity at a high level and arrests the cell in metaphase II, a specific feature of vertebrates.

The meiotic cell division shares most of the molecular regulators with mitosis. The components of the Cdk1 autoamplification loop as well as Gwl, ARPP19 and various PPPs such as PP1 and PP2A are conserved. Hence, the arrest in prophase of oocytes is commonly assimilated to a late G2 phase and the process of meiosis resumption has served as an historical model to decipher signaling pathways involved in the G2/M transition. Of these, *Xenopus* oocyte has been widely studied to decrypt the biochemical regulation of cell division. In this species, progesterone induces Cdk1 activation within 3 to 5 hours, a process visualized by GVBD (**Figure 4**). Hence, it is possible to investigate meiosis resumption from the first molecular events induced by the hormone until Cdk1 activation.

4.2. PKA, acting both at the top and at the end of the signaling pathway leading to Cdk1 activation

Xenopus prophase–arrested oocytes contain two distinct pools of inactive Cdk1: a monomeric form of Cdk1 that is already phosphorylated at T161 and a stockpile of Cdk1-cyclin B complexes phosphorylated at T14 and Y15 [70, 75, 76] (**Figure 4**). These complexes are continuously formed during the prophase arrest because of the slow accumulation of cyclin B that associates with free T161-phosphorylated Cdk1 [70, 75]. As in somatic cells, these newly formed Cdk1-cyclin B complexes are immediately phosphorylated by Myt1 at T14 and Y15 and inactivated [77–79]. Cdc25 is kept inactive, likely resulting from its S287 phosphorylation by PKA that promotes its cytoplasmic retention and from the dephosphorylated state of its Cdk1 residues [80].

4.2.1. Initiation of the signaling pathway: a PKA lock

In all vertebrates, PKA activity is responsible for the prophase arrest of oocytes. In these resting cells, PKA is kept active by elevated intracellular levels of cAMP, necessary to restrain Cdk1 activation [81–87]. In *Xenopus* oocytes, although the receptor of progesterone has not been identified, the earliest event detectable within 10 minutes upon the hormonal stimulation is a decrease of cytoplasmic cAMP concentration, immediately followed by the downregulation of PKA activity (**Figure 5**). The drop in cAMP concentration and PKA downregulation are both necessary and sufficient for unlocking a molecular cascade that activates Cdk1 3–5 h later. Forskolin, cholera toxin and IBMX, three cAMP-elevating agents, or recombinant PKA_C, are all potent inhibitors of progesterone-induced meiosis resumption [88–91]. Conversely, injection of recombinant PKA_R or a specific protein inhibitor of PKA, PKI, in the oocyte is sufficient to activate Cdk1 in the absence of progesterone [92, 93]. Hence, both cAMP and PKA, by keeping Cdk1 inactive, are the key actors of the arrest of oocytes in prophase. This implies that a substrate of PKA is responsible for the arrest in prophase under its phosphorylated form (**Figure 5**). Likewise, this PKA substrate must be dephosphorylated within 1 h after hormonal stimulation to launch the signaling pathway that ultimately authorizes Cdk1 activation 3–5 h later.

4.2.2. The core of the signaling pathway: cyclin B and Mos accumulation

The few hours long pathway connecting PKA inhibition and Cdk1 activation is not fully known. PKA inhibition controls the synthesis of new proteins from a preexisting pool of maternal mRNAs in *Xenopus* oocyte. Two newly synthesized proteins are essential for Cdk1 activation: cyclin B, in particular the B1 isoform, and the kinase Mos (**Figure 5**). Mos is a germ cell–specific kinase. It activates MEK that in turn activates MAP kinase (MAPK/ERK) that phosphorylates, among other substrates, $p90^{Rsk}$. Mos starts to accumulate to detectable levels at GVBD and activates the Mos/MAPK pathway during the whole process of meiotic maturation in all animal species analyzed so far [94–96]. Mos and cyclin B1 are both synthesized in response to progesterone or following PKI injection [94, 97]. In contrast, they do not accumulate in response to progesterone if PKA is experimentally kept active [97], hence demonstrating that their accumulation, essential in the pathway converging to Cdk1 activation, is under the negative control of PKA.

Cyclin B and Mos activate two redundant pathways ending with the activation of Cdk1. While suppressing by an antisense strategy the synthesis of one pathway, either cyclin B or

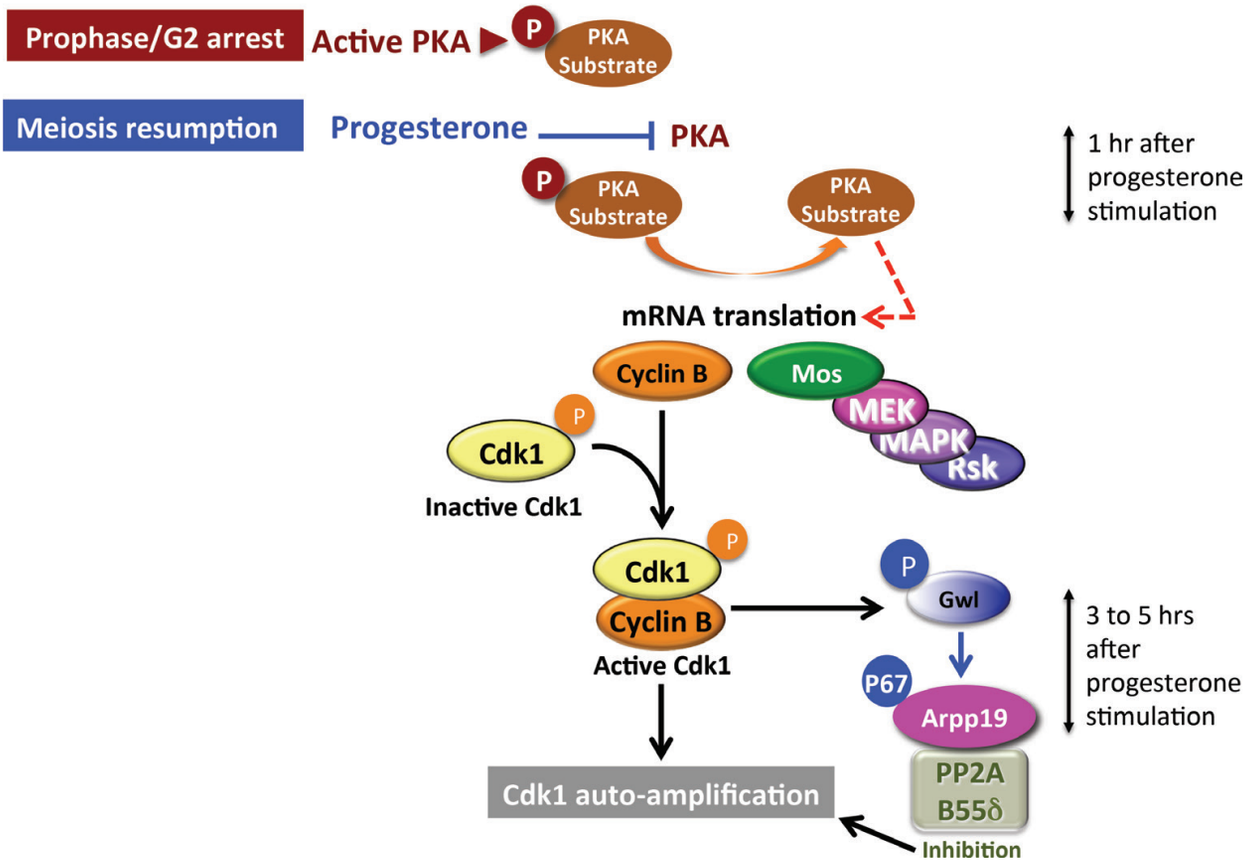

Figure 5. The signaling pathway leading to Cdk1 activation in *Xenopus* oocytes.

Mos, does not hinder meiosis resumption upon progesterone stimulation or PKI injection in *Xenopus* oocyte, inactivating simultaneously both pathways completely abolishes meiosis resumption [97, 98]. In this double knock-down condition, Cdk1 activation is restored by the replenishment of either one of them, provided by injecting oocyte with either recombinant Mos or cyclin B [97, 98]. While both proteins contribute to Cdk1 activation, cyclin B is likely the principal actor required for this process. In response to progesterone, the rate of cyclin B1 synthesis and its accumulation increase independently of Cdk1 activation [99]. On the contrary, Mos accumulates only if the protein has been primarily stabilized by its Cdk1-dependent phosphorylation [99, 100], therefore suggesting that its accumulation requires basal levels of Cdk1 activity that are not detectable in prophase oocytes. The accumulation of cyclin B1 molecules generates new Cdk1-cyclin B complexes from monomeric T161-phosphorylated Cdk1 [77, 99, 101] (**Figure 5**). Accordingly, cyclin B1 or B2 injection promotes Cdk1 activation independently of protein synthesis [97, 98], whereas overexpressing a dominant negative form of Cdk1, K33R-Cdk1, known to sequester newly synthesized cyclins, prevents progesterone-induced meiosis resumption [101]. The newly formed Cdk1-cyclin B complexes escape the Myt1-dependent inhibitory phosphorylations of Cdk1 at T14 and Y15 by an unknown mechanism [76, 77]. Hence, these newly formed Cdk1-cyclin B complexes are immediately active [77]. In this model, the Mos/MAPK pathway would contribute to Cdk1 activation by phosphorylating Myt1 and Cdc25, hence promoting their respective inhibition and activation [94, 102].

4.2.3. The endpoint of the signaling pathway: the Cdk1 autoamplification loop, a process sensitive to PKA

The few active molecules of Cdk1 generated by cyclin B1 accumulation serve as a trigger to launch the Cdk1 autoamplification loop that converts the stockpile of inactive Cdk1-cyclin B complexes into active ones in an autoaccelerated manner. This process relies entirely on the dephosphorylation of inactive Cdk1-cyclin B complexes at T14 and Y15 (**Figure 6**). As in mitosis, this process depends primarily on Cdk1 activity that phosphorylates its own regulators, Cdc25 and Myt1, resulting in their respective activation and inhibition. It is reinforced by other kinases such as the Mos/MAPK pathway, Plk1 or Aurora A, which are activated downstream of active Cdk1 and also target Cdc25 and Myt1. Importantly, PPP phosphatases that counterbalance Cdk1 activity must be inhibited as well, to avoid the dephosphorylation of Cdc25 and Myt1 (**Figure 6**). Indeed, inhibiting both PP1 and PP2A directly triggers the Cdk1 autoamplification loop independently of the upstream steps of the signaling pathway induced by progesterone, i.e. PKA downregulation and protein synthesis, showing that PPPs are part of the core machinery of the autoamplification loop [17, 103, 104]. Moreover, PP2A-B55δ inactivation is likely the key PPP involved in this process as shown by the important role of Gwl in this process: Gwl is activated upon Cdk1 activation in oocytes of a range of species (starfish, *Xenopus* and mice) (**Figures 5** and **6**) and the overexpression of a constitutive active form of Gwl is sufficient to activate Cdk1 in *Xenopus* oocytes [105, 106].

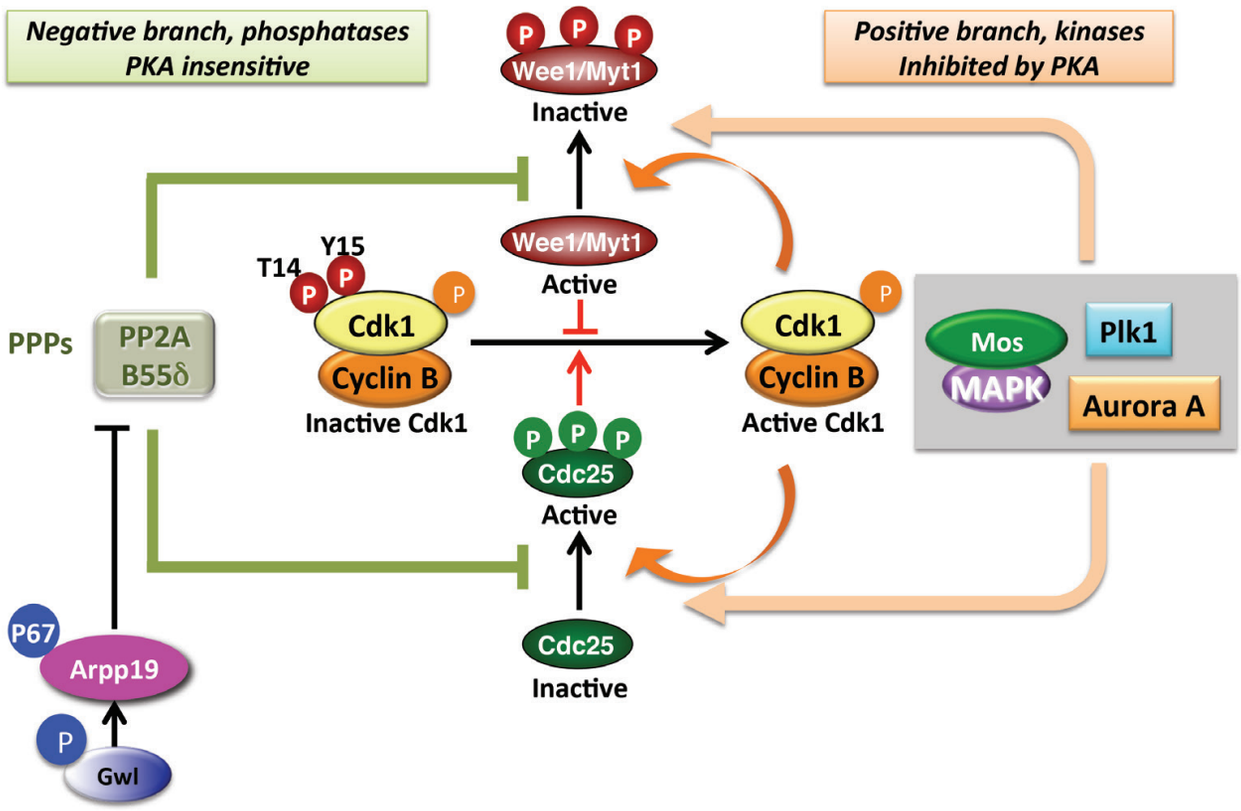

Figure 6. The Cdk1 autoamplification loop.

Consequently, the autoamplification loop can be directly activated in the oocytes using two experimental ways that bypass the upstream signaling cascade: either by inhibiting the negative branch of the loop, i.e. PPPs, by example with okadaic acid or microcystin; or by overexpressing the actors of the positive branch of the loop, such as cyclin B, Mos or Cdc25. It has to be noted that PKA is not active when the loop takes place [107, 108]. Indeed, PKA is inhibited in response to progesterone within less than 1 h and stays at a low activity level thereafter. Strikingly, an artificially elevated activity of PKA prevents all the positive actors of the Cdk1 autoamplification loop, as purified Cdk1-cyclin B complexes, cyclin B, Mos or Cdc25 to launch the loop [70, 103, 105, 109, 110]. In contrast, PKA activity does not prevent the autoamplification loop when triggered by the inhibition of PPPs [17, 104]. Thus, PKA activity is unable to control PPP activities involved in the loop but has the ability to block the positive actors of the loop by targeting unidentified substrates among them. Importantly, the control of the autoamplification loop exerted by PKA is not physiological, as this kinase is catalytically inactive at the time of Cdk1 activation [107, 108]. Thus, PKA can be pictured as a checkpoint mechanism operating under extraphysiological conditions: any abnormal elevation of PKA activity at that time will block meiotic division.

Hence, understanding the signaling pathway that triggers the G2/M transition in *Xenopus* oocytes requires the identification of the PKA substrate(s) that account for the inhibitory function of PKA at the very beginning of the signaling pathway as well as at the endpoint of this pathway, the autoamplification loop. We have undertaken this quest and found one single protein answering these issues. To our great surprise, this protein corresponds to ARPP19.

5. Under PKA control, ARPP19 is at the core of the cell decision-making: to divide or not

5.1. In search of candidate proteins corresponding to the long-sought oocyte PKA substrate

Since the 1980s, multiple attempts have been unsuccessfully undertaken to identify the substrate of PKA that blocks the initiation of the signaling cascade leading to Cdk1 activation in oocytes. In 2002, Cdc25 was shown to be phosphorylated at S287 by PKA, a process contributing to its inactivation in *Xenopus* prophase–arrested oocytes [80]. Upon progesterone stimulation, Cdc25 is dephosphorylated at S287 and phosphorylated at multiple activatory residues under the control of Cdk1, which activates its catalytic phosphatase activity and allows Cdk1 activation [80, 111]. However, Cdc25 does not fulfill the expected biochemical criteria for being the PKA substrate whose dephosphorylation triggers Cdk1 activation. In particular, S287 dephosphorylation of Cdc25 relies on protein synthesis and occurs much later than PKA inhibition, i.e. at the time of Cdk1 activation [80]. More importantly, S287 dephosphorylation depends on Cdk1 activity and therefore cannot represent the upstream event accounting for Cdk1 activation [80]. Another candidate is the kinase Wee1. In mice, Wee1 is expressed in prophase oocyte and its phosphorylation by PKA enhances its inhibitory effect toward meiosis resumption [112, 113]. However, the only kinase targeting Cdk1 at T14 and Y15 that

is expressed in *Xenopus* oocytes is Myt1, Wee1 being only expressed after the completion of the first meiotic division [114]. Thus, Wee1 cannot be the PKA substrate responsible for the prophase arrest in this species.

Going back in the 1980s, a biochemical approach using *Xenopus* prophase–arrested oocytes led to the purification of a heat- and acid-stable protein of 20 kDa, described as a PKA substrate and dephosphorylated 1 h after progesterone stimulation, downstream PKA inhibition [115]. The close similarities of the biochemical properties of this protein (heat- and acid-stability, molecular weight) with ARPP19 have drawn our attention. While the cell cycle field was focused on Gwl-ARPP19, a comeback to the origins of the ARPP19 discovery, hidden inside its fully developed name cAMP-regulated phosphoprotein 19, highlights that ARPP19 is a substrate of PKA, phosphorylated at an S residue within a PKA consensus RKP/SSLV motif conserved among most animals [21, 64, 116, 117]. Indeed, the acid-soluble proteins of the ENSA family, to which belongs ARPP19, were originally identified as a group of phosphoproteins upon dopaminergic stimulation [117–119]. The signaling pathway induced by dopamine involves cAMP production and PKA activation. Various cellular functions have been attributed to the PKA phosphorylation of these family members [118, 119]. Of these, DARPP32, a member of this family, becomes a potent inhibitor of PP1 when phosphorylated by PKA [118, 119]. ENSA/ARPP19 interacts selectively with the membrane-bound form of the presynaptic protein α-synuclein [120]. When constitutively phosphorylated by PKA, the interaction between ENSA/ARPP19 and α-synuclein is abolished, interfering with α-synuclein self-assembly in membrane, and probably accounting for the role of ENSA/ARPP19 in neurodegenerative diseases [120]. Nevertheless, no specific function related to cell division had been attributed to this particular PKA phosphorylation until we hypothesized that ARPP19 could be the substrate of PKA in prophase-arrested oocytes.

5.2. The phosphorylation of ARPP19 at S109 by PKA arrests oocytes in G2

The *Xenopus* ARPP19 sequence encompasses the residue targeted by PKA in the previous studies conducted on the ENSA family [21, 64, 117]. This residue corresponds to S109 in *Xenopus* and is part of a PKA consensus sequence (RKPS_{109}LVA) well conserved among eukaryotes. At first glance, it appears provocative to propose ARPP19, a protein recently shown to be essential for Cdk1 activation, as the PKA substrate ensuring the prophase arrest by locking the transduction cascade ending with Cdk1 activation. How to conceive that the same molecule would be negative for M-phase entry and thereafter transformed into a positive actor of cell division? This apparent paradox is circumvented by the fact that these antagonistic functions do not take place at the same period in oocytes (being negative at the top of the cascade and then positive at its endpoint), do not target the same molecular systems (early players of the cascade for the negative function, PP2A-B55δ for the positive function) and are provided by distinct kinases (PKA for the negative function, Gwl for the positive function).

Indeed, we have shown that *in vivo*, ARPP19 is phosphorylated at S109 by PKA in prophase-arrested oocytes. This residue is dephosphorylated within 1 h after progesterone stimulation

or in response to PKA inhibition provoked by PKI injection [64]. A phosphomimetic S109 mutant, S109D-ARPP19, proved to be a strong inhibitor of Cdk1 activation when injected into oocytes, not only in response to progesterone but also following PKI, Mos or cyclin B injections. However, S109D-ARPP19 does not impair the Cdk1 autoamplification loop promoted by injecting the phosphatase inhibitor, okadaic acid [64]. These results demonstrate that the PKA-dependent phosphorylation of ARPP19 at S109 restrains Cdk1 activation and is responsible for the prophase-arrest of *Xenopus* oocytes (**Figure 7**). Upon progesterone stimulation, PKA is inhibited and ARPP19 is dephosphorylated at S109. This event is necessary to initiate the signaling cascade that ultimately generates the threshold level of active Cdk1, which consequently triggers the Cdk1 autoamplification loop (**Figure 7**). Importantly, the Cdk1 autoamplification loop does no longer require the dephosphorylation of ARPP19 at S109. ARPP19 is therefore the long-sought substrate of PKA that maintains oocytes in prophase. The identity and the regulation of the phosphatase that dephosphorylates ARPP19 at S109, hence unlocking the signalization cascade converging to Cdk1 activation, as well as the direct molecular targets of phospho-S109 versus dephospho-ARRP19 that form the latch of this cascade, now deserve investigation.

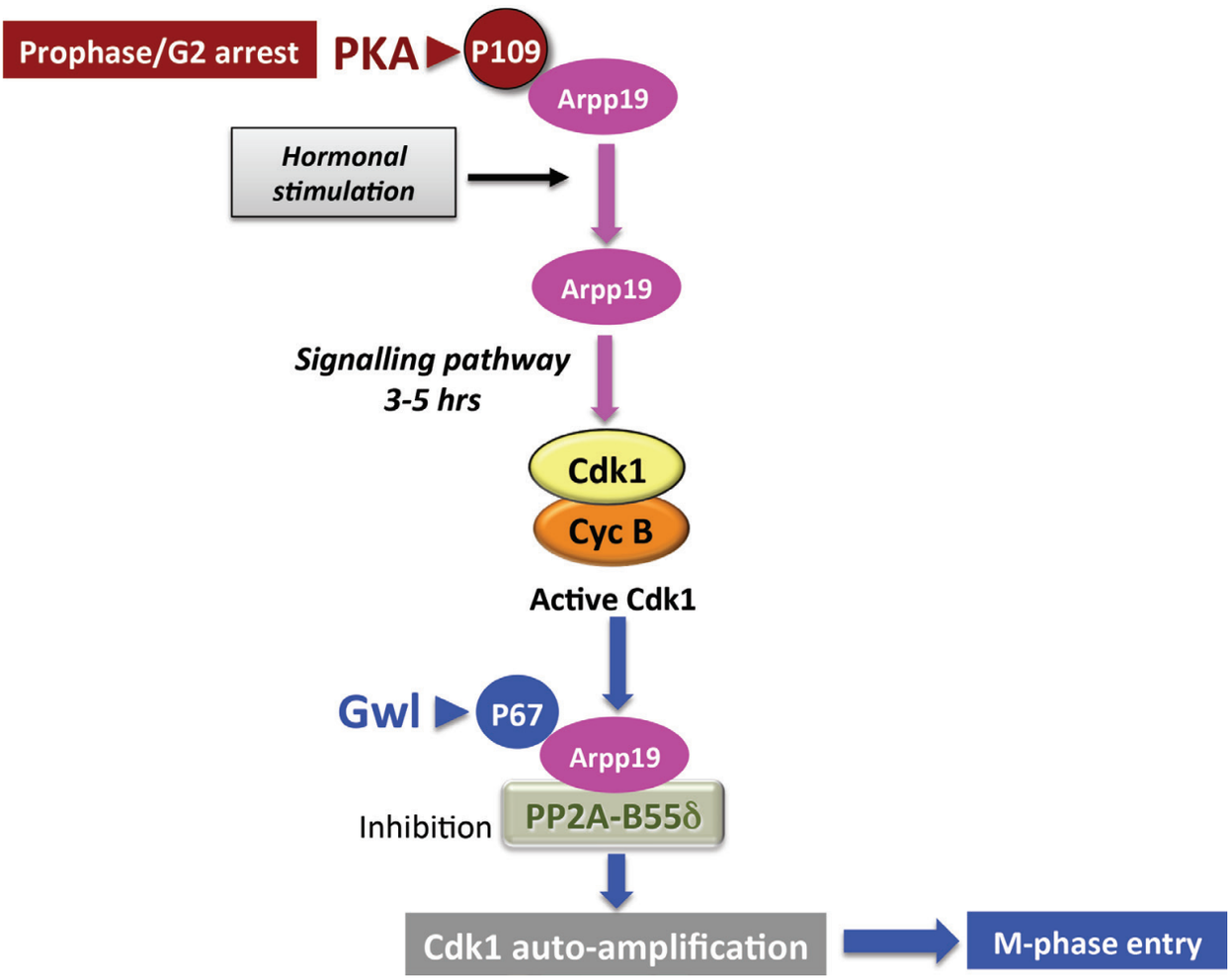

Figure 7. ARPP19 plays two crucial functions during the G2-M transition in *Xenopus* oocytes.

5.3. The phosphorylation of Arpp19 at S67 by Gwl promotes Cdk1 activation and M-phase independently of PKA activity

Strikingly, overexpressing ARPP19 in *Xenopus* oocytes produces opposing effects depending on the amount of the injected protein. High amounts of ARRP19 inhibit meiotic divisions induced by progesterone, whereas decreasing the amount of injected ARPP19 stimulates meiosis resumption [64]. This experiment reveals the antagonistic functions of ARPP19 in oocytes: it inhibits Cdk1 activation when phosphorylated by PKA (as when injected at high concentration) and is converted into a positive regulator of Cdk1 activation during meiosis resumption (as when injected at low concentration). This latter role was then investigated carefully during oocyte meiotic maturation. We showed that ARPP19 is phosphorylated at S67 by Gwl at the time of Cdk1 activation. Once phosphorylated by Gwl, ARPP19 interacts with PP2A-B55δ [110]. We then produced *in vitro* a Gwl-thiophosphorylated form of ARPP19 protein, which is resistant to dephosphorylation. Injecting this constitutively phosphorylated form of ARPP19 at S67 promoted meiosis resumption independently of progesterone and of the synthesis of either Mos or cyclin B [110]. We also generated a nonphosphorylatable ARPP19 mutant at S67 by mutating S67 into an alanine (S67A-ARPP19). The S67A-ARPP19 mutant proved to be a strong inhibitor of meiosis resumption in response to either progesterone or PKI injection but also following the overexpression of constitutive active Gwl [110]. These results clearly demonstrate that ARPP19, once phosphorylated by Gwl at S67, is involved in the Cdk1 autoamplification loop by directly inhibiting PP2A-B55δ (**Figure 7**).

Importantly, the S67-phosphorylated ARPP19 protein was able to interact with PP2A-B55δ and to activate Cdk1 in the presence of high levels of PKA activity [110]. Hence, when phosphorylated by Gwl at S67, ARPP19 inactivates PP2A-B55δ and enables the Cdk1 autoamplification loop to function independently of PKA activity, in strong agreement with the PPPs of the Cdk1 autoamplification loop being insensitive to the inhibitory action of PKA (**Figure 6**).

5.4. The functional interplay between S67 and S109 phosphorylations

When phosphorylated at S109 by PKA, ARPP19 inhibits the molecular cascade leading to Cdk1 activation. Upon Gwl phosphorylation at S67, ARPP19 becomes a potent inducer of Cdk1 activation by inactivating PP2A-B55δ. Hence, ARPP19 acts as a switch in the mechanism of Cdk1 activation depending on its phosphorylation state at S109 and S67. It was then important to determine if both phosphorylations counteract each other, explaining their antagonistic effects. We investigated whether S109 phosphorylation of ARPP19 could prevent its S67 phosphorylation by Gwl, accounting for its inhibitory action toward M-phase entry. *In vitro*, Gwl is able to phosphorylate recombinant ARPP19 at S67 independently of its phosphorylation at S109 [116, 121]. The injection of a double phosphorylated form of ARPP19 at S109 and S67 induces Cdk1 activation as well as meiosis resumption, and the protein is able to interact with PP2A-B55δ [121]. Hence, the phosphorylation of ARPP19 at S109 does neither impair its own phosphorylation by Gwl nor abolish the ability of S67-phosphorylated ARPP19 to inhibit PP2A-B55δ, resulting in Cdk1 activation through the autoamplification loop. Once Gwl is activated, the effect of S67 phosphorylation is dominant over the negative

function conferred by the S109 phosphorylation. Accordingly, the phosphomimic S109D-ARPP19 mutant is phosphorylated at S67 upon the overexpression of a constitutive active Gwl or the injection of S67-phosphorylated ARPP19 and becomes unable to block meiosis resumption under these conditions [64]. Likewise, the inhibition of PP2A-B55δ resulting from S67 phosphorylation of ARPP19 activates Cdk1 independently of PKA. Therefore, the PKA-dependent phosphorylation of ARPP19 does not exert its negative control of M-phase by antagonizing the biological ability of S67-phosphorylated ARPP19 to activate Cdk1. Moreover, the effect of S67 phosphorylation is dominant over the negative function of S109 phosphorylation.

Remarkably, ARPP19 is rephosphorylated at S109 at the time of Cdk1 activation, concomitantly with the S67 phosphorylation by Gwl [64]. This rephosphorylation of ARPP19 at S109 is promoted by a distinct kinase than PKA, not yet identified [64] (**Figure 8**). Therefore, in contrast to what was previously thought, it is not a single S67-phosphorylated form of ARPP19 that activates Cdk1, but a double S109-S67 phosphorylated form. We investigated whether S109 rephosphorylation of ARPP19 contributes to the Cdk1 autoamplification loop or is neutral. Injecting an *in vitro* S67-phosphorylated form of ARPP19 that cannot be phosphorylated at S109, the S109A mutant, is less efficient to activate Cdk1 than S67-phosphorylated ARPP19 [121]. These results show that the active form of ARPP19 at the end of the signaling pathway corresponds to a double phosphorylated form of ARPP19 at both S67 and S109, and that S109 phosphorylation confers new properties to ARPP19, contributing to Cdk1 activation in a context where S67 is already phosphorylated (**Figure 8**).

Hence, these results highlight the importance of timely synchronizing ARPP19 phosphorylations at S109 and S67 to properly activate Cdk1 in *Xenopus* oocytes. In prophase, ARPP19 phosphorylation at S109 by PKA restrains Cdk1 activation. Upon hormonal stimulation, ARPP19 is dephosphorylated at S109, which launches the signaling cascade. At its endpoint, ARPP19 is phosphorylated at both sites, S109 by an unknown kinase and S67 by Gwl. It then becomes a positive actor within Cdk1 autoamplification loop (**Figure 8**). In the future, it will be important to identify the kinase responsible for ARPP19 phosphorylation at S109 and to determine how the rephosphorylation of ARPP19 at S109 positively regulates the Cdk1 autoamplification process.

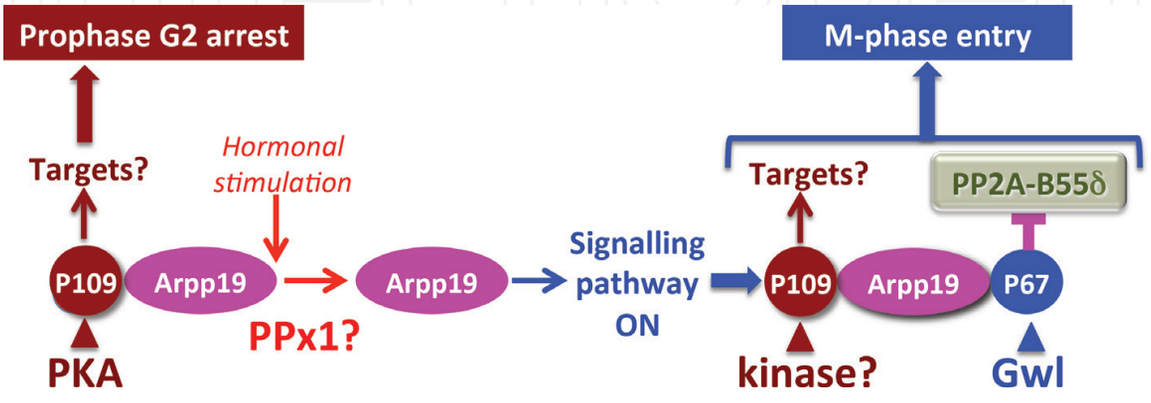

Figure 8. Synchronized phosphorylation of ARPP19 controls the G2-M transition of *Xenopus* oocytes.

The phosphorylations of ENSA/ARPP by PKA and Gwl, acting as a switch-like process to regulate PP2A activity, are found in other cell types, in particular nondividing cells. In postmitotic striatal neurons, ARPP19 is not expressed but its close relatives, ARPP16 and ARPP21, act as potent inhibitors of PP2A. ARPP16 exhibits less specificity toward PP2A isoforms than ARPP19, as it inactivates both PP2A-B55α and PP2A-B56δ [122]. In great contrast to oocytes, in which Gwl is activated as the cell undergoes meiotic divisions, MAST3, the homolog of Gwl, is active and phosphorylates ARPP16 in striatal cells [122]. The constitutive activation of the MAST3-ARPP16 module maintains PP2A continuously inhibited in the absence of stimulation, in order to avoid the dephosphorylation of PP2A substrates [122]. Upon dopaminergic stimulation, PKA is activated and then phosphorylates ARPP16, a process accompanied by its MAST3-dependent dephosphorylation [122]. Importantly, the phosphorylations of ARPP16 by PKA and MAST3 are mutually exclusive and the prior phosphorylation of ARPP16 by PKA reduces the ability of MAST3 to phosphorylate ARPP16 [123]. This reciprocal regulation of ARPP16 by PKA and MAST3 provides a switch in PP2A inactivation, under the control of cAMP-PKA. Hence, in striatal cells, the phosphorylation of ARPP16 by PKA likely attenuates PP2A inactivation promoted by MAST3-phosphorylated ARPP16, thus creating a mechanism whereby cAMP deinhibits PP2A. Therefore, a common set of molecular players, ARPP, PKA and Gwl, is used to regulate PP2A activity in response to various extracellular signals regulating cAMP, but in distinct ways depending on the cell type and/or on dividing or nondividing cells.

6. Concluding remarks

In light of these recent advances, a new model for the regulatory mechanisms controlling the G2/M transition and ending with Cdk1 activation emerged. In this model, two phosphatases would act at different times during meiosis resumption to control the signaling pathway induced by progesterone and ending with the Cdk1 autoamplification loop (**Figure 8**). Upon progesterone stimulation, PKA is downregulated and a first phosphatase (PPx1) dephosphorylates ARPP19 at S109 within 1 h. The role of PPx1 is essential for meiosis resumption as it generates a S109-dephosphorylated form of ARPP19 that licenses the signaling cascade, ending with Cdk1 activation. The intracellular pathway between S109-dephosphorylated Arpp19 and Cdk1 activation is not fully elucidated yet. However, it passes through the accumulation of two proteins: Mos and cyclin B, essential to generate a threshold amount of active Cdk1 in *Xenopus* oocytes [124]. This basal level of Cdk1 activity is not sufficient to orchestrate the structural reorganization needed for the cell to divide. But it certainly activates Gwl, leading to the phosphorylation of ARPP19 at S67 and to the inhibition of a second phosphatase, PP2A-B55δ. Surprisingly, S109 rephosphorylation of ARPP19 enhances the positive effect of S67 phosphorylation on Cdk1 activation (**Figure 8**). Thanks to PP2A-B55δ inhibition and the unknown targets of the S109 phosphorylation of ARPP19, the Cdk1 autoamplification loop becomes functional and enables the irreversible commitment of oocytes in the meiotic cell division. In this new model for Cdk1 activation, the activity of PPx1 unlocks the top of the signaling pathway in response to progesterone, whereas the inhibition of PP2A-B55δ is necessary several hours later for the Cdk1 autoamplification loop responsible for entry into M-phase.

Over the past decade, tremendous advances in our understanding of M-phase progression have been made, in particular regarding the role and the regulation of mitotic phosphatases. The concerted but opposite action of kinases and phosphatases allows for the irreversibility of M-phase transitions by controlling reversible phosphorylations, feedback loops and thresholds, primarily governed by Cdk1 and its counteracting phosphatases. At the center of the impressive number of phosphorylations occurring upon M-phase entry (over 32,000), a small protein, ARPP19, orientates the cellular decision to divide or not according to the phosphorylation of two of its residues. Determining the molecular targets of the S109-phosphorylated forms of ARPP19, as well as identifying the phosphatase that regulates the S109-phosphorylation level of ARPP19 and the kinase that collaborates with Gwl to phosphorylate S109 of ARPP19 will enlighten our understanding not only of the M-phase control but also of the mechanisms of cAMP-regulated signal transduction pathways.

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References

[1] Masui Y, Markert CL. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. The Journal of Experimental Zoology. Jun, 1971;**177**:129

[2] Wasserman WJ, Masui Y. Effects of cycloheximide on a cytoplasmic factor initiating meiotic maturation in *Xenopus* oocytes. Experimental Cell Research. 1975;**91**:381

[3] Kishimoto T. Entry into mitosis: A solution to the decades-long enigma of MPF. Chromosoma. Dec, 2015;**124**:417

[4] Reynhout JK, Smith LD. Studies on the appearance and nature of a maturation-inducing factor in the cytoplasm of amphibian oocytes exposed to progesterone. Developmental Biology. 1974;**38**:394

[5] Kishimoto T, Usui N, Kanatani H. Breakdown of starfish ovarian follicle induced by maturation-promoting factor. Developmental Biology. Jan, 1984;**101**:28

[6] Kishimoto T, Yamazaki K, Kato Y, Koide S, Kanatani H. Induction of starfish oocyte maturation by maturation-promoting factor of mouse and surf clam oocytes. The Journal of Experimental Zoology. 1984;**231**:293

[7] Hartwell LH, Culotti J, Reid B. Genetic control of the cell-division cycle in yeast. I. Detection of mutants. Proceedings of the National Academy of Sciences of the United States of America. 1970;**66**:352

[8] Nurse P, Thuriaux P, Nasmyth K. Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. Molecular & General Genetics. 1976;**146**:167

[9] Izawa D, Pines J. How APC/C-Cdc20 changes its substrate specificity in mitosis. Nature Cell Biology. Mar, 2011;**13**:223

[10] Lindon C. Control of mitotic exit and cytokinesis by the APC/C. Biochemical Society Transactions. Jun, 2008;**36**:405

[11] Virshup DM, Shenolikar S. From promiscuity to precision: Protein phosphatases get a makeover. Molecular Cell. Mar 13, 2009;**33**:537

[12] Kim HS, Fernandes G, Lee CW. Protein phosphatases involved in regulating mitosis: Facts and hypotheses. Molecules and Cells. Sep, 2016;**39**:654

[13] Queralt E, Uhlmann F. Cdk-counteracting phosphatases unlock mitotic exit. Current Opinion in Cell Biology. Dec, 2008;**20**:661

[14] Bollen M, Gerlich DW, Lesage B. Mitotic phosphatases: From entry guards to exit guides. Trends in Cell Biology. Oct, 2009;**19**:531

[15] Heim A, Rymarczyk B, Mayer TU. Regulation of cell division. Advances in Experimental Medicine and Biology. 2017;**953**:83

[16] Gowdy PM, Anderson HJ, Roberge M. Entry into mitosis without Cdc2 kinase activation. Journal of Cell Science. Nov, 1998;**111**(**Pt 22**):3401

[17] Goris J, Hermann J, Hendrix P, Ozon R, Merlevede W. Okadaic acid, a specific protein phosphatase inhibitor, induces maturation and MPF formation in *Xenopus laevis* oocytes. FEBS Letters. 1989;**245**:91

[18] Skoufias DA, Indorato RL, Lacroix F, Panopoulos A, Margolis RL. Mitosis persists in the absence of Cdk1 activity when proteolysis or protein phosphatase activity is suppressed. The Journal of Cell Biology. Nov 19, 2007;**179**:671

[19] Mochida S, Ikeo S, Gannon J, Hunt T. Regulated activity of PP2A-B55delta is crucial for controlling entry into and exit from mitosis in Xenopus egg extracts. The EMBO Journal. Aug 20, 2009;**28**:2777

[20] Gharbi-Ayachi A, et al. The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. Science. Dec 17, 2010;**330**:1673

[21] Mochida S, Maslen SL, Skehel M, Hunt T. Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. Science. Dec 17, 2010;**330**:1670

[22] Vigneron S, et al. Greatwall maintains mitosis through regulation of PP2A. EMBO Journal. Aug 13, 2009;**28**:2786

[23] White-Cooper H, Carmena M, Gonzalez C, Glover DM. Mutations in new cell cycle genes that fail to complement a multiply mutant third chromosome of drosophila. Genetics. Nov, 1996;**144**:1097

[24] Yu J, et al. Greatwall kinase: a nuclear protein required for proper chromosome condensation and mitotic progression in Drosophila. Journal of Cell Biology. Feb 16, 2004;**164**:487

[25] Yu J, Zhao Y, Li Z, Galas S, Goldberg ML. Greatwall kinase participates in the CDC2 autoregulatory loop in Xenopus egg extracts. Molecular Cell. Apr 7, 2006;**22**:83

[26] Vigneron S, et al. Characterization of the mechanisms controlling Greatwall activity. Molecular and Cellular Biology. Jun, 2011;**31**:2262

[27] Blake-Hodek KA, et al. Determinants for activation of the atypical AGC kinase Greatwall during M phase entry. Molecular and Cellular Biology. Apr, 2012;**32**:1337

[28] Vigneron S, et al. The master Greatwall kinase, a critical regulator of mitosis and meiosis. The International Journal of Developmental Biology. 2016;**60**:245

[29] Bontron S, et al. Yeast endosulfines control entry into quiescence and chronological life span by inhibiting protein phosphatase 2A. Cell Reports. Jan 31, 2013;**3**:16

[30] Talarek N, Bontron S, De Virgilio C. Quantification of mRNA stability of stress-responsive yeast genes following conditional excision of open reading frames. RNA Biology. Aug, 2013;**10**:1299

[31] Talarek N, et al. Initiation of the TORC1-regulated G0 program requires Igo1/2, which license specific mRNAs to evade degradation via the 5′-3′ mRNA decay pathway. Molecular Cell. May 14, 2010;**38**:345

[32] Juanes MA, et al. Budding yeast greatwall and endosulfines control activity and spatial regulation of PP2A(Cdc55) for timely mitotic progression. PLoS Genetics. 2013;**9**:e1003575

[33] White RE, Giffard RG. MicroRNA-320 induces neurite outgrowth by targeting ARPP-19. Neuroreport. Jul 11, 2012;**23**:590

[34] Jiang T, Zhao B, Li X, Wan J. ARPP-19 promotes proliferation and metastasis of human glioma. Neuroreport. Sep 07, 2016;**27**:960

[35] Williams BC, et al. Greatwall-phosphorylated Endosulfine is both an inhibitor and a substrate of PP2A-B55 heterotrimers. eLife. 2014;**3**:e01695

[36] Luo X, Talarek N, De Virgilio C. Initiation of the yeast G0 program requires Igo1 and Igo2, which antagonize activation of decapping of specific nutrient-regulated mRNAs. RNA Biology. Jan-Feb, 2011;**8**:14

[37] Wong PY, Ma HT, Lee HJ, Poon RY. MASTL(Greatwall) regulates DNA damage responses by coordinating mitotic entry after checkpoint recovery and APC/C activation. Scientific Reports. 2016;**6**:22230

[38] Okumura E, et al. Cyclin B-Cdk1 inhibits protein phosphatase PP2A-B55 via a Greatwall kinase-independent mechanism. The Journal of Cell Biology. Mar 17, 2014;**204**:881

[39] Burgess A, et al. Loss of human Greatwall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A balance. Proceedings of the National Academy of Sciences of the United States of America. Jul 13, 2010;**107**:12564

[40] Voets E, Wolthuis RM. MASTL is the human orthologue of Greatwall kinase that facilitates mitotic entry, anaphase and cytokinesis. Cell Cycle. Sep 29, 2010;**9**:3591

[41] Alvarez-Fernandez M, et al. Greatwall is essential to prevent mitotic collapse after nuclear envelope breakdown in mammals. Proceedings of the National Academy of Sciences of the United States of America. Oct 22, 2013;**110**:17374

[42] Cundell MJ, et al. The BEG (PP2A-B55/ENSA/Greatwall) pathway ensures cytokinesis follows chromosome separation. Molecular Cell. Nov 7, 2013;**52**:393

[43] Manchado E, et al. Targeting mitotic exit leads to tumor regression in vivo: Modulation by Cdk1, Mastl, and the PP2A/B55alpha,delta phosphatase. Cancer Cell. Dec 14, 2010;**18**:641

[44] Heim A, Konietzny A, Mayer TU. Protein phosphatase 1 is essential for Greatwall inactivation at mitotic exit. EMBO Reports. Nov, 2015;**16**:1501

[45] Ma S, et al. Greatwall dephosphorylation and inactivation upon mitotic exit is triggered by PP1. Journal of Cell Science. Apr 1, 2016;**129**:1329

[46] Wu JQ, et al. PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. Nature Cell Biology. May, 2009;**11**:644

[47] Russell P, Nurse P. cdc25+ functions as an inducer in the mitotic control of fission yeast. Cell. 1986;**45**:145

[48] Simanis V, Nurse P. The cell cycle control gene cdc2+ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. Cell. 1986;**45**:261

[49] Mueller PR, Coleman TR, Kumagai A, Dunphy WG. Myt1: A membrane-associated inhibitory kinase tht phosphorylates cdc2 on both threonine-14 and tyrosine-15. Science. 1995;**270**:86

[50] Booher RN, Holman PS, Fattaey A. Human Myt1 is a cell cycle-regulated kinase that inhibits Cdc2 but not Cdk2 activity. The Journal of Biological Chemistry. 1997;**272**:22300

[51] Fattaey A, Booher RN. Myt1: A Wee1-type kinase that phosphorylates Cdc2 on residue Thr14. Progress in Cell Cycle Research. 1997;**3**:233

[52] Liu F, Stanton JJ, Wu ZQ, PiwnicaWorms H. The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex. Molecular and Cellular Biology. 1997;**17**:571

[53] Malumbres M. Cyclin-dependent kinases. Genome Biology. 2014;**15**:122

[54] Hegarat N, Rata S, Hochegger H. Bistability of mitotic entry and exit switches during open mitosis in mammalian cells. BioEssays. Jul, 2016;**38**:627

[55] Lorca T, et al. Constant regulation of both the MPF amplification loop and the Greatwall-PP2A pathway is required for metaphase II arrest and correct entry into the first embryonic cell cycle. Journal of Cell Science. Jul 1, 2010;**123**:2281

[56] Schmitz MH, et al. Live-cell imaging RNAi screen identifies PP2A-B55alpha and importin-beta1 as key mitotic exit regulators in human cells. Nature Cell Biology. Aug 15, 2010;**12**:886

[57] Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The trinity at the heart of the DNA damage response. Molecular Cell. Jun 15, 2017;**66**:801

[58] Taylor SS, Ilouz R, Zhang P, Kornev AP. Assembly of allosteric macromolecular switches: Lessons from PKA. Nature Reviews. Molecular Cell Biology. Oct, 2012;**13**:646

[59] Dema A, Perets E, Schulz MS, Deak VA, Klussmann E. Pharmacological targeting of AKAP-directed compartmentalized cAMP signalling. Cellular Signalling. Dec, 2015; **27**:2474

[60] Sheppard JR, Prescott DM. Cyclic AMP levels in synchronized mammalian cells. Experimental Cell Research. Nov, 1972;**75**:293

[61] Kurokawa K, Kato J. Cyclic AMP delays G2 progression and prevents efficient accumulation of cyclin B1 proteins in mouse macrophage cells. Cell Structure and Function. Dec, 1998;**23**:357

[62] Dicitore A, et al. The cAMP analogs have potent anti-proliferative effects on medullary thyroid cancer cell lines. Endocrine. Jan, 2016;**51**:101

[63] Rodriguez-Collazo P, et al. cAMP signaling regulates histone H3 phosphorylation and mitotic entry through a disruption of G2 progression. Experimental Cell Research. Sep 10, 2008;**314**:2855

[64] Dupre A, Daldello EM, Nairn AC, Jessus C, Haccard O. Phosphorylation of ARPP19 by protein kinase A prevents meiosis resumption in Xenopus oocytes. Nature Communications. 2014;**5**:3318

[65] Smith LD, Ecker RE, Subtelny S. In vitro induction of physiological maturation in *Rana pipiens* oocytes removed from their ovarian follicles. Developmental Biology. Jun, 1968;**17**:627

[66] Masui Y. Relative roles of the pituitary, follicle cells, and progesterone in the induction of oocyte maturation in Rana Pipiens. The Journal of Experimental Zoology. 1967;**166**:365

[67] Schuetz A. Effect of steroids on germinal vesicle of oocytes of the frog (*Rana pipiens*) in vitro. Proceedings of the Society for Experimental Biology and Medicine. 1967;**124**:1307

[68] Fortune JE, Concannon PW, Hansel W. Ovarian progesterone levels during in vitro oocyte maturation and ovulation in *Xenopus laevis*. Biology of Reproduction. 1975;**13**:561

[69] Haccard O, et al. Naturally occurring steroids in Xenopus oocyte during meiotic maturation. Unexpected presence and role of steroid sulfates. Molecular and Cellular Endocrinology. Oct 15, 2012;**362**:110

[70] Karaiskou A, Dupre A, Haccard O, Jessus C. From progesterone to active Cdc2 in *Xenopus* oocytes: A puzzling signalling pathway. Biology of the Cell. Sep, 2001;**93**:35

[71] Inoue D, Ohe M, Kanemori Y, Nobui T, Sagata N. A direct link of the Mos-MAPK pathway to Erp1/Emi2 in meiotic arrest of *Xenopus laevis* eggs. Nature. Apr 26, 2007;**446**:1100

[72] 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. Oct 13, 2005;**437**:1048

[73] Nishiyama T, Ohsumi K, Kishimoto T. Phosphorylation of Erp1 by p90rsk is required for cytostatic factor arrest in *Xenopus laevis* eggs. Nature. Apr 26, 2007;**446**:1096

[74] Liu J, Maller JL. Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytostatic factor. Current Biology. Aug 23, 2005;**15**:1458

[75] Kobayashi H, et al. On the synthesis and destruction of A- and B-type cyclins during oogenesis and meiotic maturation in *Xenopus laevis*. The Journal of Cell Biology. Aug, 1991;**114**:755

[76] De Smedt V, et al. Thr-161 phosphorylation of monomeric Cdc2. Regulation by protein phosphatase 2C in Xenopus oocyte. Journal of Biological Chemistry. Aug 2, 2002;**277**:28592

[77] Gaffre M, et al. A critical balance between Cyclin B synthesis and Myt1 activity controls meiosis entry in Xenopus oocytes. Development. Sep, 2011;**138**:3735

[78] Ruiz EJ, Hunt T, Nebreda AR. Meiotic inactivation of Xenopus Myt1 by CDK/XRINGO, but not CDK/cyclin, via site-specific phosphorylation. Molecular Cell. Oct 24, 2008;**32**:210

[79] Okumura E, et al. Akt inhibits Myt1 in the signalling pathway that leads to meiotic G2/M-phase transition. Nature Cell Biology. Feb, 2002;**4**:111

[80] Duckworth BC, Weaver JS, Ruderman JV. G2 arrest in *Xenopus* oocytes depends on phosphorylation of cdc25 by protein kinase A. Proceedings of the National Academy of Sciences of the United States of America. Dec 24, 2002;**99**:16794

[81] Mulner O, Cartaud A, Ozon R. Cyclic AMP phosphodiesterase activities in *Xenopus laevis* oocytes. Differentiation. 1980;**16**:31

[82] Mulner O, Huchon D, Thibier C, Ozon R. Cyclic AMP synthesis in *Xenopus laevis* oocytes: Inhibition by progesterone. Biochimica et Biophysica Acta. 1979;**582**:179

[83] Sadler SE, Maller JL. Progesterone inhibits adenylate cyclase in *Xenopus* oocytes. Action on the guanine nucleotide regulatory protein. The Journal of Biological Chemistry. 1981;**256**:6368

[84] Sadler SE, Maller JL. Inhibition of *Xenopus* oocyte adenylate cyclase by progesterone: A novel mechanism of action. Advances in Cyclic Nucleotide and Protein Phosphorylation Research. 1985;**19**:179

[85] Sadler SE, Maller JL. Vivo regulation of cyclic AMP phosphodiesterase in *Xenopus* oocytes. Stimulation by insulin and insulin-like growth factor 1. The Journal of Biological Chemistry. 1987;**262**:10644

[86] Finidori J, Hanoune J, Baulieu EE. Adenylate cyclase in *Xenopus laevis* oocytes: Characterization of the progesterone-sensitive, membrane-bound form. Molecular and Cellular Endocrinology. 1982;**28**:211

[87] Finidori-lepicard J, Schorderet-slatkine S, Hanoune J, Baulieu EE. Progesterone inhibits membrane bound adenylate cyclase in *Xenopus laevis* oocytes. Nature. 1981;**292**:255

[88] Schorderet-slatkine S, Baulieu E-E. Forskolin increases cAMP and inhibits progesterone induced meiosis reinitiation in *Xenopus laevis* oocytes. Endocrinology. 1982;**111**:1385

[89] Schorderet-Slatkine S, Schorderet M, Baulieu EE. Cyclic AMP-mediated control of meiosis: Effects of progesterone, cholera toxin, and membrane-active drugs in *Xenopus laevis* oocytes. Proceedings of the National Academy of Sciences of the United States of America. 1982;**79**:850

[90] Maller JL, Butcher FR, Krebs EG. Early effect of progesterone on levels of cyclic adenosine 3′:5′-monophosphate in *Xenopus* oocytes. The Journal of Biological Chemistry. 1979;**254**:579

[91] Rime H, Haccard O, Ozon R. Activation of p34cdc2 kinase by cyclin is negatively regulated by cyclic amp-dependent protein kinase in Xenopus oocytes. Developmental Biology. May, 1992;**151**:105

[92] Maller JL, Krebs EG. Progesterone-stimulated meiotic cell division in *Xenopus* oocytes. Induction by regulatory subunit and inhibition by catalytic subunit of adenosine 3′:5′-monophosphate-dependent protein kinase. The Journal of Biological Chemistry. 1977;**252**:1712

[93] Huchon D, Ozon R, Fischer EH, Demaille JG. The pure inhibitor of cAMP-dependent protein kinase initiates *Xenopus laevis* meiotic maturation. A 4-step scheme for meiotic maturation. Molecular and Cellular Endocrinology. May, 1981;**22**:211

[94] Dupre A, Haccard O, Jessus C. Mos in the oocyte: How to use MAPK independently of growth factors and transcription to control meiotic divisions. Journal of Signal Transduction. 2011;**2011**:350412

[95] Amiel A, Leclere L, Robert L, Chevalier S, Houliston E. Conserved functions for Mos in eumetazoan oocyte maturation revealed by studies in a cnidarian. Current Biology. Feb 24, 2009;**19**:305

[96] Dumollard R, et al. Mos limits the number of meiotic divisions in urochordate eggs. Development. Mar, 2011;**138**:885

[97] Haccard O, Jessus C. Oocyte maturation, Mos and cyclins—A matter of synthesis: Two functionally redundant ways to induce meiotic maturation. Cell Cycle. Jun, 2006;**5**:1152

[98] Haccard O, Jessus C. Redundant pathways for Cdc2 activation in *Xenopus* oocyte: Either cyclin B or Mos synthesis. EMBO Reports. Mar, 2006;**7**:321

[99] Frank-Vaillant M, Jessus C, Ozon R, Maller JL, Haccard O. Two distinct mechanisms control the accumulation of cyclin B1 and mos in *Xenopus* oocytes in response to progesterone. Molecular Biology of the Cell. 1999;**10**:3279

[100] Castro A, et al. Cyclin B/cdc2 induces c-Mos stability by direct phosphorylation in Xenopus oocytes. Molecular Biology of the Cell. Sep, 2001;**12**:2660

[101] Nebreda AR, Gannon JV, Hunt T. Newly synthesized protein(s) must associate with p34cdc2 to activate MAP kinase and MPF during progesterone-induced maturation of *Xenopus* oocytes. The EMBO Journal. 1995;**14**:5597

[102] Wang R, et al. Regulation of Cdc25C by ERK-MAP kinases during the G(2)/M transition. Cell. Mar 23, 2007;**128**:1119

[103] Matten W, Daar I, Vande Woude GF. Protein kinase A acts at multiple points to inhibit *Xenopus* oocyte maturation. Molecular and Cellular Biology. 1994;**14**:4419

[104] Rime H, et al. Characterization of MPF activation by okadaic acid in *Xenopus* oocyte. Cell Differentiation and Development. 1990;**29**:47

[105] Yamamoto TM, et al. Regulation of Greatwall kinase during Xenopus oocyte maturation. Molecular Biology of the Cell. Jul 1, 2011;**22**:2157

[106] Hara M, et al. Greatwall kinase and cyclin B-Cdk1 are both critical constituents of M-phase-promoting factor. Nature Communications. 2012;**3**:1059

[107] Wang J, Cao WL, Liu XJ. Protein kinase a(PKA)-restrictive and PKA-permissive phases of oocyte maturation. Cell Cycle. Jan, 2006;**5**:213

[108] Wang J, Liu XJ. Progesterone inhibits protein kinase A (PKA) in *Xenopus* oocytes: Demonstration of endogenous PKA activities using an expressed substrate. Journal of Cell Science. Oct 1, 2004;**117**:5107

[109] Zhao Y, et al. Roles of greatwall kinase in the regulation of cdc25 phosphatase. Molecular Biology of the Cell. Apr, 2008;**19**:1317

[110] Dupre A, et al. The phosphorylation of ARPP19 by Greatwall renders the auto-amplification of MPF independently of PKA in Xenopus oocytes. Journal of Cell Science. Sep 1, 2013;**126**:3916

[111] Schmitt A, Nebreda AR. Inhibition of *Xenopus* oocyte meiotic maturation by catalytically inactive protein kinase A. Proceedings of the National Academy of Sciences of the United States of America. Apr 2, 2002;**99**:4361

[112] Han SJ, Chen R, Paronetto MP, Conti M. Wee1B is an oocyte-specific kinase involved in the control of meiotic arrest in the mouse. Current Biology. Sep 20, 2005;**15**:1670

[113] Han SJ, Conti M. New pathways from PKA to the Cdc2/cyclin B complex in oocytes: Wee1B as a potential PKA substrate. Cell Cycle. Feb, 2006;**5**:227

[114] Nakajo N, et al. Absence of Wee1 ensures the meiotic cell cycle in Xenopus oocytes. Genes & Development. 2000;**14**:328

[115] Boyer J, Asselin J, Belle R, Ozon R. Progesterone and cAMP-dependent protein kinase regulate in vivo the level of phosphorylation of two proteins (Mr 20,000 and Mr 32,000) in Xenopus oocytes. Developmental Biology. Feb, 1986;**113**:420

[116] Mochida S. Regulation of alpha-endosulfine, an inhibitor of protein phosphatase 2A, by multisite phosphorylation. The FEBS Journal. Feb, 2014;**281**:1159

[117] Dulubova I, et al. ARPP-16/ARPP-19: A highly conserved family of cAMP-regulated phosphoproteins. Journal of Neurochemistry. Apr, 2001;**77**:229

[118] Girault JA. Integrating neurotransmission in striatal medium spiny neurons. Advances in Experimental Medicine and Biology. 2012;**970**:407

[119] Girault JA. Signaling in striatal neurons: The phosphoproteins of reward, addiction, and dyskinesia. Progress in Molecular Biology and Translational Science. 2012;**106**:33

[120] Ysselstein D, et al. Endosulfine-alpha inhibits membrane-induced alpha-synuclein aggregation and protects against alpha-synuclein neurotoxicity. Acta Neuropathologica Communications. Jan 10, 2017;**5**:3

[121] Dupre AI, Haccard O, Jessus C. The greatwall kinase is dominant over PKA in controlling the antagonistic function of ARPP19 in Xenopus oocytes. Cell Cycle. 2017;**16**:1440

[122] Andrade EC, et al. ARPP-16 is a striatal-enriched inhibitor of protein phosphatase 2A regulated by microtubule-associated serine/threonine kinase 3 (Mast 3 kinase). The Journal of Neuroscience. Feb 06, 2017;**37**:2709

[123] Musante V, et al. Reciprocal regulation of ARPP-16 by PKA and MAST3 kinases provides a cAMP-regulated switch in protein phosphatase 2A inhibition. eLife. Jun 14, 2017;**6**:e24998

[124] Huchon D, Ozon R, Demaille JG. Protein phosphatase-1 is involved in *Xenopus* oocyte maturation. Nature. 1981;**294**:358

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