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Cell-cycle control during meiotic maturation

Takeo Kishimoto

The meiotic cell cycle, which is comprised of two consecutive M-phases, is crucial for the production of haploid germ cells. Although both mitotic and meiotic M-phases share cyclin-B–Cdc2/CDK1 as a key controller, there are meiosis-specific modulations in the regulation of cyclin-B–Cdc2. Recent insights indicate that a common pattern in these modulations can be found by considering the particular activities of mitogen-activated protein kinase (MAPK) during meiosis. The G_2-phase arrest of meiosis I is released via specific, MAPK-independent signalling that leads to cyclin-B–Cdc2 activation; thereafter, however, the meiotic process is under the control of interplay between MAPK and cyclin-B–Cdc2.

Address
Laboratory of Cell and Developmental Biology, Graduate School of Bioscience, Tokyo Institute of Technology, Nagatsuta 4259, Midoriku, Yokohama 226-8501, Japan
e-mail: tkishimo@bio.titech.ac.jp

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Abbreviations

APC/C	anaphase-promoting complex/cyclosome
CPEB	cytoplasmic polyadenylation element binding protein
CSF	cytostatic factor
GVBD	germinal vesicle breakdown
MAPK	mitogen-activated protein kinase
PDE3	phosphodiesterase 3
PI3K	phosphatidylinositol-3-OH kinase
PKA	cAMP-dependent protein kinase
PKB	protein kinase B
Plk1	polo-like kinase 1

Introduction

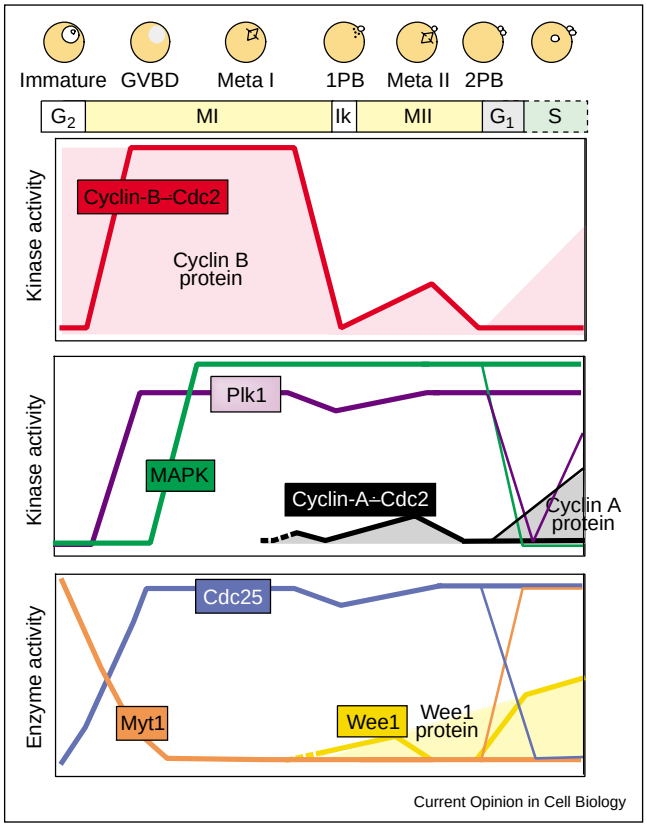
In all animals, oocytes are arrested at prophase of meiosis I (prophase I) during the growth period and resume meiosis near or at the end of growth. The prophase-I-arrested state is described as immature, and the process of resumption of meiosis is called meiotic maturation. Meiotic maturation is comprised of two consecutive M-phases, meiosis I and meiosis II; as there is no intervening S-phase, haploid gametes are produced. In many species, oocyte meiosis must arrest again at a certain stage after its resumption to await fertilisation [1]. Thus, from the viewpoint of cell-cycle control, the major questions in meiotic maturation concern the mechanisms underlying prophase I and the subsequent arrests and their release, in addition to those underlying the meiosis I-to-II transition. *Xenopus* oocytes, which await fertilisation at metaphase of meiosis II (meta II), are often used as a model system to analyse meiotic maturation [2]. Recent works, however, have led to a reconsideration of the major model for regulation of the *Xenopus* oocyte meiotic cycle, and have also resulted in considerable advances in our understanding of another historically important model system, the starfish oocyte [3], which arrests at the pronucleus stage after meiosis II in the absence of fertilisation. This review will integrate the various information on the molecular mechanism of meiotic cell-cycle control, and will propose that the process of meiotic maturation can be divided into two phases: in one of these mitogen-activated protein kinase (MAPK) is non-essential, whereas in the other it is essential.

Meiosis-specific dynamics of cell-cycle regulators

The release from the cell-cycle arrest at prophase I in immature oocytes is thought to be equivalent to the G_2/M-phase transition in mitosis. In support of this, the key event in both entries into M-phase is the activation of cyclin-B–Cdc2 kinase [4]. However, when comparing meiosis and mitosis, the states of two major cell-cycle regulators differ significantly (Figure 1). In contrast to somatic cells in G_2-phase, both cyclin A and Wee1 proteins ([5,6••]; see [2] for *Xenopus*) are undetectable or present at very low levels in prophase-I oocytes. In these fully grown immature oocytes, cyclin-B–Cdc2 is already present in an inactive form, and new synthesis of cyclins A and B is not required for meiotic resumption [7••]. Protein levels of Cdc25 [5] and Myt1 [6••] remain constant throughout meiotic maturation. Accordingly, G_2-phase in prophase-I oocytes is considered to be maintained by the dominance of Myt1 over Cdc25. A key unanswered question regarding the meiotic G_2/M-phase transition is what causes the reversal of the balance between opposing Myt1 and Cdc25 activities to initiate the activation of cyclin-B–Cdc2. Once initially activated, cyclin-B–Cdc2 can be further activated through known feedback loops with Cdc25 and Myt1.

The meiotic cycle is also characterised by the dynamics of MAPK, which is activated downstream of Mos, and of polo-like kinase1 (Plk1) ([6••,8], see [2] for *Xenopus*) (Figure 1). Both MAPK and Plk1 are inactive in immature oocytes and are activated around the time of cyclin-B–Cdc2 activation at meiotic resumption. Once activated, both kinases remain fully or almost fully active during the meiosis I-to-II transition, whereas Cdc2 is almost

Figure 1



Dynamics of cell cycle regulators through meiotic maturation. Fully grown immature oocytes, characterised by the presence of a germinal vesicle (GV), are arrested at prophase of meiosis I (prophase I). A maturation-inducing hormone (progesterone in frog; 1-methyladenine in starfish) reinitiates meiosis as indicated by GVBD, followed by two successive M-phases, meiosis I (MI) and meiosis II (MII), without an intervening S-phase, resulting in the production of haploid mature eggs. In frog and mouse oocytes, the second meiotic arrest occurs at metaphase of meiosis II (meta-II) and lasts until fertilisation. In starfish oocytes, two types of arrest can occur: a meta-I arrest, which only occurs within the ovary, and then a G_1 arrest, which lasts until fertilisation occurs. Based on the frog and starfish systems, the dynamics of cell cycle regulators are schematically shown. Cyclin B is already present in immature oocytes, whereas cyclin A and Wee1 are almost undetectable in immature oocytes and start to accumulate near the end of meiosis I (shaded areas). Cdc2 activity is carried out by cyclin-B–Cdc2 in meiosis I, and by both cyclin-B–Cdc2 and cyclin-A–Cdc2 in meiosis II. By contrast, protein levels of Cdc25, Myt1, MAPK and Plk1 remain constant throughout meiotic maturation, and the dynamics of these regulators characterise meiotic cycles. In particular, both MAPK and Plk1 are inactive in immature oocytes, are activated around the time of cyclin-B–Cdc2 activation (MAPK is activated before cyclin B-Cdc2 activation in frog, but afterwards in starfish), and remain active during the meiosis I-to-II transition and thereafter (bold lines). When fertilisation occurs, MAPK, Plk1 and Cdc25 are inactivated at exit from meiosis II, whereas Myt1 becomes active (thin lines). Ik, interkinesis period; 1PB and 2PB, the first and second polar body.

completely inactivated as a result of cyclin B degradation and then reactivated [2,3]. Both MAPK and Plk1 remain active until fertilisation occurs at meta-II in *Xenopus* or at G_1-phase in *Asterina* (starfish). Unanswered questions include whether MAPK and Plk1 are involved in cyclin-B–Cdc2 activation at the meiotic G_2/M-phase and meiosis I-to-II transitions, and how MAPK, Plk1 and Cdc2 activities are interrelated.

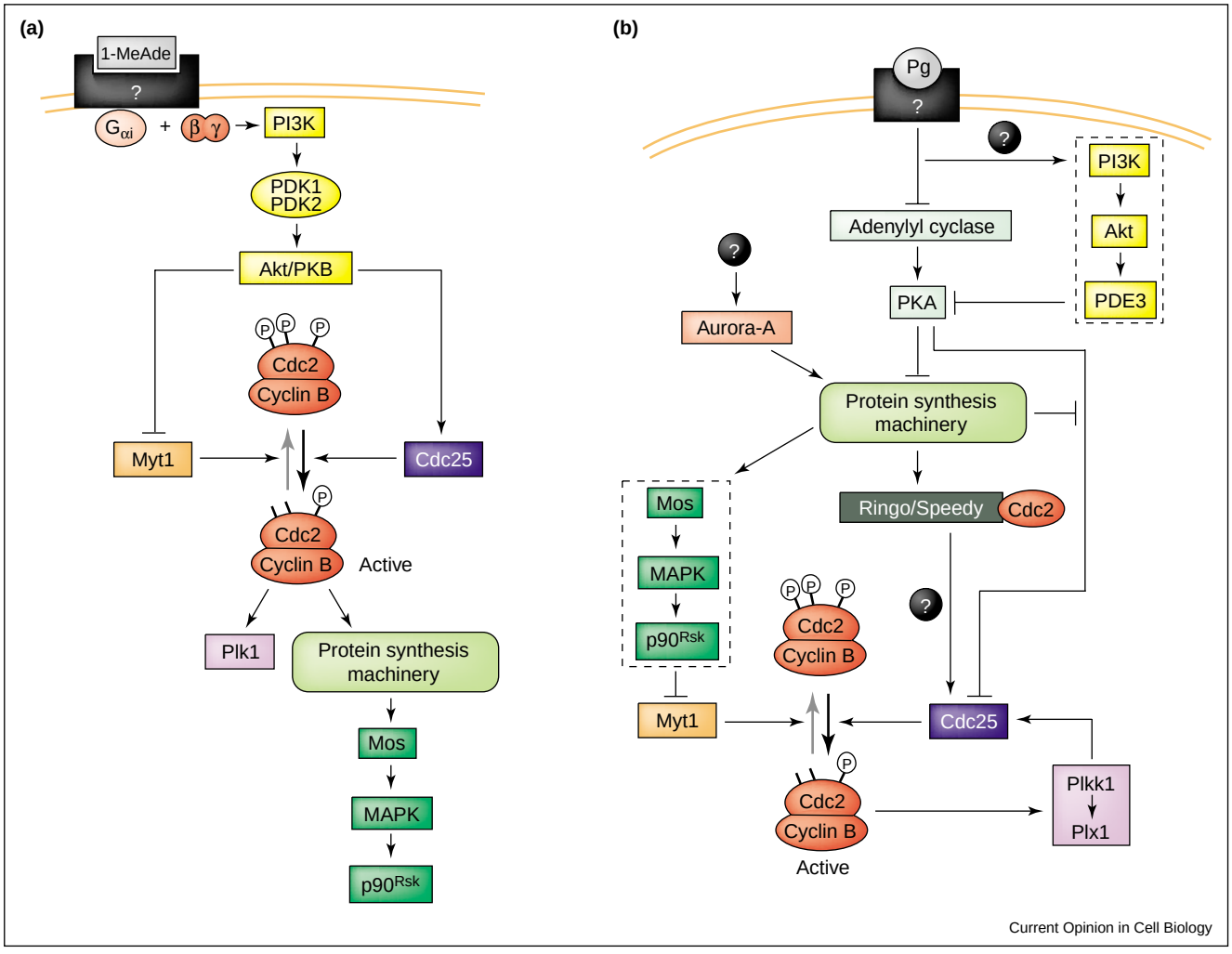
Another feature of the meiotic cell cycle is the translational activation after stimulation of meiotic resumption. In *Xenopus* oocytes, Mos synthesis begins before Cdc2 activation and germinal vesicle breakdown (GVBD), whereas in starfish and mouse oocytes it begins after GVBD [2,9]. Thereafter, during or near the end of meiosis I, protein synthesis of cyclins B and A and Wee1 starts [2,5,6••,7••] (Figure 1). A model has been presented for the mechanism of differential mRNA translation during meiotic progression [10]. In immature *Xenopus* oocytes, the cytoplasmic polyadenylation element binding protein (CPEB)–Maskin–eIF4E trimer prevents the translation of mRNA. After progesterone stimulus, Aurora-A (see below) phosphorylates CPEB on Ser174 to recruit CPSF (cytoplasmic polyadenylation specificity factor), resulting in the addition of a poly(A) tail to mRNA. Poly(A)-binding protein (PABP), which binds to the newly elongated poly(A) tail, associates with eIF4G, which binds to eIF4E displaced from Maskin. The eIF4E–eIF4G initiation complex interacts with the 40S ribosomal subunit, thereby initiating translation [11]. In this model, it is proposed that the number of CPEs in particular mRNAs might determine the differential timing of mRNA translatin; multiple (more than two) CPEBs would exclude a CPEB–CPSF interaction, but Cdc2-catalyzed phosphorylation of CPEBs causes their partial destruction, resulting in the recruitment of CPSF by a remaining CPEB [10].

Activation of Aurora-A kinase in *Xenopus* egg extracts requires phosphorylation on Thr295 (equivalent to Thr288 in human Aurora-A), which resides in the activation loop of the kinase [12]. Neither Cdc2 nor MAPK is required for activation of Aurora-A, but a member of the PKC family could be an endogenous activating kinase, as Thr295 is part of a PKC consensus motif, and apigenin (a PKC and CK2 inhibitor) can inhibit Aurora-A activation. By contrast, another report [13] indicates that, in *Xenopus* oocytes, cyclin-B–Cdc2 activity is necessary and sufficient for Aurora-A activation at meiotic resumption. Further study will be required to settle this issue.

Maintenance of prophase-I arrest

As noted above, meiotic prophase-I arrest is maintained because Myt1 is active and Cdc25 is inactive. New insights into how Cdc25 function is inhibited in prophase-I-arrested oocytes have recently been emerging. One such regulator is cAMP-dependent protein kinase (PKA) (see [2]). In *Xenopus* oocytes, the activity of adenylyl cyclase is maintained by α and/or $\beta\gamma$ subunits of Gs protein [14,15]. In mouse oocytes within the ovarian

Figure 2



MAPK is dispensable in signalling pathways leading to meiotic resumption. **(a)** Starfish oocyte model. 1-Methyladenine (1-MeAde) stimulates the release of Gβγ through an unidentified surface receptor, leading to activation of PI3K and production of PIP3. Then PDK1, along with a putative PDK2, activates Akt/PKB, which directly phosphorylates Myt1 on Ser75 to suppress its activity, and also phosphorylates and activates Cdc25. Thus the balance between Cdc25 and Myt1 activities is reversed, resulting in the initial activation of cyclin-B–Cdc2. Downstream of cyclin-B–Cdc2, Plk1 is activated independently of MAPK, and Mos synthesis is initiated independently of Plk1, leading to the activation of the Mos–MAPK–p90Rsk pathway. **(b)** *Xenopus* oocyte model. By an as-yet-unidentified pathway (it remains elusive whether the recently reported Gi-coupled receptor for progestin [26•] is a receptor for progesterone in *Xenopus* oocytes), progesterone suppresses adenylyl cyclase and may activate the PI3K–Akt–PDE3 pathway. The resulting decrease in PKA activity leads to the activation of protein synthesis. Aurora-A, which is activated through an unidentified pathway, may also participate in the translational activation. Then, in one pathway, suppression of Cdc25 by PKA is released in a protein-synthesis-dependent manner. In another pathway, newly synthesised Ringo/Speedy associates with Cdc2 and may phosphorylate and activate Cdc25. In yet another pathway, newly synthesised Mos activates MAPK–p90Rsk, resulting in the suppression of Myt1. These pathways converge on the initial activation of cyclin-B–Cdc2, although the Mos–MAPK and the Akt–PDE3 pathways (dotted box areas) are not essential. Then cyclin-B–Cdc2 is further activated, probably through a Plkk1–Plx1–dependent positive feedback loop.

follicle, Gs protein activity [16•], as well as adenylyl cyclase activity [17], are required to maintain cAMP levels, suggesting that a signal from the follicle activates Gs proteins in the oocytes. Although the physiological target of PKA in prophase-I arrest has remained elusive for 25 years, Ruderman and colleagues [18••] recently demonstrated that Cdc25 is a relevant target in *Xenopus* oocytes (Figure 2b). PKA negatively regulates Cdc25 by phosphorylating Ser287 (the equivalent of Ser216 in human Cdc25C), which results in binding of 14-3-3. It has been suggested that 14-3-3 binding does not merely mediate nuclear exclusion but directly inhibits Cdc25 function in the cytoplasm (i.e. phosphatase activity, or interaction with positive regulators or with substrate) [19]. In prophase-I-arrested *Xenopus* oocytes, the same Ser 287 of Cdc25C is also phosphorylated by the basal, but not

checkpoint-dependent, activity of the checkpoint kinases Chk1 and Cds1 [19,20]. At meiotic resumption, however, Chk1 activity does not decline significantly [19]; Cds1 activity does decrease, but the decrease depends on cyclin-B–Cdc2 activation [20]. By contrast, dephosphorylation of Ser287 on Cdc25C occurs independently of either Cdc2 or MAPK activation, suggesting that PKA serves as the main negative regulator of Cdc25 in prophase-I-arrested oocytes [18••].

Although PKA is thought to play a major role in the maintenance of prophase-I arrest, there are two observations that are not easily explained by a direct action of PKA on Cdc25. First, it is hard to explain why, after downregulation of PKA activity, protein synthesis is absolutely required for meiotic resumption in *Xenopus* oocytes (see below). Second, even PKA lacking in catalytic activity can inhibit meiotic resumption in *Xenopus* oocytes by blocking a step downstream of Mos–MAPK–p90Rsk [21•]. PKA thus appears to be involved in prophase-I arrest by blocking several steps.

Release from prophase-I arrest: hormonal receptor

In most animals, meiotic resumption from prophase-I arrest is induced by hormonal stimuli [1]. Typically, the_hormone is progesterone in frog oocytes and 1-methyladenine in starfish oocytes, both of which act on the oocyte surface [22]. Although nuclear receptors for steroid hormones were identified more than 30 years ago and also exist in *Xenopus* oocytes [23,24], the identity of the oocyte membrane receptors for the maturation-inducing hormones is not yet certain. This year two papers reported the cloning and characterisation of a new membrane progestin receptor in fish [25•] and its homologues in the frog, mouse, pig and human [26•]. This new steroid receptor is a seven-transmembrane-domain protein with the characteristics of a G-protein-coupled receptor. Upon binding to progestin, this receptor blocks the activity of adenylyl cyclase in a pertussis-toxin-sensitive manner, indicating its coupling with Gi protein. The data presented are compelling, but to establish whether this is a receptor for maturation-inducing hormone, further analysis will be required. Although results of antisense experiments that favour this hypothesis have been presented, other observations including steroid specificity are inconsistent. In particular, a pertussis-toxin-sensitive Gi protein does not mediate progesterone action in *Xenopus* oocytes [27•], and this issue remains to be fully investigated in fish oocytes.

Release from prophase-I arrest: signalling that leads to cyclin-B–Cdc2 activation

To understand how the balance between Myt1 and Cdc25 is reversed at the meiotic G_2/M-phase transition, it is essential to analyse the states of Myt1 and Cdc25 after maturation-inducing hormonal signalling, but before cyclin-B–Cdc2 activation. By blocking Cdc25 activity using an antibody, the signalling pathway that leads to cyclin-B–Cdc2 activation in starfish oocytes has been definitively clarified [28••] (Figure 2a). In the oocytes of starfish as well as the mouse, cyclin-B–Cdc2 activation at meiotic resumption does not require new protein synthesis. Although the surface receptor for 1-methyladenine has not yet been identified, a putative receptor is coupled to Gi protein, and the released Gβγ is thought to activate phosphatidylinositol-3-OH kinase (PI3K) [29,30]. Downstream of this pathway, Akt/PKB (protein kinase B) was found to be activated, and then to phosphorylate Myt1 on Ser75 and inhibit its activity [28••]. Conversely, inhibition of Akt or PDK1 (Hiraoka and Kishimoto, unpublished data) completely blocked the hormone effect. Thus Akt switches the balance between Myt1 and Cdc25, leading to the initial activation of cyclin-B–Cdc2 at meiotic resumption in starfish oocytes. In support of this conclusion, Akt can phosphorylate and activate Cdc25 directly (Okumura and Kishimoto, unpublished data). In starfish oocytes, unlike *Xenopus* oocytes (see below), Plk1 activation, Mos synthesis and the subsequent activation of MAPK and p90Rsk clearly depend on cyclin-B–Cdc2 activation, and, conversely, cyclin-B–Cdc2 activation occurs normally in the absence of their activities, while Plk1 and MAPK are independent of each other [6••,8,28••]. Thus, Akt, but neither Plk1 nor p90Rsk, functions as a trigger kinase at meiotic resumption in starfish oocytes. This is the first unravelling of a complete signalling pathway from extracellular hormonal stimulus to the cell cycle regulatory machinery for entry into M-phase.

By contrast, in *Xenopus* oocytes, which absolutely require new protein synthesis for meiotic resumption, two major parallel cytoplasmic signalling pathways that start after the decrease in PKA activity and lead to cyclin-B–Cdc2 activation have been proposed [2,18••,31] (Figure 2b). One branch induces the translation of mRNA encoding Mos, and then activation of the MEK–MAPK–p90Rsk pathway, resulting in direct phosphorylation and inactivation of Myt1 by p90Rsk [32]. The other branch leads to the activation of the Plkk1–Plx1 (Plx1 is the *Xenopus* homologue of Plk1) pathway, resulting in the triggering of Cdc25C activation through direct phosphorylation by Plx1 [33]. Thus two parallel pathways converge on the activation of cyclin-B–Cdc2, although it remains unclear how downregulation of PKA links to both branches. This two-branch model presumes that Mos–MAPK is essential for meiotic resumption, and that Plx1 is a trigger kinase for Cdc25C activation.

Although the above two-branch model for *Xenopus* meiotic resumption appears to have been well established, considerable inconsistencies have emerged recently. First, whereas MAPK can be activated by progesterone in the absence of Cdc2 activity [18••], it was demonstrated by

the use of morpholino antisense *mos* [34••] and by MEK inhibitor [31] that Mos–MAPK is not required for meiotic reinitiation after progesterone, indicating that the Mos–MAPK–p90Rsk–Myt1 pathway is not essential for cyclin-B–Cdc2 activation. Second, it was shown, at least in oocyte extracts, that activation of Plkk1 and Plx1 is downstream of Cdc2 activity and that Cdc2-induced phosphorylation of Cdc25 is a prerequisite for Cdc25 phosphorylation and activation by Plx1 [35]. Consistently, the binding of Plk1 to Cdc25 requires the priming phosphorylation of Cdc25 by a Pro-directed kinase, possibly Cdc2 [36]. These reports exclude the possibility that Plx1 is a trigger kinase for Cdc25 activation at meiotic reinitiation. Instead, Plx1 appears to contribute to positive feedback activation of Cdc25 and cyclin-B–Cdc2 [37]. These observations argue against the two-branch model in *Xenopus*. In *Xenopus* oocytes, as in starfish oocytes, it is likely that MAPK is not essential for meiotic resumption and that Plx1 does not function as a trigger kinase. In addition, it is suggested that phosphorylation of ectopically expressed cyclin B is necessary for Cdc25C to become active to dephosphorylate Cdc2 in the absence of progesterone [38], although it remains to be investigated whether progesterone-induced activation of endogenous cyclin-B–Cdc2 requires cyclin B phosphorylation.

What alternative model could be developed for *Xenopus* oocytes, in particular taking into consideration that new protein synthesis is absolutely required for meiotic resumption? After excluding Mos [34••] and cyclins A and B [7••], a possible essential candidate for a newly synthesised protein is Ringo/Speedy, which is newly synthesised after progesterone application, and which associates with and activates monomeric Cdc2 [39,40]. Although a part of monomeric Cdc2 is already phosphorylated on Thr161 in immature oocytes [41], unphosphorylated monomeric Cdc2 may be activated by Ringo (Thr161 phosphorylation impairs the activation) [42]. Another candidate could be a component involved in the dephosphorylation of Cdc25–Ser287, as this requires new protein synthesis [18••]. On the basis of these possibilities, the following model may be proposed for the signalling pathway leading to cyclin-B–Cdc2 activation at *Xenopus* meiotic resumption (Figure 2b). The decrease in PKA activity, possibly along with Aurora-A-dependent phosphorylation of CPEB, causes activation of the protein synthesis machinery. In one pathway the newly formed Ringo–Cdc2 complex may phosphorylate Cdc25, while in the other pathway Cdc25–Ser287 is dephosphorylated; both of these trigger the activation of Cdc25 to initiate cyclin-B–Cdc2 activation. Alternatively, p38γ/SAPK3 has recently been suggested to be a possible priming kinase for Cdc25 [43]. In any case, Plx1 may be implicated in the amplification of cyclin-B–Cdc2 activity but not in its initial activation, and the Mos–MAPK–p90Rsk–Myt1 pathway may have an ancillary function in the activation of cyclin-B–Cdc2 after progesterone signalling. It should be noted that a human homologue of Ringo/Speedy has been identified and may function at the G_1/S transition in somatic cells, suggesting that Ringo/Speedy may have a more general role as a priming kinase [44•].

Akt can induce meiotic resumption in *Xenopus* oocytes and this function has been suggested to be mediated by phosphodiesterase 3 (PDE3) activation, which causes a decrease in PKA activity [45,46]. However, although inhibition of Akt completely blocked insulin-stimulated meiotic resumption, it only partially (~30%) blocked progesterone-induced resumption [47•]. As PI3Kγ can induce meiotic resumption in *Xenopus* oocytes through Akt activation [48], multiple pathways, including a putative pathway from the progesterone receptor to PI3K, may participate in the decrease in PKA activity [46,47•] (Figure 2b). The possible involvement of Akt (as part of the Akt–PDE3 and/or the Akt–Myt1/Cdc25 pathways) in meiotic resumption of mouse oocytes is an intriguing question, although it has not yet been investigated.

Another report which has not been incorporated into the above models is that on the basis of a knockout experiment Cdc25B is necessary and sufficient for meiotic resumption in mouse oocytes, even though Cdc25A and Cdc25C are present [49]. Thus, Cdc25A and Cdc25C cannot compensate for the function of Cdc25B in meiotic resumption, although the absence of Cdc25C can be compensated by Cdc25A and/or Cdc25B [50].

Meiosis I-to-II transition: networks leading to cyclin-B–Cdc2 activation at entry into meiosis II

An important characteristic of the meiotic cell cycle is the immediate occurrence of meiosis II after exit from meiosis I, without an intervening S-phase. Although immature oocytes lack the ability to replicate DNA, replication competence is acquired during meiotic maturation. In *Xenopus* oocytes, Cdc6, an essential factor for recruiting the MCM helicase to the pre-replication complex, has been shown to be the only missing replication factor whose translation is necessary and sufficient to establish the oocyte's replication competence [51,52]. To repress S-phase during the meiosis I-to-II transition, it is necessary and sufficient that cyclin-B–Cdc2 is activated immediately after exit from meiosis I, bypassing inhibitory Tyr-phosphorylation of Cdc2 [53]. In the past few years, there have been considerable advances in understanding the mechanism underlying this bypass.

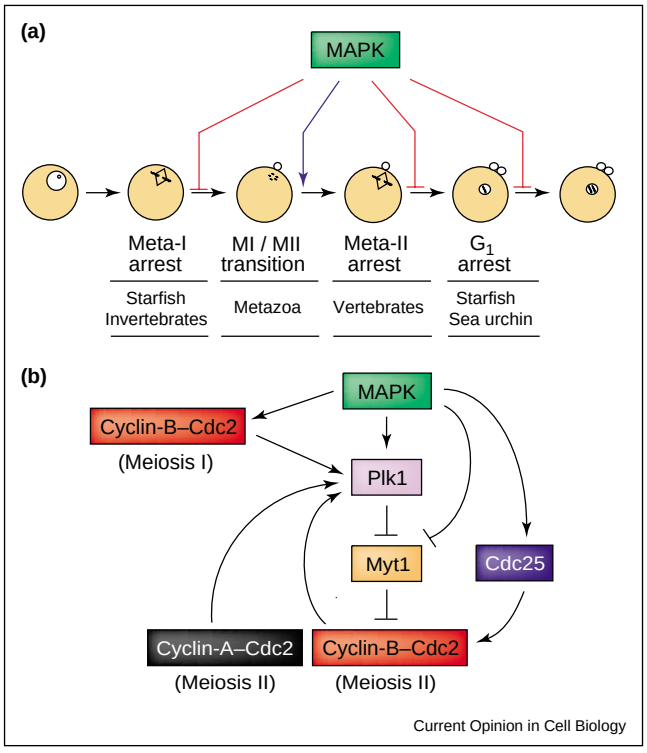
In *Xenopus*, whereas cyclins B2 and B5 are stockpiled in immature oocytes and then degraded at exit from meiosis I, new synthesis of cyclins B1 and B4 is required for entry into meiosis II [7••]. In addition, new synthesis of at least one more protein, Xkid, is required for the meiosis I-to-II transition in *Xenopus* oocytes [54•]. Xkid chromokinesin

was originally identified as a newly synthesised protein required for chromosome alignment on the metaphase plate of the spindle, and its degradation allows anaphase chromosome movement in egg extracts (see references in [54•]). However, Xkid does not degrade during meiosis I and is further required for entry into meiosis II, probably through supporting protein synthesis of cyclin B, independently of its DNA-binding domain [54•].

How then is Cdc2 that is complexed with newly synthesised cyclin B activated bypassing the inhibitory Tyr phosphorylation? At exit from meiosis I, there are two kinases, Wee1 and Myt1, responsible for the inhibitory phosphorylation. Although protein levels of Wee1 are still low at this stage (although its synthesis is already started), its activity is suppressed by the Cdc2 activity that still occurs at low levels during the interkinesis period in *Xenopus* oocytes [55]. Accordingly, the crucial issue is how to suppress Myt1 activity during the interkinesis period. Because prevention of the Mos–MAPK pathway results in the inhibitory Tyr phosphorylation of cyclin-B–associated Cdc2 [9,53], Myt1 activity is thought to be regulated downstream of MAPK. Furthermore, in mouse, *Xenopus* and starfish oocytes, inhibition of the Mos–MAPK pathway leads to entry into S-phase or progression into embryonic mitotic cycles immediately after exit from meiosis I, as if parthenogenesis had occurred [9,34••]. Thus, oocytes at the end of meiosis I appear to have the ability to undergo the mitotic cycle, but the Mos–MAPK pathway suppresses this ability and forces the meiosis I-to-II transition (Figure 3a).

The effect of the Mos–MAPK pathway is primarily mediated by p90Rsk, which is activated immediately downstream ([31,32]; Mori and Kishimoto, unpublished data). In addition to the previously indicated role of p90Rsk in directly phosphorylating and inactivating Myt1 [32], new functions downstream of MAPK (and possibly p90Rsk) have recently emerged, particularly in relation to Plk1 [6••]. At exit from meiosis I, Plk1 still has considerable activity. In starfish oocytes, this Plk1 activity depends on both MAPK and Cdc2 (in complex with either cyclin A or cyclin B). Lack of this Plk1 activity results in failure in the immediate activation of cyclin-B–Cdc2 that is required for the meiosis I-to-II transition, even when MAPK remains active. Myt1 is likely to be a target of Plk1, and Cdc25 is likely to be a target of MAPK (possibly via p90Rsk). Based on these findings [6••], the following model is now proposed for the meiosis I-to-II transition (Figure 3b). In one pathway, MAPK, along with residual cyclin-B–Cdc2 activity (the activity of which is possibly supported by MAPK at exit from meiosis I; see [55,56]) and cyclin-A–Cdc2 (which is newly assembled at entry into meiosis II), maintains Plk1 activity, which in turn maintains the suppressed state of Myt1. In the other pathway, MAPK maintains the activated state of Cdc25. Both pathways enable newly assembled cyclin-B–Cdc2 to become active, bypassing inhibitory Tyr-phosphorylation at entry into meiosis II. Thus, the direct inhibition of Myt1 by p90Rsk does not appear to be sufficient. Rather, through suppression of Myt1, Plk1 is an essential mediator of the Mos–MAPK–p90Rsk function for execution of the meiosis I-to-II transition.

Figure 3



MAPK governs meiotic maturation after its resumption. **(a)** MAPK controls the meta-I arrest in starfish [64••] and possibly many invertebrates, the meiosis I-to-II transition probably in all animals, the meta-II arrest in most vertebrates, and the G_1 arrest in echinoderms. MAPK is thus involved in the whole process after meiotic resumption, although its most conserved role might be the control of the meiosis I-to-II transition. The second and first eggs from the right end represent G_1 and S phases, respectively. **(b)** Regulation of the meiosis I-to-II transition by MAPK in starfish oocytes. The meiosis I-to-II transition without an intervening S-phase is executed by the immediate activation of cyclin-B–Cdc2, bypassing the inhibitory Tyr phosphorylation of Cdc2. This bypass is governed by MAPK, and its essential mediator is Plk1. During this transition, Plk1 activity is supported in various ways: by MAPK but not via Myt1; by cyclin B-Cdc2 whose inactivation at exit from meiosis I is delayed by MAPK; and by cyclin-A–Cdc2 and cyclin-B–Cdc2, which are activated at entry into meiosis II. Thus, Myt1 remains suppressed by Plk1 and possibly by MAPK–p90Rsk, whereas Cdc25 is supported by MAPK, resulting in the immediate activation of cyclin-B–Cdc2 at entry into meiosis II.

Second arrest by cytostatic factor

After resumption of meiosis I, the female meiotic cycle arrests again unless fertilisation occurs: the arrest is at meta-I in many invertebrates, at meta-II in most vertebrates including frog and mouse, and at G_1-phase after

meiosis II in starfish and sea urchins [1] (Figure 3a). CSF (cytostatic factor) was originally defined as having an activity responsible for the meta-II arrest in frog oocytes ([57], for review) but, as seen below, a conceptual innovation has emerged in the past few years regarding the definition of CSF.

The meta-II arrest in *Xenopus* oocytes is caused by the Mos–MAPK pathway, by way of the downstream effectors p90Rsk and Bub1 (the spindle checkpoint kinase), resulting in the inhibition of anaphase-promoting complex/cyclosome (APC/C) (see [58] for review). The meta-II arrest is now proposed to be divided into two phases: establishment and maintenance. The Mos–MAPK pathway is necessary for establishment, but once established the meta-II arrest can be maintained in the absence of MAPK–p90Rsk–Bub1 [59]. Although it has been suggested that cyclin-E–Cdk2 is involved in establishment [58], this may need further clarification (see [59,60]). On the other hand, the maintenance of the meta-II arrest is accomplished by Emi1, which binds to the substrate-binding region of Cdc20 and thereby prevents substrate binding to the APC/C [61••]. Depletion of Emi1 is sufficient to cause exit from the meta-II arrest. A major unresolved issue is why and how Emi1 escapes degradation at the meta-II arrest, because Emi1, which is phosphorylated by Cdc2, is usually destroyed at prophase by the SCFßTrCP/Slimb ubiquitin ligase to allow the activation of the APC/C [62]. It is plausible that such the unique behaviour of Emi1 at meta-II might result from the Mos–MAPK pathway.

The same Mos–MAPK pathway is necessary and sufficient for the G_1-phase arrest in mature starfish eggs that have completed meiosis II in the absence of fertilisation [8,9]. This effect is also mediated by p90Rsk, although Cdc2 is inactive (Mori and Kishimoto, unpublished data). Thus, in contrast to what occurs at the meiosis I-to-II transition, a system other than Cdc2 activity should repress DNA replication downstream of Mos–MAPK–p90Rsk, because all the necessary components for DNA replication are already present in mature starfish eggs [8]. Similarly in sea urchin eggs, which await fertilisation at the pronucleus stage (G_1-phase), MAPK is necessary and sufficient for preventing DNA replication in unfertilised eggs [63].

In addition, the Mos–MAPK pathway is also required, although not sufficient, for the meta-I arrest in starfish oocytes [64••]. Starfish oocytes undergoing maturation within the ovary, but not oocytes isolated in seawater, have been found to arrest at meta-I until spawning. The biological significance of this is to optimise the oocyte cell-cycle phase for fertilisation, because fertilisation is expected to occur immediately after spawning and polyspermy blocks function most effectively during the latter half of meiosis I. The meta-I arrest requires MAPK activity, although an increase in intracellular pH induced by spawning overrides the MAPK effect to release the meta-I arrest. The effector for the pH increase is unknown, but this finding might provide a new insight into the meta-I arrest widely observed in invertebrate oocytes. In mouse oocytes as well, Mos participates in sustaining the meta-I arrest in strain LT [65].

In summary, the Mos–MAPK pathway, or at least MAPK, is likely to be involved in every meiotic cell-cycle arrest, at meta-I, meta-II or G_1-phase, in metazoan oocytes (Figure 3a). Each of these arrests occurs after meiotic resumption, at the point at which the oocyte awaits fertilisation. Thus CSF should now be considered as a cell-cycle arrest factor that prevents parthenogenetic activation, regardless of the meiotic cycle phase.

New systems

In the frog, almost everything known about the molecular mechanisms regulating meiotic maturation has come from studies with *Xenopus laevis* [2]. In this species, however, genetic approaches are limited as a result of pseudotetraploidy. To circumvent this problem, the use of *Xenopus tropicalis*, a diploid member of the same genus, has been proposed. Two recent papers [66,67] revealed that the signalling pathways regulating meiotic maturation appear to be extremely similar in *X. laevis* and *X. tropicalis*, which makes the future use of *X. tropicalis* promising. As another genetic model system, the molecular identification of maturation-inducing factor from sperm cytoskeletal protein [68] may also promote the use of the nematode *Caenorhabditis elegans*. Another new system is more unconventional. Scholer and colleagues [69•] have recently demonstrated that mouse embryonic stem (ES) cells in culture can develop into oogonia that enter meiosis and recruit adjacent cells for folliculogenesis. Upon addition of gonadotropins to follicles, oocytes undergo apparent first meiosis, leading to formation of blastocyst-like structures resembling the parthenotes seen in *mos*-knockout mice. This ES cell system will open a novel window for the basic study of meiotic maturation, although there may be ethical implications.

Conclusions

The process of meiotic maturation can be divided into two phases, one before and one after the activation of MAPK. A key issue regarding the phase before MAPK activation is the signalling pathway that leads to meiotic resumption. At least in starfish oocytes, the missing link between the maturation-inducing hormone and cyclin-B–Cdc2 was identified last year as Akt/PKB, meaning that the signalling pathway has been almost completely unravelled. This is the latest in a series of milestones in the study of starfish meiotic maturation: historically, maturation-inducing hormone was identified as 1-methyladenine in 1969, maturation-promoting factor (MPF) was demonstrated in 1976, cyclin-B–Cdc2 was identified as

its active component in 1989, and the βγ subunit of Gi protein was demonstrated as a mediator of the hormone action in 1993, although the 1-methyladenine receptor has not yet been identified [22]. The complete signalling pathway in other systems, particularly in frog and mouse oocytes, remains elusive.

After its activation, MAPK governs almost all steps in the meiotic process, including the meiosis I-to-II transition and the second meiotic arrest before fertilisation. We can now conclude that the most conserved and crucial role of MAPK (or Mos–MAPK–p90Rsk) is to regulate the meiosis I-to-II transition that ensures the generation of haploid genomes, an aim of meiosis. One urgent issue is to understand the interplay among MAPK, Plk1 and Cdc2. Another novel aspect of MAPK (or Mos–MAPK–p90Rsk) is that it functions as CSF regardless of the arrest phase in the cell cycle. This new perception of CSF opens new questions concerning how the interfaces downstream of MAPK differ among oocytes with distinct cell-cycle arrest points.

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