Regulation of the meiotic cell cycle in oocytes

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The mitotic and meiotic cell cycle share many regulators, but there are also important differences between the two processes. The meiotic maturation of *Xenopus* oocytes has proved useful for understanding the regulation of Cdc2–cyclin-B, a key activator of G2/M progression. New insights have been made recently into the signalling mechanisms that induce G2-arrested oocytes to resume and complete the meiotic cell cycle.

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Abbreviations

CPEB	cytoplasmic polyadenylation element binding
GSK	glycogen synthase kinase
GVBD	germinal vesicle breakdown
MAPK	mitogen-activated protein kinase
MPF	maturation-promoting factor
PAK	p21-activated kinase
PKA	cAMP-dependent protein kinase

Introduction

Meiosis comprises the specialised cell divisions that produce haploid germ cells, eggs or sperm. The meiotic cell cycle consists of two consecutive divisions (M phases) in the absence of DNA replication (S phase). One of the best systems to study the biochemical mechanisms that regulate the meiotic cell cycle is probably the maturation of *Xenopus* oocytes. Fully grown *Xenopus* oocytes (also referred to as 'stage VI oocytes') can be arrested in a G2-like phase (prophase at the G2/M boundary of the first meiotic division) for months, until maturation is initiated by the hormone progesterone. The mature oocyte arrests again at the metaphase of the second meiotic division until fertilisation.

A key enzymatic activity regulating the G2/M transition in all eukaryotic cells is the maturation-promoting factor (MPF), comprising the serine/threonine protein kinase Cdc2 complexed with cyclin B. The activity of MPF is subject to a complex and tight regulation that includes both specific phosphorylation events and protein–protein interactions (for more information on the regulation of MPF in the mitotic cell cycle, see review by Catherine Takizawa and David Morgan in this issue, pp 658–665). During the meiotic cell cycle in oocytes, MPF is first activated at meiosis I, then transiently inactivated between meiosis I and II and finally reactivated at meiosis II (Figure 1). In G2-arrested *Xenopus* oocytes, there is a preformed complex of Cdc2 and cyclin B (pre-MPF), which is maintained in its inactive form by phosphorylation of Cdc2 on Thr14 and Tyr15. These inhibitory phosphorylations are probably catalysed by the Myt1 protein kinase, whereas dephosphorylation of these residues requires the Cdc25C phosphatase. Thus, the activation of MPF may be brought about either by the activation of Cdc25C, the inactivation of Myt1, or both. It should be noted that most of the stock of Cdc2 in the oocyte is monomeric, and only about 10% of the Cdc2 is associated with cyclin B in the pre-MPF complexes.

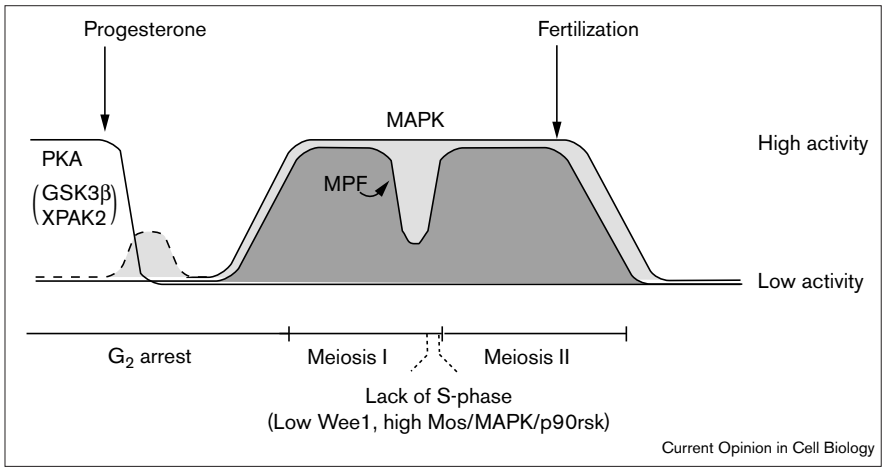
The early signalling events triggered by the interaction of progesterone with its yet unidentified membrane-bound receptor remain poorly understood, although a candidate progesterone receptor has been identified recently (J Tian, J Ruderman, personal communication). An essential requirement for the meiotic maturation is the translation of maternal mRNAs stored in the oocyte. One of these mRNAs encodes the oncoprotein Mos, a protein kinase that can phosphorylate and activate the mitogen-activated protein kinase (MAPK) kinase MEK1 (see [1] for a review). (For simplicity, in this review we use the generic name 'MAPK' to refer to *Xenopus* ERK2.) During the past two years, we have gained important insights into the nature and function of the proteins synthesised *de novo* that lead to the activation of MPF and G2/M progression in *Xenopus* oocytes. In this review, we will focus on recent progress regarding the regulation of Mos synthesis and activation, the function of the MAPK pathway, the regulation of Cdc25C and Myt1 activities and the identification of a new protein required for meiotic maturation. Table 1 summarises the proposed roles of some of the proteins acting in these pathways in the meitotic cell cycle of *Xenopus*. We will not discuss here the regulation of the meiotic cell cycle in other organisms (for a review, see [2,3]).

G2 phase arrest in oocytes

One of the early biochemical responses upon progesterone stimulation is a reduction in the level of cAMP, which is thought to translate into the inhibition of the cAMP-dependent protein kinase (PKA). Consistent with the idea that PKA plays an important role in the G2 arrest of oocytes, injection of PKA inhibitors causes maturation in the absence of progesterone, whereas overactivation of PKA potently inhibits oocyte maturation. These results suggest that PKA has an important negative role early in oocyte maturation, although it can also inhibit oocyte maturation at later stages (see [4] for a review). However, there is still no information on what the relevant PKA substrates in oocytes could be, and changes in the endogenous PKA activity during oocyte maturation have also been difficult to determine.

Recent work has identified additional regulators of the G2 arrest in *Xenopus* oocytes, which probably act late during meiotic maturation. These include Chk1, a protein kinase normally activated in response to DNA damage,

Figure 1



Meiotic maturation of *Xenopus* oocytes. Progesterone releases the G2 arrest allowing the oocytes to proceed through the two consecutive meiotic cell divisions without an intervening S phase. The mature oocytes arrest again at metaphase of meiosis II until fertilisation. Entry into M phase requires inactivation of PKA (and also possibly GSK3β and XPAK2). The exact timing of inactivation of these kinases with respect to MAPK activation is unknown. Activation of MPF is essential for entry into M phase of both meiosis I and meiosis II. MAPK is fully activated at the same time as MPF, although it might also be activated transiently at lower levels early upon progesterone treatment. Maintenance of the Mos/MAPK/p90rsk pathway, as well as the absence (or inhibition) of Wee1, is required for omission of S phase.

which inhibits Cdc25C through phosphorylation of Ser287 (see review by Walworth on pp 697–704 of this issue). Overexpression of Chk1 prevents release from G2 arrest by progesterone, whereas injection of anti-Chk1 antibodies facilitates progesterone-induced maturation [5•]. Moreover, inhibition of endogenous Chk1 facilitates oocyte maturation by overexpressed Cdc25C and a Cdc25C mutant resistant to Chk1 phosphorylation is more efficient at inducing GVBD than the wild-type Cdc25C. These results suggest that Chk1 may be involved in the physiological G2 arrest of *Xenopus* oocytes via direct phosphorylation and inhibition of Cdc25C [5•].

Phosphorylation of Ser287 in Cdc25C creates a binding site for the small, acidic 14-3-3 proteins. The binding of 14-3-3 does not significantly affect Cdc25C phosphatase activity, suggesting that it inhibits Cdc25C function by another mechanism. It has been proposed that nuclear exclusion of Cdc25C by binding to 14-3-3 may cause the G2 arrest in *Xenopus* oocytes [6•]. However, using enucleated oocytes, Cdc25C appears to be activated in the cytoplasm of maturing oocytes (T Oe, N Nakajo, N Sagata, personal communication). Thus, 14-3-3 binding may inhibit Cdc25C function in the cytoplasm, perhaps by interfering with the interaction of Cdc25C with activators such as the Polo-like kinase Plx1 and/or substrates such as Cdc2–cyclin-B. Moreover, the activity of Chk1 does not appear to be regulated during oocyte maturation (T Oe, N Nakajo, N Sagata, personal communication). This suggests that another kinase might be the major activity phosphorylating Cdc25C on Ser287 in G2-arrested oocytes; a candidate would be the checkpoint kinase Cds1/Chk2 [7]. An alternative possibility is that the phosphatase that dephosphorylates Cdc25C on Ser287 is activated during oocyte maturation and antagonises Chk1 activity. PKA has been proposed to regulate the activity of okadaic acid-sensitive serine/threonine phosphatases [8] and can also potentiate the activation of pre-MPF by okadaic acid in cell free extracts prepared for *Xenopus* oocytes (A Karaiskou, personal communication). Whether PKA might actually regulate the phosphatase that dephosphorylates Cdc25C on Ser287 remains to be investigated.

The induction of meiotic maturation by injecting anti-Myt1 antibodies in the absence of progesterone stimulation [9••], suggests that Myt1 also plays an active role in the G2 arrest of oocytes. Thus, although the inhibition of Cdc25C by the Chk1/14-3-3 pathway probably contributes to the G2 arrest, it appears that Myt1 activity plays a major role in maintaining pre-MPF in an inactive state in oocytes. Okadaic-acid-sensitive serine/threonine phosphatases also are likely to be important regulators of G2 arrest, as treatment with okadaic acid potently induces MPF activation and GVBD in oocytes [10]. The identity of these phosphatases and their targets in oocytes are still unknown, although they are likely to function at multiple levels during meiotic maturation.

Other enzymes that might also participate in cell cycle arrest in oocytes are glycogen synthase kinase (GSK)-3β and members of the p21-activated kinase (PAK) family [11,12]. GSK-3β and XPAK2 are active in G2-arrested oocytes and become inactivated during maturation. Moreover, overactivation of these protein kinases blocks progesterone-induced maturation. However, inhibition of the endogenous GSK-3β and XPAK2 activity appears to be required — but is not sufficient — to trigger meiotic progression.

Regulation of Mos synthesis and activity

Previous work has demonstrated an essential role for the Mos protein, an activator of the MEK–MAPK pathway, in progesterone-induced oocyte maturation. Mos is not detected in G2-arrested oocytes but starts to be synthesised shortly after progesterone stimulation. The level of Mos protein is initially low, probably due to rapid turnover, and is then stabilised around the time of pre-MPF

Table 1

Regulators of the meiotic cell cycle in *Xenopus* oocytes.

Regulator	Mechanism	State during meiotic maturation
	G2 arrest	
PKA	Inhibition of maturation at multiple levels	Early inactivation
PP2A	Inhibition of maturation at multiple levels	Inactivation?
Myt1	Inhibition of MPF by phosphorylation of Cdc2	Inactivation
Chk1	Phosphorylation of Cdc25C to generate a 14-3-3 binding site	Constitutively active?
14-3-3	Sequestering/inhibition of Cdc25C in the cytoplasm	Constitutively present?
GSK3β	Unknown	Inactivation
XPAK2	Inhibition of the MPF amplification loop	Late inactivation
	M phase entry	
Eg2	Activation of CPEB by phosphorylation	Synthesis + activation?
CPEB	Stimulation of Mos mRNA translation	Activation
Mos	Activation of MEK by phosphorylation	Synthesis + activation
MEK	Activation of MAPK by phosphorylation	Activation
MAPK	Activation of p90rsk by phosphorylation	Activation
p90rsk	Inhibition of Myt1 by phosphorylation	Activation
Plx1	Activation of Cdc25 by phosphorylation	Activation
Cdc25C	Activation of MPF by dephosphorylation of Cdc2	Activation
Ringo	Activation of free Cdc2	Synthesis
Cyclins	Activation of free Cdc2	Synthesis
MPF	Phosphorylation of many structural and regulatory proteins	Activation/transient inactivation

activation. This stabilisation and subsequent accumulation of the Mos protein depends on phosphorylation of Ser3 and is maintained throughout meiosis I and II (see [13] for a review). In addition, Mos can be regulated by the binding to the casein kinase II β subunit, which inhibits Mos activity [14]. Interestingly, casein kinase IIβ can also bind to and inhibit $p90^{rsk}$, a potentially physiologically relevant MAPK substrate (M Frödin, personal communication), suggesting that it might have a more general role in the negative regulation of meiotic maturation than previously suspected [15]. A new level of regulation of Mos activity through Hsp90 binding also has been proposed recently [16•]. Fisher *et al.* [16•] showed that Mos interacts in oocytes with the heat shock protein Hsp90, a chaperone known to bind to and regulate the activity of several protein kinases [17]. Injecting the oocytes with geldanamycin, an inhibitor of Hsp90, prevented Mos phosphorylation and Mos-mediated activation of MAPK, but did not affect the accumulation of Mos induced by progesterone [16•]. Work from several laboratories suggests that the MAPK activator MEK1 is an important target of Mos during the meiotic G2/M transition in oocytes (see below). Furthermore, the Mos/MAPK pathway is essential for the suppression of DNA replication between meiosis I and II, as well as for the reactivation and stabilisation of MPF in meiosis II which is required for the metaphase II arrest (also referred to as 'cytostatic factor arrest') [13].

The synthesis of Mos is preceded by and requires polyadenylation of the Mos mRNA, which in turn requires phosphorylation of the cytoplasmic polyadenylation element binding (CPEB) protein. This is an RNA-binding protein that controls the polyadenylation of several mRNAs, including those encoding Mos and several cyclins, during oocyte maturation [18]. Little is known about the signalling pathways activated by progesterone that lead to the translation of specific mRNAs. However, the Aurora/Ipl1-like serine/threonine protein kinase Eg2 [19] recently has been proposed to stimulate translation of Mos mRNA through phosphorylation of CPEB [20••]. Mendez *et al.* [20••] found that CPEB is phosphorylated early, upon progesterone stimulation, on Ser174. This phosphorylation appears to be both necessary and sufficient for the activation of CPEB, as measured by its ability to stimulate Mos mRNA polyadenylation and translation as well as oocyte maturation [20••]. Interestingly, Eg2 was able to phosphorylate CPEB on Ser174 *in vitro*, and immunodepletion of Eg2 from oocyte extracts prevented CPEB phosphorylation on Ser174.

These results indicate that Eg2 is the major CPEB kinase detected in oocyte extracts and therefore it may be a physiologically important activator of Mos mRNA translation [20••]. The new observations are in good agreement with previous results showing that overexpression of Eg2 leads to an acceleration of progesterone-induced maturation, which correlates with faster accumulation of Mos protein [21]. In contrast, work by another group [22•] is not consistent with a role for Eg2 in the early regulation of CPEB [20••]. Frank-Vaillant *et al.* [22•] reported that they cannot detect activation of Eg2 until the time of germinal vesicle breakdown (GBVD, one of the cytological markers of meiosis I) and that the activation of Eg2 during maturation is dependent on Cdc2 activation. It is hard to reconcile this discrepancy without further work. On the one hand, Eg2

might be activated early during oocyte maturation at low levels that are below detection with the techniques currently available. Alternatively, there might be additional CPEB kinases in the oocyte that are more labile than Eg2 and therefore loose their activity during the preparation of the extracts. It will be very interesting to find out how and when Eg2 is activated during oocyte maturation.

Recent work also suggests that two different signalling pathways may regulate the accumulation of Mos and cyclin B1 during oocyte maturation. Injection of $p21^{Cip}$, an inhibitor of cyclin-dependent kinases, blocks progesterone-induced GVBD as well as the activation of both MAPK and MPF. This correlates with the failure to accumulate Mos protein, whereas the level of cyclin B1 protein increases normally. Thus, it appears that, in contrast to Mos, cyclin B1 accumulation might be independent of MPF activation although it can be blocked by overexpression of PKA [23].

Previous studies also suggested that polyadenylation of the mRNAs for Mos and cyclin B1 could be differently regulated during oocyte maturation. However, they came to the conclusion that polyadenylation of the Mos mRNA precedes accumulation of the Mos protein and is independent of MPF activity, whereas polyadenylation of the cyclin B1 mRNA requires both Mos and MPF activity [24]. Taken together, these studies emphasise that mRNA polyadenylation does not equal protein accumulation. An interesting recent study suggests that addition of a synthetic polyA tail is not sufficient to upregulate translation of the Mos mRNA in the absence of progesterone stimulation. Using inhibitors of polyadenylation, the authors conclude that synthesis of Mos in oocytes requires polyadenylation of at least another mRNA, which should encode a meiotic regulator acting upstream of Mos, either by activating translation of the Mos mRNA or by stabilising the Mos protein [25].

MAPK activation has also been reported to stimulate polyadenylation of Mos mRNA [26]. This probably constitutes a positive feedback loop where MAPK activation by Mos in turn stimulates translation of Mos mRNA (see [1,4] for reviews). It is, however, unclear how this loop is initially triggered and, as with Eg2, whether the MAPK-stimulated translation of Mos might already operate early in oocyte maturation. In fact, using the MEK1 inhibitor U0126, Gross *et al.* [27••] recently showed that the accumulation of Mos during progesterone-induced maturation was not affected by the apparently complete inhibition of MEK1 and MAPK, suggesting that the initial triggering of Mos synthesis does not depend on MAPK activation. In agreement with this observation, overexpression of the MAPK phosphatase Pyst1 only partially inhibited Mos accumulation induced by progesterone [16•].

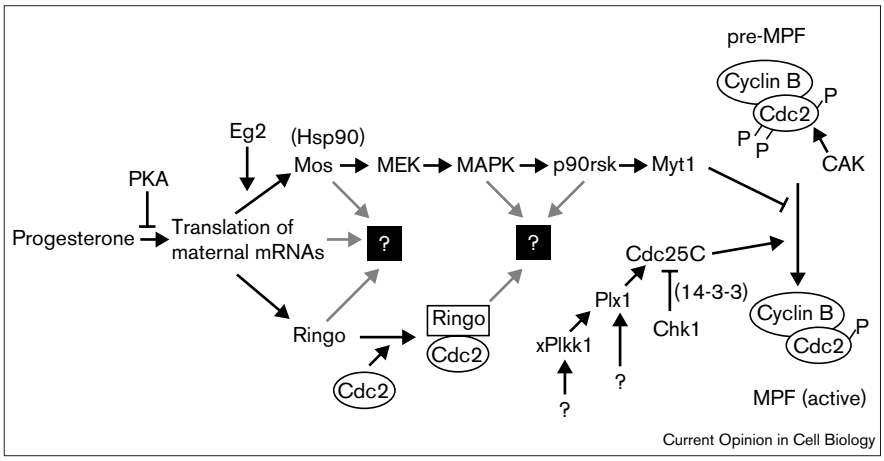
A protein kinase has recently been cloned from starfish that shows some homology to vertebrate Mos and is also synthesised during oocyte maturation concomitant with the activation of MAPK (K Tachibana, T Kishimoto, personal communication). These studies suggest that the function of Mos might be to suppress mitosis after the completion of meiosis I, so that meiosis II is allowed to ensure the production of haploid gametes. This is consistent with previous work proposing that an important role of Mos is probably to prevent parthenogenic activation [28].

On the role of MAPK in meiotic maturation

The MAPK pathway is universally activated during the meiotic maturation of vertebrate oocytes. However, the timing of and requirement for MAPK activation changes in different species. In *Xenopus* oocytes, several reports support the idea that MAPK activation is required for progesterone-induced entry into meiosis. Thus, suppressing MAPK activation by injection of anti-MEK1 antibodies, the MAPK phosphatase CL100, or the MEK inhibitor PD98059 inhibited progesterone-induced MPF activation and GVBD (see [1,4] for reviews). Recent results using the Hsp90 inhibitor geldanamycin or the MEK inhibitor U0126, however, indicate that, in the absence of detectable MAPK activation, some oocytes still undergo MPF activation and GVBD upon progesterone stimulation [27••,29]. This has also been reported by others [30,31]. These new results suggest that MAPK activation is not strictly required for GVBD in *Xenopus* oocytes and that alternative MAPK-independent pathways can trigger MPF activation. It should be pointed out, however, that geldanamycin-treated oocytes mature with much reduced efficiency [29] and U0126 only allows GVBD to occur in oocytes freshly dissected from primed frogs [27••]. This indicates that MAPK normally plays a major role in G2/M progression in oocytes, and suggests the activation of parallel (and redundant) MAPK-independent signalling pathways during oocyte maturation. Interestingly, if the oocytes that mature in the absence of MAPK activation still require Mos (an important point that has not been investigated), this would suggest the existence of other important targets (in addition to MEK1) for Mos to trigger entry into meiosis I during oocyte maturation (Figure 2).

Although U0126-treated oocytes could activate MPF and undergo GVBD upon progesterone treatment, they fail to form metaphase I spindles, reaccumulate cyclin B and to hyperphosphorylate the Cdc27 component of the anaphase-promoting complex. Instead, these oocytes appear to enter S phase with subsequent DNA replication, in the absence of MAPK activity [27••]. These results imply that MAPK activation may not be essential for entry into meiosis I but that it is required for suppression of S phase and entry into meiosis II. Gross *et al.* [27••] also showed that ectopic expression of a constituitively active form of $p90^{rsk}$ is sufficient to rescue the absence of MAPK activity in U0126-treated oocytes. The results imply that $p90^{rsk}$ is a physiologically relevant substrate for MAPK during oocyte maturation. This is also consistent with recent reports proposing an essential role for $p90^{rsk}$ as a mediator of the cytostatic factor activity responsible for metaphase II arrest in mature oocytes [32•,33•].

Figure 2



Signalling mechanisms activated by progesterone to regulate the meiotic cell cycle in *Xenopus* oocytes. Progesterone stimulates *de novo* synthesis of the Mos protein kinase, which requires binding to Hsp90 for activation. Mos can activate the MAPK/p90^{rsk} pathway leading to the inactivation of Myt1, which, in turn, is responsible for the inhibitory phosphorylation of Cdc2 on Tyr14 and Thr15. These two Cdc2 residues are dephosphorylated by the Cdc25C phosphatase. Cdc25C is phosphorylated and activated by Plx1, which in turn can be phosphorylated and activated by xPlkk1. It is not known how xPlkk1 is activated during oocyte maturation and there may be additional Plx1 activators in oocytes. Cdc25C phosphorylation by Chk1 allows binding of 14-3-3, which has been proposed to maintain Cdc25C in an inactive form during G2 arrest. PKA has a negative role early in meiotic maturation but it might also function at later stages. The kinase activity that phosphorylates Cdc2 on Thr161 (known as CAK [Cdk-activating kinase] I) is thought to be constituitively active during the meiotic cell cycle. Synthesis of Ringo (also known as 'Speedy') is also necessary for progesterone-induced pre-MPF activation. Ringo's function is likely to involve direct activation of free Cdc2. The targets of Ringo-activated Cdc2 in oocytes are unknown. Both Mos and Ringo might have additional roles in the meiotic cell cycle. It also is likely that MAPK and p90rsk phosphorylate additional substrates in oocytes that may function at different levels during meiotic maturation. Other proteins synthesised *de novo* (for example, cyclins) also might play important roles at various stages during meiotic maturation. Cdc25C and Myt1 might also be regulated by additional (unidentified) protein kinases. For simplicity, the existence of multiple positive feedback loops that ensure the coordinated all-or-nothing response of the oocytes are not indicated.

The efficient activation of p90^{rsk} by MAPK requires their interaction through a docking site located at the carboxy-terminal end of p90^{rsk} [34,35]. MAPK can associate with p90^{rsk} in G2 of meiosis I, but not in meiosis II. The dissociation of the complex is due to the phosphorylation of p90^{rsk} [36•]. In contrast, an amino-terminally truncated p90^{rsk} mutant (comprising the MAPK docking site) constitutively interacts with MAPK. Overexpression of this p90^{rsk} mutant allows MAPK to become activated in the absense of Cdc2–cyclin-B in response to progesterone, indicating that the activation of MAPK normally precedes the activation of pre-MPF [36•]. Moreover, overexpression of the MAPK docking site does not appear to prevent signalling through p90^{rsk} but interferes with the phosphorylation and activation of Plx1, a potential activator of the Cdc25C phosphatase [36•]. Although this pathway that links the activation of MAPK and Plx1 during oocyte maturation requires new protein synthesis, it does not require the activation of MPF. It is unclear, however, whether this pathway might be activated early during maturation or might be part of the feedback loops that operate at later stages.

During meiotic maturation, the stock of MAPK in oocytes becomes fully phosphorylated and activated at about the same time as MPF (see [1,4]). More recently, however, MAPK phosphorylation has been detected as early as 15 minutes after progesterone treatment, by using an anti-phospho-MAPK antibody that allows detection of low levels of phosphorylated MAPK [16•,29]. This observation has led to the proposal that MAPK might be activated in a biphasic manner during meiotic maturation [16•]. The early and transient activation of MAPK is geldanamycin insensitive (and hence probably Mos independent), although it might still require protein synthesis as with the later MAPK activation mediated by Mos (see above). It remains to be demonstrated that the cross-reactivity with the anti-phospho-MAPK antibody does correlate with early MAPK activation in oocytes. If this is the case, it will be very interesting to find out more on the mechanism of activation and possible function of MAPK early in oocyte maturation.

A recent study has investigated the inactivation of MAPK in oocytes and found that it is mediated by at least two phosphatases, an unidentified tyrosine phosphatase and a PP2A-like threonine phosphatase. Moreover, the rate of threonine and tyrosine dephosphorylation remains constant during meiotic maturation, indicating that the two phosphatases are probably constituitively active. This suggests that MAPK activation is mainly due to the increase in MEK1 activity during oocyte maturation [37].

Regulation of Cdc25C and Myt1 in oocytes

During oocyte maturation, activation of pre-MPF correlates with dephosphorylation of Cdc2 on Tyr15 and probably also on Thr14. Thus, the activation of Cdc25C or the inhibition of Myt1 should be sufficient to activate pre-MPF, and indeed both events occur upon progesterone treatment at about the time of pre-MPF activation. Myt1 is a dual-specificity kinase of the Wee1 family that is associated with membranes. Although both Wee1 and Myt1 can efficiently phosphorylate and inhibit Cdc2,

there are several observations implying Myt1 as the physiologically relevant kinase responsible for meiotic G2 phase arrest. Most importantly, Wee1 is not detectable in G2-arrested oocytes and is only synthesised late during oocyte maturation [9••,38]. In contrast, Myt1 is present in G2 oocytes and is hyperphosphorylated during oocyte maturation. Hyperphosphorylation of Myt1 on its carboxy-terminal noncatalytic region correlates with its inactivation during mitosis [39–41,42•]. In addition, ectopic expression of low levels of Wee1 efficiently block oocyte maturation, whereas overexpression of Myt1 can only delay maturation, suggesting that Myt1, but not Wee1, can be specifically downregulated during meiotic maturation [9••]. In fact, the absence of Wee1 has been proposed to be important for the omission of the S phase between the two meiotic divisions [9••] (see below).

What are the signalling pathways that lead to Cdc25C activation and Myt1 inactivation during oocyte maturation? A potential link between the MAPK pathway and the activation of pre-MPF could be $p90^{rsk}$, a well-known substrate of MAPK that was found to specifically associate with the carboxy-terminal noncatalytic region of Myt1 [41]. Phosphorylation by $p90^{rsk}$ decreases the ability of Myt1 to downregulate Cdc2–cyclinB *in vitro*. As $p90^{rsk}$ is activated at the same time as MAPK, $p90^{rsk}$ is a good candidate to downregulate Myt1 during oocyte maturation (Figure 2). Since inhibition of the endogenous Myt1 can trigger meiotic maturation in the absence of progesterone stimulation [9••], Myt1 should be responsible for maintaining the Cdc2–cyclin-B complexes in an inactive form in G2-arrested oocytes.

In contrast to Wee1, which is localised to the nucleus, human Myt1 localises to the endoplasmic reticulum and Golgi complex in tissue culture cells [43]. This membrane localisation depends on a hydrophobic motif that is carboxy-terminal to the kinase domain. It was recently reported that overexpression of both active and kinase-inactive forms of human Myt1 can delay cell cycle progression. Moreover, Myt1 can associate with Cdc2–cyclin-B complexes and the interaction requires the carboxy-terminal 63 amino acids of Myt1 [41,42•,44•]. These results suggest that, in addition to phosphorylating and inhibiting the catalytic activity of Cdc2–cyclin B complexes, Myt1 could also sequester MPF in the cytoplasm, thereby preventing its entry into the nucleus. Both mechanisms may be important for preventing G2/M progression, but it is still unknown whether Myt1 operates at both levels in *Xenopus* oocytes has not been investigated, because the localisation of Myt1 in *Xenopus* oocytes. It should be noted that although *Xenopus* Myt1 is also associated with membranes, the carboxy-terminal regulatory domain is significantly divergent between the human and *Xenopus* Myt1 proteins that have been cloned (see [4] for a review).

Other pathways are also likely to act in parallel and lead to the activation of the Cdc25C phosphatase, thus contributing to the rapid burst of MPF activity that drives the oocyte into M phase. Plx1 can phosphorylate and activate Cdc25C *in vitro* and is activated at the same time as Cdc25C during oocyte maturation. Inhibiting Plx1 delays the activation of Cdc25C upon progesterone stimulation [45], while expression of a constituitively active form of Plx1 (with Ser128 and Thr201 replaced by aspartic acid residues) in oocytes triggers Cdc25C and MPF activation [46], in the absence of progesterone stimulation. These results suggest that Plx1 could function as a triggering kinase that directly phosphorylates and activates Cdc25 during progesterone-induced oocyte maturation. Other results indicate that Plx1 may be regulated by MPF (directly or indirectly) and participate in the MPF/Cdc25C autoamplification loop [45,47,48]. The protein kinase xPlkk1 recently has been identified as a candidate activator for Plx1 because it can phosphorylate Plx1 *in vitro* and is also activated at the same time as Plx1 during oocyte maturation [49]. xPlkk1 is probably also regulated by phosphorylation, suggesting that there might be a protein kinase cascade targeting Cdc25C activity through Plx1 (Figure 2). Immunodepletion experiments using oocyte extracts that can enter meiosis I upon addition of a PKA inhibitor indicate that xPlkk1 is unlikely to be the only Plx1 activator in oocytes, however (Y-W Qian, JL Maller, personal communication).

In G2-arrested oocytes, Cdc25C is predominantly found in the cytoplasm associated with 14-3-3 proteins. The complex dissociates following progesterone treatment, which may allow Cdc25C to accumulate in the nucleus [6•]. However, as discussed above, it is still not clear how and where Cdc25C becomes activated during the meiotic cell cycle in oocytes.

Xe-p9 and the recruitment of substrates to Cdc2–cyclin-B

Xe-p9 is a member of the Suc1/Cks family of proteins that directly associate with cyclin-dependent kinase complexes and can regulate their function (see [50] and references therein). Immunodepletion of Xe-p9 prevents interphase *Xenopus* egg extracts from entering M phase [50]. Xe-p9 is required for the phosphorylation of the anaphase-promoting complex component Cdc27 by Cdc2–cyclin-B [50]; also, it strongly stimulates the phosphorylation of Cdc25C, Myt1 and Wee1 by Cdc2–cyclin B *in vitro* [51•]. These observations suggest that Xe-p9 might act as a targeting subunit for Cdc2–cyclin B substrates. It is not clear whether Xe-p9 is normally involved in oocyte maturation, although Xe-p9 overexpression can inhibit the activation of pre-MPF in oocytes [36•]. It remains to be investigated at what level an excess of Xe-p9 might interfere with the signalling pathways that target tyrosine dephosphorylation of the Cdc2 subunit of pre-MPF. Interestingly, addition of recombinant p13Suc1 to an oocyte extract slows down the kinetics of okadaic-acid-induced activation of Cdc2–cyclin-B by Cdc25C, even though Cdc25C is still hyperphosphorylated, suggesting that Suc1/Cks family proteins might be

required for targeting active (hyperphosphorylated) Cdc25C to pre-MPF complexes [48].

Absence of S phase in the meiotic cell cycle

One important characteristic of the meiotic cell cycle is the occurrence of two consecutive M phases without an intervening S phase, which is essential for the generation of haploid germ cells (Figure 1). In *Xenopus* oocytes, the mechanism of S phase omission involves meiosis-specific factors such as Mos, a MAPK activator that reactivates Cdc2 and suppresses S phase after meiosis I [28]. Recent work suggests that the inhibition of DNA replication by the Mos/MAPK pathway might be mediated by $p90^{rsk}$ [27••].

It also has been shown recently that the lack of the mitotic inhibitor Wee1 is important for S phase omission [9••]. Wee1 is not present in G2-arrested oocytes but becomes synthesised during maturation [9••,38]. The new work shows that Wee1 is specifically downregulated during oogenesis, probably by translational repression of the maternal mRNA [9••]. Moreover, ectopic expression of Wee1 in oocytes blocks the meiotic maturation after meiosis I and induces DNA replication. These results, together with the observation that Wee1 is not present during meiosis I in mice, starfish and yeast, suggest that the absence of Wee1 in meiosis I may be a conserved mechanism to ensure the absence of S phase between the two meiotic divisions [9••].

Using cell-free extracts prepared from *Xenopus* oocytes, which reproduce the meiosis I to meiosis II transition [52•], some Wee1 protein has been detected at the meiosis I exit but its activity is apparently suppressed by the low level of Cdc2 activity remaining at this stage. This residual Cdc2 activity has been proposed to be required for suppressing entry into S phase during the two meiotic divisions [52•]. Taken together, the results suggest that a mechanism underlying the inhibition of DNA replication during the meiotic cell cycle is the absence of inhibitory Tyr15 phosphorylation on Cdc2, which allows MPF to be reactivated. This can be achieved through the regulation of Myt1 (probably mediated through the Mos/MAPK/$p90^{rsk}$ pathway) and either the absence or the inhibition of Wee1.

Ringo, a novel Cdc2 activator that triggers meiotic G2/M progression

The observation that progesterone-induced oocyte maturation was inhibited by injection of a kinase-inactive Cdc2 mutant or an anti-Cdc2 antibody led to the suggestion that activation of free Cdc2 was required for meiotic maturation [53]. The most likely candidates to activate free Cdc2 would be A or B type cyclins, several of which are newly synthesised during oocyte maturation. However, there is evidence that synthesis of the currently known cyclins is not required for progesterone-induced activation of MPF and entry into meiosis I of oocytes (H Hochegger and T Hunt, personal communication). These results suggest that synthesis of other unidentified Cdc2-activating proteins is required to trigger oocyte maturation.

Recently, a novel protein termed 'Ringo' (*r*apid *in*ducer of *G2*/M in *o*ocytes) was identified in an expression screen based on its ability to induce *Xenopus* oocyte maturation in the absence of progesterone stimulation [54••]. The same protein was independently cloned by another group in a screen for proteins that complements a *rad1* mutant of *Schizosaccharomyces pombe* and termed 'Speedy' [55•]. Overexpression of Ringo or Speedy in *Xenopus* oocytes potently triggers pre-MPF activation and GVBD [54••,55•], even in the presence of protein synthesis inhibitors [54••]. Moreover, antisense ablation of Ringo mRNA inhibits MPF activation and GVBD induced by progesterone, indicating that Ringo synthesis is required for oocyte maturation. Experiments using *Xenopus* cell-free extracts and baculovirus-expressed proteins suggest that Ringo probably functions by directly activating free Cdc2 in the oocytes [54••]. This is consistent with the observation that histone H1 kinase activation (a marker for Cdc2 activity) precedes the activation of MAPK in Ringo-injected oocytes. Ringo-induced maturation also can be blocked by overexpression of kinase-inactive Cdc2 mutant in the oocytes [54••]. Ringo/Speedy also binds to Cdk2 upon co-expression in oocytes [55•] and can activate both endogenous Cdk2 in oocytes [55•] and bacterially produced Cdk2 protein *in vitro* (A Karaiskou, AR Nebreda, unpublished data).

Thus, Ringo appears to represent a novel type of cyclin-dependent kinase regulatory partner that does not have significant homology to cyclins at the amino acid sequence level. In this sense, Ringo resembles the Cdk5 activator p35 [56]. The available information suggest that Ringo may be a crucial protein that needs to be synthesised *de novo* upon progesterone stimulation to initiate the meiotic maturation of oocytes (Figure 2). Ringo's synthesis may also explain the requirement for the activation of free Cdc2 during progesterone-induced maturation [53]. It will be interesting to further characterise Ringo's role in oocyte maturation — for example, to identify substrates of Cdc2–Ringo complexes — as well as other possible roles of Ringo in cell cycle regulation.

Conclusions

Significant progress has been made towards understanding the regulation of the meiotic cell cycle in *Xenopus* oocytes (Table 1). New insights have been gained into how the meiotic protein kinase Mos becomes activated as well as on the timing and function of MAPK during meiosis re-initiation. The kinase Eg2 is an excellent candidate to upregulate Mos mRNA translation via phosphorylation of CPEB, but it is unclear whether Eg2 is activated early during oocyte maturation. Binding to Hsp90 has been identified as an additional level of regulation for the Mos protein kinase. A very early phosphorylation of MAPK has been proposed but it is not clear how this could be triggered, as it might be independent of Mos synthesis. A link between MAPK and Cdc2–cyclin-B activation has been identified, via the $p90^{rsk}$-induced phosphorylation and inactivation of the Cdc2 inhibitory kinase Myt1. There is

also evidence, however, that alternative pathways may be activated during maturation to bypass the requirement for MAPK. The identification of these signalling pathways and whether they might be Mos independent represent challenging tasks for the future.

In contrast, although it has been known for many years that a decrease in PKA activity is necessary for maturation, the connections between PKA and other signalling molecules that regulate oocyte maturation are still unclear.

The past two years also have yielded important information on how the natural G2 arrest is maintained in oocytes, with Myt1 activity probably playing a critical role. A potentially general mechanism also has been proposed — that is, absence of the mitotic inhibitor Wee1 — to account for the absence of S phase between the two meiotic divisions. Finally, a new protein (Ringo/Speedy) has been identified whose synthesis appears to be required for *Xenopus* oocyte maturation; this protein can bind to and activate free Cdc2 and Cdk2.

It will be very interesting to investigate if the molecular mechanisms that regulate the meiotic cell cycle in *Xenopus* oocytes also are conserved in male meiosis, as well as in other organisms such as *Caenorrhabditis elegans*, *Drosophila*, starfish and mouse.

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Now in press

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