

The interplay between cyclin-B–Cdc2 kinase (MPF) and MAP kinase during maturation of oocytes

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Summary

Throughout oocyte maturation, and subsequently during the first mitotic cell cycle, the MAP kinase cascade and cyclin-B–Cdc2 kinase are associated with the control of cell cycle progression. Many roles have been directly or indirectly attributed to MAP kinase and its influence on cyclin-B–Cdc2 kinase in different model systems; yet a principle theme does not emerge from the published literature, some of which is apparently contradictory. Interplay between these two kinases affects the major

events of meiotic maturation throughout the animal kingdom, including the suppression of DNA replication, the segregation of meiotic chromosomes, and the prevention of parthenogenetic activation. Central to many of these events appears to be the control by MAP kinase of cyclin translation and degradation.

Key words: Cyclin B, Cdc2, MAP kinase, MPF, Oocyte meiosis

Introduction

In the animal kingdom, oocytes arrest the cell cycle at G₂ phase after replicated chromosomes have undergone meiotic pairing and recombination. Then they grow to their maximal size, which can take years in some species. G₂ arrest is terminated by specific signals, often hormones, that cause release from cell cycle arrest, and progression into and sometimes completion of the meiotic cell cycles. Collectively, these events are termed oocyte maturation. Shortly after identification of the intracellular maturation-promoting factor (MPF) responsible for onset of oocyte maturation as a complex formed by cyclin B and Cdc2 kinase (Labbé et al., 1989; Draetta et al., 1989; Gautier et al., 1990; Yamashita et al., 1992), several groups discovered that another kinase, MAP kinase, is also activated during oocyte maturation (Haccard et al., 1990; Ferrell et al., 1991; Shibuya et al., 1992; Verlhac et al., 1993) and inactivated after fertilization in all species. This suggested that this kinase has a maturation-specific role. A representation of the changes in cyclin B protein level, and the activities of cyclin-B–Cdc2 kinase and MAP kinase throughout *Xenopus* oocyte maturation is shown in Fig. 1.

In most organisms, fully grown oocytes contain a stockpile of pre-formed cyclin-B–Cdc2 kinase, which is maintained in an inactive form (pre-MPF) by inhibitory phosphorylations on Thr14 and Tyr15 of Cdc2. In addition, work on *Xenopus* oocytes and oocyte extracts has revealed a conserved cascade of kinases – Mos, MEK and MAP kinase – whose activation is critical for ordering of events during meiosis and is required for the first mitotic cell cycle (Sagata, 1997; Ferrell, 1999). Most efforts have been devoted to understanding how MAP kinase affects the initial Cdc2 kinase activation that causes germinal vesicle breakdown (GVBD; i.e. the breakdown of the large nucleus), but both kinases are also intimately involved in the control of subsequent events in meiotic maturation,

including suppression of DNA replication between meiosis I and meiosis II, meiotic chromosome segregation and prevention of parthenogenetic activation. Here, we analyse the interplay between cyclin-B–Cdc2 kinase and MAP kinase in all these aspects of meiotic maturation. Firstly we focus on activation of MPF, before considering the fundamental processes of meiosis and, finally, the transition into the mitotic cell cycle.

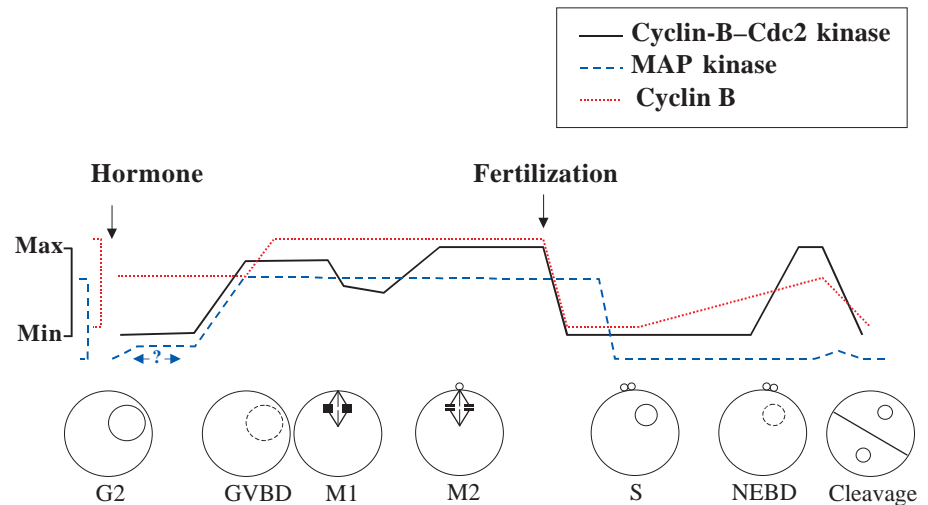
Germinal vesicle breakdown

Oocytes of most species speed up cyclin B translation when stimulated to enter the maturation process, even though they contain pre-formed, inactive cyclin-B–Cdc2 kinase phosphorylated on Tyr15 (pre-MPF) – with the notable exception of goldfish, whose fully grown oocytes do not contain cyclin B (Katsu et al., 1993). In some species, including starfish and mouse, translation of cyclin B is enhanced only after GVBD, and protein synthesis is not required for GVBD (Picard et al., 1985; Galas et al., 1993; Hashimoto and Kishimoto, 1988). In other species, however, including *Xenopus* (Kobayashi et al., 1991; de Moor and Richter, 1997), protein synthesis is required for GVBD; at least one species of cyclin B (cyclin B1) is present in very low amounts in G₂-arrested oocytes, and its enhanced translation/accumulation appears to start before GVBD and does not require GVBD (Fisher et al., 1998a). After recruitment of Cdc2, newly synthesized cyclin B might thus play a role in GVBD.

Is cyclin B translation required for GVBD in *Xenopus* oocytes?

Minshull et al., using an antisense oligonucleotide strategy to evaluate the requirement for cyclin B1 synthesis in progesterone-dependent GVBD, concluded that oocytes do not

Fig. 1. Changes in *Xenopus* oocytes in the levels of cyclin B protein, and the cyclin-B-Cdc2 kinase and MAP kinase activities throughout the course of meiotic maturation. For species-specific variations, see the main text. The question mark indicates that early phosphorylation of MAP kinase has been seen (Fisher et al., 1999; Fisher et al., 2000), but whether or not this represents active MAP kinase is unknown. GVBD, germinal vesicle breakdown; M1, meiosis I; M2, meiosis II; NEBD, nuclear envelope breakdown.



require cyclin translation (Minshull et al., 1991). In addition, *Xenopus* oocytes become independent of protein synthesis for GVBD at 0.65 GVBD₅₀ (Wasserman and Masui, 1975; 65% of the time by which 50% of oocytes have undergone GVBD after progesterone stimulation). Since cyclin B1 has not yet accumulated to a significant level at this time in progesterone-stimulated oocytes (Kobayashi et al., 1991), it is unlikely to be required for GVBD in *Xenopus*. However, at least one other cyclin B, cyclin B4, is synthesized during progesterone-induced maturation (Ferby et al., 1999). Dephosphorylation of Tyr15 of pre-MPF occurs and is sufficient, even in the absence of protein synthesis, to induce GVBD in oocytes receiving a small amount (1/20 in volume) of cytoplasm taken from donor oocytes after GVBD (Wasserman and Masui, 1975). In this kind of experiment, however, the donor cytoplasm might contain some active compound that contributes to GVBD, but is produced after GVBD in donor oocytes and not present (at least in an active form) before GVBD.

At least one argument nevertheless suggested that translation of a cyclin or cyclin-like molecule is required for progesterone-stimulated MPF activation and GVBD in *Xenopus* oocytes. Sagata and co-workers demonstrated that ectopic expression of a dominant negative mutant of Cdc2, expected to bind cyclins but to produce inactive complexes, suppresses progesterone-dependent MPF activation and GVBD (Furuno et al., 1994). This was also demonstrated by Hunt and co-workers, who furthermore showed that microinjection of a monoclonal antibody against Cdc2 that prevents its activation with cyclins had the same effect (Nebreda et al., 1995). These results suggested that newly synthesized protein(s), probably an unidentified cyclin or cyclin-like molecule(s), associates with Cdc2 to activate MPF during progesterone-induced maturation of *Xenopus* oocytes. Alternatively, progesterone could induce release of a cyclin-like molecule, sequestered in G₂ phase oocytes, for association with Cdc2 (see below). Recently, however, Nebreda and colleagues described a novel non-cyclin Cdc2-binding and -activating protein, ringo, whose translation is necessary for meiotic maturation of *Xenopus* oocytes (Ferby et al., 1999). Injection of ringo protein in the absence of protein synthesis induces GVBD and MPF activation. This does not exclude the possibility that a cyclin is required for GVBD in *Xenopus* but does provide an alternative explanation for the

above results. Nevertheless, a more general explanation can also be proposed. The idea that a pool of oocytes represents subpopulations that have different intrinsic sensitivities to biochemical parameters is not usually considered, but evidence suggests that this is the case (see below). GVBD might therefore require cyclin B translation to a greater or lesser degree in different oocytes. Such population differences could go some way towards resolving apparent contradictions in the published literature.

The requirement for MAP kinase activation in GVBD

MAP kinase activation is not required for the initial activation of MPF in oocytes of species such as starfish or mouse, which activate MAP kinase only after GVBD. In other species, by contrast, MAP kinase is activated before GVBD and might stimulate GVBD. This is the case in *Xenopus* oocytes, which either do not reinitiate meiosis after G₂ arrest (Kosako et al., 1994; Gotoh et al., 1995) or delay this process (Fisher et al., 1999; Gross et al., 2000), if MAP kinase activation is inhibited. One possible function of MAP kinase in the G₂-M transition is to prevent inhibitory phosphorylation of Cdc2. When purified active cyclin-B-Cdc2 is injected into immature starfish or *Xenopus* oocytes at subthreshold levels for GVBD induction, its histone H1 kinase activity is not maintained in recipient immature oocytes but decreases because of inhibitory phosphorylation of Cdc2 (Picard et al., 1991; Okumura et al., 1996; Abrieu et al., 1997a). This implies that the inactivator of cyclin-B-Cdc2, probably Myt1 kinase (at least in *Xenopus*, Wee1, the other Cdc2 inhibitory kinase, is not expressed in the G₂-arrested oocyte) is highly active in immature oocytes. Interestingly, microinjected cyclin-B-Cdc2 kinase is not inactivated in oocytes containing prematurely activated MAP kinase (Abrieu et al., 1997a). Rather, it rapidly triggers GVBD by activating the MPF amplification loop, in which cyclin-B-Cdc2 kinase directly or indirectly stimulates its own activatory phosphatase, Cdc25, and inactivates the inhibitory Tyr15 kinase. These results suggest that MAP kinase downregulates the mechanism that inactivates cyclin-B-Cdc2 kinase in G₂-arrested *Xenopus* oocytes. In this case, Myt1 is a potential target for the MAP kinase cascade (Palmer et al., 1998), and in the absence of MAP kinase activity Myt1 is only phosphorylated to intermediate levels (Fisher et al., 1999).

A further potential contribution of MAP kinase to cyclin-B–Cdc2 kinase activation at GVBD is subcellular relocalization due to phosphorylation of cyclin B1, either directly – MAP kinase can phosphorylate cyclin B1 in vitro (Izumi and Maller, 1991) – or indirectly. Phosphorylation of certain serine sites is essential for the biological activity of injected cyclin B1 in *Xenopus* oocytes and inhibits its retention in the cytoplasm (Li et al., 1997), thus leading to its nuclear localization. Nevertheless cyclin B1 is present in only very low amounts in G₂-arrested oocytes, and the germinal vesicle is not essential for MPF activation. Therefore, if cyclin B1 phosphorylation is important for GVBD, it may be to release sequestered, inactive cyclin B1, which could promote MPF activation. Whether or not cyclin B1 is phosphorylated in the absence of MAP kinase is unknown.

Although MAP kinase can certainly promote cyclin-B–Cdc2 activity, there is a slight problem: the MAP kinase cascade appears to be inactive if Cdc2 activation is blocked by various means. Furthermore, MAP kinase activation is dispensable for Cdc2 activation and GVBD in a variety of species. The clearest demonstration of this is in mice, in which MAP kinase activation occurs well after Cdc2 activation and does not occur at all in *Mos*^{-/-} mice (Verlhac et al., 1996). The meiotic defect in *Mos*^{-/-} oocytes, however, is not one of Cdc2 activation and GVBD but rather that microtubules revert to an interphase-like state between the two M phases. Different subpopulations of oocytes that have different sensitivities to Cdc2 kinase might occur; quantitative differences in the capacity for cyclin B synthesis and dependence on MAP kinase activation do occur between different populations of *Xenopus* oocytes (Thibier et al., 1997; Fisher et al., 1999). Thus, in different species and in different populations of the same species, some oocytes might require MAP kinase activation to inactivate Cdc2-inhibitory kinases, to regulate cyclin B localization or to stimulate synthesis of cyclins (see below), in order to generate sufficient Cdc2 kinase activity to undergo the G₂-M transition, whereas others might not.

The role of cyclin-B–Cdc2 kinase in MAP kinase activation

MAP kinase controls cyclin-B–Cdc2 activity; the converse is also true. In oocytes of all species, full activation of MAP kinase occurs only once Cdc2 is active. Indeed, dominant negative Cdc2 mutants or inactivating Cdc2 antibodies block the majority, if not all, of MAP kinase activation in *Xenopus* oocytes (Nebreda et al., 1995). Furthermore, MAP kinase activation is downstream of Cdc2 activation in starfish and mouse oocytes. Since MAP kinase is controlled by activatory phosphorylation, active Cdc2 probably stimulates, either directly or indirectly, the upstream activator Mos – or possibly an equivalent kinase in systems in which Mos has not been discovered. A contribution from a Mos-independent mechanism cannot be excluded, since active cyclin-B–Cdc2 kinase can activate MAP kinase even in the absence of detectable Mos (Minshull et al., 1994; Guadagno and Ferrell, 1998), although in *Xenopus* oocytes protein synthesis is absolutely required for MAP kinase activation, and suppression of Mos translation also inhibits MAP kinase activation in both mouse and *Xenopus* oocytes. Mos is therefore the best candidate for a cyclin-B–Cdc2-activated stimulator of MAP kinase activation during meiosis. More than one mechanism

might therefore control MAP kinase activation. Recent results support such a model: enzymatic activation of Mos in *Xenopus* oocytes requires both interaction of Mos with Hsp90 and its phosphorylation at Ser3 (Fisher et al., 2000). Several potential Ser3 kinases exist, including MAP kinase and cyclin-B–Cdc2, for which Mos Ser3 fulfils consensus-site requirements. MAP kinase cannot be the only kinase responsible for Mos Ser3 phosphorylation, since phosphorylation and activation of Mos occur even if MAP kinase is maintained inactive by expression of the Pyst1 MAP kinase phosphatase. Cyclin-B–Cdc2 is thus the best candidate. MAP kinase may therefore be activated early to a low level independently of cyclin-B–Cdc2, whereas activation of the latter activates Mos around the time of GVBD, promoting high-level MAP kinase activation (Fisher et al., 1999; Fisher et al., 2000).

The role of MAP kinase in cyclin B translation

In all investigated cases, there is a strong temporal correlation between the level of MAP kinase activation and cyclin B translation. In *Spisula*, starfish, mouse and *Xenopus*, increased cyclin B synthesis begins soon after MAP kinase activation. In enucleated starfish oocytes, both MAP kinase activation and an increase in cyclin B synthesis are suppressed, although Cdc2 kinase is readily activated in response to the maturation-inducing hormone 1-methyladenine (Galas et al., 1993; Abrieu et al., 1997a). In mouse oocytes, inhibition of the Mos-dependent MAP kinase cascade by ablation of Mos mRNA suppresses accumulation of newly translated cyclin B1 (O'Keefe et al., 1991). A requirement for MAP kinase activity in cyclin B synthesis may at least partially explain why, when the synthesis or activity of Mos is specifically inhibited at GVBD in *Xenopus* oocytes, MPF activity remains low after meiosis I, and the oocytes fail to enter meiosis II (Furuno et al., 1994; Daar et al., 1991; Kanki and Donoghue, 1991).

The increase in the rate of cyclin B translation when oocytes are released from arrest at G₂-prophase of the first meiotic cell cycle might be due to unmasking of its mRNAs and depends on a cytoplasmic polyadenylation element (CPE) in the 3' untranslated region (UTR). Indeed, microinjection of high levels of cyclin B1 3' UTR RNA induces cyclin B1 synthesis in *Xenopus* oocytes. Moreover, the presence of a cyclin B1 3' UTR inhibits translation of a fused reporter construct RNA (de Moor and Richter, 1999). This indicates that the CPEs of the cyclin B1 3' UTR are translational-masking elements in the immature oocyte and that cytoplasmic polyadenylation might be essential for efficient translation of cyclin B1 during meiotic maturation of *Xenopus* oocytes. MAP kinase activation appears to be required for unmasking of the cytoplasmic polyadenylation element in the 3' UTR of cyclin B under physiological conditions, since polyadenylation does not occur in response to progesterone if MAP kinase is inhibited by the compound PD98059 (Howard et al., 1999). However, MPF-induced cytoplasmic polyadenylation of cyclin B1 mRNA can occur in microinjected *Xenopus* oocytes in the presence of injected MAP kinase phosphatase MKP1, which eliminates the majority of MAP kinase activation (Howard et al., 1999).

Frank-Vaillant et al. have reported that cyclin B1 accumulates to normal levels in oocytes microinjected with the Cdk inhibitor p21^{CIP1}, which suppresses activation of both Cdc2 kinase and MAPK, as well as GVBD (Frank-Vaillant et al., 1999). This is surprising, because polyadenylation of cyclin

B1 mRNA requires both MAP kinase and Cdc2 kinase activities in *Xenopus* oocytes (Ballantine et al., 1997; de Moor and Richter, 1997; Howard et al., 1999). If confirmed (it is unclear whether this work discriminated between cyclin B1 and cyclin B4: see Ferby et al., 1999), these results would indicate that progesterone-dependent stimulation of cyclin B1 translation is partly independent of MAP kinase, Cdc2 kinase and therefore polyadenylation of cyclin B1 mRNA in *Xenopus* oocytes. However, part of the accumulation of cyclin B1 to a high level could also be accounted for by the absence of cyclin B1 degradation, which normally occurs after GVBD and is mediated by the anaphase promoting complex (APC), which is dependent on Cdc2 kinase activation.

In studies using the Hsp90 inhibitor geldanamycin to prevent activation of the Mos-dependent MAP kinase cascade, oocytes of most females accumulated cyclin B1 to a low level, even when they underwent GVBD, in spite of MAP kinase suppression (Fisher et al., 1999). Cyclin B accumulated to a similar level at GVBD in studies using the MAP kinase inhibitor UO126 (Gross et al., 2000), although it did not attain normal levels after GVBD. In the latter experiments the interpretation was that MAP kinase is responsible for inactivation of cyclin degradation, although, as discussed below, it seems likely that the influence of MAP kinase on cyclin B accumulation is also one of stimulation of cyclin B translation after GVBD.

Taken together, the available reports support the view that MAP kinase is not strictly required for stimulation of cyclin B translation, which might occur through a polyadenylation-independent mechanism. However, throughout the animal kingdom, MAP kinase exerts a positive feedback control on cyclin B translation in oocytes induced to escape from G₂ arrest. This is clearly one mechanism by which MAP kinase assists in the activation of MPF. Although not required for GVBD in all oocytes of one species or between species, this control is very important for the subsequent flow of meiotic events.

Suppression of DNA replication in the first meiotic cell cycle

In all investigated animals (including *Spisula*, *Patella*, starfish, *Xenopus* and mouse), new synthesis of cyclin B does not appear to be required after GVBD for oocytes to complete the first meiotic cell cycle, including emission of the first polar body. This is particularly evident in the case of starfish and *Spisula* oocytes, which escape G₂ arrest and complete the first meiotic cell cycle in the complete absence of protein synthesis (Picard et al., 1985; Hunt et al., 1992). Paradoxically, the marked increase in cyclin B translation does not lead to a significant increase in cyclin B protein levels after GVBD in starfish and *Xenopus* oocytes. This may be because oocytes lack a spindle assembly checkpoint in the first meiotic cell cycle and activate the cyclin-degradation machinery shortly after GVBD, before a bipolar spindle is assembled. Consequently, newly synthesized cyclin B completely replaces stockpiled cyclin B by the first meiotic metaphase (Galas et al., 1993; Furuno et al., 1994; Kanki and Donoghue, 1991; Ohsumi et al., 1994). Why should oocytes enhance translation of cyclin B after GVBD if at the same time stockpiled cyclin B is being destroyed? There is reason to believe that this is to prevent DNA replication.

Meiosis is characterized by the absence of DNA replication between meiosis I and meiosis II, a necessary condition to maintain the diploid number of chromosomes in spite of fertilization. As in mitosis (Adachi and Laemmli, 1994; Hayles et al., 1994), cyclin-B-Cdc2 kinase inhibits formation of pre-replication complexes and entry into S phase at the first meiotic interphase. Indeed inhibition of the assembly of endogenous cyclin-B-Cdc2 complexes readily induces premature DNA replication in the oocyte, shortly after emission of the first polar body, at a time corresponding to the transition from the first to the second meiotic cell cycle in normally maturing oocytes (Picard et al., 1996). These results strongly suggest that enhanced translation of cyclin B after GVBD is part of the mechanism that suppresses DNA replication in the oocyte between meiosis I and II. In these experiments, MAP kinase activation still occurred at the first prometaphase, and there was no detectable reduction in activity. Thus, inactivation of MAP kinase is unnecessary for DNA replication between meiosis I and II in starfish. In *Xenopus*, suppression of MAP kinase activation during meiosis I induces premature DNA replication after meiosis I (Furuno et al., 1994; Gross et al., 2000), and expression of a dominant negative Cdc2 mutant gives the same result. Thus, suppression of MAP kinase probably acts indirectly, by downregulating cyclin-B-Cdc2 kinase activity. This could involve both suppression of cyclin B translation and Myt1/Wee1 inactivation. The kinase p90^{rsk} is likely to be the effector of MAP kinase in these processes, because even in the absence of MAP kinase activation, expression of a constitutively active form of p90^{rsk} allows cyclin B to accumulate at a high level and suppresses entry into S phase (Gross et al., 2000).

Suppression of DNA replication between meiosis I and meiosis II may not be the only target that requires enhanced cyclin B synthesis after GVBD. Indeed, inhibition of protein synthesis frequently results in the formation of an enormous first polar body in the starfish *Asterias rubens*, indicating some defect in regulation of the size and anchoring of the first meiotic spindle in oocyte cortex (Galas et al., 1993). Female *Mos*^{-/-} mice have a similar phenotype (Choi et al., 1996), which suggests that the Mos-MAP-kinase pathway is involved.

Control of meiotic chromosome segregation and metaphase arrest

The role of MAP kinase in control of the APC and the exit from metaphase arrest

Cyclins are degraded by the proteasome after ligation of ubiquitin by the APC-cyclosome complex. Cyclin degradation is necessary for exit from M phase, although not for the metaphase-anaphase transition at which chromatid axes separate. However, almost all of the data concerning the relationship between cyclin degradation and chromatid separation at exit from M phase concern mitotic M phase, and it is not known whether the same mechanisms are involved in segregation of homologues during meiosis I and of chromatid axes during meiosis II.

Meiosis II most closely resembles mitosis both mechanically – it is the chromatid axes that separate, and this is followed by cytokinesis, passage into interphase and reformation of the nucleus – and biochemically – cyclin-B-Cdc2 inactivation is

definitive. In vertebrates, maturing oocytes produce a cytostatic factor (CSF), which is defined as an activity present in the cytoplasm taken from unfertilized eggs that induces metaphase arrest when transferred into one blastomere of a two-cell embryo (Masui and Markert, 1971). CSF might also be the activity that causes vertebrate oocytes to arrest at metaphase II. Mature mouse oocytes fail to arrest at the second meiotic metaphase when *Mos* is disrupted by gene targeting (Colledge et al., 1994; Hashimoto et al., 1994). Conversely, microinjection of *Mos* mRNA or protein into one blastomere of a two-cell frog embryo induces cleavage arrest at metaphase. Several experiments indicate that MAP kinase mediates the CSF activity of *Mos*: microinjection of thiophosphorylated (irreversibly activated) MAP kinase (Haccard et al., 1993) or its constitutively activated target $p90^{\text{rsk}}$ (Gross et al., 1999) into one blastomere of a two-cell embryo induces a metaphase arrest similar to that induced by *Mos*, and *Xenopus* egg extracts can apparently be released from metaphase II arrest by addition of specific MAP kinase phosphatases (Minshull et al., 1994), even though, surprisingly, depletion of $p90^{\text{rsk}}$ is ineffective (Bhatt and Ferrell, 1999). MAP kinase, therefore, at least in part through activation of $p90^{\text{rsk}}$, might prevent the operation at metaphase II of an APC-dependent process that controls degradation of M-phase cyclins and sister chromatid segregation. Nevertheless, the assay for CSF is the ability to induce a mitotic arrest in a two-cell embryo, for which the *Mos*-MAP-kinase- $p90^{\text{rsk}}$ pathway is clearly essential. What does this tell us about the nature of endogenous factor causing arrest at metaphase?

Under physiological conditions (Fig. 2), release from metaphase II arrest is mediated by Ca^{2+} /Calmodulin-dependent kinase II, which in the presence of Plx1 kinase activity (Descombes and Nigg, 1998) triggers rapid and complete degradation of M-phase cyclins and sister chromatid segregation (Lorca et al., 1991; Lorca et al., 1993; Morin et al., 1994). Cyclin-B-Cdc2 kinase (MPF) is the initial trigger for APC activation in mitosis and is necessary for degradation of mitotic cyclins (Felix et al., 1990); cyclin-A-Cdc2 kinase is ineffective at promoting APC activation (Luca et al., 1991; Lorca et al., 1992). In meiosis also, cyclin-B-Cdc2 kinase may activate APC initially, since extensive turnover of stockpiled cyclin B occurs shortly after complete activation of MPF after GVBD in meiosis I, even though in

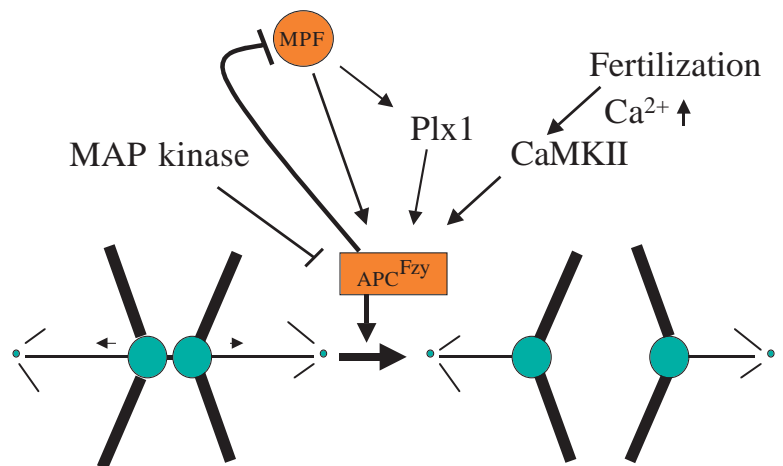
meiosis II full-scale cyclin degradation does not occur until activation of CaM kinase II.

MAP kinase does not appear to inactivate the cyclin-degradation pathway, but can prevent cyclin-B-Cdc2 kinase from promoting cyclin degradation in the first place (Abrieu et al., 1996; Vorlauffer and Peters, 1998) possibly by targeting APC. However, although MAP kinase activity is required to inhibit activation of APC-dependent proteolysis when the spindle assembly checkpoint (which prevents APC-mediated degradation from occurring before a bipolar spindle has formed in mitosis) is activated, and it is maximally active at the point in meiosis at which APC-mediated degradation is switched off, a putative inhibitor of APC can be activated (by okadaic acid) at metaphase II in the absence of active MAP kinase (Vorlauffer and Peters, 1998). Thus, under some conditions, MAP kinase activity may not be essential for the inhibition of APC. Furthermore, active APC is detectable in metaphase-II-arrested oocytes even though cyclin degradation is at best rather limited (Thibier et al., 1997; Vorlauffer and Peters, 1998). This suggests that MAP kinase might not inactivate APC per se in meiosis II.

In its mitotically phosphorylated form, APC binds to Cdc20/Fizzy (Shteinberg et al., 1999), a factor required for its meiotic and mitotic activation (Lorca et al., 1998). However, even in the presence of Fizzy, a large pool of cyclin B is resistant to APC-dependent proteolysis in oocytes arrested at the second meiotic metaphase (Thibier et al., 1997) even though immunoprecipitated APC is active. Although other interpretations are possible (see below), a plausible explanation is that this is due to the presence of an inhibitor that restricts the localization of APC or the availability of its substrates. The putative inhibitor might require MAP kinase activity to maintain its effect in metaphase-II-arrested oocytes (Minshull et al., 1994). Since MAP kinase or p38 kinase is also involved in the spindle-assembly checkpoint (Wang et al., 1997; Takenaka et al., 1998), this putative inhibitor could perform mitotic and meiotic roles.

In mouse and *Xenopus* oocytes, cyclin B is to some extent continually degraded and synthesized even at metaphase II (Kubiak et al., 1993; Thibier et al., 1997). One explanation for the CSF-arrested-like state is that the MAP-kinase-stimulated process prevents active APC from ubiquitinating its substrates in metaphase II, and/or MAP-kinase-dependent

Fig. 2. The network of biochemical events controlling segregation of sister chromatids in the second meiotic cell cycle of *Xenopus* oocytes. Chromatids under tension undergo segregation when cohesion between sisters at centromeres is lost at the onset of anaphase II. Loss of cohesion is indirectly controlled by and requires the activity of the APC^{Fzy} complex. In a mitotic cell cycle, cyclin-B-Cdc2 activates APC^{Fzy} after a lag phase, possibly with the help of Plx1, a kinase downstream of MPF. In the second meiotic cell cycle of vertebrates, CaM kinase II is activated at fertilization and releases APC^{Fzy} from inhibition, which is probably dependent on MAP kinase. Activation of APC^{Fzy} triggers both segregation of sister chromatids and inactivation of MPF through degradation of cyclin B. This in turn will inactivate the MAP kinase cascade, because the activity of *Mos* is controlled by MPF.



protein synthesis outweighs ubiquitin-dependent degradation prior to full activation of the degradation machinery. The CaMKII-dependent trigger for full APC-mediated degradation does not require inactivation of MAP kinase, which occurs significantly later (Lorca et al., 1991; Watanabe et al., 1991). If MAP kinase activity maintains an inhibitor of degradation, the CaMKII signal overrides this inhibition. This might explain why invertebrate oocytes, which are generally fertilized earlier than metaphase II, do not arrest at meiotic metaphase II, even when the MAP kinase cascade is still active: the trigger to exit meiosis has already been pulled. What mechanism triggers the metaphase-to-anaphase transition in meiosis I and what prevents it from triggering this transition at the metaphase arrest in meiosis II, and how does this relate to MAP kinase and Cdc2 kinase activity? For the time being, these questions are unanswered.

The nature of CSF

CSF has unfortunately been equated with activity of the Mos–MAP-kinase–p90^{msk} cascade, which is a necessary component of CSF from meiotic oocytes and is by itself sufficient to promote the mitotic arrest of injected blastomeres at the first mitosis (Sagata et al., 1989; Haccard et al., 1990; Bhatt and Ferrell, 1999) that functionally defines CSF (Masui and Markert, 1971). It is less clear that CSF in meiotic oocytes is identical to this kinase cascade – for example, in mouse and *Xenopus* oocytes, fluctuations in CSF activity occur at the exit from metaphase II, while MAP kinase is maximally active (Ciemerych and Kubiak, 1999). It is also not clear that CSF is responsible for the arrest of vertebrate oocytes at metaphase II of meiosis, although Occam's razor would argue that this is likely to be the case.

Biochemically, the physiological metaphase arrest of oocytes in meiosis and the experimental arrest of blastomeres in mitosis, as well as in vitro extracts prepared from unfertilized eggs, can be defined by two very unusual characteristics: they have extremely high levels of activity of the MAP kinase cascade, and they both require extensive mobilisation of Ca²⁺ from internal stores, or the addition of high amounts of Ca²⁺ in the case of cell-free extracts. An explosion in levels of free Ca²⁺ occurs in eggs at fertilization in both vertebrates and invertebrates, and even occurs at fertilization in plants (Ridgway et al., 1977; Steinhardt et al., 1977; Busa and Nucitelli, 1985; Digonnet et al., 1997). Inactivation of MAP kinase apparently bypasses the requirement for high exogenous levels of Ca²⁺ in vitro for the release of the arrest, and oocytes from *Mos*^{-/-} mice do not require the Ca²⁺ transient induced in vivo by fertilization to escape from metaphase II arrest. Moreover, in invertebrate oocytes that arrest at metaphase I, such as in ascidians, the signal for release from the arrest is identical to that of vertebrate oocytes at metaphase II – a large Ca²⁺ transient associated with fertilization – and a high MAP kinase activity is associated with metaphase I arrest (Russo et al., 1998).

A tempting hypothesis is thus that MAP kinase activation blocks Ca²⁺ mobilization within the unfertilized egg – a situation that can be reversed under physiological conditions only by the dramatic surge of Ca²⁺ associated with fertilization. Thus, control of Ca²⁺ regulation might be the basis for CSF. Even in the mitotic cell cycle, increasing Ca²⁺

buffering capacity inhibits cyclin degradation, which suggests that small local transients of Ca²⁺ are required for this process and exit from metaphase (Lindsay et al., 1995). In contrast, mitotic cells do not contain high levels of MAP kinase activity at metaphase, and mobilization of Ca²⁺ from internal stores may be sufficient to satisfy the requirement for a Ca²⁺ transient that results in activation of calmodulin-dependent targets (Groigno and Whitaker, 1998), including CaMKII, and exit from metaphase.

If CSF is the same as the activity that causes metaphase II arrest, one might wonder why it does not cause arrest at metaphase I in vertebrates. In *Xenopus* oocytes, CSF develops just after GVBD (Masui and Markert, 1971). Since *Xenopus* oocytes do not arrest at metaphase I, the metaphase I spindle might be refractory to CSF arrest. To suppress cyclin degradation, MAP kinase must be activated before cyclin-B–Cdc2 kinase has switched on cyclin degradation (Abrieu et al., 1996), and this may not be the case in the first meiotic cell cycle. Yet this cannot explain the failure of MAP kinase to promote arrest at the first meiotic metaphase. Indeed experimental activation of MAP kinase before cyclin-B–Cdc2 kinase leads to arrest of oocytes at metaphase of meiosis II, not meiosis I, as does injection of cytoplasm from metaphase-II-arrested oocytes. Furthermore, the timing of the onset of anaphase I is normal in oocytes from LT/Sv mice (which arrest temporarily at metaphase I) when fused to wild-type oocytes arrested at metaphase II (Ciemerych and Kubiak, 1998). It seems likely, on the basis of the evidence discussed below, that in vertebrates the metaphase-anaphase transition of meiosis I is controlled by mechanisms different from that of meiosis II.

The trigger for the metaphase-anaphase transition in meiosis I

Full activation of APC-dependent proteolysis by CaM kinase II at fertilization (Lorca et al., 1993) drives vertebrate oocytes into the second meiotic anaphase without a detectable lag phase; however, cyclin degradation occurs well before the first meiotic metaphase in both vertebrate and invertebrate oocytes (Galas et al., 1993; Furuno et al., 1994; Ohsumi et al., 1994; Thibier et al., 1997), and there is no degradation-resistant cyclin pool in meiosis I (Thibier et al., 1997). Thus, if degradation is the trigger for separation of homologous chromosomes at meiosis I, compartmental regulation of APC must differ between meiosis I and meiosis II. Although APC-mediated degradation of the glue-material that maintains cohesion of sister chromatids is essential for the metaphase-anaphase transition in mitosis and meiosis II, whether it is in fact necessary for the metaphase-anaphase transition of meiosis I is another question.

In both fission and budding yeast, a central role for cohesins in preventing premature separation of chromatids has been demonstrated during both mitosis and meiosis (Klein et al., 1999; Watanabe and Nurse, 1999). In animal cells, 95% of the 14S cohesin complex, containing homologues of yeast cohesins and condensins, dissociates from chromosomes at the onset of mitotic prophase (Losada et al., 1998), whereas a small amount is still detectable until anaphase I (Losada et al., 2000). The initial dissociation is due to phosphorylation and not degradation. In yeast cells, the cohesin Rec8p is required for prevention of sister chromatid separation at meiotic anaphase

I, is lost from chromosome arms at anaphase I (which thus allows recombination), but remains at kinetochores until anaphase II (Klein et al., 1999). The kinetochore localization of Rec8p is mediated by a meiosis-specific factor, Slk19p (Kamieniecki et al., 2000). No equivalent work has been documented for meiosis in animal cells, although one would expect a similar meiosis-specific regulation of cohesins/condensins. Interestingly, translation of Eg7/XCAP-D2, a component of 13S condensin required for chromosome condensation in cell-free extracts, starts at around GVBD in *Xenopus* oocytes, in parallel with translation of cyclin B1, and its phosphorylation by cyclin-B–Cdc2 kinase activates DNA-supercoiling activity (Cubizolles et al., 1998). Given that polyadenylation of late-activated mRNAs, including the cyclin B1 and possibly Eg7 mRNAs, might require MAP kinase activation, condensation/cohesion of paired meiotic chromosomes may thus indirectly depend on MAP kinase activation, which occurs at prometaphase in oocytes of all species.

Several recent papers suggest that MAP kinase and Cdc2 kinase positively regulate the metaphase – anaphase transition of meiosis I. Firstly, active MAP kinase localizes to the midzone of the elongated spindle at early anaphase I in porcine oocytes and is required for chromosome separation and cytokinesis (Lee et al., 2000). This is interesting in the light of the similar active MAP kinase localization during mitotic M phase in somatic cells (Shapiro et al., 1998; Zecevic et al., 1998). However, it is cyclin B translation that is rate limiting for meiosis I in mouse oocytes (Polanski et al., 1998), and the Mos–MAP-kinase cascade is non-essential, which suggests that, in porcine oocytes, MAP kinase is required simply to stimulate cyclin B synthesis. Newly formed cyclin-B–Cdc2 kinase (due to cyclin B translation) may therefore control kinetochore attachment to microtubules, since this appears to be the trigger for the metaphase-anaphase transition of meiosis I (Brunet et al., 1999).

MAP kinase and cyclin-B–Cdc2 kinase thus appear to regulate homologous chromosome separation during the transition between meiosis I and meiosis II, at the same time as suppressing DNA replication. The role of MAP kinase may be to regulate the level or localization of cyclin-B–Cdc2 kinase activity. The Mos–MAP-kinase cascade might have an additional, cyclin-B–Cdc2-kinase-independent effect on microtubules and chromatin since these are reorganized in oocytes from *Mos*^{-/-} mice between meiosis I and meiosis II even though cyclin-B–Cdc2-kinase is reactivated with normal timing at metaphase II (Verlhac et al., 1996). However, once the activity of APC towards its substrates is turned off or reduced during this transition, MAP kinase may play a role in maintenance of this state, potentially by acting on an inhibitor or by maintaining high-level cyclin B translation, until the normal trigger for the metaphase-anaphase transition of meiosis II occurs.

Role of MAP kinase in prevention of parthenogenetic activation

Throughout the animal kingdom, MAP kinase activity remains high during the second meiotic cell cycle and drops in fertilized or parthenogenetically activated eggs after the drop in cyclin-B–Cdc2 activity and shortly before DNA

synthesis of the first mitotic cell cycle. It does not reappear during the next few cell cycles, which proceed more rapidly than the first and in the absence of gap phases (Picard et al., 1996; Shibuya et al., 1992; Verlhac et al., 1994). In *Xenopus*, for example, early cell division proceeds in the presence of only very low levels of MAP kinase activity (Hartley et al., 1994; Labonne et al., 1995). Why is MAP kinase inactivated and maintained at a low level in the rapidly proceeding cell cycle of the early embryo?

Because oocytes are already equipped with the machinery required for DNA replication by the end of the first meiotic cell cycle but do not initiate DNA replication before the drop in MAP kinase activity in natural conditions, it has been proposed that downregulation of MAP kinase might be necessary and sufficient for entry of mature oocytes into the first round of DNA replication (Tachibana et al., 1997). Indeed, inactivation of MAP kinase by microinjection of a specific phosphatase initiates DNA synthesis in the absence of fertilization in oocytes of the starfish *Asterina pectinifera* (which normally arrest at G₁ phase after the completion of meiosis), whereas constitutive experimental activation of MAP kinase represses the initiation of DNA synthesis following fertilization.

Several other laboratories have confirmed that suppressing downregulation of MAP kinase suppresses entry of fertilized eggs into the first mitosis – not only in other starfish species but also in *Xenopus* and presumably quite generally in the animal kingdom. However, the most general effect of maintaining MAP kinase activity in fertilized eggs appears to be prevention of the G₂-to-M phase rather than the G₁-to-S phase transition (Abrieu et al., 1997b). If eggs are first allowed to inactivate MAP kinase following fertilization, and MAP kinase is then inappropriately reactivated, the cell cycle may arrest at metaphase (see below), at least in vertebrates (Haccard et al., 1993; Sagata et al., 1989; MacNicol et al., 1995). This is, however, not observed in starfish and other invertebrates, which arrest at G₂ phase in the following cell cycle (Fisher et al., 1998a). In the starfish *Marthasterias glacialis*, for example, fully mature oocytes arrest at G₂ phase after completion of meiosis, not G₁ phase, and thus readily replicate DNA in the absence of fertilization and MAP kinase inactivation (Picard et al., 1996). In this species, suppression of MAP kinase downregulation at fertilization prevents subsequent cyclin-B–Cdc2 kinase activation and its consequence, entry into the first mitosis (Abrieu et al., 1997b; Fisher et al., 1998a). In *Xenopus* also, suppression of MAP kinase downregulation after fertilization induces G₂ phase arrest (Abrieu et al., 1997b; Walter et al., 1997; Bitangcol et al., 1998; Chau and Shibuya, 1998). In cell cycle extracts, MAP kinase specifically prevents cyclin-B–Cdc2 kinase activation in the first mitotic cell cycle and has no effect on activation of cyclin-A–Cdc2. Prevention of cyclin-B–Cdc2 kinase activation is associated with tyrosine phosphorylation of Cdc2 and requires Wee1 (Murakami et al., 1999; Walter et al., 2000), which is not present in the meiotic cell cycle. This sharply contrasts with the facilitatory role of MAP kinase in cyclin-B–Cdc2 kinase activation in the first meiotic cell cycle.

Conclusions

In the animal kingdom, many connections between MAP

Cyclin-B–Cdc2 kinase

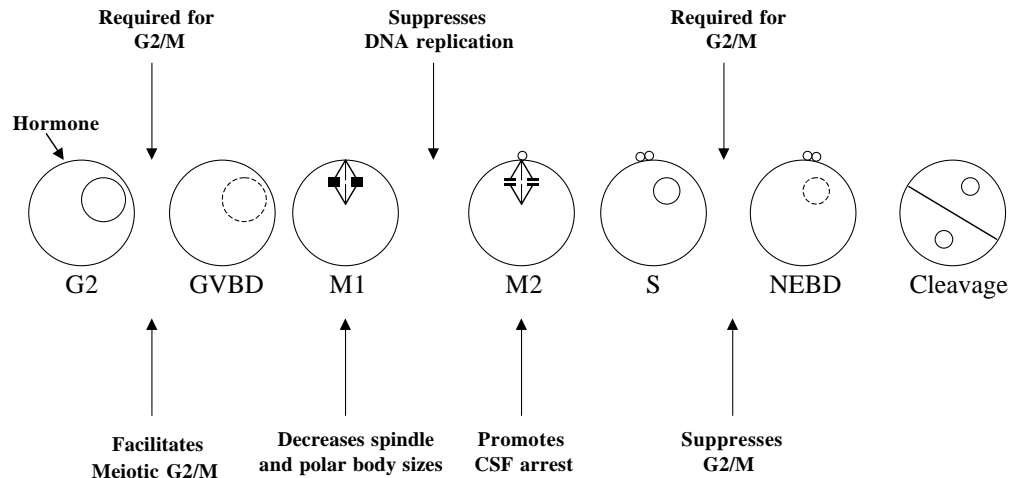


Fig. 3. A summary of events under the control of cyclin-B–Cdc2 kinase (top) and/or MAP kinase (bottom) during meiotic maturation of oocytes in the animal kingdom (arrest at meiosis II occurs only in vertebrates). CSF, colony-stimulating factor; GVBD, germinal vesicle breakdown; M1, meiosis I; M2, meiosis II; NEBD, nuclear envelope breakdown.

MAP kinase

kinase and Cdc2 kinase cascades are established during oocyte maturation. Both kinases are intimately associated in the control of the flow of events in meiotic maturation (Fig. 3). The connections between them are summarized in Fig. 4.

A major and universal role of MAP kinase in oocyte maturation seems to be to control the levels of B-type cyclins. Maintenance of cyclin-B–Cdc2 kinase levels is fundamental in preventing DNA replication between the first and the second meiotic divisions, a necessary condition to maintain the diploid number of chromosomes in spite of fertilization. In *Xenopus* oocytes, and probably other oocytes, MAP kinase contributes to this regulation by stimulating cyclin B translation and formation of active cyclin-B–Cdc2 kinase complexes in meiosis I and potentially also by inhibiting APC–cyclosome-mediated degradation in meiosis II. Both MAP kinase and cyclin-B–Cdc2 kinase may contribute to chromosome pairing in meiosis I, but their inactivation is not required for the metaphase-anaphase at meiosis I transition or for the transition into meiosis II. MAP kinase is necessary for the induction of metaphase arrest and CSF, both of which require large Ca^{2+} transients for release. Paradoxically, in all oocytes, MAP kinase inactivation following fertilization is essential to allow cyclin-B–Cdc2 reactivation and completion of the first mitotic cell cycle. Although interactions between MAP kinase and cyclin-B–Cdc2 kinase

are conserved across species, differences in requirements for MAP kinase activity at different points in the meiotic cell cycle exist and may stem from quantitative differences in the sensitivities of the different systems to different levels of cyclin-B–Cdc2 kinase. Although $p90^{rsk}$ appears to be downstream of MAP kinase in several meiotic processes, we still do not know the substrates of MAP kinase cascade, which highlights a recurrent problem in signal transduction biology: the difficulty of identifying genuine *in vivo* biochemical targets of protein kinases in biological processes.

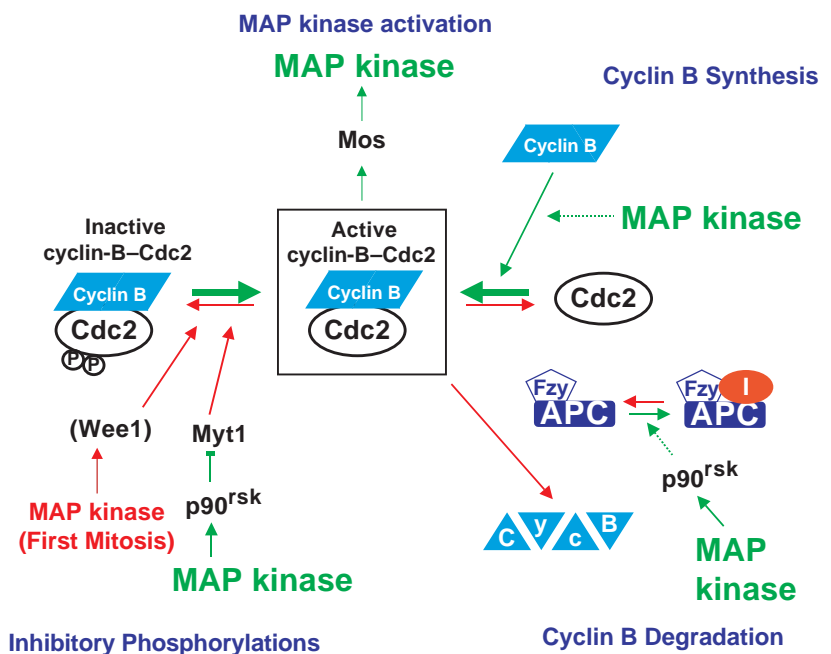


Fig. 4. The interactions between MAP kinase and cyclin-B–Cdc2 kinase in oocytes. Steps leading to activation of MAP kinase or cyclin-B–Cdc2 kinase are shown in green; inhibitory steps are shown in red. APC, anaphase-promoting complex; I, inhibitor.

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