

# Meiotic cell cycle in *Xenopus* oocytes is independent of cdk2 kinase

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**In vertebrates, M phase-promoting factor (MPF), a universal G<sub>2</sub>/M regulator in eukaryotic cells, drives meiotic maturation of oocytes, while cytostatic factor (CSF) arrests mature oocytes at metaphase II until fertilization. Cdk2 kinase, a G<sub>1</sub>/S regulator in higher eukaryotic cells, is activated during meiotic maturation of *Xenopus* oocytes and, like Mos (an essential component of CSF), is proposed to be involved in metaphase II arrest in mature oocytes. In addition, cdk2 kinase has been shown recently to be essential for MPF activation in *Xenopus* embryonic mitosis. Here we report injection of *Xenopus* oocytes with the cdk2 kinase inhibitor p21<sup>Cip</sup> in order to (re)evaluate the role of cdk2 kinase in oocyte meiosis. Immature oocytes injected with p21<sup>Cip</sup> can enter both meiosis I and meiosis II normally, as evidenced by the typical fluctuations in MPF activity. Moreover, mature oocytes injected with p21<sup>Cip</sup> are retained normally in metaphase II for a prolonged period, whereas those injected with neutralizing anti-Mos antibody are released readily from metaphase II arrest. These results argue strongly against a role for cdk2 kinase in MPF activation and its proposed role in metaphase II arrest, in *Xenopus* oocyte meiosis. We discuss the possibility that cdk2 kinase stored in oocytes may function, as a maternal protein, solely for early embryonic cell cycles.**

**Keywords:** cdk2 kinase/metaphase II arrest/Mos/MPF activation/*Xenopus* oocyte meiosis

## Introduction

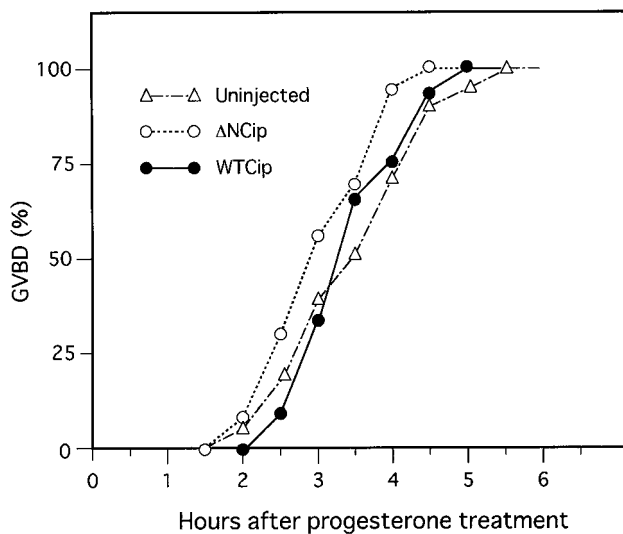
In almost all animal species, immature oocytes are arrested naturally in the first meiotic prophase (prophase I) or the late G<sub>2</sub> phase (Masui and Clarke, 1979; Murray and Hunt, 1993). This meiotic arrest is released usually by hormonal stimulation and is followed by activation of maturation- or M phase-promoting factor (MPF) (Masui and Markert, 1971). MPF, which is a universal G<sub>2</sub>/M regulator in eukaryotic cells and consists of a complex of cdc2 kinase and cyclin B (Nurse, 1990), drives the oocyte to enter not only meiosis I but also meiosis II, with a transient decrease in its activity between the two meiotic divisions (Gerhart *et al.*, 1984; Furuno *et al.*, 1994).

In many vertebrate species, mature oocytes (or unfertilized eggs) are arrested again at the second meiotic metaphase or metaphase II until fertilization (Sagata, 1996). In progesterone-induced *Xenopus* oocyte maturation, metaphase II arrest of mature oocytes is caused by an endogenous cytoplasmic factor called cytostatic factor (CSF) (Masui and Clarke, 1979). The *c-mos* proto-oncogene product Mos is shown to be an essential component of CSF (Sagata *et al.*, 1989), and its CSF function most probably is mediated by the mitogen-activated protein kinase (MAPK) (Haccard *et al.*, 1993; Kosako *et al.*, 1994). The Mos–MAPK pathway is thought to stabilize MPF, thereby enabling mature oocytes to arrest at metaphase II (reviewed in Sagata, 1996). However, some other new protein(s), besides Mos, has also been suggested to be required for metaphase II arrest in *Xenopus* oocytes (Yew *et al.*, 1992; Furuno *et al.*, 1994).

Cdk2 kinase, a cognate kinase of cdc2 kinase, functions in the G<sub>1</sub>/S transitions or for DNA replication in higher eukaryotic cells (Fang and Newport, 1991; reviewed in Sherr, 1993; Hunter and Pines, 1994). Interestingly, this kinase (and its associated partner cyclin E) has been shown to be synthesized *de novo* and accumulated several-fold during *Xenopus* oocyte maturation (Gabrielli *et al.*, 1992; Rempel *et al.*, 1995), a process that lacks both G<sub>1</sub> and S phases (Sagata, 1996). Moreover, inhibition of this synthesis during maturation by cdk2 antisense oligonucleotides has been shown to prevent metaphase II arrest in mature oocytes (Gabrielli *et al.*, 1993). These results have led to the important proposal that cdk2 kinase (and cyclin E) must be the new protein that, besides Mos, is required for metaphase II arrest in mature *Xenopus* oocytes (Gabrielli *et al.*, 1993; Rempel *et al.*, 1995). For such an important proposal to be validated, however, yet another method to inhibit cdk2 kinase specifically may be required, since oligonucleotide injection often gives non-specific effects in *Xenopus* oocytes (cf. Smith *et al.*, 1990).

In addition to the proposed meiotic role and the well-established role for DNA replication in the somatic cell cycle, cdk2 kinase has also recently been shown to be essential for MPF activation in *Xenopus* embryonic mitosis: specific inhibition or depletion of cdk2 kinase in cell-free egg extracts prevented MPF activation, while readdition of it to the extracts allowed MPF activation (Guadagno and Newport, 1996). This direct linkage of cdk2 kinase (a G<sub>1</sub>/S regulator) to positive regulation of MPF (a G<sub>2</sub>/M regulator) may well be universal in mitosis in higher eukaryotic cells (see discussions in Guadagno and Newport, 1996, and in this paper). If so, it will be very important to know whether cdk2 kinase is also essential for MPF activation in meiotic maturation (or the meiotic cell cycle) in *Xenopus* oocytes.

In the present study, we took advantage of p21<sup>Cip</sup>, the cyclin-dependent kinase inhibitor, to (re)examine any role



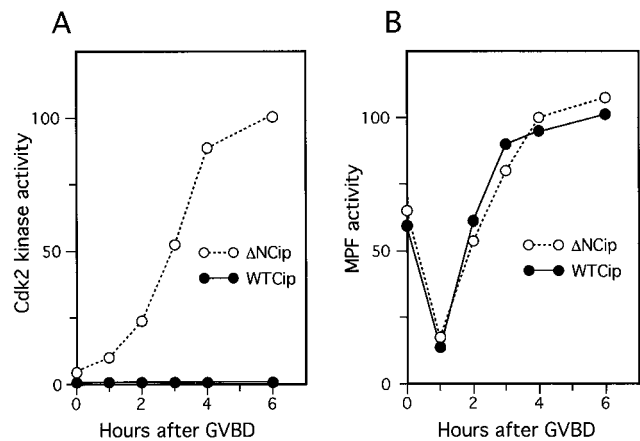
**Fig. 1.** Effect of cdk2 kinase inhibition on the initiation of meiotic maturation in *Xenopus* oocytes. Thirty prophase I-arrested immature oocytes were uninjected or injected with either  $\Delta$ NCip or WTCip at an internal concentration of 100 nM. Thirty minutes later, they were treated with progesterone, cultured and scored for the percentage GVBD every 30 min. The kinetics of GVBD did not differ significantly among the three groups of oocytes; this result was reproducible in three other independent experiments.

for cdk2 kinase in the meiotic maturation of *Xenopus* oocytes. Surprisingly, our results argue strongly against a role for cdk2 kinase in MPF activation and its proposed role in metaphase II arrest, in *Xenopus* oocyte meiosis. Based on these and other results, we discuss the role of cdk2 kinase in oocyte meiosis and embryonic mitosis.

## Results

### Effect of cdk2 kinase inhibition on MPF activation and initiation of maturation

To inhibit the total cdk2 kinase activity in oocytes, we utilized (human) p21<sup>Cip</sup> protein, an inhibitor of several cyclin-dependent kinases such as cdk2 and cdc2 kinases (Elledge and Harper, 1994); at appropriate concentrations, this protein can specifically inhibit cdk2 kinase activity both *in vivo* and *in vitro* (Harper *et al.*, 1995; Guadagno and Newport, 1996). We prepared glutathione *S*-transferase (GST)–wild-type p21<sup>Cip</sup> fusion protein (WTCip) and, as a control, GST–(N-terminal half-truncated) p21<sup>Cip</sup> fusion protein ( $\Delta$ NCip); the latter is known to be unable to bind to and inhibit cdk2 kinase (Chen *et al.*, 1995; Harper *et al.*, 1995). First, we examined whether cdk2 kinase activity is required for MPF activation and initiation of maturation in progesterone-treated oocytes. We injected prophase I (or G<sub>2</sub>)–arrested immature oocytes with either  $\Delta$ NCip or WTCip at an internal concentration of 100 nM, a concentration sufficient to inhibit cdk2 kinase activity completely (Guadagno and Newport, 1996; see also below). Upon progesterone treatment, uninjected control oocytes and those injected with either  $\Delta$ NCip or WTCip (or GST alone; data not shown) all underwent germinal vesicle breakdown (GVBD; a hallmark of initiation of maturation), essentially with the same kinetics (Figure 1); they all also underwent MPF activation essentially normally, or shortly before GVBD (data not shown, but

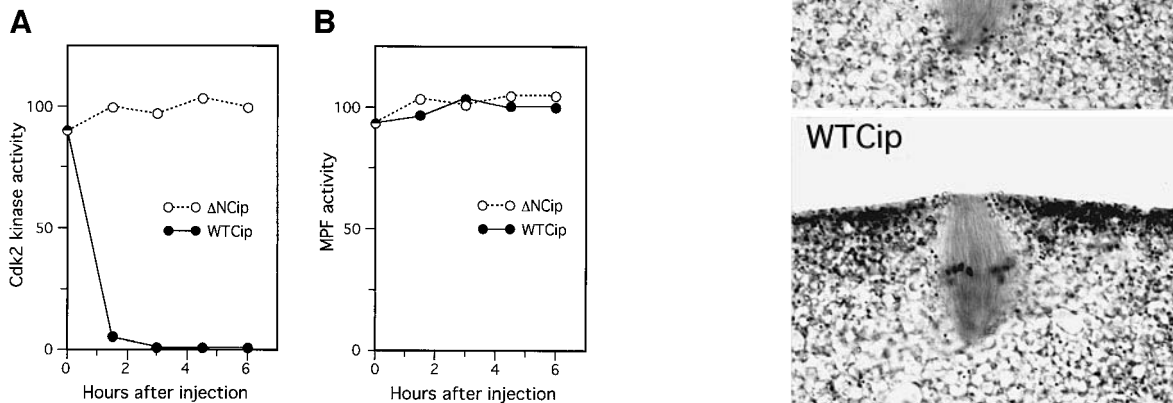


**Fig. 2.** Effect of cdk2 kinase inhibition on MPF reactivation (and entry into meiosis II) in maturing oocytes. More than 30 oocytes were injected with either  $\Delta$ NCip or WTCip and treated with progesterone. Five oocytes were collected every 1 h after GVBD (time 0 at GVBD), and subjected to H1 kinase assays for either cdk2 kinase (A) or MPF (B). Both cdk2 kinase activity (A) and MPF activity (B) are represented in an arbitrary unit; although the ordinates in (A) and (B) are shown by a similar scale, the activity of cdk2 kinase relative to MPF was 50- to 100-fold less in  $\Delta$ NCip-injected oocytes 4–6 h after GVBD. Essentially similar results to those presented here were obtained in three other independent experiments.

see the high MPF activities at GVBD in Figure 2B). Thus, cdk2 kinase activity was not required for either MPF activation or initiation of maturation in *Xenopus* oocytes. These results are surprising if we consider the essential role of cdk2 kinase in MPF activation in *Xenopus* embryonic mitosis (Guadagno and Newport, 1996) (but might not necessarily be surprising if we note its considerably low kinase activities around the time of MPF activation and GVBD during maturation; Gabrielli *et al.*, 1992; Rempel *et al.*, 1995; see also below).

### Effect of cdk2 kinase inhibition on MPF reactivation and entry into meiosis II

We next examined whether cdk2 kinase activity is necessary for reactivation of MPF and entry into meiosis II during maturation, which normally occur 1–2 h after GVBD and approximately at the time of the large cdk2 kinase activation during maturation (Furuno *et al.*, 1994; Rempel *et al.*, 1995). To do this, we injected oocytes with either  $\Delta$ NCip or WTCip, treated them with progesterone and harvested them every 1 h after GVBD; we then subjected their extracts to either total histone H1 kinase assays (for measuring MPF activity; Furuno *et al.*, 1994) or H1 kinase assays of cdk2 kinase (after immunoprecipitation with anti-cdk2 kinase antibody; for the specificity and efficiency of this immunoprecipitation, see Materials and methods). In  $\Delta$ NCip-injected oocytes (and uninjected control oocytes; not shown), cdk2 kinase activity increased progressively after 1 h of GVBD, attaining a 50-fold activity at 6 h after GVBD (Figure 2A), essentially as reported previously (Gabrielli *et al.*, 1992; Rempel *et al.*, 1995). By contrast, in WTCip-injected oocytes, cdk2 kinase activity remained extremely low throughout 6 h after GVBD; we could estimate that, in these oocytes, >98% of the cdk2 kinase activity was inhibited by WTCip (at 4–6 h after GVBD). In both types of oocytes (and the uninjected control oocytes; not shown), however, MPF



**Fig. 3.** Effect of cdk2 kinase inhibition on metaphase II arrest in mature oocytes. Thirty mature oocytes (5 h after GVBD) were co-injected with EGTA and either  $\Delta$ NCip or WTCip (see Materials and methods). Five oocytes were collected every 1.5 h after the injection, and subjected to H1 kinase assays for either cdk2 kinase (A) or MPF (B). Both cdk2 kinase activity and MPF activity are represented as described in the legend of Figure 2. Essentially similar results to those presented here were obtained in two other independent experiments. Mature oocytes 6 h after the injection were also subjected to cytological examination (C).

activity showed essentially the same kinetics: it decreased transiently at 1 h, re-increased greatly at 2–3 h (representing entry into meiosis II; Furuno *et al.*, 1994) and then further increased gradually until 6 h after GVBD (Figure 2B). Thus, very interestingly, cdk2 kinase activity was also not required for MPF reactivation and entry into meiosis II in maturing oocytes, despite the fact that it was greatly activated during these periods. Taken together, the present results strongly indicate that, unlike that in embryonic mitosis (Guadagno and Newport, 1996), MPF activation in *Xenopus* oocyte meiosis does not depend on cdk2 kinase activity.

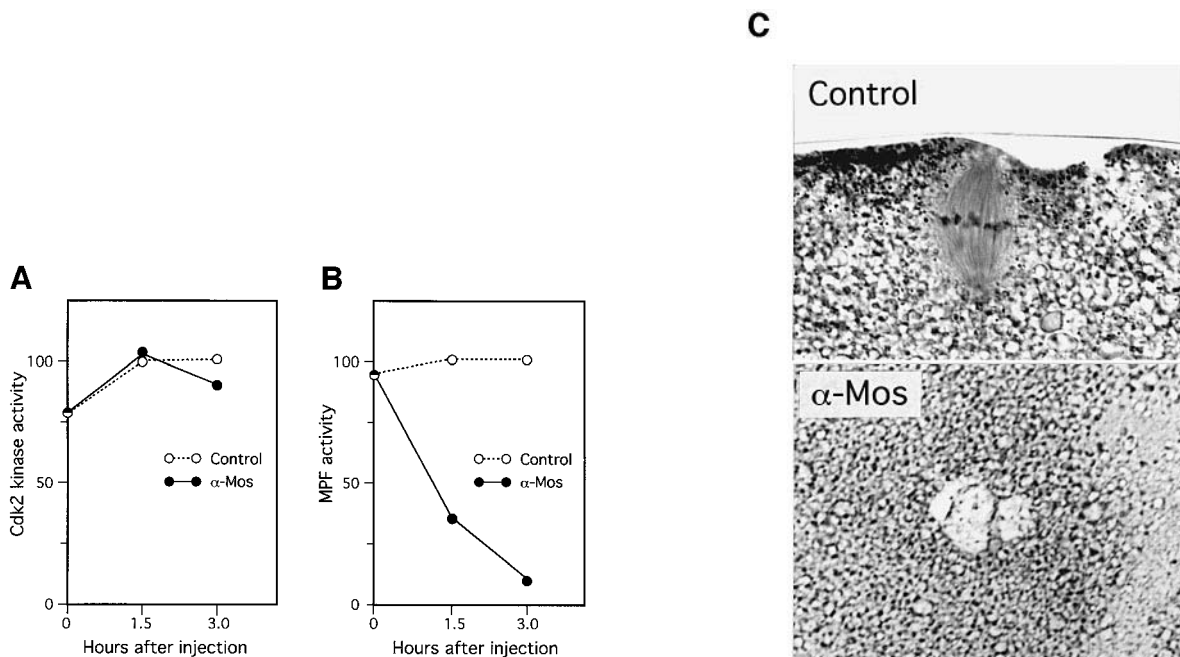
#### **Effect of cdk2 kinase inhibition on metaphase II arrest**

The high MPF activities, even at 4–6 h after GVBD, in WTCip-injected oocytes (Figure 2B) suggest that cdk2 kinase activity may not be essential for metaphase II arrest in mature oocytes (usually, metaphase II arrest occurs from ~3 h after GVBD; Furuno *et al.*, 1994). This notion, if correct, contrasts with the previous proposal that cdk2 kinase is essential and functions (together with Mos) for metaphase II arrest in mature oocytes (Gabielli *et al.*, 1993; Rempel *et al.*, 1995). [We initially attempted to confirm this proposal, by injecting oocytes with cdk2 antisense oligonucleotides as reported (Gabielli *et al.*, 1993), but we could not achieve the same results. Although the reported cdk2 antisense oligonucleotides (Gabielli *et al.*, 1993) and our newly designed antisense oligonucleotides (nucleotide positions 6–25, from the start codon; cf. Paris *et al.*, 1991) both inhibited the synthesis of cdk2 kinase almost completely and (at most) 60% of its kinase activity (due to the presence of pre-existing cdk2 kinase) during maturation, none of them could prevent mature oocytes from arresting at metaphase II, as judged both

biochemically and cytologically (our unpublished data).] Therefore, this time, we injected mature oocytes (5 h after GVBD and arrested in metaphase II) with either WTCip or  $\Delta$ NCip (under conditions where artificial activation of the mature oocytes does not occur by the action of injection; see Materials and methods). Cdk2 kinase activity in WTCip-injected mature oocytes decreased drastically (to 6%) 1.5 h after the injection and, thereafter, to negligible levels (<2%) until 6 h after the injection, while that in  $\Delta$ NCip-injected oocytes remained high throughout 6 h after the injection (Figure 3A). By contrast, not only in  $\Delta$ NCip-injected but also in WTCip-injected oocytes, MPF activity remained high (without any significant changes) throughout 6 h after the injection (Figure 3B), strongly suggesting the persistence of metaphase II arrest in the WTCip-injected oocytes. To confirm this more directly, we cytologically examined mature oocytes 6 h after injection with either WTCip or  $\Delta$ NCip. Both types of oocytes (and the oocyte before injection; not shown) showed a well-formed bipolar spindle with highly condensed chromosomes, just under the animal cortical layer (Figure 3C), which is typical of metaphase II arrest in mature oocytes (Gard, 1992; Furuno *et al.*, 1994). Thus, WTCip could not release mature oocytes from metaphase II arrest, despite its almost complete inhibition of cdk2 kinase activity. These results argue strongly that, in contrast to the previous proposal (Gabielli *et al.*, 1993; Rempel *et al.*, 1995), cdk2 kinase is not required for metaphase II arrest in mature oocytes.

#### **Release from metaphase II arrest in Mos-ablated mature oocytes is independent of cdk2 kinase activity**

Finally, we examined whether release from metaphase II arrest occurs in mature oocytes depleted of Mos, an



**Fig. 4.** Effect of Mos inhibition on metaphase II arrest and cdk2 kinase activity in mature oocytes. Twenty mature oocytes (5 h after GVBD) were co-injected with EGTA and either neutralizing anti-Mos antibody ( $\alpha$ -Mos) or control rabbit IgG (Control), each at 200 ng/oocyte. Five oocytes were collected at 0, 1.5 and 3 h after the injection, and subjected to H1 kinase assays for either cdk2 kinase (A) or MPF (B). Both cdk2 kinase activity and MPF activity are represented as described in the legend of Figure 2. Essentially similar results to those presented here were obtained in two other independent experiments. Mature oocytes 3 h after the injection were also subjected to cytological examination (C).

essential component of CSF (Sagata *et al.*, 1989), and whether this occurs independently of cdk2 kinase activity. To do this, we injected mature oocytes (5 h after GVBD) with either neutralizing anti-Mos antibody (Furuno *et al.*, 1994) or control rabbit IgG, and harvested them 1.5 and 3 h later to measure MPF and cdk2 kinase activities. As was expected (Sagata *et al.*, 1989; Furuno *et al.*, 1994), in anti-Mos antibody-injected oocytes, MPF activity dropped progressively to 35 and 10% at 1.5 and 3 h, respectively, after the injection, while in control IgG-injected oocytes it remained high and constant (Figure 4B). When examined cytologically, the anti-Mos antibody-injected oocyte (3 h after the injection) exhibited a lobed nucleus somewhat deep in the cytoplasm (which is typical of an activated interphase egg; Hausen and Riebesell, 1991), while the control IgG-injected oocyte retained a metaphase II spindle (Figure 4C); thus, Mos ablation released the mature oocyte from metaphase II arrest. In contrast to these results, it was found that cdk2 kinase activity remained high throughout 3 h after the injection in both Mos-ablated and control oocytes (Figure 4A). Thus, very interestingly, metaphase II arrest was released independently of cdk2 kinase activity in Mos-ablated oocytes. These results, together with those with WTCip injection (Figure 3), not only confirm the previously described role for Mos (Sagata *et al.*, 1989; Furuno *et al.*, 1994), but also argue against the proposed role for cdk2 kinase (Gabielli *et al.*, 1993; Rempel *et al.*, 1995) in metaphase II arrest in mature *Xenopus* oocytes.

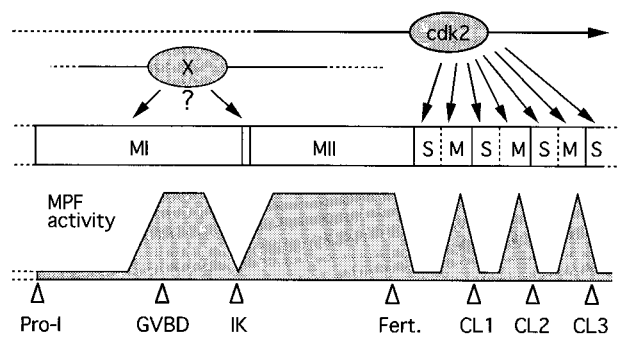
## Discussion

The present results, utilizing p21<sup>Cip</sup>, provide unexpected findings that cdk2 kinase activity is required for neither MPF activation (and reactivation) nor metaphase II arrest in the meiotic maturation of *Xenopus* oocytes. p21<sup>Cip</sup>,

under the present conditions, inhibited >98% of the cdk2 kinase activity (but virtually no activity of cdc2 kinase, a catalytic component of MPF; see Figures 2A and 3B). p21<sup>Cip</sup> could also have inhibited other endogenous proteins, such as cdk4 kinase and PCNA (Elledge and Harper, 1994; Chen *et al.*, 1995), that might be present in maturing oocytes. However, the apparently (both biochemically and cytologically) normal meiotic cell cycle in p21<sup>Cip</sup>-injected oocytes strongly suggests that cdk2 kinase may not play any major role in this type of cell cycle.

Our results on the role of cdk2 kinase in metaphase II arrest in mature *Xenopus* oocytes clearly contradict the previous results and proposal (Gabielli *et al.*, 1993; Rempel *et al.*, 1995). This contradiction, however, is unlikely to be due to the use of different cdk2 kinase inhibitors (p21<sup>Cip</sup> versus antisense oligonucleotides) (see Results). Currently, we do not know the reason(s) for the contradiction. However, the fact that metaphase II arrest persists in cdk2 kinase-inhibited oocytes (Figure 3) while cdk2 kinase activity persists in Mos-inhibited (and hence metaphase II-released) oocytes (Figure 4) argues strongly that, unlike Mos (Sagata *et al.*, 1989), cdk2 kinase is dispensable for metaphase II arrest in mature *Xenopus* oocytes. [The persistence of cdk2 kinase activity in Mos-inhibited (and hence MPF-inactivated) oocytes is interesting, however, since this indicates that cdk2 kinase activation is independent of MPF activity, as previously shown in cell-free *Xenopus* egg extracts (Gabielli *et al.*, 1992).] In mice, Mos is also involved in metaphase II arrest in mature oocytes (O'Keefe *et al.*, 1989; Colledge *et al.*, 1994; Hashimoto *et al.*, 1994); so far, however, neither the activity of cdk2 kinase in mouse oocyte maturation nor its involvement in metaphase II arrest has been known.

The apparent lack of a requirement for cdk2 kinase



**Fig. 5.** Model of the role for cdk2 kinase in the meiotic and mitotic cell cycles in animal eggs. In almost all species, immature oocytes are arrested naturally in the first meiotic prophase (prophase I, Pro-I); this arrest is released by a variety of stimuli, depending on the species (Masui and Clarke, 1979). The time of fertilization (Fert.) also differs depending on the species (Sagata, 1996) and the time shown here (metaphase II) is for vertebrate species, including *Xenopus*. In many species, early embryonic cell cycles after fertilization are abbreviated and each consist of only S and M phases (Murray and Hunt, 1993). Presumably, cdk2 kinase is synthesized and accumulated in oogenesis and/or during oocyte maturation (depending on the species) (cf. Gabrielli *et al.*, 1992; Rempel *et al.*, 1995; this study), and becomes used in the early embryonic cell cycles for both DNA replication (in S phase) and MPF activation (in M phase) (cf. Fang and Newport, 1991; Guadagno and Newport, 1996; Hartley *et al.*, 1996). In the meiotic cell cycle in oocytes, however, cdk2 kinase may not play any role in MPF activation (this study) (but presumably plays a key role in DNA replication in the so-called 'pre-meiotic' S phase, which occurs long before prophase I arrest; not shown); instead, in this cell cycle, some (perhaps meiosis-specific) protein(s) (X) might play a role in MPF activation in both meiosis I (MI) and meiosis II (MII) (in *Xenopus*, Mos might correspond to X). The present model may well apply at least to those many animal species in which embryonic cell cycles are very rapid and depend largely on maternal factors. GVBD, germinal vesicle breakdown; IK, interkinesis (in which DNA replication does not occur); CL1, first cleavage; CL2, second cleavage; CL3, third cleavage.

activity in MPF activation (Figure 1) and its reactivation (Figure 2) in *Xenopus* oocytes is very intriguing, because cdk2 kinase recently has been linked directly to positive regulation of MPF in (cell-free) *Xenopus* embryonic mitosis (Guadagno and Newport, 1996) (we recently have confirmed this linkage by using intact embryos; our unpublished data). *Xenopus* embryonic mitosis is unique in its rapidity and independence of the preceding DNA replication (Newport and Dasso, 1989; Murray and Hunt, 1993). However, the regulation mechanism of mitosis is generally well conserved during evolution (Maller, 1990; Nurse, 1990; Nigg, 1995), and cdk2 kinase (associated A-type cyclin) has been shown to have high activities in the G<sub>2</sub>/M transitions (as well as in the S phases) in mammalian somatic cells (Pagano *et al.*, 1992, 1993; Rosenblatt *et al.*, 1992; Tsai *et al.*, 1993). Thus, the linkage of cdk2 kinase to MPF activation (found in *Xenopus* embryonic mitosis) may be universal in mitosis in higher eukaryotic cells. If so, our results suggest that the mechanism of MPF activation in oocyte meiosis may differ largely, at least in part, from that in mitosis. Indeed, MPF activation (and reactivation) in *Xenopus* oocytes, unlike that in mitosis, is known to depend, at least in part, on the meiosis-specific protein Mos and its downstream kinase MAPK (reviewed in Sagata, 1997). In oocyte meiosis (in many species), therefore, some specific protein(s) might compensate for the cdk2 kinase function to regulate MPF activity positively (Figure 5).

If cdk2 kinase plays no major role in the meiotic cell cycle in (maturing) oocytes, what would its role be? In higher eukaryotic cells, cdk2 kinase plays a key role in DNA replication (Sherr, 1993; Hunter and Pines, 1994). In *Xenopus*, as in many other species, DNA replication does not occur during oocyte maturation (or the meiosis I–meiosis II transition), but occurs immediately after fertilization (Sagata, 1996). Cdk2 kinase stored in *Xenopus* oocytes is, in fact, very stable and persists long after fertilization (Hartley *et al.*, 1996; Howe and Newport, 1996; our unpublished data) and, in each early embryonic cell cycle, cdk2 kinase has high activities in both S and M phases (Hartley *et al.*, 1996). Thus, almost certainly, most of the cdk2 kinase stored in oocytes will contribute directly to DNA replication (Fang and Newport, 1991) (and MPF activation; Guadagno and Newport, 1996) in the early embryonic cell cycles (Figure 5). We cannot yet rigorously exclude a subtle role(s) for cdk2 kinase in oocyte maturation *per se*. However, as discussed here, its major role in oocytes may be best explained by the developmental context, or by preparation for early embryonic cell cycles.

## Materials and methods

### GST–p21<sup>Cip</sup> fusion proteins

A cDNA encoding human p21<sup>Cip</sup> (Harper *et al.*, 1995) was subjected to PCR to yield a cDNA fragment encoding either the whole wild-type p21<sup>Cip</sup> (WTCip) or N-terminal half (amino acid positions 1–86)-truncated p21<sup>Cip</sup> (ΔNCip); the 5' primer used (containing an artificial *Bam*HI site) was either 5'-GCCGGATCCCCATGTCAGAACCGGCTGGGG-3' (for WTCip) or 5'-GCCGGATCCCCATGGATGAGTTGGGAGGAGGCA-GGC-3' (for ΔNCip), while the 3' primer (also containing a *Bam*HI site) was 5'-GCCGGATCCTCTAGAGCTTCTCTTGGAGAAGATCAGC-3' for both WTCip and ΔNCip. After digestion with *Bam*HI, the PCR products were subcloned into a *Bam*HI-digested pGEX-3X plasmid vector (encoding GST), transfected into *Escherichia coli*, and GST–WTCip and GST–ΔNCip fusion proteins were purified by glutathione–Sepharose chromatography (Pharmacia), as described previously (Furuno *et al.*, 1994). The purified proteins were concentrated by Centricon-30 (Amicon), buffer-exchanged with 10 mM HEPES (pH 7.5)–50 mM NaCl, and the concentration was determined by Coomassie blue staining using bovine serum albumin (BSA) as a standard.

### Antibodies

Polyclonal antibody was raised in rabbits against the synthetic peptide THPPFRDVSRRTPHLI, corresponding to the last 16 amino acids of *Xenopus* cdk2 kinase (formerly Eg1; Paris *et al.*, 1991) and coupled to keyhole limpet hemocyanin, by standard methods. Upon immunoprecipitation and Western blotting analyses of *Xenopus* oocyte extracts, this antibody recognized a single band of 33 kDa or cdk2 kinase very specifically (or only in the absence of competing peptide antigens); moreover, the antibody was able to immunoprecipitate as much as 80–90% of the total cdk2 kinase present in oocytes that were either uninjected or injected with p21<sup>Cip</sup>, as revealed by Western blotting analysis of the immunoprecipitates with either anti-PSTAIR antibody (Yamashita *et al.*, 1991) or anti-cdk2 kinase antibody (our unpublished data). Preparation of neutralizing anti-Mos antibody was described previously (Furuno *et al.*, 1994).

### H1 kinase assays

Five oocytes were homogenized in EB buffer (10 μl/oocyte), centrifuged briefly, and 5 μl of the clear supernatant (45 μl in total) was subjected to histone H1 kinase assays to measure the total MPF activity, as described previously (Furuno *et al.*, 1994). Ten μl of the remaining supernatant was diluted with 300 μl of RIPA buffer and then subjected to immunoprecipitation with anti-cdk2 kinase antibody (in the presence or absence of competing peptide antigens), as described (Nishizawa *et al.*, 1993). The immunoprecipitates were then assayed for H1 kinase activity by the addition of 25 μl of a cdk2 kinase-assaying buffer (Gabrielli *et al.*, 1993) supplemented with 200 μg/ml BSA, 200 μM

ATP, 500 µg/ml histone H1 and 5 µCi of [ $\gamma$ - $^{32}$ P]ATP, at 25°C for 30 min. Phosphorylated histone H1 was separated by SDS-PAGE, dried and fixed as described (Furuno *et al.*, 1994), and then quantified for  $^{32}$ P incorporation by BAS-1000 Image Analyzer (Fuji).

### Microinjection and cytological examination

Preparation and culture of oocytes, induction of oocyte maturation by progesterone and cytological examination of fixed oocytes were all described previously (Sagata *et al.*, 1989; Furuno *et al.*, 1994). Forty nl of 2 µM test protein (WTCip or  $\Delta$ NCip) was microinjected into oocytes (giving rise to an internal concentration of ~100 nM of the protein), as described (Sagata *et al.*, 1989). For microinjection of mature oocytes, test protein was co-injected with 40 nl of 10 mM EGTA, to prevent artificial activation of the mature oocyte by the action of injection. Under these conditions, co-injection of neutralizing anti-Mos antibody (200 ng/oocyte) efficiently released mature oocytes from metaphase II arrest (after 1–2 h of the injection), while that of control rabbit IgG did not (see Results); these results not only show the validity of the present method but also confirm the previously described role for Mos in metaphase II arrest (see text).

### Acknowledgements

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