Okadaic Acid Mimics a Nuclear Component Required for Cyclin B-cdc2 Kinase Microinjection to Drive Starfish Oocytes into M Phase

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Abstract. G2-arrested oocytes contain cdc2 kinase as an inactive cyclin B–cdc2 complex. When a small amount of highly purified and active cdc2 kinase, prepared from starfish oocytes at first meiotic metaphase, is microinjected into *Xenopus* oocytes, it induces activation of the inactive endogenous complex and, as a consequence, drives the recipient oocytes into M phase. In contrast, the microinjected kinase undergoes rapid inactivation in starfish oocytes, which remain arrested at G_2. Endogenous cdc2 kinase becomes activated in both nucleated and enucleated starfish oocytes injected with cytoplasm taken from maturing oocytes at the time of nuclear envelope breakdown, but only cytoplasm taken from nucleated oocytes becomes able thereafter to release second recipient oocytes from G2 arrest, and thus contains M phase-promoting factor (MPF) activity. Both nucleated and enucleated starfish oocytes produce MPF activity when type 2A phosphatase is blocked by okadaic acid. If type 2A phosphatase is only partially inhibited, neither nucleated nor enucleated oocytes produce MPF activity, although both do so if purified cdc2 kinase is subsequently injected as a primer to activate the endogenous kinase. The nucleus of starfish oocytes contains an inhibitor of type 2A phosphatase, but neither active nor inactive cdc2 kinase. Microinjection of the content of a nucleus into the cytoplasm of G2-arrested starfish oocytes activates endogenous cdc2 kinase, produces MPF activity, and drives the recipient oocytes into M phase. Together, these results show that the MPF amplification loop is controlled, both positively and negatively, by cdc2 kinase and type 2A phosphatase, respectively. Activation of the MPF amplification loop in starfish requires a nuclear component to inhibit type 2A phosphatase in cytoplasm.

CELLS in M phase contain a dominant factor, called M phase-promoting factor (MPF)[1] which is able upon transfer to drive G_2 cells into M phase (Masui and Markert, 1971; Kishimoto et al., 1982) or to induce prematurely some cytological features of M phase in G_1 cells (Johnson and Rao, 1970). Purification of MPF activity from vertebrate and invertebrate oocytes at meiotic metaphase led to isolation of a single active component, identified as cdc2 kinase, a stoichiometric complex between cyclin B and cdc2. It corresponds to the major M phase-specific H_1 histone kinase, not only in oocytes and early embryos, but also in yeast (reviewed in Nurse, 1990; Dorée, 1990). Possibly, cdc2 kinase is not the single component with MPF activity in intact cells, for several reasons. Firstly, reducing conditions are required to preserve enzymatic activities during fractionation, which could conceivably separate associated proteins from the cyclin B–cdc2 complex (Meikrantz et al., 1991). Secondly, factors sharing MPF activity in common with cdc2 kinase may undergo inactivation during homogenization or fractionation of cell extracts. In agreement with this view, at least one component with MPF activity could be separated from cdc2 kinase when homogenates were treated with adenosine (3'-O-thio)-triphosphate (γS-ATP), to produce thiophosphorylated proteins resistant to phosphatases (Dorée et al., 1989; Yamashita and Maller, 1990; Kuang et al. 1991). Efficiency of γS-ATP treatment was enhanced when part of protein phosphatase activity was removed by ammonium sulfate precipitation as the first step of MPF purification (Hermann et al., 1983; Cyert and Kirschner, 1988).

Nonetheless, biochemical identification of MPF as cdc2 kinase is satisfactory, because genetic analysis did not demonstrate any cell cycle control gene acting downstream of $cdc2^+$, which appears to control ultimately the G_2 to M phase transition in fission yeast (see Nurse, 1990 for review). Furthermore, addition of active cdc2 kinase to extracts prepared from interphase cells has been shown to trigger many mitotic events, including the shortening of centrosome-nucleated microtubules, associated with an increased turnover of tubulin in microtubules (Verde et al., 1990), and the inhibition of intracellular membrane fusion (Tuomikoski et al., 1989). Finally, cdc2 kinase has been shown to phosphorylate mitosis-specific sites on lamins B (Peter et al., 1990a), vimentin (Chou et al., 1990), nonmuscle caldesmon (Yamashiro et al., 1991), nucleolar proteins (Peter et al.,

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1. *Abbreviation used in this paper:* MPF, M phase-promoting factor.

1990b; Belenguer et al., 1990), and H_1 histone (Langan et al., 1989). Thereby, cdc2 kinase plays a key role at M phase in disassembly of both the lamina and the remaining network of intermediate filaments, alteration in the organization of the microfilament cytoskeleton, disassembly of the nucleolus, and chromosome condensation.

However, there is a more characteristic property of MPF, not easily accessible to either genetical or biochemical analysis. MPF was initially defined as a factor in maturing oocytes which, upon microinjection, can drive a G_2-arrested oocyte into M phase. Serial transfer in which the recipient of a transfer becomes the donor of the next one remains successful even in the absence of protein synthesis (Wasserman and Masui, 1975; Dorée, 1982; Gerhart et al., 1984). This property is referred to as MPF amplification.

In this work, we began to dissect the MPF amplification loop. More specifically, we investigated whether microinjection of a small amount of cdc2 kinase is sufficient to produce more cdc2 kinase activity in recipient oocytes. This was found to hold true in *Xenopus*, but not starfish oocytes. In the latter case, a factor sequestered in the germinal vesicle (GV), the large nucleus of G_2-arrested oocytes, was found to be required for cdc2 kinase activation. It could be bypassed by specific inhibition of type 2A phosphatase, suggesting that cdc2 kinase and an unidentified inhibitor of type 2A phosphatase act synergistically to induce MPF amplification.

Materials and Methods

Oocytes, Enucleations, and Microinjections

The starfishes *Astropecten aranciacus* and *Marthasterias glacialis* were collected during the breeding season near Banyuls, France. Prophase-arrested oocytes were prepared free of follicle cells by washing them several times in artificial Ca^{2+}-free sea water and finally transferred to natural sea water. *Xenopus* oocytes were prepared free of follicle cells either by manual dissection with forceps or collagenase treatment. We performed enucleations as previously described (Picard et al., 1988a) by sucking the germinal vesicle into a micropipette. Microinjections were performed according to Hiramoto (1974).

Cdc2 Kinase Preparation

Starfish cdc2 kinase (the cyclin B–cdc2 heterodimer) was prepared to apparent homogeneity as reported previously (Labbé et al., 1989a, 1991). Briefly, an homogenate was prepared from oocytes at first meiotic metaphase. The high-speed supernatant was passed through a DEAE-cellulose column and eluted at 200 mM NaCl. The eluate was applied to a column of Sepharose covalently bound to the yeast protein $p13^{suc1}$. After washing the column, the kinase was eluted with $p13^{suc1}$ in excess. The eluted kinase was applied on a Mono S column, and eluted at 330 mM NaCl. Its specific activity was $\sim$8 μmol of phosphate transferred to H1 histone per minute per milligram of proteins.

H_1 Histone Kinase Activities

Assays of purified H_1 histone kinase were performed as previously described (Labbé et al., 1989a, 1991). Assays of H_1 histone kinase in oocytes was as follows: microinjected oocytes were rapidly washed (<10 s) in an excess of buffer containing 50 mM β-glycerophosphate, 15 mM EGTA, 10 mM $MgCl_2$, and 0.7 mM DTT at pH 7.3; then the oocytes were taken in a small volume of buffer (2 μl for starfish oocytes, 50 μl for *Xenopus* oocytes) and frozen in liquid nitrogen. Immediately after thawing, which disrupted the oocytes, an identical volume of a mixture containing 40 mM Hepes, pH 7.3, 10 mM $MgCl_2$, 100 μM γ-^{32}P-ATP (10^3 cpm/pmol) and 2 mg/ml H_1 histone (Boehringer, Mannheim, Germany) was added. After various incubation times (from 0 to 10 min) at 23°C, the reaction was stopped by addition of Laemmli's buffer, then the proteins were separated by

SDS-PAGE. Autoradiography was performed on dried gels with X-OMAT-S films (Eastman Kodak Co., Rochester, NY), then parts of the gels were cut and counted by liquid scintillation. The amount of cdc2 kinase that catalyzes the transfer of 1 picomol of phosphate from ATP to H_1 histone per minute was taken as unit.

Immunoblotting

After electrophoresis, proteins were transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH). Before incubation with affinity-purified polyclonal antibodies raised in rabbits against bacterially produced cyclin B (Labbé et al., 1989a) or against the EGVPSTAIREISLLKE peptide (PSTAIR), a sequence conserved in cdc2 homologues, blots were treated with Tris-buffered saline (25 mM Tris, pH 7.5, 200 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$) containing 3% polyvinylpyrrolidone and 0.05% Tween 20. After antibody binding, the blots were washed; a second antibody directed against rabbit immunoglobulins and conjugated with alkaline phosphatase was then added. The blots were washed again and transferred in alkaline phosphatase buffer (100 mM Tris, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$ containing 66 μl of nitroblue tetrazolium and 66 μl 5-bromo-4-chloro-3 indolylphosphate for 20 ml of buffer). The staining reaction was stopped in 25 mM Tris, pH 7.5, 200 mM NaCl, 1 mM EDTA.

Phosphatase Activities

[^{32}P]Casein was prepared by incubating casein (0.2 mg) with the catalytic subunit of cAMP-dependent protein kinase from rabbit skeletal muscle (Beavo et al., 1974) for 4 h at 25°C in 100 μl of a solution containing 40 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, and 100 μM ATP (2,000 cpm/pmol). After incubation the mixture was extensively dialyzed overnight at 4°C against 50 mM Tris, pH 7.0, 150 mM NaCl, 1 mM EDTA, and 1 mM DTT.

Homogenates were prepared by freezing and thawing from 30 oocytes (packed volume, $\sim$0.2 μl) in 8 μl of the above buffer. To each homogenate was added 5 μl of the [^{32}P]casein solution, and incubation was allowed to proceed at room temperature for various times (from 0 to 20 min). Reactions were stopped by adding 1 vol of 2% SDS. Then proteins were separated by SDS-PAGE. Autoradiography was performed on dried gels.

Inhibitor 1 was an active thiophosphorylated peptide of inhibitor 1 (residues 9–41) prepared according to Aitken and Cohen (1982). Okadaic acid was dissolved in dimethylsulfoxide to give a 5 mM solution, and further diluted in aqueous buffer before use.

Results

Cdc2 Kinase Microinjection Is Sufficient to Activate a Preformed Cyclin B–cdc2 Complex in Xenopus But Not Starfish Oocytes

Cdc2 kinase was purified from starfish oocytes at first meiotic metaphase. It contained two polypeptides, cor-
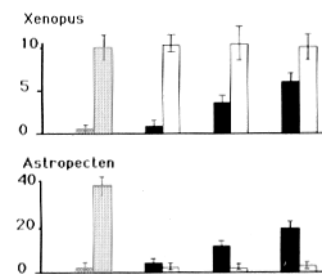
Figure 1. Changes of H1 histone kinase activities in recipient oocytes after microinjection of various amounts of purified cdc2 kinase. The upper panel refers to *Xenopus* oocytes, the lower one to starfish oocytes. H_1 histone kinase activities, expressed as picomoles of P transferred per minute per microliter of cytoplasm (oocyte volumes were taken as 1 μl for *Xenopus* and 8 nl for *Astropecten*), were measured from 5–10 individual oocytes for each experiment. Homogenates were prepared from individual oocytes either 10–15 s after microinjection (■) or 45 min (starfish) and 2 h (*Xenopus*) later (□). In control experiment (no cdc2 kinase injected), H_1 histone kinase activities were measured in homogenates prepared before hormonal stimulation (▨) or at the time of GVBD after hormonal stimulation (▤).
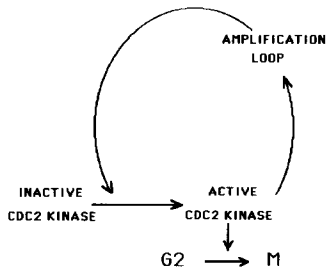
Figure 2. The cdc2 kinase amplification loop.

responding to intact cyclin B and p34^{cdc2} (Labbé et al., 1989a). Its MPF activity was tested by microinjection of 50 nl of solution into *Xenopus* oocytes (internal volume, ~1 μl). We found that an activity of 10 U/μl in the pipette was sufficient to promote germinal vesicle breakdown (GVBD), which occurred after cdc2 kinase activity had raised to ~10 U/μl in the recipient oocytes (Fig. 1). The same result was obtained whether or not protein synthesis occurred in the recipient oocytes, implying that amplification of histone H1 kinase entailed activation of preformed (but inactive) cdc2 kinase. In confirmation of this, we isolated the inactive cyclin B-p34^{cdc2} complex from G$_2$-arrested oocytes and found that the concentration of p34^{cdc2} and cyclin B was the same in unactivated and activated oocytes (data not shown; see also Gautier and Maller, 1991). Together, these results show that microinjection of active cdc2 kinase in *Xenopus* oocytes is sufficient to activate an amplification loop that promotes the conversion of an inactive cyclin B-p34^{cdc2} complex into its active form (Fig. 2).

By contrast, oocytes of the starfish *Astropecten aranciacus* did not undergo GVBD when the same preparation of purified cdc2 kinase was delivered to their cytoplasm by
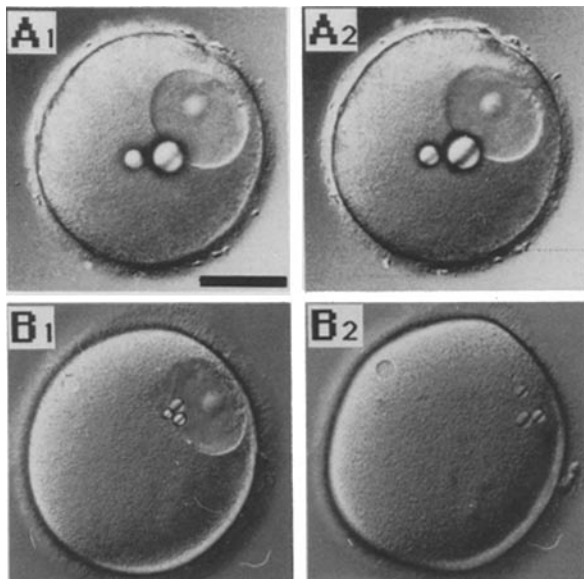

Figure 3. Cdc2 kinase induces the G$_2$ to M phase transition when microinjected into the GV, but not the cytoplasm of starfish oocytes. Oocyte A was microinjected with 1.5 nl of cdc2 kinase into the cytoplasm, oocyte B was microinjected with 0.4 nl of the same kinase preparation into the GV. Micrographs were taken immediately after microinjections (A_1, B_1), and 75 min (B_2) and 2 h after microinjection (A_2), respectively. Bar, 100 μm.

microinjection (Fig. 3 *A*), although like the frog oocytes, they contained a preformed cyclin B-p34^{cdc2} inactive complex (Strausfeld et al., 1991). Even high amounts of the purified cdc2 kinase, corresponding to about one half the total amount that would be formed upon hormonal oocyte activation, did not cause GVBD (Fig. 1), whereas direct transfer of one-tenth that amount (in terms of histone H1 kinase activity) of crude cytoplasm taken at the time of GVBD caused rapid GVBD in the recipient oocytes. We considered that the important difference was the admixture in the latter case of components from the germinal vesicle. To confirm this view, enucleated oocytes were stimulated by transfer of cytoplasm taken at the time of GVBD from hormone-stimulated oocytes. As expected, cdc2 kinase activation occurred in recipient oocytes (Fig. 4, sequence A_1-A_5). In contrast, neither cdc2 kinase activation nor GVBD occurred in second recipient oocytes when they received cytoplasm taken from enucleated oocytes (Fig. 4, E_1-E_2). Finally GVBD occurred and endogenous kinase was activated when oocytes received a small amount of the purified cdc2 kinase directly into the GV (Fig. 3 *B*). Together, the above results show that microinjection in the cytoplasm of native cdc2 kinase is not sufficient to activate the amplification loop in starfish oocytes, and suggest that its mixing with a nuclear factor originating from the GV is required to protect it from rapid inactivation upon transfer in G$_2$-arrested oocytes. Since [^{35}S]methionine-labeled starfish cyclin B does not undergo proteolysis when microinjected into starfish oocytes (data not shown), cdc2 kinase inactivation is not due to degradation of its cyclin B subunit. Possible mechanisms of inactivation include phosphorylation on inhibitory sites and dephosphorylation of stimulating sites on either the cdc2 or the cyclin B subunits (see Discussion).

Inhibition of Type 2A Phosphatase Mimics the Nuclear Factor Required for Microinjected cdc2 Kinase to Release Starfish Oocytes from G$_2$ Arrest

There are several indications that serine-threonine phosphatases play an important part in maintaining the G$_2$ arrest of oocytes. For example Lee et al. (1991) showed that INH, an inhibitor of MPF activation in *Xenopus*, corresponds to a type 2A phosphatase. Moreover, okadaic acid (OA), the powerful inhibitor of protein phosphatases types 1 and 2A obtained from dinoflagellates (see Cohen et al., 1990, for review) promotes oocyte maturation in several animal species (Goris et al., 1989; Rime and Ozon, 1990). This suggested that perhaps the component from the germinal vesicle might be a type 2A phosphatase inhibitor.

In starfish, OA induces MPF activation when injected to an intracellular concentration of 1-2 μM. This high concentration is required to titrate type 2A phosphatase, which is in the micromolar range in starfish oocytes (Picard et al., 1989). As a first test of the above hypothesis, we tried injecting purified cdc2 kinase into oocytes in the presence of low doses of akadaic acid. Indeed, we found that 0.3 μM OA potentiated GVBD in response to cdc2 kinase (Fig. 5). This potentiation could be overcome by microinjection of the catalytic subunit of type 2A phosphatase (data not shown), and could not be mimicked by inhibitors 1 and 2, which block the activity of type 1 phosphatase (Table I).

To test the idea that the GV contained an inhibitor of type 2A phosphatase, casein was phosphorylated by A kinase and
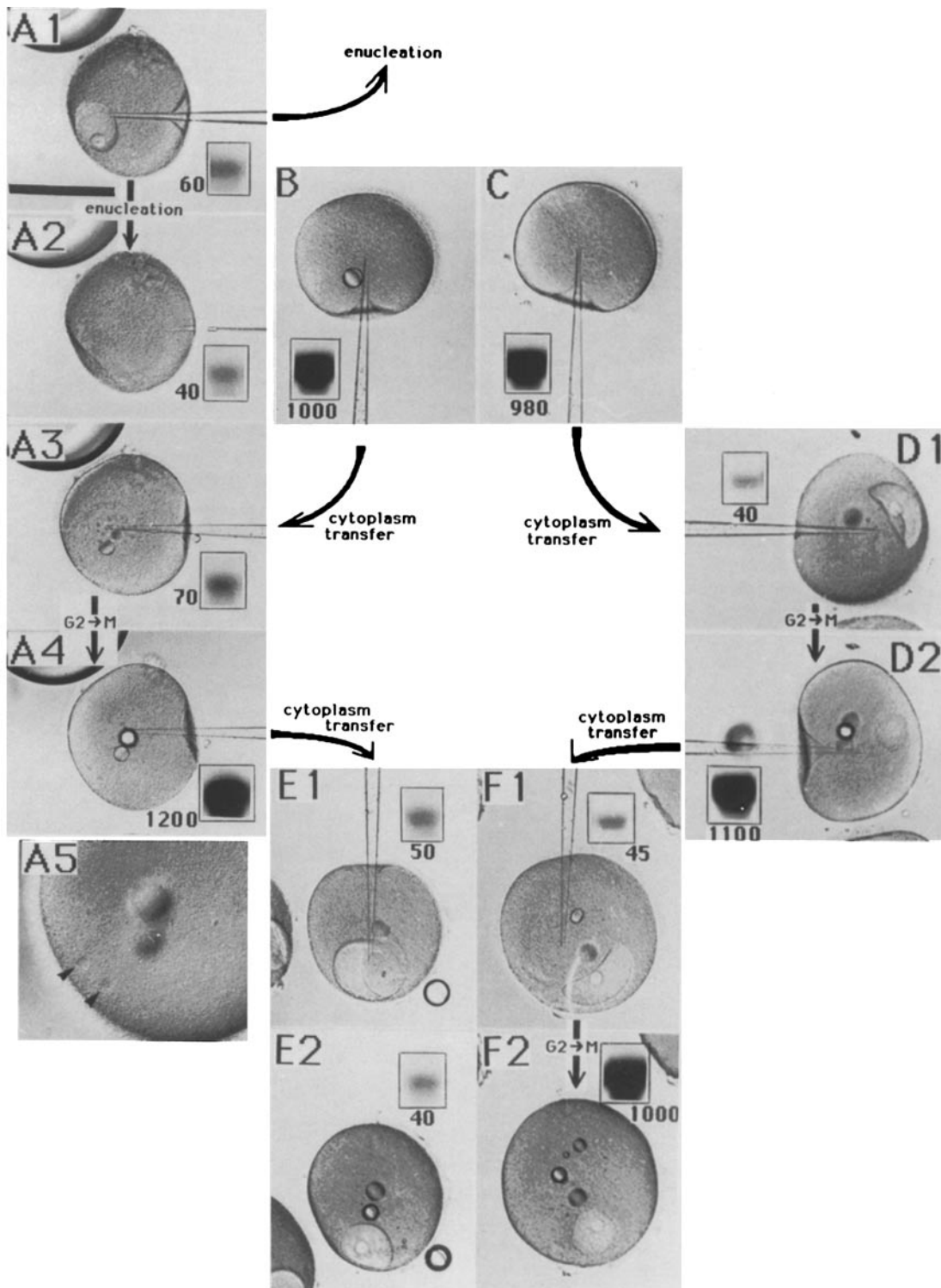
Figure 4. MPF amplification in a recipient oocyte requires a nuclear factor transferred with the donor cytoplasm. Oocyte A_2 was obtained by enucleating oocyte A_1. 20 min after enucleation (A_3) it received cytoplasm, taken 30 min after hormonal stimulation from a maturing oocyte (B). 30 min after the first cytoplasm transfer, cytoplasm was taken from the first recipient, now used as donor (A_4) and transferred into the cytoplasm of an intact oocyte (E_1), which remained arrested at G_2 even 2 h later (E_2). When the same sequence of experiments was made using a nucleated oocyte as first recipient (D_1, D_2), GVBD readily occurred in the second recipient (F_2) 20 min after cytoplasm transfer, shown in F_1. In each panel, the inset shows H_1 histone activity (estimated by the darkness of the H_1 histone spot from autoradiograms and the corresponding number of eluted counts per minute: see Materials and Methods). A_5 is a magnification of A_4; it shows that transfer of cytoplasm taken from the maturing oocyte B has induced, not only H_1 histone kinase activation, but also the nucleation of unusually big meiotic asters (*arrowheads*). Bar, 200 μm.
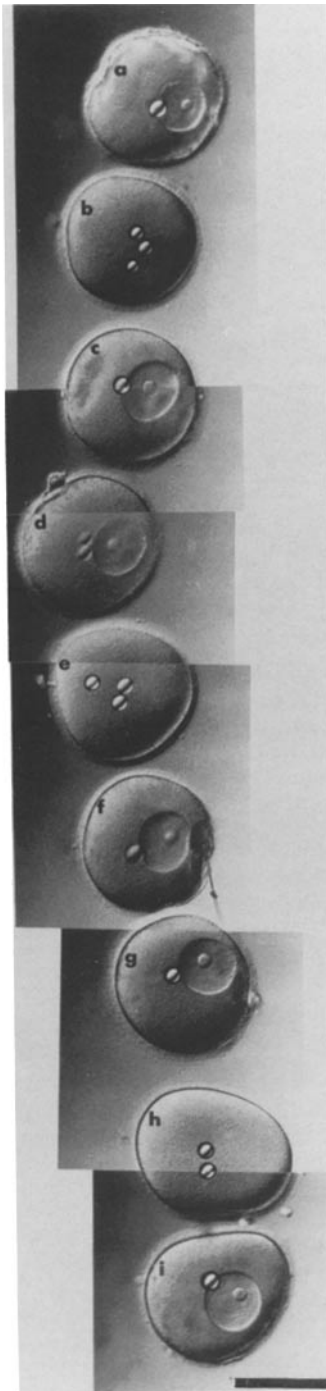
Figure 5. Microinjection of cdc2 kinase induces the G_2-to-M phase transition only if recipient starfish oocytes have been previously microinjected with an infraliminar amount (0.3 μM) of okadaic acid. Oocytes *a, b, d, e, g,* and *h* were microinjected with 50 pl of 50 μM OA (final intracellular concentration, $\sim$0.3 μM). Oocytes *b, c, e, f, h,* and *i* were then microinjected with 0.4 nl of cdc2 kinase, purified to apparent homogeneity (100 pmol of P transferred to H_1 histone per minute per microliter). Micrographs were taken 2 h after the last microinjection: only oocytes *b, e,* and *h* have been released from G_2 arrest. Bar, 200 μm.

Table I. Coinjection of Inhibitor 1 and 2 Does Not Help cdc2 Kinase to Release Starfish Oocytes from G2 Arrest

Coinjected compounds (*all 0.3 vM*)	Number of recipient oocytes with GVBD / Number of injected oocytes
None	0/10
Okadaic acid	10/10
Inhibitor 1	0/10
Inhibitor 2	0/10
Inhibitor 1 plus inhibitor 2	0/10

G_2-arrested oocytes of the starfish *Astropecten aranciacus* were injected, first with each phosphatase inhibitor to the indicated intracellular concentration, then (5 min later) with 0.4 nl of cdc2 kinase (specific activity: 10 picomol P transferred to H1 histone per minute per microliter). Oocytes with GVBD were scored 1 h later (percentages did not change subsequently).
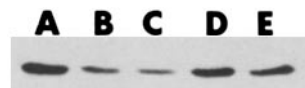
shown), suggesting that the GV may only indirectly exert a negative control on the activity of type 2A phosphatase.

We next tested whether microinjection of the content of the GV could induce oocyte maturation by itself, and it did (Fig. 7). In this experiment, cdc2 kinase activity was stimulated $\sim$20-fold in the recipient oocyte (Table II), although the GV does not contain any detectable H_1 histone kinase activity (data not shown), lacks cyclin B, and contains only a faint amount of cdc2 (Fig. 8). Moreover, such activation could be blocked by the simultaneous injection of p13^{suc1} (Table II), which is well known to block the activation of cdc2 kinase (Dunphy and Newport, 1989). Thus, oocyte activation appears to require the activation of cdc2 kinase, and the germinal vesicle contains an essential component of the amplification loop, which perhaps negatively controls type 2A phosphatase activity.

Discussion

The MPF amplification process is currently viewed as a regulatory loop of posttranslational events by which a low amount of microinjected cdc2 kinase acts as a primer, resulting in production of more cdc2 kinase from an inactive endogenous pool in G_2-arrested oocytes. In agreement with this view, we found that microinjection of active cdc2 kinase, prepared to apparent homogeneity from starfish oocytes, is sufficient to activate the MPF amplification loop in *Xenopus* oocytes.

Nonetheless, cdc2 kinase failed to drive the inactive cyclin B–cdc2 precursor into its active form when microinjected

used as a substrate to compare the phosphatase activities of homogenates of enucleated and intact starfish oocytes. Dephosphorylation of casein was completely inhibited by 3 nM OA, and not significantly by 100 nM inhibitor 1 in diluted homogenates. Therefore, it was due almost exclusively to type 2A phosphatase, as previously reported (Cormier et al., 1990). As expected, the phosphatase activity of the enucleated oocytes was reproducibly higher than that of intact oocytes (Fig. 6). Nonetheless, no inhibition of type 2A phosphatase could be detected when the GV contents were directly assayed on purified type 2A phosphatase (data not

A B C D E

*Figure 6.* Type 2A phosphatase activity is higher in extracts prepared from enucleated than from nucleated oocytes. Phosphatase activities were compared by monitoring the amount of residual [^{32}P]casein on autoradiograms after incubating for 20 min at 25°C extracts prepared from 30 oocytes, nucleated or enucleated, in the presence of [^{32}P]casein (see Materials and Methods). *A,* nucleated oocytes; *B,* enucleated oocytes; *C,* enucleated oocytes plus 100 nM inhibitor 1; *D,* enucleated oocytes plus 3 nM okadaic acid; *E,* the amount of [^{32}P]casein added at zero time in each of the above experiments was run on the same gel (control).
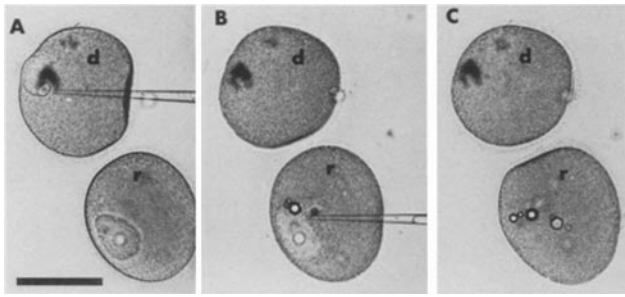
Figure 7. Microinjection of the content of one germinal vesicle induces the G2-to-M phase transition in recipient oocytes. (*A*) The content of a GV is being sucked from a donor oocyte (*d*). (*B*) It is being microinjected into the cytoplasm (near the GV in this experiment, but this is not necessary for successful transfer) of a recipient oocyte (*r*). (*C*) 1 h later GVBD had occurred in the recipient oocyte. Bar, 200 μm.

into oocytes of the starfish *Astropecten aranciacus*. Rather, the injected kinase underwent rapid inactivation in recipient oocytes. This suggests that another component exerts a negative control and may neutralize the priming effect of injected cdc2 kinase.

Why does purified cdc2 kinase undergo inactivation when it is microinjected into G$_2$-arrested starfish oocytes? As already emphasized, it is not due to degradation of its cyclin B subunit. It is also unlikely that cyclin dephosphorylation could be sufficient to inactivate cdc2 kinase, since extensive activation can be triggered in extracts prepared from G$_2$-arrested amphibian or starfish oocytes—which contain the dephosphorylated form of cyclin—either by depleting ATP
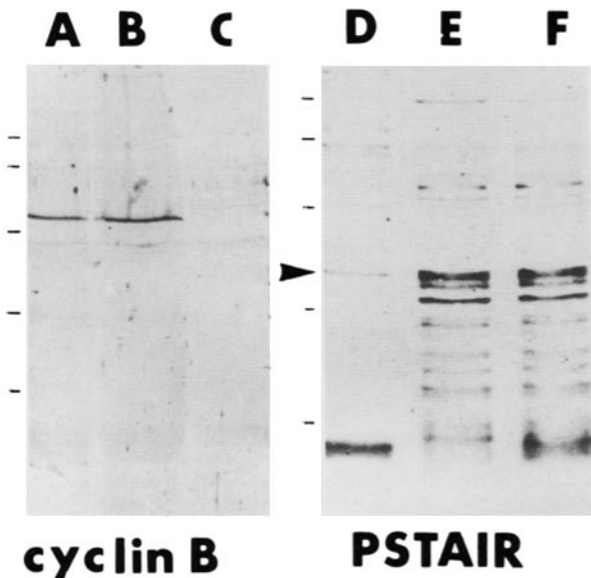
Figure 8. Cyclin B is localized exclusively in the cytoplasm of G$_2$-arrested starfish oocytes, and only a faint amount of cdc2 is in the GV. Western blots were made from 10 intact oocytes (lanes *A* and *F*), 10 enucleated oocytes (lanes *B* and *E*) or 60 whole GV (lanes *C* and *D*) using polyclonal antiserums directed against starfish cyclin B, or a 16-amino acid peptide corresponding to the PSTAIR domain of p34^{cdc2}. The arrowhead points to p34^{cdc2} on the PSTAIR immunoblot.

Table II. Microinjection of the Content of a GV Is Sufficient to Activate cdc2 Kinase and to Release Starfish Oocytes from G$_2$ Arrest

Microinjected material	Cdc2 kinase activity*		GVBD
	t_0	t_{50}	
GV alone	15 ± 6	330 ± 70	10/10
GV plus p13^{suc1}	16 ± 5	18 ± 9	0/10

G$_2$-arrested oocytes were injected either with the content of a single GV alone, or with both a GV and 10 μM p13^{suc1}. H$_1$ histone kinase activities were measured in homogenates prepared from individual oocytes, either before or 50 min after microinjections. GVBD occurred earlier than 50 min after microinjection of GV material alone. It never occurred when p13^{suc1} was coinjected. Cdc2 kinase activities are expressed as femtomoles of P transferred to H$_1$ histone per minute per oocyte (each result obtained from 10 oocytes).

(Labbé et al., 1988*a, b*, 1989*b*) or by adding phosphatases of broad specificity in the absence of ATP (Jessus et al., 1990; Pondaven et al., 1990). Furthermore, in the absence of ATP, the mitotic inducer cdc25$^+$ readily activates the cyclin B–cdc2 complex prepared from G$_2$-arrested oocytes (Kumagai and Dunphy, 1991; Strausfeld et al., 1991). Therefore, inactivation is most likely due to rephosphorylation of the microinjected kinase on inhibitory sites of its cdc2 subunit (Labbé et al., 1989*b*; Gautier et al., 1989; Dunphy and Newport, 1989; Morla et al., 1989; Picard et al., 1989; Gould and Nurse, 1989), and inhibition of type 2A phosphatase antagonizes this process in the cytoplasm of G$_2$-arrested oocytes.

The inhibitory kinases that phosphorylate cdc2 have not been identified in oocytes, but there is genetic evidence in fission yeast that at least two protein kinases negatively control cdc2 kinase activity. One is p107^{wee1}, which is able to phosphorylate both tyrosine and serine/threonine residues, at least in vitro (Featherstone and Russel, 1991). The other is p^{mik1}, whose specificity is not known (Lundgren et al., 1991). The wee1$^+$ gene product, at least, is negatively controlled by another protein kinase, p^{niml}. Interestingly, mutants in *ppa2*, a gene which encodes a homologue of type 2A phosphatase, have the same phenotype as mutants in *wee1*$^+$ and *mik1*$^+$ and enter M phase at a reduced size (Kinoshita et al., 1990). This supports the view that type 2A phosphatase antagonizes protein kinases, whose function is to cancel the inhibitory effect of p107^{wee1} and p^{mik1} on cdc2 kinase activity. If such a network of inhibitory controls exists in G$_2$-arrested oocytes, it could explain why partial inhibition of type 2A phosphatase potentiates GVBD in response to cdc2 kinase microinjection, and why complete inhibition of type 2A phosphatase is sufficient to induce GVBD in the absence of any other treatment. The alternative hypothesis that inhibition of type 2A phosphatase might increase the activity of a putative cdc2 phosphatase (Dorée et al., 1989) is now unlikely, since the unphosphorylated cdc25$^+$ gene product appears sufficient to induce cdc2 dephosphorylation and activation, at least in vitro (Strausfeld et al., 1991).

Several years ago, we proposed that an unidentified factor originating from the GV was required in addition to cdc2 kinase for production of transferable MPF activity in starfish oocytes (Picard et al., 1988*b*; see also Kishimoto et al., 1981; Picard and Dorée, 1984). Indeed, we had found that cdc2 kinase reaches its maximal activity after hormonal stimulation well before cytoplasm becomes able to induce

GVBD in recipient oocytes (Labbé et al., 1987). Moreover, cytoplasm taken from stimulated enucleated oocytes was found to lack transferable MPF activity, although they produced as much cdc2 kinase activity as nucleated oocytes. More recently, we showed that enucleated oocytes can produce MPF activity upon hormonal stimulation if type 2A phosphatase is partially depressed (by about one-third) by microinjecting 0.3 μM OA. In fact, MPF activity is produced even in the absence of hormonal stimulation in enucleated oocytes if they receive a dose of OA sufficient to titrate completely type 2A phosphatase (Dorée et al., 1991). Since blocking type 1 phosphatase only with both inhibitor 1 or 2 had no such effect, the above experiments demonstrated that specific inhibition of type 2A phosphatase suppresses the requirement for the GV component in the process of MPF amplification. In agreement with this view, we found in the present work that partial inhibition of type 2A phosphatase in recipient oocytes potentiates GVBD in response to cdc2 kinase, as does its admixture with components of the GV.

The active component of the GV has not yet been identified. We found, however, that type 2A phosphatase activity is higher in homogenates made from enucleated than from nucleated oocytes. Moreover, microinjection of GV material activates by itself cdc2 kinase in starfish oocytes, as does that of 1 μM OA. This may indicate that the GV contains an element that interacts physically with type 2A phosphatase to depress its activity. Alternatively the GV may only indirectly control type 2A phosphatase activity via a loop of as yet unidentified regulatory events. Whatever the explanation, the inhibitory element would shuttle in the cytoplasm before GVBD since cytoplasm becomes able to induce GVBD in recipient oocytes just before the first cytological marks of nuclear envelope disorganization ("GV fading") in donor oocytes. We should notice that the GV component would have escaped detection if its translocation had occurred before hormonal stimulation. Perhaps such a translocation occurs earlier in species like *Xenopus*, which can produce MPF in the absence of GV (Smith and Ecker, 1969; Dabauvalle et al., 1988). Removal of the GV has also been shown to delay or to suppress appearance of transferable MPF activity after hormonal stimulation in two different amphibian species (Gautier, 1987; Skoblina et al., 1984) suggesting that a requirement for a GV component in the process of MPF amplification is not restricted to starfish oocytes. Identification of this GV component will be a major step in understanding the amplification loop for MPF amplification.

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