In vivo regulation of MPF in Xenopus oocytes

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Summary

Entry into M phase in the eukaryotic cell cycle is controlled by the oscillating activity of MPF. The active component of MPF is now known to be the p34cdc2 protein kinase originally found in yeast. The p34cdc2 protein kinase displays a characteristic M-phase-specific histone H1 kinase activity when it interacts with cyclins, which are proteins that oscillate through the cell cycle and are thought to regulate p34cdc2 activity. Cyclins can induce M phase when introduced into fully grown Xenopus oocytes and cyclin may play a role in normal oocyte maturation. Small Xenopus oocytes do not mature in response to the hormonal triggers which act on stage 6 oocytes. We introduced cyclin into stage 4 (small) Xenopus oocytes and showed that it activates MPF in these cells, probably by interacting with endogenous p34cdc2 kinase. We made labelled extracts from cyclin-mRNA-injected stage 4 oocytes and used them to show differential stability of clam cyclins A and B at oocyte maturation. The relative stability of the two forms of cyclin related directly to their ability to stabilize crude MPF preparations from injected stage 6 oocytes.

Key words: Xenopus oocyte, in vivo regulation, MPF, cell cycle, cyclin.

Introduction

Full-grown amphibian oocytes arrested in late G2 of meiosis I, must mature to the second meiotic metaphase before fertilization is possible. Oocyte maturation is induced by an action of progesterone at the oocyte surface. This leads to appearance of a cytoplasmic maturation-promoting factor (MPF), which induces nuclear membrane breakdown and the subsequent meiotic events. MPF was first identified by injecting cytoplasm from maturing oocytes into oocytes not exposed to steroid hormone; recipients underwent maturation (Masui and Markert, 1971; Smith and Ecker, 1971). Using this assay, MPF subsequently was identified in mitotically cleaving embryonic cells (Wasserman and Smith, 1978; Gerhart et al. 1984) as an activity that peaked at each M phase and then disappeared. These and several additional studies on diverse cell types have established MPF as a universal M-phase-inducing factor in eukaryotic cells (Gerhart et al. 1985).

In spite of intense effort for some time, MPF has only recently been characterized. Lohka et al. (1988) obtained a highly purified egg fraction exhibiting MPF activity which contained two predominant proteins, one of 34 x 10^3 and one of 45 x 10^3 Mr, respectively. The 34K protein has been identified as the Xenopus laevis homolog of a fission yeast protein encoded by the gene cdc2+ which is required for the G2-M transition in the yeast mitotic cell cycle. This protein is a serine/threonine that exhibits a strong preference for histone H1 as a substrate; histone H1 kinase activity cycles during cell division coincident with MPF activity (review by Smith, 1989). The second protein has been identified as a Xenopus cyclin (Gautier et al. 1990).

Cyclins were originally identified because of periodic synthesis and degradation during mitotic divisions in cleaving sea urchin and clam embryos (Evans et al. 1983).

A number of studies have shown that p34cdc2 levels remain constant throughout the mitotic cell cycle (Draetta et al. 1989; labbe et al. 1989; Meijer et al. 1989; Moreno et al. 1989), suggesting that the periodic synthesis and turnover of cyclin regulates the periodic bursts of MPF activity. In support of this, cyclin is known to associate with p34cdc2 to form active H1 kinase (Draetta et al. 1989; Meijer et al. 1989), inhibition of cyclin synthesis prevents activation of MPF activity in egg extracts (Minshull et al. 1989), and preventing the destruction of cyclin prevents inactivation of MPF activity and maintains M-phase arrest (Murray et al. 1989; Luca and Ruderman, 1989).

Further, Murray and Kirschner (1989) have shown directly that synthesis of cyclin is the only requirement for activation of MPF activity in a Xenopus egg extract. The activation of MPF during meiosis I appears to be more complicated.

Full-grown (stage 6) Xenopus oocytes normally activate MPF in response to progesterone by a process that
progestrone induces the synthesis of cyclin, which then leads to active MPF. However, it has been clear for some time that stage 6 oocytes contain a precursor form of MPF, pre-MPF, which can be activated and amplified post-translationally by small amounts of active MPF (Gerhart et al. 1984). Furthermore, Cyert and Kirschner (1988) have reported that pre-MPF can activate spontaneously in an oocyte extract, requiring only ATP for activation. These observations suggest that stage 6 oocytes already contain both p34<sup>cdc2</sup> and cyclin in inactive form (see Barrett et al. 1990). In this regard, Westendorf et al. (1989) have demonstrated that clam oocytes contain a pool of sequestered cyclin, which is released by the stimulus (fertilization) that induces maturation.

Reports that MPF could be continually amplified by serial transfers of cytoplasm containing MPF through multiple recipients initially implied that oocytes contain a store of inactive MPF (Reynhout and Smith, 1974; Drury et al. 1975). Based on this biological assay, Taylor and Smith (1987) demonstrated that pre-MPF existed in stage 4 oocytes as small as 750 µm in diameter, but not in smaller oocytes. This suggests that one or more components of MPF are not yet present in early stage 4 oocytes. The present study was initiated to test this possibility. The results show that p34<sup>cdc2</sup> is present in oocytes as small as stage 3. Further, injection of cyclin mRNA into oocytes at all stages induces active MPF as assayed by the breakdown of the oocyte nucleus. Surprisingly, stable MPF activity can be extracted from oocytes that overexpress cyclin protein by homogenization in simple saline buffers. These results are discussed relative to the role of cyclin in regulating MPF activity in vivo.

Materials and methods

**Antibodies and cDNA clones**

Antisera against p34<sup>cdc2</sup> used in these studies was provided by Dr G. Draetta of the Cold Spring Harbor Laboratory. Plasmids pAXH(+) carrying a clam cyclin A cDNA, and pCD102 encoding clam cyclin B were gifts from Dr J. Ruderman of Duke University.

**Manipulation and injection of oocytes**

Oocytes at all stages of oogenesis used in these studies were manually defolliculated, and maintained in OR 2 medium (Wallace et al. 1973). When maturation was induced, oocytes were incubated in 10 µg/ml progesterone in OR2. Protein synthesis was arrested by incubating oocytes in 10 µg/ml cycloheximide in OR2 for 30min prior to injection of materials, and in the continuous presence of cycloheximide thereafter. The injection of either donor cytoplasm or cytosolic extracts (50nl) was performed in healing medium (Ford and Gurdon, 1977) with or without cycloheximide. Following injection, the oocytes were incubated in this medium for an additional 30 min to promote healing, and then transferred to OR 2.

**Preparation of transcripts**

Transcripts were synthesized with SP6 RNA polymerase according to the conditions optimized by Krieg and Melton (1984) with the following modifications: 7mGpppG (Pharmacia) was included in the reaction at 1 mM, the concentration of GTP was 100 µM, and that of SP6 polymerase was 120 units/100µl. The reaction was incubated at 40°C for 2h before treatment with DNase. After extraction and ethanol precipitation, the reaction was passed over a NICK column (Pharmacia) equilibrated with 0.3M sodium acetate, pH 5.2, and precipitated again with ethanol. These conditions routinely yielded about 2 µg of translatable RNA/reaction. The RNA pellet was finally resuspended in H2O at a concentration of 0.5 to 1 mg ml<sup>-1</sup>.

**Western blots**

A piece of ovary was removed from frogs anesthetized by hypothermia, and the ovary was digested in 2% collagenase in 0.1M NaPO<sub>4</sub>, pH 7.4, at room temperature. This treatment has been shown to completely remove follicle cells from oocytes (Horrell et al. 1987). The oocytes were rinsed extensively in OR2, and sized with an ocular micrometer. 5 to 10 oocytes were homogenized in 0.5 to 1 ml buffer containing 50 mM NaCl, and 0.5 mM PMSF. The homogenates were centrifuged at 14,000 g and the supernatant was precipitated with 4 vol acetone. Pellets were dissolved by boiling in Laemmli buffer and electrophoresed on 10% polyacrylamide. The gels were transferred to nitrocellulose overnight at 30 volts in a Trans blotter (Bio-RAD). The blots were blocked in 3% BSA and probed with a 1000:1 dilution of antibody to p34<sup>cdc2</sup> in 3% BSA in Tris-saline. The antibody was detected with [125]I protein A from ICN at a final specific activity of 0.5 µCi/ml<sup>-1</sup> in 1 mM NaCl, Tris pH 8.0.

**Translation of synthetic mRNAs**

Oocytes incubated in OR2 were injected individually with 5 to 15 nl delivering 5 to 10 ng of cyclin mRNA per oocyte. The oocytes were transferred to OR2 containing 100 µCi/ml<sup>-1</sup> [35S]methionine (trans label) from ICN, and incubated for 6 to 7h. The oocytes were processed as described above for electrophoresis, or extracts were prepared as described below. Gels were impregnated with Amplify (Amersham), and used for fluorography. It is usually possible to develop fluorographs from a single stage 4 oocyte with an overnight exposure under these conditions. In vitro translation was performed with a nuclease-treated reticulocyte lysate (Promega) using the recommended conditions, except that about 1/10 of the recommended volume of extract was loaded on gels for electrophoresis.

**Scoring for GVBD**

Stage 6 oocytes were scored for GVBD by the appearance of a white spot in the animal hemisphere, confirmed by dissection after fixation of oocytes in 0.5 n perchloric acid (PCA). In some cases, oocytes treated with cycloheximide failed to demonstrate a white spot, even if GVBD had occurred. Therefore, in these experiments, oocytes were incubated until a white spot had appeared in oocytes not incubated in cycloheximide, usually 3 to 4 h, after which they were fixed in 0.5 n PCA and dissected. Stage 4 oocytes also do not exhibit a white spot indicative of GVBD and these oocytes were always dissected in PCA at a time equivalent to GVBD (white spot) in stage 6 oocytes injected with extracts.
Preparation of extracts

For the preparation of cyclin containing extracts from stage 4 oocytes, 50 to 100 stage 4 oocytes of 650 to 700 μm diameter were injected with 5 to 10 ng of cyclin mRNA. These oocytes were then incubated in trans label until companion unlabelled stage 4 oocytes had undergone GVBD (usually about 4 to 6 h). At this time the oocytes were rinsed once in extraction buffer containing 50 mM NaCl, 10 mM Tris pH 7.4. The oocytes were collected and allowed to settle to the bottom of a microcentrifuge tube. As much excess extraction buffer as possible was removed, and the oocytes were homogenized with a heat-sealed micropipet tip. This homogenate was then centrifuged for 5 min at 14,000 g in a microcentrifuge. The supernatant was collected and used directly, or was frozen on dry ice and stored at -70°C. Before injection, an extract was always briefly centrifuged to remove debris. Extracts from stage 6 oocytes were prepared using the same procedures described for stage 4 oocytes with the exception that only 10 to 20 cells were used.

Results

Cyclin activates MPF in small oocytes

Cyclins are characterized as being either type A or type B depending on their degree of sequence homology to the two cyclins identified in clams (Swenson et al. 1989). Either type A or B cyclins are reported to induce maturation when the respective mRNAs are microinjected into stage 6 Xenopus oocytes (Swenson et al. 1986; Pines and Hunt, 1987; Westendorf et al. 1989). Initial studies to test the effects of cyclin on MPF activation in stage 4 oocytes showed that both mRNAs (5 ng/oocyte) induced GVBD when injected into oocytes of about 750 μm in diameter. Additional experiments were performed in which cyclin A mRNA only was injected into oocytes as small as 650 μm in diameter. In this case also, GVBD was observed in 98% of the recipient oocytes. In order to verify that the injected mRNA actually resulted in active MPF, cyclin A mRNA was injected into the smallest oocytes and, after GVBD had occurred, cytoplasm from these oocytes was injected into stage 6 oocytes treated with cycloheximide. The induction of GVBD in the absence of protein synthesis is considered diagnostic for the presence of MPF activity (Gerhart et al. 1984, 1985). As shown in Table 1, a high percentage of the stage 6 recipients underwent GVBD in response to the injected cytoplasm.

Cyclin alone is not known to induce GVBD in the absence of p34cdc2. Thus, the simplest explanation to explain the results above is that stage 4 oocytes already contain p34cdc2 protein. To test this directly, protein isolated from stage 3 to 6 oocytes was electrophoresed on polyacrylamide gels, transferred to nitrocellulose and reacted with antibody to p34cdc2. Fig. 1 demonstrates that the protein is present in oocytes of all stages examined. Based on the relative intensity of the bands, the p34cdc2 protein appears to accumulate through oogenesis.

Extracts from cyclin-A-mRNA-injected oocytes induce GVBD in stage 6 recipients

Murray and Kirschner (1989) demonstrated that cyclin protein translated in a reticulocyte lysate was sufficient to induce MPF activity when added to a cell-free egg extract. However, our attempts to induce GVBD in stage 6 oocytes by injecting cyclin-mRNA-directed reticulocyte lysates have been unsuccessful (data not shown). One possibility for the failure of cyclin-mRNA-directed lysates to activate MPF in vivo might be that an insufficient mass of cyclin was produced in the lysates. Alternatively, it is possible that these lysates do not direct appropriate post-translational modification of the protein required for interaction with p34cdc2 in vivo. To test this possibility, we prepared an extract from stage 4 oocytes previously injected with cyclin mRNA.

Table 1. Cyclin mRNA induction of MPF in oocytes

<table>
<thead>
<tr>
<th>Treatment of recipients</th>
<th>% GVBD in recipients incubated in:</th>
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<tbody>
<tr>
<td>Progesterone</td>
<td>OR2 (30)</td>
</tr>
<tr>
<td>Cyclin A mRNA</td>
<td>OR2+Cyclohex (20)</td>
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<th>Injection of cytoplasm from:</th>
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<tbody>
<tr>
<td>Stage 6</td>
</tr>
<tr>
<td>Stage 6+prog.</td>
</tr>
<tr>
<td>Stage 6+cyclin A mRNA</td>
</tr>
<tr>
<td>Stage 4+cyclin A mRNA</td>
</tr>
<tr>
<td>Stage 6+cyclin A mRNA</td>
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</tbody>
</table>

Stage 6 and stage 4 oocytes (650–700 μm in diameter) were injected individually with 5 ng cyclin A mRNA. For cytoplasmic transfers, recipient stage 6 oocytes were injected with 10 nl cytoplasm from donor oocytes. Stage 6+cyclin mRNA injected refers to oocytes incubated in progesterone (10 μg/ml−1) and exhibiting a white spot (usually 6–7 hours after steroid exposure). Stage 6+cyclin mRNA injected refers to oocytes taken at the time of GVBD (about 3–4 hours after injection). Stage 6 refers to control oocytes (no steroid exposure). Stage 4+cyclin mRNA refers to donors used at a time when companion mRNA-injected oocytes had undergone GVBD as determined by dissection (about 6 hours after injection). For injection of extracts, stage 4 or stage 6 oocytes previously injected with 5 ng cyclin A mRNA were homogenized at the time of GVBD and the extracts (see Materials and methods) were frozen on dry ice for use the next day. Recipients (stage 6) were injected with 50 nl of the thawed extract. The total number of recipient oocytes assayed in each experiment is indicated in parentheses.

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Cyclin A synthesis in these oocytes to be as high as 7 ng oocyte−1. Furthermore, since cyclin mRNA generates MPF activity in these cells, it seems likely that any pre-requisite post-translational modifications have occurred.

Fig. 3 shows a series of dilutions of a radioactive
extract from cyclin-A-mRNA-injected stage 4 oocytes; the extract had been frozen and thawed and incubated on ice for 30 min (the average duration of experiments). The cyclin A protein appears to be completely stable under these conditions. When the extract was injected into cycloheximide-treated stage 6 oocytes, GVBD was induced in 3 to 4 h (Table 1), while progesterone-treated control oocytes exhibited GVBD in about 7 h. The precocious induction of GVBD by cyclin compared to progesterone has been observed previously in experiments involving injection of different amounts of cyclin mRNA (Westendorf et al. 1989) and presumably is a function of cyclin protein concentration. In support of this, the stage 4 extract retained full activity (GVBD at 3 h after injection) at a dilution of 1:4, but then began to decrease. At a 1:8 dilution, GVBD occurred in 25% of the oocytes at 6 h after injection, in 50% of the oocytes at 8.5 h with a 1:16 dilution, and in 12.5 h in 16% of the oocytes after a 1:32 dilution. Further dilutions resulted in loss of activity.

The extraction of relatively stable MPF activity from cyclin-mRNA-injected stage 4 oocytes by homogenization in a simple buffer was unexpected since similar approaches with maturing (non-injected) stage 6 oocytes have not been successful. To determine if this was due to the overexpression of cyclin protein and not some other difference between stage 4 and stage 6 oocytes, stage 6 oocytes injected with cyclin A mRNA were homogenized and the crude extracts were tested for MPF activity. The data from these experiments are presented in Tables 1 and 2. Extracts prepared from cyclin-A-mRNA-injected oocytes when 50% (GVBD$_{50}$) and 100% (GVBD$_{100}$) of the oocytes exhibited GVBD was capable of inducing GVBD in recipients, even when the recipient oocytes had been treated with cycloheximide. Similar results were obtained when stage 6 oocytes injected with cyclin B mRNA were homogenized at GVBD$_{50}$, but no MPF activity was present in extracts prepared at GVBD$_{100}$ (Table 2).
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Table 2. MPF activity in extracts from cyclin B-injected stage 6 oocytes

<table>
<thead>
<tr>
<th>Source of extract</th>
<th>% GVBD in recipients</th>
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<tbody>
<tr>
<td>Cyclin A mRNA-injected oocytes at GVBD50</td>
<td>96 (30)</td>
</tr>
<tr>
<td>Cyclin B mRNA-injected oocytes at GVBD50</td>
<td>76 (23)</td>
</tr>
<tr>
<td>Progestosterone-treated oocytes at GVBD50</td>
<td>0 (10)</td>
</tr>
<tr>
<td>Cyclin A mRNA-injected oocytes at GVBD100</td>
<td>97 (23)</td>
</tr>
<tr>
<td>Cyclin B mRNA-injected oocytes at GVBD100</td>
<td>0 (23)</td>
</tr>
<tr>
<td>Progestosterone-treated oocytes at GVBD100</td>
<td>0 (10)</td>
</tr>
</tbody>
</table>

Stage 6 oocytes were injected with 5 ng of cyclin A or cyclin B mRNA, or treated with progesterone, and the oocytes were then incubated until either 50% (GVBD50) or 100% (GVBD100) of the oocytes exhibited GVBD. At this time, the oocytes were used for the preparation of extracts as described in Materials and methods and Table 1. Stage 6 recipients were each injected with 50 nl of extract. In all cases, the maximum percentage of GVBD was observed within 3 hours after injection while control stage 6 oocytes treated with progesterone exhibited GVBD in about 8 hours. The number of oocytes assayed in each experiment is shown in parentheses.

Discussion

Extraction of stable MPF from cyclin-mRNA-injected oocytes

The most surprising result from the current study is that a relatively stable MPF activity can be obtained from cyclin-mRNA-injected oocytes by homogenization in simple buffer containing EGTA, protease and phosphatase inhibitors. Previous attempts to extract MPF under these conditions have been uniformly unsuccessful (Drury, 1978; Wu and Gerhart, 1980), although Felix et al. (1989) have obtained an activity that is stable for a few hours at room temperature by homogenizing unfertilized Xenopus eggs in acetate buffer containing EGTA. The requirement for phosphatase inhibitors has been especially crucial in extraction and purification strategies, although the reason for this is not yet clear (see later). The presence of EGTA is required to prevent calcium-induced inactivation of cytostatic factor (review by Masui and Shibuya, 1987), which acts to stabilize MPF activity in metaphase-arrested eggs (Murray et al. 1989). Cytostatic factor (CSF) appears to be equivalent to the c-mos proto-oncogene product (Sagata et al. 1989a). This raises the obvious question, why can MPF activity be extracted with relative ease from oocytes that overexpress cyclin protein?
Fig. 4. Cyclin A is stable after injection into oocytes. An extract was prepared from stage 4 oocytes (700 μm diameter) previously injected with 5 ng/oocyte cyclin A mRNA and 50 nl/oocyte was injected into stage 6 recipients incubated in cycloheximide. Each lane represents the equivalent of protein from 4 oocytes; equal radioactivity was loaded in each lane. Variability of label recovered from each group of oocytes was about 7%. Lane 1, 1 μl of reticulocyte lysate to which cyclin A mRNA was added (see Fig. 3); lane 2, 200 nl of original extract (4 oocyte equivalents); lane 3, oocytes processed at time 0; lane 4, oocytes processed at 1 h post-injection; lane 5, oocytes processed at 2 h post-injection; lane 6, oocytes processed at 3.5 h post-injection (30 min after 100% GVBD was observed in this population of oocytes). Arrow shows position of cyclin A protein.

One explanation is that oocytes that have completed maturation normally contain relatively small amounts of cyclin, and these are reduced further during the multiple and time-consuming chromatographic steps used for purification (Lohka et al. 1988). This might explain why recovery of MPF has been about 1% of the starting activity. There are several reasons for believing this. For example, Meijer et al. (1989) have reported that the ratio of p34cdc2 to cyclin in cleaving sea urchin embryos is about 15:1. Gautier et al. (1989) have reported further that only about 10% of the p34cdc2 kinase actually functions as MPF during Xenopus oocyte maturation, suggesting insufficient levels of cyclin to interact with all p34cdc2. In addition, MPF purified from starfish oocytes by a procedure requiring multiple chromatographic steps (recovery about 1%) contained only p34cdc2 but not cyclin, while use of a rapid purification procedure on maturing starfish oocytes yielded about a 50% recovery and the MPF contains both the cdc2 protein as well as cyclin B in a 1:1 stoichiometric ratio (Labbe et al. 1989).

The above discussion suggests that maintenance of high cyclin levels during extraction and purification procedures is a major prerequisite for the recovery of maximal p34cdc2 kinase, hence MPF activity. By the same token, we suggest that the overproduction of cyclin protein in mRNA-injected oocytes generates a high cyclin to p34cdc2 ratio, facilitating the relatively simple extraction of MPF activity from small numbers of oocytes. The mechanism by which cyclin functions in this regard is not clear. However, several studies have now demonstrated that p34cdc2 kinase is active as MPF only when it is dephosphorylated (Gautier et al. 1989;
Dunphy and Newport, 1989; Labbe et al. 1989). Thus, the simplest suggestion is that interaction of cyclin with active p34\textsuperscript{cdc2} kinase prevents phosphorylation and inactivation of the kinase.

**The role of cyclin in MPF activation**

There appears to be little doubt that the periodic synthesis and degradation and cyclin regulates MPF activity during mitosis as well as during the progression from meiosis I to meiosis II. Since the induction of meiosis I in *Xenopus* oocytes requires protein synthesis, it is reasonable to suggest that progesterone stimulation leads to the synthesis of cyclin, which then interacts with stored p34\textsuperscript{cdc2} to produce active MPF. However, while newly synthesized cyclin has been detected in cleaving embryos (Murray and Kirschner, 1989), it has not been observed prior to completion of the first meiotic division (Minshull et al. 1989). On the other hand, recent evidence indicates that *Xenopus* oocytes contain a store of cyclin B already complexed with p34\textsuperscript{cdc2} (see Barrett et al. 1990), analogous to the situation previously reported in clam oocytes (Westendorf et al. 1989). In this case, the protein synthesis requirement for MPF activation during meiosis I presumably would involve synthesis of a protein that somehow activates the stored p34\textsuperscript{cdc2}-cyclin complex. A logical candidate for this activator is the c-mos protein; it is synthesized during oocyte maturation prior to MPF activation and injection of synthetic c-mos RNA induces oocyte maturation (Sagata et al. 1989b; Watanabe et al. 1989). How, then, does injection of cyclin mRNA (or cyclin protein) also activate MPF and induce oocyte maturation?

An answer to this question depends to some extent on the answer to another question, what constitutes MPF activity? Biochemically, MPF is viewed as a complex of p34\textsuperscript{cdc2} kinase and cyclin, with the former actually exhibiting MPF activity and the latter acting to regulate that activity (Draetta et al. 1989; Meijer et al. 1989; Murray and Kirschner, 1989: Westendorf et al. 1989). Biologically, MPF is defined as an activity that induces GVBD in the absence of progesterone and in the absence of protein synthesis (Cyert and Kirschner, 1988). Thus, in a system in which both components preexist, injection of either individually might be expected to exhibit MPF activity provided not all of the stored components exist in a stable complex.

Stage 4 oocytes contain pre-MPF, which can be activated by injection of small amounts of active MPF and which can be amplified by repeated serial transfers of cytoplasm (Taylor and Smith, 1987). Presumably this means that stage 4 oocytes contain both p34\textsuperscript{cdc2} and cyclin, as appears to be the case in stage 6 oocytes. Thus, assuming that the level of p34\textsuperscript{cdc2} in both stage 4 and stage 6 oocytes is in excess relative to cyclin, the protein translated from injected cyclin mRNA would interact with p34\textsuperscript{cdc2}, initiating oocyte maturation. As pointed out by Westendorf et al. (1989), the cytoplasmic environment is modified in a number of ways during maturation and these changes could result in the activation of stored pre-MPF. The practical implication of such a suggestion is that amplification of MPF as assayed biologically would be a consequence of the induction of maturation as opposed to a direct action of small amounts of active MPF or stored pre-MPF.

The above suggestion that injected cyclin mRNA leads to active MPF by interaction with free p34\textsuperscript{cdc2} raises certain questions regarding the role of c-mos protein in both the activation and stabilization of MPF activity. It is not clear that stage 6 oocytes induced to mature by the product of injected cyclin mRNA ever develop CSF since it is not certain such oocytes progress to metaphase II (Swenson et al. 1986). However, oocytes induced to mature by injection of small amounts of active MPF do arrest at metaphase II. Presumably, if c-mos synthesis is required for such arrest, then injected MPF must also induce the translation of endogenous c-mos mRNA. On the other hand, c-mos protein presumably is not present in stage 4 oocytes (Watanabe et al. 1989) and we have observed that injection of c-mos mRNA into stage 4 oocytes does not activate MPF (Xu and Smith, unpublished data). Nevertheless, a relatively stable MPF activity can be obtained from stage 4 oocytes injected with cyclin mRNA. This implies that c-mos protein does not act directly on p34\textsuperscript{cdc2} or cyclin. Perhaps c-mos acts on the *Xenopus* homolog of another gene product known to be involved in p34\textsuperscript{cdc2} function in yeast such as wee1+ or nii1+ (Russell and Nurse, 1987a,b). To be consistent with the present study, we need postulate that such proteins are present only in stage 6 oocytes. In such a case, the stage 4 oocyte may turn out to be a useful system to address many of these kinds of questions since events leading to MPF activation can be separated from other progesterone-induced changes leading to maturation in stage 6 oocytes.

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