[REVIEW]

Toward Modeling of a General Mechanism of MPF Formation during Oocyte Maturation in Vertebrates

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ABSTRACT—Vertebrate oocytes arrested at meiotic prophase I are still immature even when they reach their fully grown stage. For the acquisition of fertilizability, the fully grown oocytes must undergo oocyte maturation, during which the meiosis is released from prophase I arrest and stops again at metaphase II until inseminated. The resumption of meiosis from prophase I to metaphase II is induced by the action of maturation-promoting factor (MPF). The molecular structure of MPF is common to all eukaryotes, but the mechanisms of its formation and activation vary in cell types and in species. In this review, I summarize the mechanisms of MPF formation during oocyte maturation in two amphibian species, *Xenopus laevis* and *Rana japonica*. In *Xenopus*, immature oocytes are equipped with inactive MPF (pre-MPF) sufficient for completing oocyte maturation, and therefore only its activation is required after hormonal stimulation. In contrast, immature *Rana* oocytes contain no pre-MPF. Therefore, MPF must be newly formed during oocyte maturation, as is the case in fishes and other amphibians (toads and newts). The mechanism of MPF formation in *Xenopus* therefore seems to be different to that in other lower vertebrates. However, I wish to propose a new mechanism of MPF formation that might be appropriate for all species of lower vertebrates, inclusive of *Xenopus*, based on the novel concept that pre-MPF is an artifact produced under unnatural conditions and is not an essential molecule for initiating oocyte maturation, in contradiction to the generally believed notion that pre-MPF is actively stocked for the initiation of oocyte maturation. The standpoint that pre-MPF is not indispensable for initiating oocyte maturation might provide a new insight into the mechanism of MPF formation during oocyte maturation, allowing us to model a comprehensive mechanism applicable to all vertebrates, including mammals.

INTRODUCTION

The life of many multicellular organisms begins with the union (fertilization) of germ cells (the eggs and the spermatozoa). The processes during which the spermatozoa and eggs are produced are called spermatogenesis and oogenesis, respectively. These processes are indispensable for organisms that use sexual reproduction to maintain the species beyond the limited life of individuals. In contrast to the somatic cells that compose and maintain the organisms, the germ cells function to extend life to the next generation. The eggs and spermatozoa are highly differentiated cells, but in some aspects they are undifferentiated cells that retain the ability to become various kinds of cells. The germ cells thus mysteriously have the properties of being both differentiated and undifferentiated, and with the aid of these cells, organisms can maintain the species. In addition to the contribution of germ cells to the continuity of life, the recombination of genes during oogenesis and spermatogenesis and the blending of genes by fertilization give diversity to organisms. Consequently, the germ cells are responsible for both the continuity and the diversity of life. Investigations into the mechanisms by which the germ cells are produced and fertilized should lead to an understanding of the mechanisms that assure two major contradictory characteristics of life: being able to reproduce those equal to the self and to produce those different from the self.

Oocytes are produced in ovaries by the entry of mitotically proliferating oogonia into meiosis. Oocytes stop their meiotic cell cycle at prophase I (prophase I arrest), during which they grow due to the accumulation of substances necessary for early embryonic development (vitellogenesis). In many vertebrates, fully grown postvitellogenic oocytes under...
prophase I arrest are unable to be fertilized until they mature (Fig. 1). In response to hormonal stimulation, fully grown immature oocytes resume meiosis and proceed to metaphase II, at which time their meiosis stops again (metaphase II arrest) until they are inseminated through the activity of a cytostatic factor (CSF) produced in the oocyte cytoplasm (Masui and Clarke, 1979; Masui, 1985). Metaphase II-arrested oocytes are called mature oocytes or eggs and can undergo embryonic development when fertilized. During the course of maturation, oocytes undergo drastic morphological changes associated with progression of the meiotic cell cycle, among which breakdown of the oocyte nuclear envelope (germinal vesicle breakdown, GVBD) occurring at the prophase/metaphase transition is usually regarded as a hallmark of the progress of oocyte maturation (Fig. 1).

Oocyte maturation is induced by the sequential actions of three substances (Nagahama et al., 1995): gonadotropic hormone (GTH) secreted from the pituitary gland, maturation-inducing hormone (MIH) synthesized in and secreted from follicle cells surrounding the oocytes, and maturation-promoting factor (MPF) produced and activated in the oocyte cytoplasm (Fig. 2). GTH comprises two species, a luteinizing hormone (LH) and a follicle-stimulating hormone (FSH), each of which consists of two glycoproteinous subunits. FSH is responsible for oocyte growth (vitellogenesis), and LH triggers oocyte maturation. MIH is a steroid (ex., progesterone in amphibia) and interacts with a membrane-bound receptor on the oocyte surface. The MIH signal received on the surface is presumably transduced to the cytoplasm with the aid of GTP-binding proteins (G-proteins). The MIH signal finally results in the formation and activation of MPF. In contrast to GTH and MIH, the action of MPF on the promotion of oocyte maturation is universal, displaying no species-specificity. MPF also acts as the dominant inducer of the mitotic M-phase in all eukaryotes (Kishimoto, 1988; Nurse, 1990). MPF is a complex of Cdc2 (a catalytic subunit) and cyclin B (a regulatory subunit), and its activity is controlled by inhibitory phosphorylation of Cdc2 on threonine 14/tyrosine 15 (T14/Y15) by Myt1 (Fattaey and Booher, 1997; Wells et al., 1999) and activating phosphorylation on threonine 161 (T161) by cyclin-dependent kinase activating kinase (CAK, Harper and Elledge, 1998; Larochelle et al., 1998) after a complex formation of Cdc2 and cyclin B.

MPF is stored in immature oocytes as an inactive form (named pre-MPF), although its amount greatly differs from species to species (Yamashita et al., 2000). For example, the oocytes of the African clawed frog _Xenopus laevis_ contain an abundant amount of pre-MPF, which is sufficient for completing oocyte maturation; mouse oocytes contain a limited amount of pre-MPF, which is sufficient for GVBD but not for the subsequent processes of oocyte maturation; and the oocytes of fish and amphibia other than _Xenopus_ contain no pre-MPF. In striking contrast to those oocytes containing enough pre-MPF for completing oocyte maturation, MPF must be newly produced to initiate oocyte maturation in those species having an insufficient supply of pre-MPF, although even in those oocytes equipped with abundant pre-MPF, MPF is formed through the de novo synthesis of cyclin B induced by MIH during maturation under normal conditions (Taieb et al., 1997). At least superficially, the molecular mechanisms of MPF formation during oocyte maturation seem to vary from species to species, despite its ubiquitous structure in all eukaryotes.

In this review, I first summarize the typical two different mechanisms of MPF formation during oocyte maturation, giving an example of two amphibian species, _Xenopus laevis_ and _Rana japonica_. The former can breed all the year round under artificial culture conditions, whereas the latter breeds only once a year, in spring. I then reevaluate the mechanism.
of MPF formation in *Xenopus*, in consideration of the natural role of pre-MPF in initiating oocyte maturation. Finally, I propose a hypothetical mechanism common to lower vertebrates comprising fish and amphibians, including *Xenopus*, in the expectation that, in the future, it will serve as a clue for the understanding of a more comprehensive mechanism of MPF formation during oocyte maturation in vertebrates, including mammals.

**MPF FORMATION DURING XENOPUS OOCYTE MATURATION**

**Mechanism of pre-MPF activation**

Fully grown immature *Xenopus* oocytes are equipped with pre-MPF that comprises cyclin B1-bound Cdc2 and cyclin B2-bound Cdc2 (cyclin B2 is 5-times more abundant than B1). Besides the cyclin B-bound Cdc2, immature *Xenopus* oocytes contain at least 10-times more monomeric Cdc2 molecules than do those bound to cyclin B (Kobayashi *et al.*, 1991). The cyclin B-bound Cdc2 is phosphorylated on both T14/Y15 and T161. T14/Y15 is phosphorylated by Myt1 (Mueller *et al.*, 1995; Palmer *et al.*, 1998), and T161 is phosphorylated by CAK (Fesquet *et al.*, 1993; Poon *et al.*, 1993; Solomon *et al.*, 1993; Fisher and Morgan, 1994). Progesterone is received on a receptor present on the oocyte surface (its biochemical entity still being unknown). After the transduction, probably mediated by G-proteins and cyclic AMP (cAMP), the progesterone signal stimulates Cdc25 and inhibits Myt1 to dephosphorylate T14/Y15, yielding active MPF that consists of cyclin B-bound Cdc2 phosphorylated only on T161 (Fig. 3). The active MPF stimulates Cdc25, and the resultant active Cdc25 activates pre-MPF in turn. This positive feedback loop assures rapid increases in MPF activity, facilitating the initiation of oocyte maturation. When a small amount of active MPF is injected...
Mos as an inhibitor of Xenopus oocyte maturation

New proteins called "initiators" must be synthesized for initiating amphibian oocyte maturation in response to progesterone stimulation, as in the case of oocyte maturation in fishes and mammals except for the mouse (Masui and Clarke, 1979). In this review, I describe Mos, p33\(^{ringo}\) and cyclin B1 as the most likely candidates for initiators.

Mos, a serine/threonine protein kinase encoded by c-mos protooncogene, is synthesized soon after progesterone stimulation by translational activation of the dormant (masked) mRNA. Introduction of c-mos mRNA or recombinant Mos protein into immature Xenopus oocytes induces maturation, and, conversely, the ablation of its mRNA by antisense oligonucleotides suppresses progesterone-induced maturation (Sagata et al., 1988, 1989; Yew et al., 1992; Roy et al., 1996). These findings indicate that Mos is an inhibitor of Xenopus oocyte maturation. The function of Mos seems to be mediated by MAP kinase (MAPK), since Mos, as a MAPK kinase kinase, activates MAPK through the activation of MAPK kinase (also called MEK) (Nebreda and Hunt, 1993; Posada et al., 1993; Shibuya and Ruderman, 1993). Consistent with this, artificial activation of MAPK by injection into immature Xenopus oocytes of constitutive active forms of either Ste11 (a yeast MAPK kinase, Gotoh et al., 1995), MAPK kinase (Gotoh et al., 1995; Huang et al., 1995) or MAPK (Haccard et al., 1995) forces the oocytes to mature. In addition, progesterone-induced Xenopus oocyte maturation is delayed or sometimes inhibited by injection of a neutralizing anti-MAPK kinase antibody (Kosako et al., 1994) or mRNA encoding the MAPK inhibitory phosphatase CL100 (Gotoh et al., 1995). It has also been suggested that a positive feedback loop consisting of the Mos/MAPK pathway and MPF facilitates initiation of oocyte maturation (Gotoh et al., 1991; Matsuda et al., 1992; Gotoh and Nishida, 1995; Howard et al., 1999). Consequently, Mos appears to initiate Xenopus oocyte maturation with the aid of MAPK activity (Fig. 3). Contradictory to this notion, however, the activation of MAPK coincides with the increase in MPF activity, although a low level of transient activation of MAPK occurs 15-30 min after progesterone treatment (Fisher et al., 1999a, 2000). The coincidence of MAPK activation and pre-MPF activation suggests that MAPK facilitates, rather than initiates, pre-MPF activation (Sagata, 1997). A recent finding that some batches of Xenopus oocytes can mature in the absence of MAPK activation (Fisher et al., 1999a; Gross et al., 2000) also implies that MAPK is not directly involved in initiating oocyte maturation. It is therefore plausible that Mos makes a contribution to the initiation of oocyte maturation without MAPK activity. I will discuss the general role of the Mos/MAPK pathway during amphibian oocyte maturation in a later section.

Translational control of c-mos mRNA

The de novo synthesis of Mos after progesterone stimulation is a prerequisite for initiating oocyte maturation in Xenopus. Similar to many maternal mRNAs synthesized during oocyte growth, the c-mos mRNA exists as a dormant form in immature oocytes. This dormancy is probably achieved by the binding of ubiquitous masked proteins such as Y-box proteins (Matsumoto et al., 1996) and of masked proteins specific to the mRNA. Progesterone must liberate the c-mos mRNA from the masked elements, making it available to the translational machinery. The biochemical pathways that link progesterone stimulation to the unmasking of c-mos mRNA are largely unknown, but there has recently been significant progress toward elucidation of the mechanism for translational activation of c-mos mRNA via a change in poly (A) status, an elongation of the poly (A) tail by cytoplasmic polyadenylation (Hake and Richter, 1997). Translation of c-mos mRNA is regulated by cytoplasmic polyadenylation, which requires two cis sequences in the 3' untranslated region (3' UTR), the highly conserved hexanucleotide, AAUAAA, and a nearby U-rich sequence called the cytoplasmic polyadenylation element (CPE) (Fox et al., 1992; Gebauer and Richter, 1997). The sufficiency and the necessity of the hexanucleotide and CPE of c-mos mRNA for progesterone-induced Xenopus oocyte maturation have been confirmed by antisense oligonucleotide-directed selective amputation of these sequences and by the introduction of a prosthetic RNA, which restores c-mos polyadenylation signals by base-pairing to the amputated mRNA (Sheets et al., 1995). Using similar techniques, it has also been demonstrated that translational activation of c-mos mRNA requires a long poly(A) tail per se rather than the process of polyadenylation itself (Barkoff et al., 1998).

Polyadenylation of mRNA containing the CPE on its 3' UTR is mediated by a CPE-binding protein (CPEB) (Hake and Richter, 1994). Injection of a neutralizing antibody against CPEB inhibits progesterone-induced Xenopus oocyte maturation by preventing polyadenylation and translation of c-mos mRNA, strongly suggesting that CPEB-mediated polyadenylation of c-mos mRNA is essential for inducing Xenopus oocyte maturation (Stebbins-Boaz et al., 1996). In addition to the function of translational activation (unmasking) of masked mRNAs, CPEB may also function as a masking protein itself for dormant mRNAs stored in immature oocytes, since injection of large amounts of the CPE into oocytes can initiate translation of the dormant mRNAs that contain the CPE, probably due to the titration of the masking protein CPEB (Stutz et al., 1998; de Moor and Richter, 1999; Barkoff et al., 2000). CPEB directly binds to maskin, a protein that can also bind directly to the cap-binding translation initiation factor eIF-4E (Stebbins-
Boaz et al., 1999). It is presumed that the dissociation of maskin from elF-4E allows elF-4G to bind to elF-4E, which brings elF-3 and the 40 S ribosomal subunit to the mRNA to initiate translation via cap-ribose methylation (Kuge et al., 1998; Keiper and Rhoads, 1999; Stebbins-Boaz et al., 1999).

p33\textsuperscript{ringo} (or Speedy) as an initiator of Xenopus oocyte maturation

Besides c-mos mRNA, Xenopus oocyte maturation requires polyadenylation of at least one other mRNA (Barkoff et al., 1998), suggesting that molecules other than Mos also function as an initiator of Xenopus oocyte maturation. In fact, it has been demonstrated that a newly synthesized protein(s) that associates with Cdc2 (like cyclins) is involved in the initiation of Xenopus oocyte maturation by the finding that progesterone-induced maturation is inhibited by the microinjection of either a mutant Cdc2 that can bind cyclins but lacks protein kinase activity or an anti-Cdc2 monoclonal antibody that blocks the kinase activation by cyclins (Nebreda et al., 1995).

p33\textsuperscript{ringo} has been isolated as a protein that can effectively induce maturation under the condition of inhibited protein synthesis when injected into immature Xenopus oocytes (Ferby et al., 1999). Injection of p33\textsuperscript{ringo} induces GVBD considerably faster than does progesterone treatment, and in this case, the activation of pre-MPF occurs before MAPK activation. The synthesis of p33\textsuperscript{ringo} is up-regulated during progesterone-induced oocyte maturation, and ablation of endogenous p33\textsuperscript{ringo} mRNA using antisense oligonucleotides inhibits progesterone-induced oocyte maturation. This protein binds to monomeric Cdc2 (but not to cyclin B-bound Cdc2) and activates it. These results lead to the conclusion that p33\textsuperscript{ringo} is one of the initiators of Xenopus oocyte maturation (Fig. 3). However, the relationship between Mos and p33\textsuperscript{ringo} remains to be elucidated. Independent of p33\textsuperscript{ringo}, a protein that shows 98% identity to p33\textsuperscript{ringo} (named Speedy) has been isolated as a rapid inducer of Xenopus oocyte maturation (Lenormand et al., 1999). However, Speedy is reported to bind to and activate Cdk2 but not Cdc2. At present, it is uncertain whether p33\textsuperscript{ringo} and Speedy are identical or whether they are different with extremely high homology.

Cyclin B1 as an initiator of Xenopus oocyte maturation

Like c-mos mRNA, translation of cyclin B1 mRNA is activated during Xenopus oocyte maturation (Kobayashi et al., 1991; de Moor and Richter, 1997). When cyclin B1 (as well as cyclins A and B2) is injected into immature Xenopus oocytes, it induces GVBD through the binding to monomeric Cdc2 and the subsequent activation of the cyclin B1-bound Cdc2 (Westendorf et al., 1989; Huchon et al., 1993). These results suggest that cyclin B1 is also an initiator of Xenopus oocyte maturation. Nevertheless, this notion has not been accepted for the following reasons: 1) immature Xenopus oocytes stockpile cyclin B as a form of pre-MPF (Gautier and Maller, 1991), 2) ablation of mRNAs for cyclins A, B1 and B2 by antisense oligonucleotides injected into immature Xenopus oocytes had no inhibitory effect on progesterone-induced oocyte maturation (Minshull et al., 1989), and 3) cytoplasmic polyadenylation of cyclin B1 mRNA occurs at or shortly after GVBD (Ballantyne et al., 1997). Recently, however, it has been shown that artificial translational activation of endogenous cyclin B1 mRNA by the injection of a high dose of CPE brings about GVBD without progesterone and Mos (de Moor and Richter, 1999). Moreover, a study using a dominant negative Cdc2 demonstrated that cyclin-like protein(s) must be synthesized during Xenopus oocyte maturation induced by progesterone (Nebreda et al., 1995), although it might be p33\textsuperscript{ringo} (Ferby et al., 1999). In this context, the most remarkable finding recently reported is that progesterone induces cyclin B1 synthesis without any activities of Mos, MAPK and MPF. This strongly suggests that progesterone-induced cyclin B1 synthesis is not a result of MPF activation but a physiological trigger of pre-MPF activation (Frank-Vaillant et al., 1999). Taken together, cyclin B1 can be regarded as one of the initiators of Xenopus oocyte maturation. I will further discuss this possibility in a later section.

As in the case of c-mos mRNA, the 3' UTR of cyclin B1 mRNA contains CPEs and the increase in its translation is correlated with the increase in its poly(A) tail, from \textasciitilde30 nucleotides in immature oocytes to \textasciitilde250 nucleotides in mature eggs (Sheets et al., 1994). In contrast to c-mos mRNA, however, the protein level of cyclin B1 can increase in the absence of polyadenylation or cap-ribose methylation of mRNA and is independent of MAPK activity (Ballantyne et al., 1997; Kuge et al., 1998; Frank-Vaillant et al., 1999; Howard et al., 1999), indicating that translation of c-mos and cyclin B1 mRNAs is controlled by different mechanisms. In Drosophila embryogenesis, translation of cyclin B mRNA is repressed by the binding of Pumilio to the specific sequence present in the 3'UTR of mRNA, in cooperation with Nanos (Dalby and Glover, 1993; Asaoka-Taguchi et al., 1999). Xenopus oocytes contain a Pumilio homolog that binds specifically to a definite 3'UTR sequence (different from the CPE) of cyclin B1 mRNA (S. Nakahata, Y. Katsu, N. Nagahama, M. Yamashita, unpublished). It is highly likely that Pumilio, in addition to CPEB, is involved in translational control of cyclin B1 mRNA.

Other proteins involved in the initiation of Xenopus oocyte maturation

Besides the candidate initiators Mos, p33\textsuperscript{ringo} and cyclin B1, other proteins are also reported to be involved in the initiation of Xenopus oocyte maturation. In relation to the reception of progesterone signals on the oocyte surface and transduction of the signals to the cytoplasm, Eg2 kinase is of particularly interest, since it can phosphorylate the specific residues of CPEB that are essential for the polyadenylation of c-mos mRNA (Andrèsson and Ruderman, 1998; Mendez et al., 2000). The \( \beta \) subunit of casein kinase II is thought to be a physiological repressor of Mos during the early phase of Xenopus oocyte maturation, and it might function to determine the threshold level of Mos (Chen and Cooper, 1997). Hsp70 and Hsp90 are suggested to be required for the accumulation and activation of Mos during the early phase of Xe-
MPF FORMATION DURING RANA OOCYTE MATURATION

Mechanism of MPF formation

In the previous section, I have stated that active MPF is formed by T14/Y15 dephosphorylation of Cdc2 that comprises pre-MPF in Xenopus. In lower vertebrates (fish and amphibians), however, this mechanism is rather exceptional. In fact, the mechanism that was first discovered in goldfish is commonly adopted in many fishes and amphibians (Hirai et al., 1992; Kajiura et al., 1993; Katsu et al., 1993; Tanaka and Yamashita, 1995; Yamashita et al., 1995; Kondo et al., 1997; Ihara et al., 1998; Sakamoto et al., 1998). Here, I summarize this mechanism, giving an example of the Japanese brown frog Rana japonica. In striking contrast to Xenopus, immature Rana oocytes contain only monomeric Cdc2, and neither cyclin B1 nor B2 is detectable (pre-MPF thereby being absent in the immature oocytes). After stimulation by progesterone, both cyclins B1 and B2 are synthesized from the stored mRNA and bind to the preexisting Cdc2. The protein content of cyclin B2 is two-times greater than that of B1, and the ratio of monomeric Cdc2 to cyclin B-bound Cdc2 is about 6 to 1. The binding of Cdc2 to cyclin B (the formation of MPF) enables CAK to phosphorylate cyclin B-bound Cdc2 on T161 and to activate MPF. Neither T14/Y15 phosphorylation nor its dephosphorylation thereafter is involved in MPF activation in this species (Fig. 4). Hence it follows that MPF is formed only after hormonal stimulation and activated by T161 phosphorylation in this species.

Cyclin B as an initiator of Rana oocyte maturation

The necessity and the sufficiency of either cyclin B1 or

Fig. 4. MPF formation during Rana oocyte maturation. Immature oocytes contain monomeric Cdc2, and cyclin B is synthesized by translational activation of the masked mRNA after progesterone stimulation received by the surface receptor. After the complex formation of the preexisting Cdc2 and the \textit{de novo} synthesized cyclin B, Cdc2 is activated by CAK-catalyzed T161 (T) phosphorylation but not by Cdc25-catalyzed T14/Y15 (Y) dephosphorylation. Progesterone also induces Mos synthesis. The resultant Mos promotes cyclin B synthesis by itself and stabilizes MPF via MAPK activity, but these are neither necessary nor sufficient for initiating oocyte maturation in this species, although the Mos/MAPK pathway makes a contribution to metaphase II arrest as CSF.
B2 for inducing GVBD in Rana have been confirmed by the following findings (Ihara et al., 1998): 1) progesterone-induced GVBD is inhibited only when both cyclins B1 and B2 mRNAs are deleted from immature oocytes by microinjection of the antisense RNAs, and 2) injection of either cyclin B1 or B2 alone is able to induce GVBD. Cyclin B is thus an initiator of oocyte maturation in this species. As in the case of Xenopus, does Mos play a role in initiating maturation in Rana? To address this issue, the role of the Mos/MAPK pathway during Rana oocyte maturation was investigated (Yoshida et al., 2000a). MAPK is activated in accordance with MPF activation during Rana oocyte maturation. However, injection of c-mos mRNA is unable to initiate Rana oocyte maturation despite full-activation of endogenous MAPK in the injected oocytes. Consistent with this, ectopic activation of MAPK in immature Rana oocytes with constitutive active Ste11 protein also fails to initiate maturation. Even when Mos synthesis and MAPK activation are inhibited, Rana oocytes can mature in response to progesterone. According to the results obtained so far, it is concluded that Mos does not serve to initiate oocyte maturation via the MAPK pathway during Rana oocyte maturation (Fig. 4). Essentially the same result was obtained using immature goldfish oocytes (Kajura-Kobayashi et al., 2000), in which pre-MPF is also absent as it is in Rana.

General role of Mos/MAPK

In spite of the general role of Mos/MAPK in metaphase II arrest as CSF in all vertebrates (Sagata, 1996), its role as an initiator is restricted to Xenopus. At least in vertebrates, it seems to be dependent on the amount of pre-MPF in immature oocytes whether the Mos/MAPK can act as an initiator or not (Yamashita, 1998); the Mos/MAPK pathway can act as an initiator in Xenopus oocytes that contain an abundant amount of pre-MPF, whereas it cannot act in oocytes that contain a limited amount of or no pre-MPF. The pre-MPF-dependent difference in the function of Mos/MAPK implies that the final target of Mos/MAPK is pre-MPF itself (a complex of cyclin B and Cdc2). This notion and the following results from experiments in which the activities of Mos and MAPK were artificially manipulated during Rana oocyte maturation have suggested that the general role of Mos/MAPK is to induce cyclin B synthesis by Mos itself and to stabilize cyclin B-bound Cdc2 (MPF) by the Mos-induced MAPK activity, the latter role of which is probably comparable to the CSF activity (Yoshida et al., 2000a): 1) artificial activation of the Mos/MAPK pathway in immature Rana oocytes induces cyclin B1 synthesis, although the level is so low that GVBD is not induced, 2) even under the condition of inhibited MAPK activity, ectopic expression of Mos induces cyclin B synthesis, indicating that Mos can induce cyclin B synthesis without MAPK activity, and 3) ectopic activation of MAPK enhances progesterone-induced GVBD and inhibition of MAPK activity retards it.

RECONSIDERATION OF MPF FORMATION IN XENOPUS

Biological significance of pre-MPF

Now, it becomes clear that Xenopus oocytes exceptionally exhibit a mechanism of MPF formation that is different from that in other amphibians. The most remarkable difference between Xenopus and other amphibians is the presence or the absence of pre-MPF in immature oocytes. Then, why do only Xenopus oocytes contain pre-MPF? What is the role of pre-MPF? The general understanding of the biological significance of pre-MPF is that it is an indispensable stock actively accumulated for inducing oocyte maturation. Indeed, the mechanism of Xenopus oocyte maturation proposed to date is dependent on this conception. However, a finding that queries this conception has been obtained from Rana oocytes.

Immature Rana oocytes during the natural breeding season in spring have no pre-MPF. However, immature oocytes in frogs that have been kept for a long time after their natural breeding season (until summer) contain a small amount of pre-MPF that consists mainly of cyclin B2-bound Cdc2 (Ihara et al., 1998). This finding suggests that pre-MPF appears only in old immature oocytes, in contrast to the original notion that pre-MPF is a prerequisite for oocyte maturation. A leaky translation of cyclin B (particularly of B2) mRNA may occur in old oocytes. In this case, cyclin B-bound Cdc2 must be phosphorylated on T14/Y15 to inhibit its precocious activation, resulting in the accumulation of pre-MPF in the old oocytes. If this is in fact the case, then pre-MPF may not be an indispensable component for inducing maturation in normal oocytes but a redundancy that has resulted from the inhibition of precocious maturation of old oocytes. In other words, pre-MPF is a waste, rather than a stock, accumulated in the oocytes.

Role of pre-MPF in immature Xenopus oocytes

To examine whether pre-MPF is actually necessary for inducing Xenopus oocyte maturation, frogs in which oocytes had matured in response to progesterone were first selected, and then the pre-MPF contents in the oocytes were measured (Yoshida et al., 2000b). Despite the fact that all of the oocytes were able to mature when treated with progesterone, the pre-MPF contents were highly variable in the frogs and some oocytes did not have detectable levels of pre-MPF, indicating that even the oocytes with a low level of or no pre-MPF can mature in response to progesterone. The relationship between the pre-MPF contents and the time required to reach 50% GVBD also showed that the oocytes with a low level of pre-MPF can mature with a similar time course to those with a high level of pre-MPF, although some oocytes equipped with the maximum dose of pre-MPF can mature faster. These results indicate that pre-MPF is unnecessary for Xenopus oocytes to mature in response to progesterone.

The climate of the natural habitat of Xenopus in Africa is mild; the temperature in their natural habitat ranges from below 10 to 25°C, and their breeding is restricted to the rainy season, which comes once or twice a year (Tinsley and Kobel, 1996). On the other hand, frogs for use in experiments are
kept under artificial conditions with a constant (and relatively high) water temperature, which enables us to obtain oocytes and eggs all the year round but disorders their natural annual reproductive cycle. Furthermore, prior to the experiments, they are usually treated with pregnant mares’ serum gonadotropin (PMSG), a technique known as priming to improve the synchrony of the response to progesterone; otherwise *Xenopus* oocytes are highly heterogenous in responsiveness according to the individual (Smith, 1989). Pre-MPF might be an artifact due to unnatural conditions, such as culture with a constant and high water temperature and priming with PMSG. In a wild population, immature *Xenopus* oocytes may have no pre-MPF, as is the case in other amphibians.

**New hypothetical mechanism of MPF formation**

On the basis of the concepts that fundamentally similar pathways operate during oocyte maturation in *Xenopus*

![Diagram of MPF formation](image)

**Fig. 5.** A hypothetical mechanism of MPF formation common to all amphibians (and fish). Basic pathways (A) and modified pathways due to the presence of pre-MPF (B) are shown. Molecules formed only after progesterone stimulation are boxed. A) Cyclin B synthesis induced by progesterone is indispensable for promoting oocyte maturation in all amphibians, as is the case in fishes. The synthesized cyclin B binds to Cdc2 to form MPF. The resultant MPF stimulates cyclin B synthesis, allowing continued MPF formation. Progesterone also induces Mos synthesis. The synthesized Mos facilitates MPF formation and increases the synchronicity of oocyte maturation, but its presence itself is not essential for initiating oocyte maturation.

**PERSPECTIVES**

In this paper, I have proposed a new model for MPF formation during oocyte maturation in fish and amphibians (Fig. 5). Its gist is that progesterone-induced cyclin B synthesis is the fundamental pathway for inducing MPF formation in lower vertebrates, including *Xenopus*, contradictory to the generally believed notion that cyclin B synthesis is unnecessary for initiating oocyte maturation in this species. This model should be verified by further studies, but I believe that the mechanism of *Xenopus* oocyte maturation must be reevaluated from the new standpoint that pre-MPF is not essential for inducing oocyte maturation. In addition, the functions of several proteins that have been believed to be indispensable for initiating *Xenopus* oocyte maturation to date must be reexamined using immature *Xenopus* oocytes that are responsive to progesterone but contain only a small amount of or no pre-MPF (It should be emphasized that these oocytes exist and might be natural.).

It is also worth examining whether this model is applicable to oocyte maturation in mammals, since new protein synthesis is required for initiating oocyte maturation in many mammals (except for the mouse), a situation similar to that in...
lower vertebrates (Laboratory mice might bear unnatural characteristics similar to *Xenopus*.). Moreover, cyclin B is stimulated to be synthesized during oocyte maturation in all species examined so far, including mammals, and the contents of pre-MPF in immature oocytes are very low in many mammals (Christmann et al., 1994; Chesnel and Eppig, 1995; Naito et al., 1995; de Vantéry et al., 1996; Lévesque and Sirard, 1996; Hue et al., 1997; Goudet et al., 1998) and sometimes undetectable in the cow (Wu et al., 1997). These facts call for investigation of the role of newly synthesized cyclin B in initiating oocyte maturation in mammals, as well as in promoting oocyte maturation beyond GVBD, as was investigated previously in the mouse (Hampl and Eppig, 1995; Polanski et al., 1998). I hope that the hypothetical mechanism presented here provides the first step toward the modeling of a more comprehensive mechanism of MPF formation during oocyte maturation that is suitable for all vertebrates, including fish, amphibians and mammals.

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**REFERENCES**


Andrésson T, Ruderman JV (1998) The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. EMBO J 17: 5627–5637


Christmann L, Jung T, Moor RM (1994) MPF components and mitotic competence in growing pig oocytes. Mol Reprod Dev 38: 85–90


de Moor CH, Richter JD (1999) Cytoplasmic polyadenylation elements mediate masking and unmasking of cyclin B1 mRNA. EMBO J 18: 2294–2303


Gotoh Y, Nishida E (1995) Activation mechanism and function of the
MAP kinase cascade. Mol Reprod Dev 42: 486–492
Hake LE, Richter JD (1994) CPEB is a specificity factor that mediates cytoplasmic polyadenylation during Xenopus oocyte maturation. Cell 79: 617–627
Hake LE, Richter JD (1997) Translational regulation of maternal mRNA. Biochim Biophys Acta 1332: M31–M38
must associate with p34<sup>cdk2</sup> to activate MAP kinase and MPF during progesterone-induced maturation of Xenopus oocytes. EMBO J 14: 5597–5607


Palmer A, Gavin AC, Nebreda AR (1998) A link between MAP kinase and p34<sup>cdk2</sup>/cyclin B during oocyte maturation: p90<sup>mt</sup> phosphorylates and inactivates the p34<sup>cdk2</sup> inhibitory kinase Myt1. EMBO J 17: 5037–5047

Palmer A, Nebreda AR (2000) The activation of MAP kinase and p34<sup>cdk2</sup>/cyclin B during the meiotic maturation of Xenopus oocytes. Prog Cell Cycle Res 4: 131–143


Solomon M, Harper JW, Shuttleworth J (1993) CAK, the p34<sup>cdk2</sup> activating kinase, contains a protein identical or closely related to p40<sup>MO15</sup>. EMBO J 12: 3133–3142

Stebbins-Boaz B, Cao Q, de Moor CH, Mendez R, Richter JD (1999) Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. Mol Cell 4: 1017–1027

Stebbins-Boaz B, Hake LE, Richter JD (1996) CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNA and is necessary for oocyte maturation in Xenopus. EMBO J 15: 2582–2589


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