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[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

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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 amphibians) 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 amphibians 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

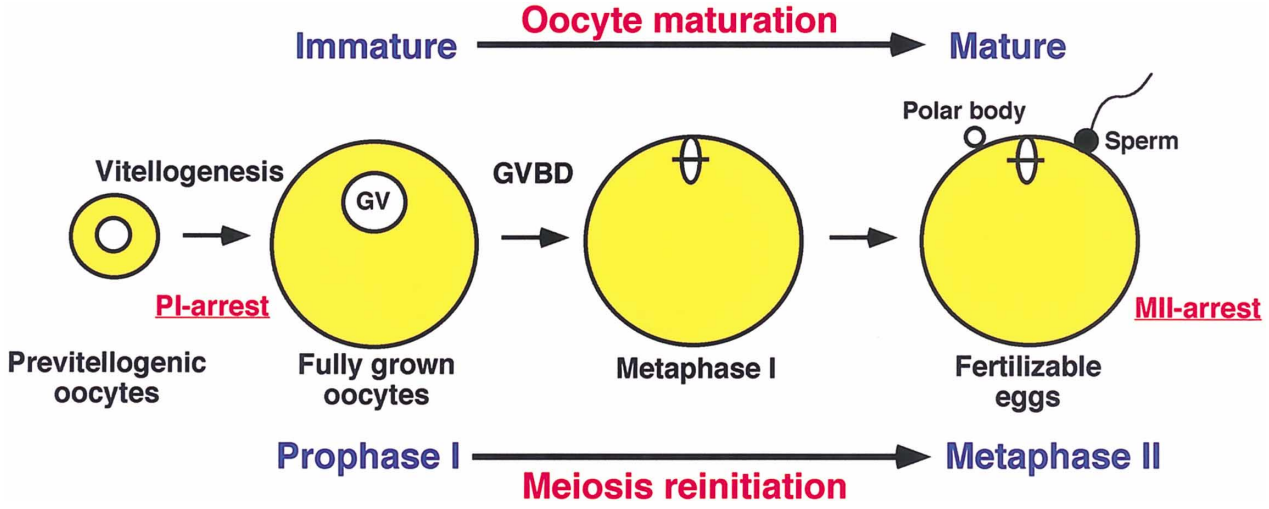

Fig. 1. Oocyte maturation and meiotic cell cycle. During oogenesis in vertebrates, oocytes stop their meiosis at two stages, prophase I and metaphase II (PI- and MII-arrest). Oocytes under PI-arrest (characterized by a huge nucleus called a germinal vesicle, GV) grow due to the accumulation of yolk (vitellogenesis) and become fully grown but are still immature. Upon hormonal stimulation, the immature oocytes are released from the PI-arrest, and after undergoing germinal vesicle breakdown (GVBD) and passing through metaphase I, they reach metaphase II, where they are naturally inseminated. MII-arrested oocytes are usually called mature oocytes or eggs.

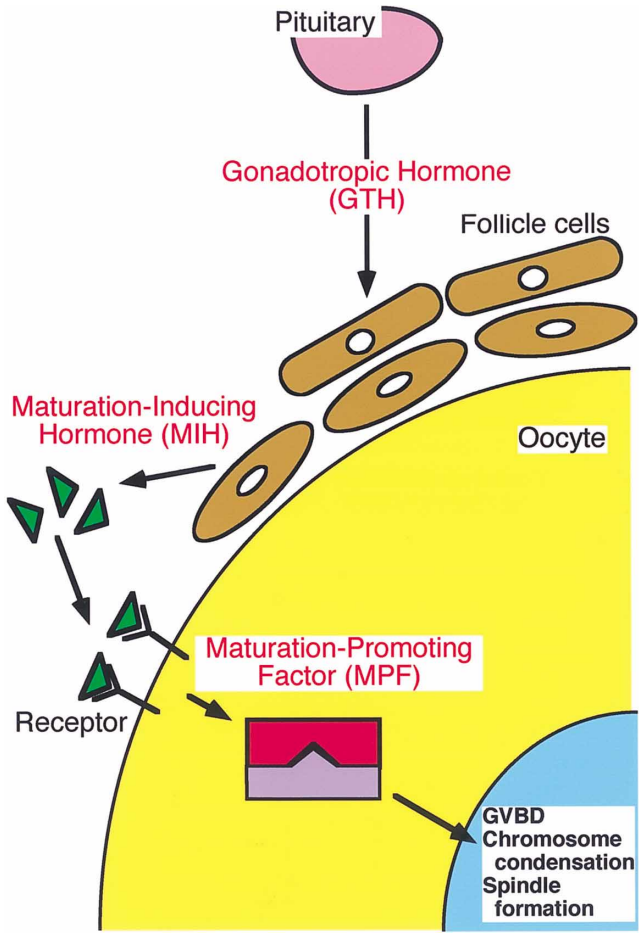

Fig. 2. Three major mediators for inducing oocyte maturation. Under the influence of GTH secreted from the pituitary gland, follicle cells surrounding the oocytes produce and secrete MIH, which is received by a receptor on the oocyte surface. The MIH signal stimulates the oocyte to form and activate MPF, which finally triggers all of the changes accompanying oocyte maturation, such as germinal vesicle breakdown (GVBD), chromosome condensation and spindle formation.

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

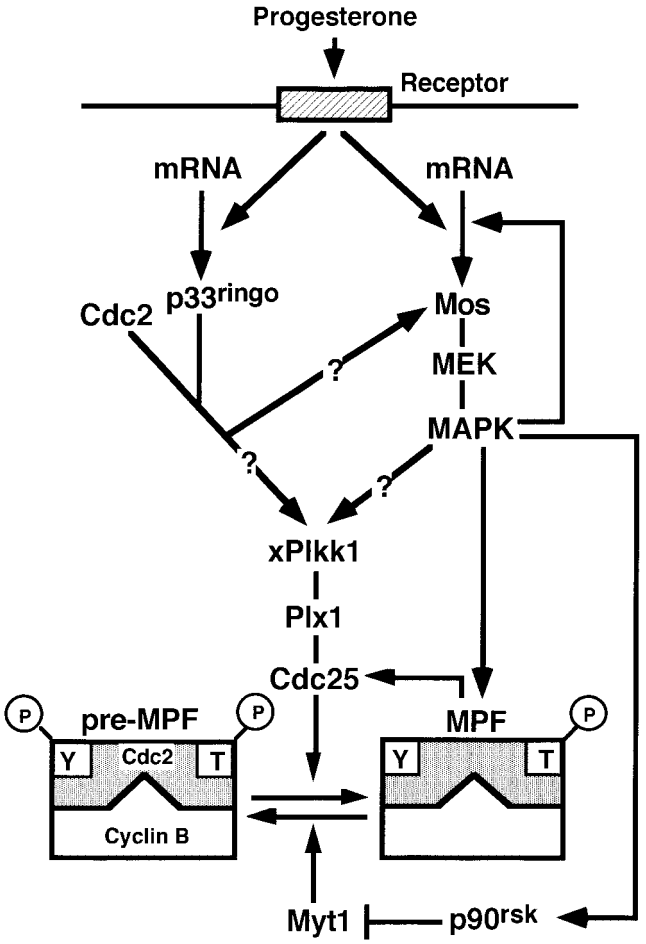

Fig. 3. MPF formation (pre-MPF activation) during *Xenopus* oocyte maturation. Cdc2 molecules that comprise pre-MPF are phosphorylated on both T14/Y15 (Y) and T161 (T). The progesterone signal received by the surface receptor induces the translation-mediated syntheses of Mos and $p33^{ringo}$, resulting in the Cdc25-catalyzed pre-MPF activation through T14/Y15 (Y) dephosphorylation of cyclin B-bound Cdc2. The inhibition of Myt1 via the Mos/MAPK/$p90^{rsk}$ pathway also facilitates pre-MPF activation. Positive feedback loops consisting of the MPF-induced Cdc25 activation and the MAPK-induced Mos synthesis allow oocytes to activate pre-MPF rapidly. The Mos/MAPK pathway is also responsible for stabilizing MPF as CSF.

into immature *Xenopus* oocytes, the oocytes mature without any protein synthesis due to the activity of MPF formed through the Cdc25-catalyzed T14/Y15 dephosphorylation of pre-MPF (Izumi and Maller, 1995). This means that MPF formation (the complex formation of Cdc2 and cyclin B) has already been completed in fully grown immature *Xenopus* oocytes, and upon progesterone stimulation, only its activation by T14/Y15 dephosphorylation is required to initiate oocyte maturation (Fig. 3). Hence, the most important event for the initiation of *Xenopus* oocyte maturation is how the activation of Cdc25 (and/or the inhibition of Myt1) is triggered upon hormonal stimulation.

Mos as an initiator 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 initiator 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 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 eIF-4E allows eIF-4G to bind to eIF-4E, which brings eIF-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^{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^{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^{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^{ringo} is up-regulated during progesterone-induced oocyte maturation, and ablation of endogenous p33^{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^{ringo} is one of the initiators of *Xenopus* oocyte maturation (Fig. 3). However, the relationship between Mos and p33^{ringo} remains to be elucidated. Independent of p33^{ringo}, a protein that shows 98% identity to p33^{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^{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^{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 ≈30 nucleotides in immature oocytes to ≈250 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^{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 (Andrésson and Ruderman, 1998; Mendez *et al.*, 2000). The β 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-*

nopus oocyte maturation (Liu *et al*., 1999; Fisher *et al*., 2000). Since immature oocytes are thought to be arrested at the G2 phase, the Chk1/Cdc25/14-3-3 protein pathway might also operate in *Xenopus* oocytes (Nakajo *et al*., 1999; Yang *et al*., 1999), as in the mitotic cell cycle in somatic cells (Nilsson and Hoffmann, 2000). The activation of pre-MPF requires the activation of Cdc25 and the inhibition of Myt1 (Fig. 3). A polo-like kinase (Plx1) and its activating kinase (xPlkk1) are involved in Cdc25 activation (Abrieu *et al*., 1998; Qian *et al*., 1998a, b; Karaïskou *et al*., 1999), and the inhibition of Myt1 kinase is mediated by $p90^{rsk}$, a protein kinase that is phosphorylated and activated by MAPK and phosphorylates and inhibits Myt1 (Palmer *et al*., 1998; Gavin *et al*., 1999). Other kinases reported to be involved in the initiation of *Xenopus* oocyte maturation include p21-activated kinase (Faure *et al*., 1999), glycogen synthase kinase-3 (Fisher *et al*., 1999b), phosphatidylinositol 3-kinase (Muslin *et al*., 1993a), cAMP-dependent kinase (Daar *et al*., 1993; Matten *et al*., 1994), and Raf-1 (Muslin *et al*., 1993b; for review, see also Palmer and Nebreda, 2000).

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

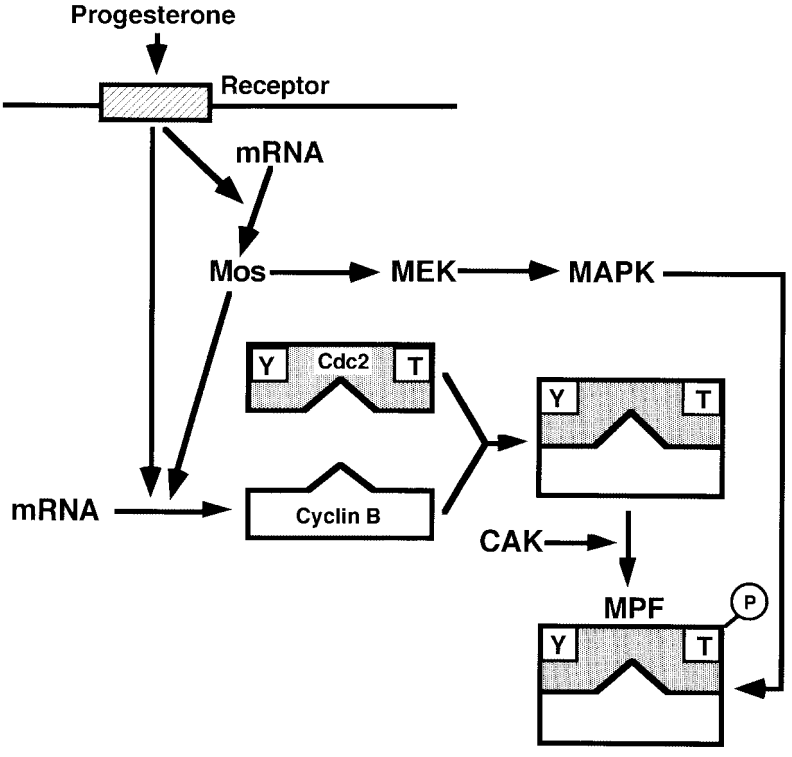

Fig. 4. MPF formation during *Rana* oocyte maturation. Immature oocytes contain monomeric Cdc2, and cyclin B is synthesized by translational activation of the masked mRNA after progesterone stimulation received by the surface receptor . After the complex formation of the preexisting Cdc2 and the *de 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 (Kajiura-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* and in other amphibians and that pre-MPF is not a pivotal molecule for inducing oocyte maturation, I propose a new hypothetical mechanism of the initiation of oocyte maturation in amphibians (Fig. 5). In this model, cyclin B synthesis induced by progesterone is a prerequisite for initiating oocyte maturation in all amphibians (and fish). The synthesized cyclin B binds to monomeric Cdc2 to form MPF. The activity of the newly formed MPF stimulates further translation of cyclin B to enhance MPF formation. Progesterone also induces Mos synthesis, and the resulting Mos plays a subsidiary role in the initiation of oocyte maturation by facilitating cyclin B protein accumulation through the stimulation of translation by Mos itself. The extent to which the translation of cyclin B mRNA is dependent on Mos may differ between *Xenopus* and other amphibians. Mos also contributes to MPF formation by stabilizing MPF via activation of MAPK, and the activated MAPK indirectly assists the MPF formation through the enhancement of Mos synthesis, although these functions of Mos/MAPK are unnecessary for initiating oocyte maturation. The progesterone-induced intracellular state that expedites T14/Y15 dephosphorylation of Cdc2 through the Mos/MAPK/p90^{rsk} pathway or through a pathway as yet unknown may allow oocytes to form MPF more easily. MPF thus continues to be formed, and when the level of MPF reaches the threshold, oocytes undergo GVBD. When pre-MPF is present in immature oocytes, the accumulated cyclin B protein acts as a starter to stimulate autocatalytic full-activation of pre-MPF, and thereby oocyte maturation would be induced by only a low level of MPF newly produced by cyclin B (Fig. 5). Thus, it is certain that pre-MPF facilitates MPF formation and increases the synchronicity of oocyte maturation, but its presence itself is not essential for initiating oocyte maturation.

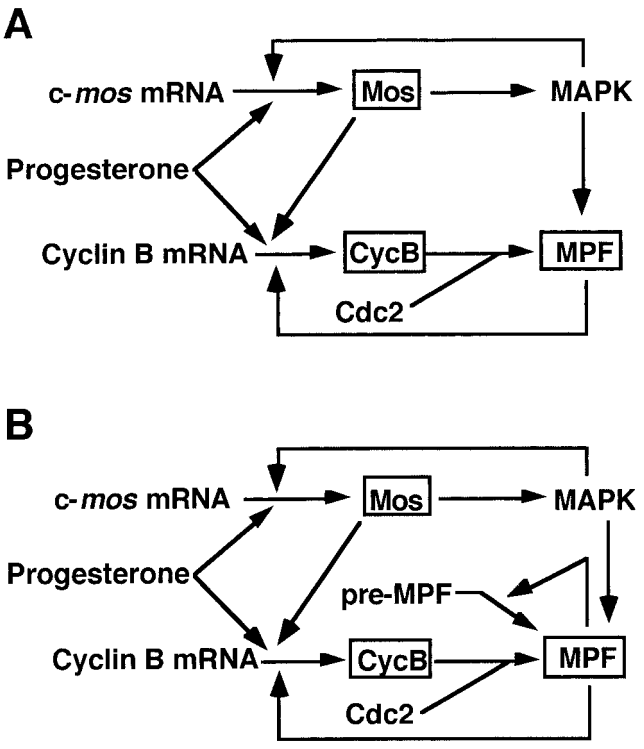

Fig. 5. A hypothetical mechanism of MPF formation common to all amphibians (and fish). Basic pathways (A) and modified pathways due to the presence of pre-MPF (B) are shown. Molecules formed only after progesterone stimulation are boxed. A) Cyclin B synthesis induced by progesterone is indispensable 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 promotes cyclin B synthesis and the Mos-induced MAPK activation contributes to MPF formation through the stimulation of Mos synthesis and the stabilization of MPF. B) When pre-MPF is present in immature oocytes, it facilitates maturation through its autocatalytic activation that is initiated by a starter dose of cyclin B synthesized under the condition of progesterone stimulation. Even in the absence of pre-MPF, however, the oocytes can mature due to the activity of MPF newly formed from the *de novo* synthesized cyclin B and the preexisting Cdc2.

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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