Redundant pathways for Cdc2 activation in *Xenopus* oocyte: either cyclin B or Mos synthesis

Olivier Haccard & Catherine Jessus⁺

Laboratoire de Biologie du Développement, UMR CNRS 7622, UPMC, Paris, France

***Xenopus* oocytes are arrested in meiotic prophase I. Progesterone induces the resumption of meiotic maturation, which requires continuous protein synthesis to bring about Cdc2 activation. The identification of the newly synthesized proteins has long been a goal. Two plausible candidates have received extensive study. The synthesis of cyclin B and of c-Mos, a kinase that activates the mitogen-activated protein kinase pathway in oocytes, is clearly upregulated by translational control in response to progesterone. Recent studies suggest that ablation of either c-Mos or cyclin B synthesis by antisense oligonucleotides does not block meiotic maturation. Here, however, we show that when both pathways are simultaneously inhibited, progesterone no longer triggers maturation; adding back either c-Mos or cyclin B restores meiotic maturation. We conclude that the specific synthesis of either B-type cyclins or c-Mos, induced by progesterone, is required to induce meiotic maturation. The two pathways seem to be functionally redundant.**

Keywords: Cdc2; cyclin B; MAPK; Mos; *Xenopus* oocyte

EMBO reports (2006) 7, 321–325. doi:10.1038/sj.embor.7400611

INTRODUCTION

During oogenesis, the cell cycle arrests at prophase of the first meiosis. Release from this arrest depends on the activation of the cyclin B–Cdc2 kinase or M-phase promoting factor (MPF). In prophase-arrested oocytes, the Myt1 kinase keeps existing cyclin B–Cdc2 complexes inactive by phosphorylating Cdc2 on residues T14 and Y15. During meiotic maturation, pre-MPF is activated by Cdc25 phosphatase, which dephosphorylates T14 and Y15 (Karaïskou *et al*, 2001).

In *Xenopus* oocytes, progesterone triggers release from the prophase arrest. The signalling pathway induced by the hormone and leading to Cdc2 activation still remains unclear. It requires a decrease of the cyclic AMP-dependent protein kinase (PKA) activity (Eyers *et al*, 2005). One of the downstream effects of PKA

inhibition is to promote the synthesis of new proteins necessary for the meiotic maturation process. However, the identity of these essential proteins remains unsettled; they could induce MPF activation through two non-exclusive mechanisms: either by binding and directly activating Cdc2 or by controlling the balance of Cdc25 and Myt1 activities. Most of the Cdc2 protein is present as a monomer in the cytoplasm, whereas a minor fraction is associated with cyclins B2 and B5, forming the inactive pre-MPF (Hocheegger *et al*, 2001). A significant amount of monomeric Cdc2 is already phosphorylated on T161, and is thus available for direct activation by cyclin binding (De Smedt *et al*, 2002). Indeed, *de novo* synthesis of cyclins is induced by progesterone, resulting in the accumulation of B1 and B4 cyclins (Hocheegger *et al*, 2001). Furthermore, it has been shown that the accumulation of cyclin B1 triggered by progesterone requires the inactivation of PKA and is independent of MPF activity (Frank-Vaillant *et al*, 1999). This simple picture has been complicated by the discovery of a novel Cdc2-binding protein named Ringo/Speedy, the translation of which is found by some authors to be necessary for germinal vesicle breakdown (GVBD; Ferby *et al*, 1999; Lenormand *et al*, 1999). But as Ringo/Speedy exercises its effects by binding and activating Cdc2, the basic model is the same as for cyclins: in response to progesterone, newly synthesized partners form a small pool of active MPF complexes by associating with monomeric Cdc2. A small amount of active MPF can bring about Cdc25 activation and Myt1 inactivation, thereby establishing a positive feedback loop (Karaïskou *et al*, 1999). Cyclin B or Ringo/Speedy synthesis thus seems to be a key event for the initiation of meiotic maturation in *Xenopus* oocytes. However, antisense oligonucleotides designed to target the destruction of B-type cyclin messenger RNAs (B1, B2, B4, B5) do not block MPF activation in response to progesterone (Hocheegger *et al*, 2001).

Another way to trigger MPF activation is to reverse the balance between the two opposing enzymes that regulate Cdc2 activity, Myt1 and Cdc25 (Uto *et al*, 2004). Newly synthesized proteins could activate Cdc25 phosphatase or inhibit Myt1 protein kinase, causing the activation of the pre-MPF stockpile without requiring synthesis of any Cdc2-binding partner. Such a candidate exists in Mos, an S/T kinase, the synthesis of which is upregulated in response to progesterone (Sagata *et al*, 1988). Mos is a direct activator of mitogen-activated protein kinase (MEK), which in turn activates mitogen-activated protein kinase (MAPK; Posada

Laboratoire de Biologie du Développement, UMR CNRS 7622, UPMC, case 24, 9 quai Saint-Bernard, 75005 Paris, France

⁺Corresponding author. Tel: +33 1442 72642; Fax: +33 1442 73472;

E-mail: jessus@ccr.jussieu.fr

Received 28 July 2005; revised 15 November 2005; accepted 15 November 2005; published online 16 December 2005

et al, 1993). Injection of Mos (or constitutively active forms of its downstream kinases) induces meiotic maturation in the absence of progesterone (Sagata *et al*, 1988; Haccard *et al*, 1995; Huang *et al*, 1995; Gross *et al*, 2001). Moreover, it has been shown that Myt1 is inhibited by either Rsk, an MAPK substrate, or Mos (Palmer *et al*, 1998; Peter *et al*, 2002), providing a link between the Mos/MAPK cascade and MPF activation. However, several studies convincingly show that neither the activation of MAPK nor Mos synthesis is necessary for progesterone-induced MPF activation in *Xenopus* oocytes (Gross *et al*, 2000; Dupre *et al*, 2002). Therefore, the identification of the newly synthesized proteins required for Cdc2 activation in *Xenopus* oocytes remains an open question.

We began to suspect that the identification of these necessary new proteins was unsuccessful because progesterone uses more than one pathway to induce meiotic maturation. Progesterone turns on both the Mos/MAPK pathway and the synthesis of cyclin B, both of which promote Cdc2 activation. If one pathway were to fail, then progesterone would still be able to trigger Cdc2 activation. It is possible that each individual pathway is dispensable, provided that the other one remains functional. To address this hypothesis, we inhibited the Mos/MAPK cascade and cyclin B synthesis either separately or concomitantly and investigated whether progesterone was still able to induce meiotic maturation. The results show that either pathway is sufficient to trigger meiotic maturation, whereas ablation of both completely blocks the action of progesterone.

RESULTS

MPF activation requires cyclin B and Mos synthesis

Mos synthesis was inhibited by morpholino oligonucleotides, as described by Dupre *et al* (2002). To inhibit all B-type cyclin synthesis, we used a combination of two oligonucleotides called cyc8 and B5-2 (Hochegger *et al*, 2001). We first targeted each pathway separately and then both of them concomitantly.

The injection of morpholino antisense against Mos did not prevent GVBD (Fig 1A). Cdc2 kinase was activated, as monitored using histone H1 as substrate, by the dephosphorylation of the inhibitory Y15 residue and by the electrophoretic mobility of cyclin B2 (Fig 1B). The levels of Mos in injected oocytes were estimated by western blotting and by phosphorylation of its secondary target, MAPK. Fig 1A shows that the oligonucleotide injection efficiently inhibited Mos accumulation and the phosphorylation of MAPK. We thus confirmed that Mos ablation does not prevent GVBD and Cdc2 activation induced by progesterone in *Xenopus* oocytes (Dupre *et al*, 2002).

Next, oocytes were injected with oligonucleotides to block the synthesis of all B-type cyclins. Fig 1 shows that GVBD still occurred in response to added progesterone, Cdc2 and MAPK were activated and Mos was accumulated at GVBD, as described by Hochegger *et al* (2001). Antisense oligonucleotides efficiently prevented cyclin B synthesis, as ascertained by the absence of cyclin B1 accumulation (Fig 1B).

We next injected a combination of oligonucleotides to block the synthesis of Mos and cyclin B, and added progesterone. Both pathways were efficiently impaired (Fig 1B): Mos accumulation was not detected, MAPK activation was prevented and cyclin B1 synthesis was inhibited. In the simultaneous absence of cyclin B and Mos synthesis, GVBD and Cdc2 activation were blocked (Fig 1). No maturation was observed even 24 h after progesterone

Fig 1 | Contribution of cyclin B and Mos synthesis to progesterone-induced maturation. Oocytes were injected with buffer, Mos morpholino antisense (AS-Mos), cyclin B antisense (AS-cyclins) or a mixture of both (AS-cyclins/Mos). After 18 h, progesterone (Pg) was added. (A) Time course of germinal vesicle breakdown (GVBD). (B) Oocytes were homogenized at GVBD, or 24 h after antisense injection when GVBD did not occur. Lysates were assayed for Cdc2 activity using histone H1 as substrate (H1; top panel) or immunoblotted with the indicated antibodies. P-MAPK, phosphorylated mitogen-activated protein kinase.

stimulation, suggesting that Cdc2 activation requires either cyclin B or Mos synthesis.

To test whether the function of Mos is exclusively mediated by MAPK, MAPK activation was prevented by U0126, a pharmacological inhibitor of MEK, together with inhibition of cyclin B synthesis by antisense oligonucleotides. Under these conditions, progesterone failed to trigger GVBD and Cdc2 activation (supplementary Fig S1 online). We conclude that progesterone recruits either the MAPK pathway, downstream of Mos, or cyclin B synthesis to induce Cdc2 activation and GVBD. Each of these pathways is dispensable, provided that the other is functional.

Mitotic cyclins rescue MPF activation

Our results indicate that both cyclin B synthesis and the Mos/MAPK pathway act in parallel to activate Cdc2. If this hypothesis is correct, the replenishment of either one pathway or the other should restore Cdc2 activation and GVBD. To test this, we first injected oocytes with a combination of oligonucleotides to prevent Mos and cyclin B synthesis and then injected recombinant cyclin A. The injection of cyclin A restored GVBD and Cdc2 activation, despite the inhibition of endogenous cyclin B synthesis and MAPK activation (Fig 2).

Similar results were obtained using cyclin B protein (supplementary Fig S2 online). Moreover, inhibiting protein synthesis with cycloheximide did not affect the ability of either cyclin A or B to rescue Cdc2 activation in the absence of cyclin B synthesis and active MAPK (Fig 2; supplementary Fig S2 online). This result shows that the overexpression of a mitotic cyclin is sufficient to restore the defect owing to the abolishment of cyclin and Mos synthesis on Cdc2 activation, independently of the synthesis of any other protein.

Mos injection rescues MPF activation

We then tested whether activating the Mos/MAPK pathway is able to restore Cdc2 activation in oocytes in which endogenous Mos

Fig 2 | Exogenous cyclin A induces germinal vesicle breakdown (GVBD) and M-phase promoting factor activation in the absence of cyclin B and Mos synthesis. Oocytes were either injected or not injected with a mixture of Mos morpholino antisense and cyclin B antisense (AS-cyclins/Mos). After 18 h, they were either incubated or not incubated with cycloheximide (CHX) for 1 h. Time course of GVBD was monitored after (A) progesterone (Pg) addition or (B) cyclin A injection. (C) Analysis as in Fig 1B. P-MAPK, phosphorylated mitogen-activated protein kinase.

and cyclin B synthesis was impaired. Oocytes were first injected with a combination of antisense oligonucleotides against Mos and cyclin B, and then injected with *Xenopus* Mos protein. Whereas antisense oligonucleotides abrogated meiotic maturation induced by progesterone, recombinant Mos protein activated MAPK and induced Cdc2 activation and GVBD, despite the absence of cyclin B synthesis (Fig 3).

We next examined whether the re-activation of the Mos/MAPK pathway in Mos- and cyclin B-ablated oocytes rescues Cdc2 activation in the absence of protein synthesis. Cyclin B and Mos synthesis was inhibited by antisense oligonucleotides and oocytes were incubated in the presence of cycloheximide for 1 h, before injection with the *Xenopus* Mos protein. In most of the cases (four out of five experiments performed with different females), Mos was unable to induce GVBD and Cdc2 activation in the absence of protein synthesis, although MAPK was activated under these conditions (Fig 3A–C). In one experiment, Mos restored Cdc2 activation and GVBD in the absence of protein synthesis (Fig 3D,E), indicating that the requirement of the synthesis of new proteins varies between oocytes from different females.

On the basis of this, the most likely candidate for a ‘Mos helper’ would be Ringo/Speedy, a Cdc2-binding protein synthesized in response to progesterone and which is able to induce meiotic maturation (Ferby *et al*, 1999; Lenormand *et al*, 1999). The injection of mRNA encoding Ringo/Speedy induced meiotic maturation even in oocytes in which the synthesis of Mos and cyclin B was blocked by the appropriate antisense oligonucleotides (supplementary Fig S3A online). To address the necessity for

Fig 3 | Mos induces germinal vesicle breakdown (GVBD) and M-phase promoting factor activation in the absence of cyclin B and Mos synthesis. Oocytes were either injected or not injected with a mixture of Mos morpholino antisense and cyclin B antisense (AS-cyclins/Mos). After 18 h, they were either incubated or not incubated for 1 h with cycloheximide (CHX) and then stimulated with progesterone (Pg) or injected with *Xenopus* Mos recombinant protein. (A,B,D) Time course of GVBD. (C,E) Analysis as in Fig 1B. (A–C) Mos did not restore Cdc2 activation and GVBD in the absence of protein synthesis. (D,E) Mos restored Cdc2 activation and GVBD in the absence of protein synthesis. P-MAPK, phosphorylated mitogen-activated protein kinase.

the translation of endogenous Ringo/Speedy mRNA, Ringo/Speedy antisense oligonucleotides were microinjected in oocytes in combination with either anti-Mos morpholinos or oligonucleotides against cyclin B. In all combinations, progesterone was still able to induce GVBD and Cdc2 activation (supplementary Fig S3B online). These results indicate that Ringo/Speedy is not required for meiotic maturation when Mos or cyclin B synthesis is prevented.

PKI effects require cyclin B and Mos synthesis

We investigated whether cyclin B synthesis and the activation of the Mos/MAPK cascade were under the control of PKA down-regulation. To analyse the pathways controlled by a decrease in PKA activity independently of progesterone, meiotic maturation was triggered by PKI, the specific inhibitor of PKA. PKI injection induced GVBD and Cdc2 activation as progesterone does (Fig 4). Oocytes were next injected with antisense oligonucleotides against all B-type cyclins and later with PKI. In the absence of cyclin B synthesis, PKI was still able to lead to GVBD and activate both Cdc2 and the MAPK pathway (Fig 4A,B), indicating that PKA inhibition elicits Cdc2 activation independently of cyclin B synthesis.

Fig 4 | Inhibition of cyclin B and Mos synthesis prevents germinal vesicle breakdown (GVBD) and M-phase promoting factor activation induced by PKI (inhibitor of cyclic AMP-dependent protein kinase A). Oocytes were either injected or not injected with (A,B) cyclin B antisense (AS-cyclins), (C,D) Mos antisense (AS-Mos), (E,F) a mixture of both (AS-cyclins/Mos) or (C,D) incubated in the presence of U0126. At 18 h after oligonucleotide injection or 1 h after incubation with U0126, progesterone (Pg) was added or recombinant PKI injected. (A,C,E) Time course of GVBD. (B,D,F) Analysis as in Fig 1B. P-MAPK, phosphorylated mitogen-activated protein kinase.

We next inhibited the Mos/MAPK pathway either by injecting antisense morpholinos against Mos or by incubating oocytes with U0126. Each pretreatment prevented the activation of MAPK, but Cdc2 activation and GVBD occurred in response to PKI injection (Fig 4C,D). PKA downregulation therefore triggers Cdc2 activation independently of the Mos/MAPK pathway.

Finally, oocytes were injected with antisense morpholinos against Mos together with antisense oligonucleotides against all B-type cyclins and then stimulated by the injection of PKI. Under these conditions, Cdc2 was not activated and GVBD did not occur (Fig 4E,F). These data indicate that PKA inhibition triggers Cdc2 activation either through the stimulation of cyclin B synthesis or through the activation of the Mos/MAPK cascade, as does progesterone.

DISCUSSION

In *Xenopus* oocyte, Cdc2 activation depends on the synthesis of new proteins triggered by progesterone (Wasserman & Masui,

1975). For many years, the search for candidate synthesized proteins has been an important goal, and the most attractive candidates, B-type cyclins and Mos, have been extensively studied. However, recent studies show that neither the ablation of the expression of all cyclin B isoforms nor the ablation of the Mos protein, or the inhibition of its unique target, MAPK, prevents the resumption of meiotic maturation (Gross *et al*, 2000; Hochegger *et al*, 2001; Dupre *et al*, 2002). In this study, however, we show that Cdc2 activation induced by progesterone is completely abolished when cyclin B synthesis and the Mos/MAPK pathway are simultaneously impaired. The replenishment of at least one of these pathways restores Cdc2 activation. Moreover, we show that Cdc2 activation induced by a direct inhibition of PKA also depends on at least one of these pathways. Altogether, these results indicate that progesterone triggers cyclin B or Mos synthesis through the inhibition of PKA activity and that Cdc2 activation requires either the Mos/MAPK pathway or cyclin B synthesis. Each of these pathways can bypass the deficiency of the other. How can cyclin B synthesis or activation of the Mos/MAPK pathway carry out MPF activation?

Two mechanisms could be put forth for MPF activation. The first one relies on the recruitment of monomeric Cdc2. A small increase in the amount of cyclin B available could be sufficient to bind and activate Cdc2 already phosphorylated on T161, and therefore to generate a threshold level of Cdc2 activity that is able to trigger MPF autoamplification. A second possible mechanism relies on the balance between the activities of Myt1 kinase and Cdc25 phosphatase. The activation of Cdc25 correlated with an inactivation of Myt1 may allow the formation of a threshold amount of MPF from the pre-MPF store, initiating the autoamplification loop.

Among the pathways initiated by progesterone, one clearly depends on newly synthesized cyclin B1 molecules that associate with monomeric Cdc2. How do the newly formed Cdc2–cyclin complexes escape the inhibitory phosphorylations of Cdc2 catalysed by Myt1? One possibility is that progesterone suppresses Myt1 kinase in parallel to its stimulation of cyclin B synthesis. The mechanism of Myt1 downregulation would have to be independent of the Mos/MAPK pathway and of protein synthesis, as cyclin B can trigger the activation of MPF when protein synthesis and MAPK activation are blocked. Another explanation is that Myt1 is largely inactive in full-grown prophase oocytes. Indeed, it has been proposed that Myt1 kinase is highly active during the oocyte growth period, allowing the accumulation of pre-MPF, whereas it might be sequestered in full-grown oocyte containing a pre-MPF stockpile (Karaïskou *et al*, 2004).

The Mos/MAPK pathway can bypass lack of new cyclin B synthesis. In one experiment, it was observed that Mos was able to promote MPF activation in the absence of synthesis of any other protein. This suggests that depending on the female, some protein(s) required for Mos to activate MPF might be more or less accumulated during the final period of oogenesis. This newly synthesized protein might correspond to a different Cdc2-activating partner, although our results rule out Ringo/Speedy as a possible candidate for this role. What is the molecular link between the Mos/MAPK pathway and MPF activation? The only known connection is the Myt1 kinase, which has been reported to be downregulated either by Rsk or Mos (Palmer *et al*, 1998; Peter *et al*, 2002). Under physiological conditions, the inhibition of

Myt1 by the Mos/MAPK cascade may facilitate the formation of active complexes between Cdc2 and newly synthesized cyclins. However, the Mos/MAPK pathway is able to promote MPF activation in the absence of cyclin B synthesis. Under these conditions, the only possibility to form MPF is to dephosphorylate Cdc2 of the pre-MPF store by Cdc25. The inhibition of Myt1 catalysed by the Mos/MAPK cascade cannot promote the conversion from pre-MPF to active MPF unless Mos also stimulates Cdc25.

Although it is established that progesterone stimulates cyclin B1 synthesis through PKA inhibition independently of MPF, the strong accumulation of Mos requires a stabilizing phosphorylation catalysed by Cdc2 (Frank-Vaillant *et al*, 1999; Castro *et al*, 2001). Consequently, in contrast to cyclin B1 accumulation, MAPK activation only takes place when MPF activation is already initiated. The existence of this differential regulation suggests that the physiological pathway induced by progesterone depends on cyclin B synthesis, and that the Mos/MAPK cascade contributes to MPF activation once Mos stabilization is achieved. When cyclin B synthesis is impaired, some rescue mechanism could recruit the Mos/MAPK pathway and allow it to complement the deficiency in cyclin B synthesis. Despite the advances in detailed understanding of how progesterone causes oocyte maturation in *Xenopus*, one still cannot write down a completely coherent chain of molecular events. Our results imply a functional redundancy that both confuses the analysis and probably enhances the physiological robustness of the process in real life.

METHODS

Preparation and handling of oocytes. Reagents, unless otherwise specified, were from Sigma (Lyon, France). Oocytes were obtained from unprimed *Xenopus laevis* females (CNRS, Rennes, France). The external medium contained 2 μ M progesterone, 20 μ M cycloheximide and 50 μ M U0126 (Promega, Charbonnière, France). Each oocyte was injected with 25 ng B5-2 antisense, 75 ng cyc8 antisense, 84 ng Mos antisense morpholinos, 25 ng MBP-Mos, 5 ng cyclin A and 12 ng PKI. Then, they were lysed in ten volumes of extraction buffer (supplementary information online) and centrifuged at 15,000g for 10 min at 4 °C.

Antibodies and western blotting. Two oocytes equivalent were separated by SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose and probed with anti-*Xenopus* Mos, anti-*Xenopus* cyclin B1, anti-*Xenopus* cyclin B2, anti-Y15-phosphorylated Cdc2 (P-Cdc2) and anti-active (phosphorylated) MAPK (P-MAPK) antibodies. Appropriate horseradish peroxidase-labelled secondary antibodies were shown by chemiluminescence.

Kinase assays. To measure Cdc2 kinase activity, histone H1 assays were carried out on p13^{suc1} Sepharose pull-down from extracts (three oocytes equivalent) as described (Jessus *et al*, 1991).

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank Dr A. Dupre, Dr T. Hunt and Professor R. Ozon for advice and critical reading of the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique, the Université Pierre et Marie Curie, the Association pour la Recherche sur le Cancer (no. 3571 to C.J.) and Ligue Nationale contre le Cancer (Equipe Labellisée 2003 to C.J.).

REFERENCES

- Castro A, Peter M, Magnaghi-Jaulin L, Vigneron S, Galas S, Lorca T, Labbe JC (2001) Cyclin B/cdc2 induces c-Mos stability by direct phosphorylation in *Xenopus* oocytes. *Mol Biol Cell* **12**: 2660–2671
- De Smedt V, Poulhe R, Cayla X, Dessauge F, Karaïskou A, Jessus C, Ozon R (2002) Thr-161 phosphorylation of monomeric Cdc2. Regulation by protein phosphatase 2C in *Xenopus* oocytes. *J Biol Chem* **277**: 28592–28600
- Dupre A, Jessus C, Ozon R, Haccard O (2002) Mos is not required for the initiation of meiotic maturation in *Xenopus* oocytes. *EMBO J* **21**: 4026–4036
- Eyers PA, Liu J, Hayashi NR, Lewellyn AL, Gautier J, Maller JL (2005) Regulation of the G(2)/M transition in *Xenopus* oocytes by the cAMP-dependent protein kinase. *J Biol Chem* **280**: 24339–24346
- Ferby I, Blazquez M, Palmer A, Eritja R, Nebreda AR (1999) A novel p34(cdc2)-binding and activating protein that is necessary and sufficient to trigger G(2)/M progression in *Xenopus* oocytes. *Genes Dev* **13**: 2177–2189
- Frank-Vaillant M, Jessus C, Ozon R, Maller JL, Haccard O (1999) Two distinct mechanisms control the accumulation of cyclin B1 and Mos in *Xenopus* oocytes in response to progesterone. *Mol Biol Cell* **10**: 3279–3288
- Gross SD, Schwab MS, Taieb FE, Lewellyn AL, Qian YW, Maller JL (2000) The critical role of the MAP kinase pathway in meiosis II in *Xenopus* oocytes is mediated by p90(Rsk). *Curr Biol* **10**: 430–438
- Gross SD, Lewellyn AL, Maller JL (2001) A constitutively active form of the protein kinase p90Rsk1 is sufficient to trigger the G2/M transition in *Xenopus* oocytes. *J Biol Chem* **276**: 46099–46103
- Haccard O, Lewellyn A, Hartley RS, Erikson E, Maller JL (1995) Induction of *Xenopus* oocyte meiotic maturation by MAP kinase. *Dev Biol* **168**: 677–682
- Hochegger H, Klotzbucher A, Kirk J, Howell M, le Guellec K, Fletcher K, Duncan T, Sohail M, Hunt T (2001) New B-type cyclin synthesis is required between meiosis I and II during *Xenopus* oocyte maturation. *Development* **128**: 3795–3807
- Huang W, Kessler D, Erikson R (1995) Biochemical and biological analysis of Mek1 phosphorylation site mutants. *Mol Biol Cell* **6**: 237–245
- Jessus C, Rime H, Haccard O, Van LJ, Goris J, Merlevede W, Ozon R (1991) Tyrosine phosphorylation of p34^{cdc2} and p42 during meiotic maturation of *Xenopus* oocyte. Antagonistic action of okadaic acid and 6-DMAP. *Development* **111**: 813–820
- Karaïskou A, Jessus C, Brassac T, Ozon R (1999) Phosphatase 2A and polo kinase, two antagonistic regulators of cdc25 activation and MPF auto-amplification. *J Cell Sci* **112**: 3747–3756
- Karaïskou A, Dupre A, Haccard O, Jessus C (2001) From progesterone to active Cdc2 in *Xenopus* oocytes: a puzzling signalling pathway. *Biol Cell* **93**: 35–46
- Karaïskou A, Lepretre AC, Pahlavan G, Du Pasquier D, Ozon R, Jessus C (2004) Polo-like kinase confers MPF autoamplification competence to growing *Xenopus* oocytes. *Development* **131**: 1543–1552
- Lenormand JL, Dellinger RW, Knudsen KE, Subramani S, Donoghue DJ (1999) Speedy: a novel cell cycle regulator of the G2/M transition. *EMBO J* **18**: 1869–1877
- Palmer A, Gavin AC, Nebreda AR (1998) A link between MAP kinase and p34cdc2 cyclin B during oocyte maturation: p90(rsk) phosphorylates and inactivates the p34(cdc2) inhibitory kinase Myt1. *EMBO J* **17**: 5037–5047
- Peter M, Labbe JC, Doree M, Mandart E (2002) A new role for Mos in *Xenopus* oocyte maturation: targeting Myt1 independently of MAPK. *Development* **129**: 2129–2139
- Posada J, Yew N, Ahn NG, Vande Woude GF, Cooper JA (1993) Mos stimulates MAP kinase in *Xenopus* oocytes and activates a MAP kinase kinase *in vitro*. *Mol Cell Biol* **13**: 2546–2553
- Sagata N, Oskarsson M, Copeland T, Brumbaugh J, Vande Woude GF (1988) Function of c-mos proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature* **335**: 519–525
- Uto K, Inoue D, Shimuta K, Nakajo N, Sagata N (2004) Chk1, but not Chk2, inhibits Cdc25 phosphatases by a novel common mechanism. *EMBO J* **23**: 3386–3396
- Wasserman WJ, Masui Y (1975) Effects of cycloheximide on a cytoplasmic factor initiating meiotic maturation in *Xenopus* oocytes. *Exp Cell Res* **91**: 381–388