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CORRECTION

Correction: Polo-like kinase confers MPF autoamplification competence to growing *Xenopus* oocytes (doi:10.1242/dev.01050)

Anthi Karaiskou, Anne-Claire Leprêtre, Golbahar Pahlavan, David Du Pasquier, René Ozon and Catherine Jessus

Development was made aware by a reader of potential duplication of data in Fig. 2A, Fig. 5C, Fig. 6 and Fig. 7A of Development (2004) **131**, 1543-1552 (doi:10.1242/dev.01050).

The journal contacted the authors who said that some of the bands in western blots were duplicated during figure compilation. After discussion with Anthi Karaiskou and Catherine Jessus, Development referred this matter to Université Pierre et Marie Curie (UPMC, now Sorbonne Université), who investigated and cleared the authors of any wrongdoing. The UPMC committee decided that the conclusions of the paper were not affected by the errors and recommended correction of the paper (full reports available at: http://www2.cnrs.fr/sites/communique/fichier/rapport_conclusions.pdf and http://www2.cnrs.fr/sites/communique/fichier/rapport_analyse_detaillee.pdf). Development's editorial policies state that: "Should an error appear in a published article that affects scientific meaning or author credibility but does not affect the overall results and conclusions of the paper, our policy is to publish a Correction…" and that a Retraction should be published when "…a published paper contain[s] one or more significant errors or inaccuracies that change the overall results and conclusions of a paper…". Development follows the guidelines of the Committee on Publication Ethics (COPE), which state: "Retraction should usually be reserved for publications that are so seriously flawed (for whatever reason) that their findings or conclusions should not be relied upon". The standards of figure assembly and data presentation in this paper fall short of current good scientific practice. However, given that the investigating committee at UPMC declared that the conclusions of the paper were not affected by the errors, the appropriate course of action – according to COPE guidelines – is to publish a Correction, which Development has made as detailed as possible.

Readers should note that the policy of the UPMC is that authors should retain original data for 10 years; the paper falls outside this period. The authors were unable to find all the original data, but replicates of experiments carried out at the same time showing the same results were found for most blots and several new figure panels have been assembled.

In Fig. 2A, no data for the cyclin B2 blot could be found. The authors say that as the same results are also shown in Fig. 1B, and Figs 3 and 6, the cyclin B2 blot can be removed without affecting the conclusions. The new Fig. 2A is shown below. Development has not seen the original data for these results.

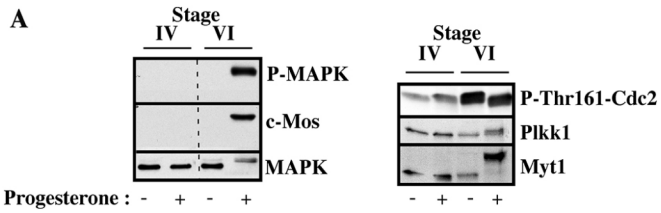

Fig. 2. Expression of the regulators of Cdc2 activation during oogenesis. (A) Prophase oocytes at stage IV (750-800 µm), stage V (900-11,000 µm) and stage VI (≥1200 µm) were incubated or not in the presence of progesterone and collected 18 hours afterwards. Oocyte extracts were western blotted with antibodies against the active phosphorylated form of MAPK (P-MAPK), Mos, MAPK, the Thr161-phosphorylated form of Cdc2 (P-Thr161-Cdc2), Plkk1 and Myt1.

Fig. 4B had unmarked splicing that the authors would like to correct. Original data could not be found for these blots but results from a replicate experiment carried out at the same time are shown.

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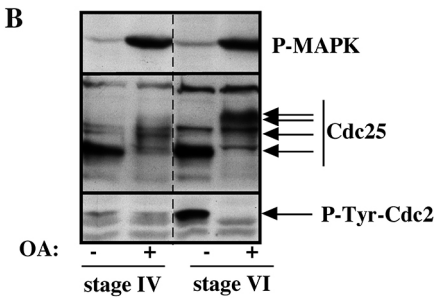

Fig. 4. Okadaic acid is unable to induce MPF auto-amplification in stage IV oocyte extracts. Stage IV and stage VI oocyte extracts were incubated in the presence (+) or in the absence (–) of 1 µM okadaic acid (OA) and an ATP-regenerating system for 3 hours. (B) Western blots revealed with antibodies against the active phosphorylated form of MAPK (P-MAPK), Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2).

For Fig. 5C, most of the original blots were found and the corrected figure panel is shown below. Note that original data were not found for the Cdc2 kinase activity, so this autoradiogram has been removed. The authors state that this does not affect the conclusions as Cdc2 activity is reflected by its tyrosine phosphorylation level and the graph in Fig. 5B. Lines indicating where the blots in Fig. 5A have been spliced have also been added; however, Development has not seen the original data for Fig. 5A.

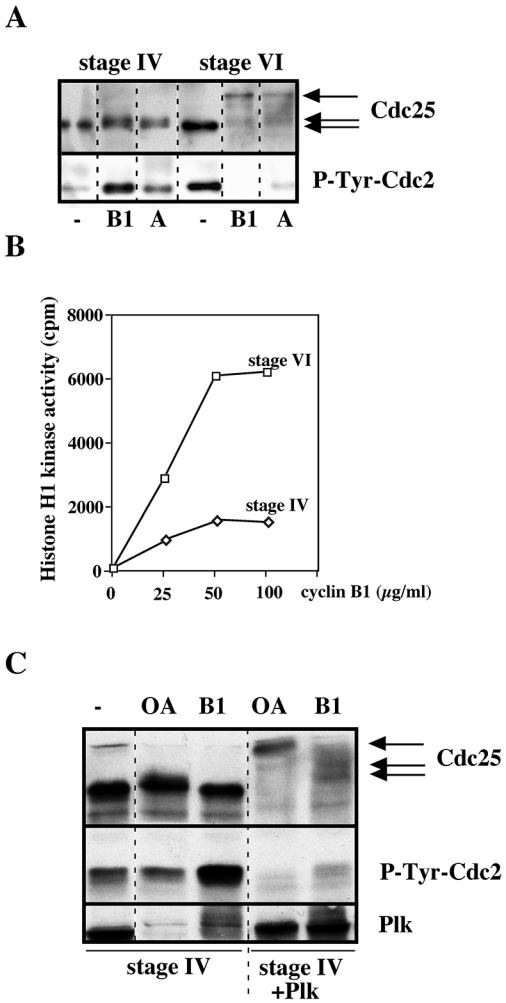

Fig. 5. Presence of Plk1 restores MPF auto-amplification induced by cyclins or okadaic acid in stage IV oocyte extracts. (A) Stage IV and stage VI oocyte extracts were incubated in the presence or in the absence of either His-cyclin B1 (B1) or GST-cyclin A (A) and ATP for 3 hours. They were western blotted with antibodies against Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2). (B) Stage IV and stage VI oocyte extracts were incubated for 3 hours in the presence of increasing amounts of human His-cyclin B1 and were then assayed for H1 kinase activity of Cdc2. (C) Stage IV oocytes were injected with human Plk1 mRNA. After overnight incubation, oocyte extracts were prepared and supplemented with 1 µM okadaic acid (OA) or His-cyclin B1 (B1) and ATP. Three hours later, extracts were western blotted with antibodies directed against Myc (indicating the presence of the Myc-tagged Plk protein), Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2).

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In Fig. 6, original blots were found for all panels except P-MAPK and the corrected figure with lines indicating splices is shown.

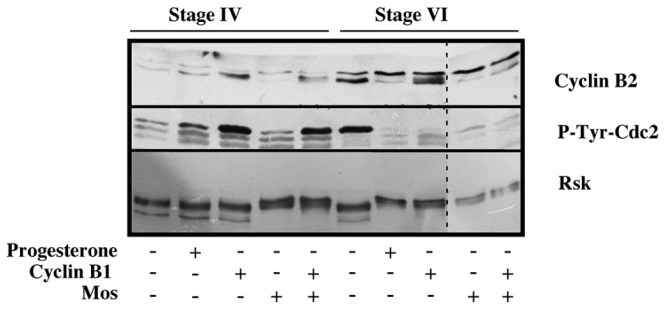

Fig. 6. Stage IV and stage VI oocytes were stimulated by progesterone or injected with either GST-cyclin B or *Xenopus* Mos protein or both GST-cyclin B and Mos. Oocytes were collected and western blotted with antibodies against cyclin B2, the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2) and Rsk.

In Fig. 7A, replicate results from the same experiment were found. No original data were found for the H1 kinase activity, so this has been removed. The authors state that absence of these data does not affect the conclusions because Cdc2 activity is reflected by its tyrosine 15 phosphorylation level. Lines indicating where the blots in Fig. 7B have been spliced have also been added. The new figure is shown here.

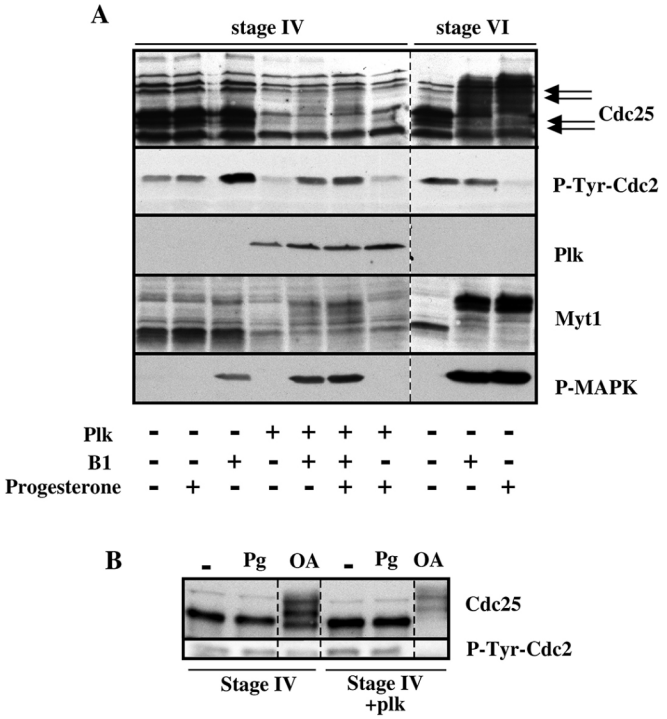

Fig. 7. *In vivo* rescue of MPF auto-amplification by Plk1 in stage IV oocytes. (A) Stage IV oocytes were injected or not with Plk1 mRNA. After overnight incubation, they were either incubated in the presence or not of progesterone, or injected with His-cyclin B1. Stage VI oocytes were used as control. Extracts were western blotted with antibodies against Cdc25, the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2), Myc (indicating the presence of the Myc-tagged Plk protein), Myt1 and the active phosphorylated form of MAPK (P-MAPK). (B) Stage IV oocytes were injected or not with Plk1 mRNA. After overnight incubation, they were either incubated in the presence or not of progesterone (Pg), or injected with okadaic acid (OA). Extracts were western blotted with antibodies against Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2).

As a result of corrections to these figures, readers of *Development* (2004) **131**, 1543-1552 (doi:10.1242/dev.01050) should ignore reference to H1 kinase activity on p. 1549. The second paragraph should now read: 'The high H1 kinase activity generated by cyclin B1 addition in the presence of Plk1 indicates that, despite the partial phosphorylation of Cdc2, the cyclinB1-Cdc2 neocomplexes are mainly active (Fig. 5C).' Text in the fourth paragraph should read: 'Constitutively active Plk1 expression did not allow stage IV oocytes to respond to progesterone, as indicated by the absence of Cdc25 and Myt1 electrophoretic shift and the maintenance of Tyr15 phosphorylation of Cdc2 (Fig. 7A).'

The authors apologise to the journal and readers for these errors.

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Development refers readers to other notices related to the UPMC investigation, published in our sister journal, Journal of Cell Science:

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Polo-like kinase confers MPF autoamplification competence to growing *Xenopus* oocytes

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Summary

During oogenesis, the *Xenopus* oocyte is blocked in prophase of meiosis I. It becomes competent to resume meiosis in response to progesterone at the end of its growing period (stage VI of oogenesis). Stage IV oocytes contain a store of inactive pre-MPF (Tyr15-phosphorylated Cdc2 bound to cyclin B2); the Cdc25 phosphatase that catalyzes Tyr15 dephosphorylation of Cdc2 is also present. However, the positive feedback loop that allows MPF autoamplification is not functional at this stage of oocyte growth. We report that when cyclin B is overexpressed in stage IV oocytes, MPF autoamplification does not occur and the newly formed cyclin B-Cdc2 complexes are inactivated by Tyr15 phosphorylation, indicating that Myt1 kinase remains active and that Cdc25 is prevented to be activated. Plx1 kinase (or polo-like kinase), which is required for Cdc25 activation and MPF autoamplification in full grown oocytes is not expressed at the protein level in small stage IV oocytes. In order to determine if Plx1 could be the missing regulator that prevents MPF autoamplification, polo kinase was overexpressed in stage IV oocytes. Under these conditions, the MPF-positive feedback loop was restored. Moreover, we show that acquisition of autoamplification competence does not require the Mos/MAPK pathway.

Key words: Cdc2, Cyclin, Cdc25, Plx1, *Xenopus* oocyte

Introduction

Oocytes in the vertebrate ovary are arrested at the prophase of first meiotic division. During this long period of prophase arrest, the oocytes grow by accumulating a variety of molecules that are required for early embryonic development. The prophase block is released when oocyte growth is completed, at time of ovulation. Full-grown oocytes resume the first meiotic division and arrest at the metaphase of the second meiotic division, a process known as meiotic maturation.

In amphibians, oocyte growth is divided into six stages, from stage I (50 μm in diameter) to the full-grown stage VI oocyte (≥1.2 mm in diameter) (Dumont, 1972). A steroid hormone, progesterone, triggers meiotic maturation at time of ovulation. Only stage VI oocytes are responsive to progesterone. The unresponsiveness of smaller oocytes to progesterone prevents premature meiotic maturation and fertilization, a mechanism contributing to the success of the embryonic development. Activation of MPF (M-phase promoting factor or Cdc2-cyclin B complex) promotes the first meiotic division in response to progesterone. Full-grown oocytes contain pre-MPF where Cdc2 is maintained inactive by Thr14 and Tyr15 phosphorylation. Progesterone induces the abrupt activation of MPF by a post-transcriptional mechanism ending to the activatory dephosphorylation of Cdc2 by Cdc25 phosphatase. This transduction pathway is not well understood. It depends on a drop in cAMP, as well as on synthesis of new proteins. A progesterone receptor is already functional in small stage IV oocytes: a decrease in cAMP can be induced by progesterone in stage IV oocytes (Mulner et al., 1983; Sadler and Maller, 1983). Therefore, the incompetence of small oocytes to undergo meiotic maturation in response to progesterone lies downstream the cAMP step, presumably at the level of MPF activation.

MPF activation in full-grown oocytes depends on the conversion of pre-MPF into MPF by Cdc25 phosphatase, by an 'auto-amplification' mechanism (Masui and Markert, 1971). This mechanism is initiated by the formation of a small amount of active MPF, that could be generated in response to progesterone by binding of newly synthesized A- or B-cyclins or Cdk-stimulatory partners, such as the Ringo/Speedy protein, to monomeric Cdc2 (Ferby et al., 1999; Kobayashi et al., 1991; Lenormand et al., 1999). Indeed, progesterone is able to trigger cyclin B1 synthesis upstream MPF activation (Frank-Vaillant et al., 1999) and an excess of monomeric Cdc2 is present in the prophase oocyte (De Smedt et al., 2002; Kobayashi et al., 1991). This small amount of active MPF would involve cyclin phosphorylation (Peter et al., 2002b) and should escape the inactivating phosphorylation of Thr14/Tyr15 of Cdc2 achieved by Myt1 kinase (Mueller et al., 1995). Cdc2 associated with either Ringo/Speedy or cyclin A appears to be less sensitive to inhibition by Myt1 (Devault et al., 1992; Karaiskou et al., 2001). Moreover, Myt1 activity is downregulated by progesterone (Mueller et al., 1995). Several kinases have been proposed as responsible for phosphorylation and downregulation of Myt1 activity: Rsk and Mos in *Xenopus* oocyte, Akt in Asterina oocyte, and recently the Plk1 in

somatic cells (Nakajima et al., 2003; Okumura et al., 2002; Palmer et al., 1998; Peter et al., 2002a). Once a catalytic amount of active MPF is formed, it initiates a positive feedback loop by phosphorylating and activating Cdc25, allowing the activating dephosphorylation of pre-MPF to start. The activating phosphorylations of Cdc25 are achieved by at least two kinases: Cdc2 and the *Xenopus* Plk1 homolog, Plx1, a kinase itself under the control of Cdc2-cyclin B (Abrieu et al., 1998; Karaiskou et al., 1999). Plx1 represents the major protein of the family of Polo-like kinases in *Xenopus* oocyte (Duncan et al., 2001; Kumagai and Dunphy, 1996). An okadaic acid sensitive phosphatase, probably a type 2A, negatively controls Cdc25 phosphorylation and activation, by opposing to Plx1 action (Karaiskou et al., 1999).

The origins of the inability of small oocytes to support MPF activation can therefore depend on various mechanisms: inability to generate a small amount of active MPF triggering the auto-amplification mechanism, and/or inability of the positive feedback loop to function.

Pre-MPF as well as Cdc25, are present in incompetent stage IV oocytes (Furuno et al., 2003; Rime et al., 1995). Although entry into M-phase can be triggered in the growing oocytes by microinjection of cytoplasm taken from matured stage VI oocytes (Hanocq-Quertier et al., 1976; Sadler and Maller, 1983; Taylor and Smith, 1987), Tyr15 dephosphorylation of endogenous Cdc2 is not complete (Rime et al., 1991), suggesting that the auto-amplification mechanism is not fully functional. Moreover, when cyclins are injected into stage IV oocytes, they associate with endogenous free Cdc2, and the illegitimate complexes undergo phosphorylation on Tyr15 (Rime et al., 1995). This result indicates a failure in small oocytes to generate active neo-complexes that could trigger the auto-amplification mechanism.

The aim of our study was to study the molecular mechanisms that prevent MPF to be activated in small oocytes. We first analyzed the expression level of the main proteins involved in MPF auto-amplification. This study revealed that Plx1, a kinase required for the auto-amplification mechanism, is absent in incompetent oocytes. We then studied the molecular mechanisms required for MPF activation during late oogenesis. The formation of the MPF neocomplexes was studied by overexpressing mitotic B or A cyclins; the functionality of the MPF feedback loop was approached by inhibiting PP2A. Our results show that MPF activation is locked during oogenesis at the level of both the generation of the active MPF trigger and the positive feedback loop between Cdc2 and Cdc25. Plx1 kinase, a central player at both levels, represents a crucial limiting factor, accounting for incompetence of small oocytes to re-enter meiosis in response to progesterone.

Materials and methods

Materials

Xenopus laevis females were purchased from CNRS (Rennes, France). Reagents, unless otherwise specified, were from Sigma.

Preparation of recombinant proteins and mRNAs

Sea urchin GST-Cyclin B (Lorca et al., 1992), human GST-cyclin A (Faivre et al., 2001) and *Xenopus* MBP-Mos (Roy et al., 1996) were expressed in bacteria. Human His-cyclin B1-Δ90 was expressed in baculovirus-infected insect cells and purified as described (Kumagai and Dunphy, 1995). *Xenopus* Myt1 protein was expressed by in vitro transcription-translation system (Abrieu et al., 1998) (TNT Coupled Reticulocyte Lysate System, Promega) in the presence of [^{35}S]methionine (Dupont NEN). Constitutively active (T210D) myc-GFP-Plk mRNA (kindly provided by Dr E. Nigg) and mouse Mos mRNA (kindly provided by Dr M.-H. Verlhac) were produced by in vitro transcription system (MEGAscript kit, Ambion).

Preparation and handling of oocytes

Xenopus oocytes were prepared as described (Jessus et al., 1987). Oocytes of different sizes were sorted on binocular using a micrometer and according to Dumont (Dumont, 1972): stage IV oocytes (750-800 μm in diameter), stage V (1000 μm in average) with two subpopulations: V_1 (900-1000 μm) and V_2 (1000-1100 μm) and stage VI (≥1200 μm in diameter). Oocytes were injected with reagents at the following concentrations in the pipette: sea urchin GST-Cyclin B (0.1 mg/ml), human His-Cyclin B1 (0.3 mg/ml), *Xenopus* MBP-Mos (0.5 mg/ml), mRNA encoding mouse Mos (1 mg/ml), mRNA encoding Plk1 (1 mg/ml), okadaic acid (10 μM, ICN). The microinjection volume was 25 nl per stage IV oocyte and 50 nl per stage VI oocyte. Progesterone was used at 1 μM. GVBD was monitored by the appearance of a white spot at the animal pole for stage VI oocytes and after fixation in 5% TCA and dissection for stage IV-V oocytes. After microinjections, oocytes of different sizes were collected 2-4 hours after GVBD in stage VI oocytes. Oocytes were collected by groups of 7 for stage VI, 14 for stage V and 21 for stage IV, and lysed at 4°C in 70 μl of EB (80 mM β-glycerophosphate, pH 7.3, 20 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT), supplemented with protease inhibitor mixture (Sigma, P8340) and 1 μM okadaic acid (ICN). This ratio of homogenization was chosen after a Bradford estimation of total protein amount present in oocytes of different sizes and resulted in the same protein concentration in oocyte extracts (2.5 mg/ml). Lysates were centrifuged at 15,000 ***g*** at 4°C for 15 minutes, and frozen at –80°C. Western blot analysis and kinase activity assays were performed by using the same lysate.

High speed oocyte extracts

High-speed stage VI oocyte extracts were prepared as described (Karaiskou et al., 1998). For the stage IV oocyte extracts, the same protocol was used except that 3 stage IV oocytes were used instead of 1 stage VI oocyte. The prophase high-speed extracts were incubated for 3 hours, at room temperature, in the presence of ATP-regenerating system (10 mM creatine phosphate, 80 μg/ml creatine phosphokinase, 1 mM ATP, 1 mM $MgCl_2$) and various effectors were used: 1 μM okadaic acid (ICN), 50 μg/ml recombinant human His-cyclin B1, 75 μg/ml recombinant human GST-cyclin A. Reaction was stopped by adding 1 mM Na-orthovanadate and 1 μM okadaic acid at 4°C. Samples were collected for western blot analysis and/or for Cdc2 kinase assays.

Western blotting

Samples of 50 μg of proteins (equivalent to 2 stage VI oocytes and 6 stage IV oocytes) were electrophoresed on 12.5% SDS-PAGE Anderson or 12% Laemmli systems (Anderson et al., 1973; Laemmli, 1970) and transferred to nitrocellulose filters (Schleicher and Schuell) using a semi-dry blotting system (Millipore). The following antibodies were used: polyclonal antibodies against Cdc2 phosphorylated on Tyr15 or on Thr161 (Cell Signaling Technology), monoclonal anti-Cdc2 (Kobayashi et al., 1994), polyclonal anti-*Xenopus* Cdc25 (Izumi et al., 1992), polyclonal anti-Plkk1 antibody (Qian et al., 1998a), polyclonal anti-Myt1 (produced by Eurogentec, Belgium) (Mueller et al., 1995), polyclonal anti-*Xenopus* cyclin B1 and cyclin B2 (De Smedt et al., 2002), polyclonal anti-*Xenopus* Plx1 (Descombes and Nigg, 1998), monoclonal antibody against phosphorylated MAPK (New England Biolabs), polyclonal anti-ERK1 and anti-*Xenopus* Mos (Santa Cruz Biotechnologies), and polyclonal anti-Rsk1 (Santa Cruz Biotechnologies).

The primary antibodies were detected with appropriated horseradish peroxidase-conjugated second antibodies (Jackson ImmunoResearch Laboratories) and the Western Blot Chemoluminescence Renaissance kit (Perkin Elmer Life Sciences).

Kinase assays

Cdc2 kinase activity was assayed in extracts (equivalent to 2 stage VI oocytes or 6 stage IV oocytes) in the presence of 0.5 mM PKI peptide,1 μCi of [γ^{32}P]ATP (Dupont, NEN), 100 μM ATP and 0.2 mg/ml histone H1 (Boehringer) in 50 μl of kinase buffer (50 mM Tris-HCl, pH 7.2, 15 mM $MgCl_2$, 5 mM EGTA, 1 mM DTT). Kinase reactions were stopped by adding Laemmli buffer (Laemmli, 1970) and boiling, followed by electrophoresis and autoradiography.

RT-PCR

RNA was extracted from oocytes at various stages using the Rneasy kit (Qiagen). Reverse transcription and PCR amplification were performed on 1.5 μg RNA (Onestep RT-PCR kit Qiagen). The thermal cycling conditions were 55°C for 30 minutes, 95°C for 15 minutes, 35 cycles at 94°C for 1 minute, 55°C for 1 minute and 72°C for 1.5 minutes. The 5′ and 3′ primers used were respectively 5′-GTCGCATATGGCTCAAGTGGCCGGTAAGAAAC-3′ and 5′-CT-GGATGGCGATCTCCATGGTCATCTTATCC-3′ for Plx1, 5′-ACAT-TTTTCAAGCAGTGTTTTAAA-3′ and 5′-AGGGAGATGCCCTT-GTCTCAGCTG-3′ for Myt1. The amount of Myt1 transcripts was previously reported to remain constant during oogenesis (Furuno et al., 2003). A PCR on 1.5 μg RNA without reverse transcription using Plx1 primers was performed as a negative control. Reaction products were fractionated on 1.5% agarose gels, stained with ethidium bromide and photographed.

Results

Expression levels of regulators of the MPF auto-amplification loop during late oogenesis

Small oocytes (≤1.1 mm in diameter) do not undergo GVBD in response to progesterone (Dumont, 1972; Hanocq-Quertier et al., 1976; Sadler and Maller, 1983). We analyzed GVBD and measured Cdc2 activity after progesterone treatment of oocytes of different sizes (Fig. 1A). As expected, the only oocytes responding to progesterone were the stage VI population (Fig. 1A).

We then analyzed the expression pattern of the main MPF auto-amplification loop regulators during oogenesis (stages IV-VI), by loading identical protein quantities (50 μg) (Fig. 1B, Fig. 2A). Although the amount of Cdc2 protein is present at a constant level throughout stages IV to VI, the level of pre-MPF or inactive cyclin B2-Cdc2, progressively accumulates during late oogenesis, as revealed by the expression of cyclin B2, and the Tyr15 and Thr161 phosphorylation levels of Cdc2 (Fig. 1B, Fig. 2A). Cyclin B1 is undetectable in stage IV oocytes and expressed at a very low level in prophase oocytes of stages V or VI (Fig. 1B). This indicates that cyclin B synthesis occurs from stage IV to stage VI, leading to an accumulation of inactive cyclin B-bound Cdc2. The ratio of free Cdc2 versus cyclin B-Cdc2 is therefore higher in stage IV oocytes than in stage VI oocytes.

The two main regulators of the MPF positive feedback loop, the inhibitory kinase Myt1 and the activating Cdc25 phosphatase, are present in constant concentrations throughout stages IV to VI (Fig. 1B, Fig. 2A). Phosphatase 2A (PP2A), one of the main negative regulators of the MPF auto-amplification loop in prophase oocytes, is also present throughout stages IV to VI (data not shown). Thus, during late oogenesis, pre-MPF is accumulating, whereas the protein levels of its direct regulators remain stable.

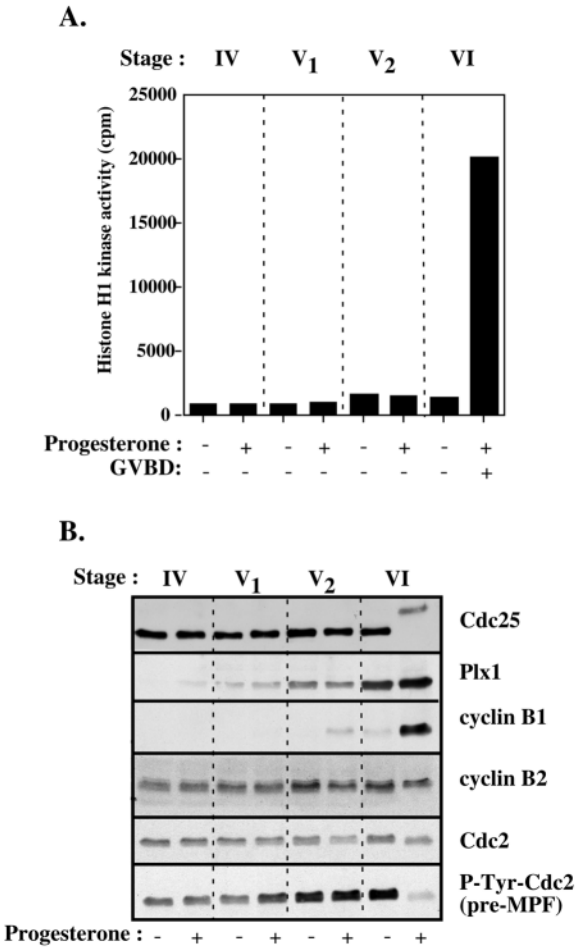

Fig. 1. Expression of the regulators of Cdc2 activation in stage IV oocytes. Prophase oocytes at stage IV (750-800 μm), stage V_1 (900-1000 μm), stage V_2 (1000-1100 μm) and stage VI (≥1200 μm) were incubated or not in the presence of progesterone and collected 18 hours afterwards. Occurrence of GVBD is indicated by (+). Oocyte extracts were either assayed for H1 kinase activity of Cdc2 (A) or western blotted (B) with antibodies against Cdc2, cyclin B1, cyclin B2, Cdc25, Plx1 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2).

We next examined the protein expression of Plx1 kinase, which directly phosphorylates Cdc25 and is required in the MPF auto-amplification loop (Abrieu et al., 1998; Karaiskou et al., 1999; Kumagai and Dunphy, 1996; Qian et al., 1998a). Plx1 was not detected in stage IV oocytes (Fig. 1B). Its level progressively increased during late oogenesis to a maximum in stage VI oocytes (Fig. 1B). RT-PCR analysis revealed that Plx1 mRNA is present in stage IV oocytes, as well as in stage VI oocytes (Fig. 2B), showing that Plx1 expression is regulated at a translational level during the end of oogenesis. Plx1 therefore represents a good candidate as a limiting factor accounting for meiotic incompetence. It is noteworthy that Plkk1 kinase, direct activator of Plx1 (Qian et al., 1998b), is already present in stage IV oocytes (Fig. 2A).

We also tested if the protein expression of the above-mentioned MPF auto-amplification players could be modified

after progesterone treatment in incompetent oocytes (Fig. 1B, Fig. 2A). As expected, after progesterone treatment, only stage VI full-grown oocytes exhibited phosphorylations of MPF regulators: activating phosphorylation of Cdc25, Plx1, Plkk1, cyclin B2 and inhibitory phosphorylation of Myt1 (Fig. 1B, Fig. 2A). Moreover, in response to progesterone, stage VI oocytes accumulated high levels of cyclin B1 proteins; interestingly, a slight increase of cyclin B1 was reproducibly observed in stage V_2 oocytes, in the absence of H1 kinase activation (Fig. 1B).

To characterize the molecular context of incompetent oocytes further, we analyzed the presence of the different elements of the Mos/MAPK/Rsk pathway, known to contribute positively to the kinetics of MPF activation during entry into meiosis I (Dupre et al., 2002; Fisher et al., 1999; Gross et al., 2000). Stage IV oocytes contain similar levels of MAPK and Rsk as stage VI oocytes (Fig. 2A, Fig. 3). However, incompetent stage IV oocytes were incapable of either accumulating Mos kinase, the initiator of the cascade, or activating MAPK and Rsk in response to progesterone (Fig. 2A, Fig. 3).

The MAPK/ Rsk pathway is able to be activated by Mos in stage IV oocytes but has no interaction with the Cdc2 pathway

Stage IV oocytes fail to accumulate Mos protein in response to progesterone (Fig. 2A, Fig. 3). As MAPK protein is expressed at a similar concentration in incompetent and competent oocytes (Fig. 2A), it was interesting to investigate if the MAPK pathway was functional, i.e. inducible by Mos, and able to lead to Cdc2 activation. We microinjected mouse Mos mRNA into stage IV and stage VI oocytes and then proceeded to western blot analysis. The choice of the mouse species was dictated by the necessity to distinguish the endogenous Mos from the ectopically produced protein. Fig. 3 shows that activating phosphorylations of MAPK and Rsk (visualized by its electrophoretic retardation) occurred in response to Mos microinjection in stage IV oocyte, in the same way as in stage VI oocyte. This result shows that Mos expression is sufficient to turn on the MAPK pathway in incompetent oocyte.

We then analyzed if Mos expression can lead to MPF activation in small oocytes, as in full-grown oocytes. Activating the MAPK pathway was not sufficient to trigger the activating Tyr15 dephosphorylation of Cdc2, Cdc25 hyperphosphorylation and the activating phosphorylation of cyclin B2 (Fig. 3). Moreover, cyclin B1 did not accumulate in response to Mos injection in stage IV oocytes (Fig. 3) and Myt1 activity was not downregulated by the activation of the MAPK pathway in these oocytes (see later, Fig. 6). Altogether, these results show that the molecular link between the MAPK pathway and Cdc2 is not functional.

We next asked if providing Mos to stage IV oocyte together with progesterone could allow MPF activation. Mouse Mos mRNA was injected into stage IV or stage VI oocytes in the absence or presence of progesterone, and the activation state of the MPF auto-amplification loop was monitored. Even in the presence of both an activated MAPK pathway and progesterone, the incompetent stage IV oocytes did not accumulate cyclin B1 and fail to activate MPF, as monitored by the phosphorylation status of the Tyr15 of Cdc2 and the phosphorylation levels of Cdc25 and cyclin B2 (Fig. 3). We also investigated if in a stage IV oocyte, activation of the MAPK pathway by injected Mos mRNA can trigger endogenous Mos protein accumulation, as in full-grown oocytes (Faure et al., 1998). Fig. 3 shows that stage IV oocytes do not accumulate Mos protein in response to active MAPK and Rsk, indicating that the positive feedback loop of active MAPK on Mos translation is not functional in small oocytes or that upstream stabilizing events preventing Mos degradation do not occur in stage IV oocytes.

PP2A inhibition does not trigger MPF auto-amplification loop in stage IV oocyte extracts

Okadaic acid, an inhibitor of PP2A and PP1, is known to induce MPF activation when injected in stage VI oocytes but is inefficient in stage IV oocytes, indicating that a PP2A/PP1 target involved in MPF autoamplification is not operating in small oocytes (Felix et al., 1990; Goris et al., 1989; Izumi et al., 1992; Rime et al., 1995). Okadaic acid

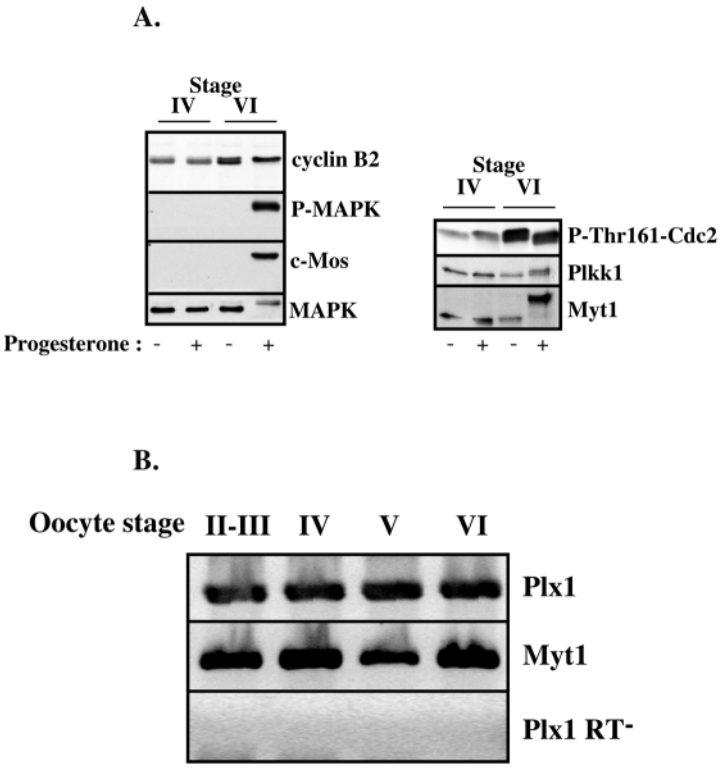

Fig. 2. Expression of the regulators of Cdc2 activation during oogenesis. (A) Prophase oocytes at stage IV (750-800 μm), stage V (900-11,000 μm) and stage VI (≥1200 μm) were incubated or not in the presence of progesterone and collected 18 hours afterwards. Oocyte extracts were western blotted with antibodies against cyclin B2, the active phosphorylated form of MAPK (P-MAPK), Mos, MAPK, the Thr161-phosphorylated form of Cdc2 (P-Thr161-Cdc2), Plkk1 and Myt1. (B) RT-PCR analysis of Plx1 transcripts. Total RNA of oocytes at various stages was subjected to RT-PCR analysis using Plx1 or Myt1 oligonucleotides as primers. Myt1 was used as the loading control. Without reverse transcription (Plx1 RT^-), no amplification products were detectable using Plx1 primers.

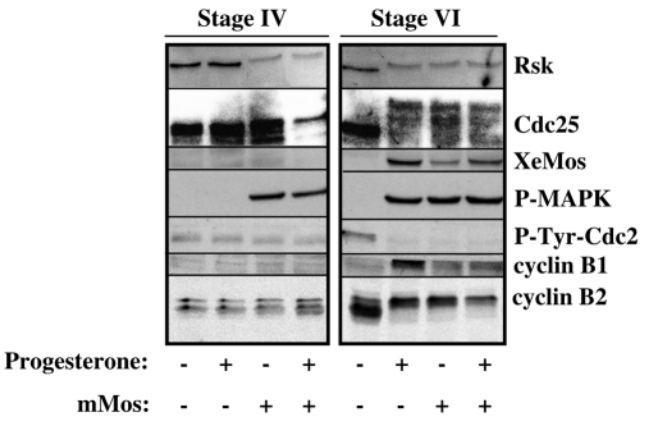

Fig. 3. Mos is able to activate MAPK and Rsk in stage IV oocytes but does not promote MPF activation. Stage IV and stage VI oocytes were injected with mouse Mos mRNA. After overnight incubation, they were incubated or not in the presence of progesterone. Stage IV oocytes were collected 4 hours after GVBD occurred in stage VI oocytes. Oocyte extracts were western blotted with antibodies against Rsk, Cdc25, *Xenopus* Mos (XeMos), the active phosphorylated form of MAPK (P-MAPK), the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2), cyclin B1 and cyclin B2.

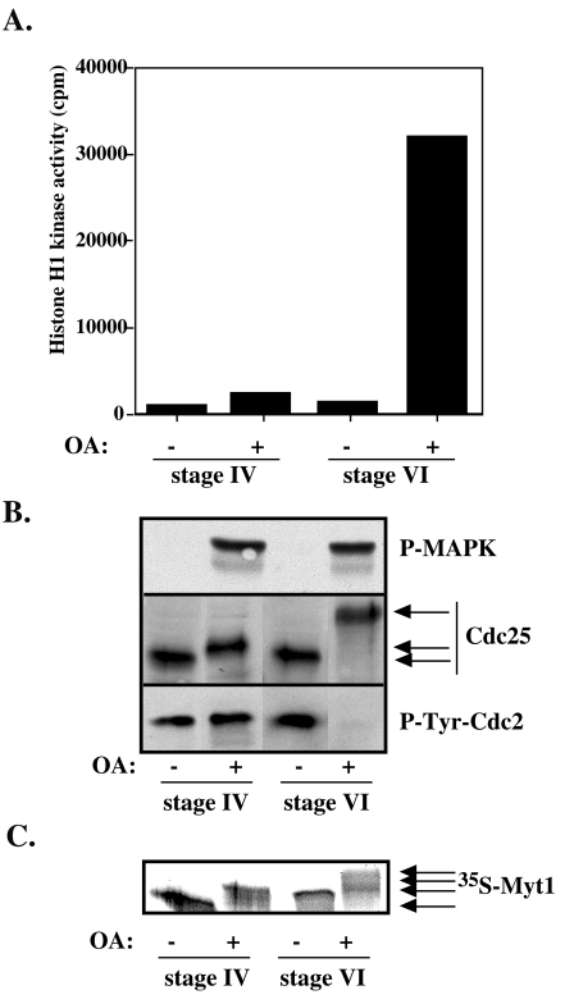

Fig. 4. Okadaic acid is unable to induce MPF auto-amplification in stage IV oocyte extracts. Stage IV and stage VI oocyte extracts were incubated in the presence (+) or in the absence (–) of 1 μM okadaic acid (OA) and an ATP-regenerating system for 3 hours. (A) Histone H1 kinase assay of Cdc2. (B) Western blots revealed with antibodies against the active phosphorylated form of MAPK (P-MAPK), Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2). (C) Oocyte extracts were supplemented with [^{35}S]methionine labeled Myt1 at time of OA addition. Extracts were then submitted to SDS-PAGE and to autoradiography.

is also able to trigger Cdc2 activation in different cell-free systems derived from full-grown oocytes (Huang and Ferrell, 1996; Karaiskou et al., 1998; Karaiskou et al., 1999; Nebreda et al., 1995; Nebreda and Hunt, 1993). We have previously shown that addition of 0.5 μM okadaic acid, a concentration targeting PP2A, in a cell-free system derived from prophase stage VI oocytes, results in MPF autoamplification: abrupt onset of the Cdc2 positive feedback loop, including Cdc25 hyper-phosphorylation and Cdc2 activation, an event requiring ATP and Plx1 kinase activity (Karaiskou et al., 1998; Karaiskou et al., 1999). To study the mechanism that locks MPF activation in incompetent oocytes, we therefore used the powerful system of extracts that allows the molecular dissection of MPF autoamplification. Okadaic acid was added in stage IV or stage VI oocyte high-speed extracts, in the presence of ATP. After 3 hours of incubation, the state of the MPF auto-amplification loop was monitored by measuring Cdc2 kinase activity and analyzing Cdc25 phosphatase and Myt1 kinase phosphorylation states, or directly the Tyr15 phosphorylation of Cdc2 (Fig. 4). In control stage VI oocyte extract, okadaic acid triggered Cdc2 activation and Cdc25 and Myt1 hyper-phosphorylation, as expected (Karaiskou et al., 1998; Karaiskou et al., 1999). By contrast, PP2A inhibition in stage IV oocyte extract did not result in substantial MPF activation, as shown by the H1 kinase assay, and did not induce Tyr15 dephosphorylation of Cdc2 nor Cdc25 and Myt1 hyper-phosphorylation (Fig. 4). Increasing the periods of incubation up to 5 hours, or okadaic acid concentration up to 2 μM never led to MPF activation and Cdc25 and Myt1 phosphorylation. Nevertheless, okadaic acid addition in incompetent oocyte extracts resulted in a small but reproducible electrophoretic mobility shift of both Cdc25 and Myt1 (Fig. 4). Significantly, MAPK was activated by okadaic acid addition in stage IV and stage VI oocyte extract (Fig. 4B), showing that the PP2A-sensitive equilibrium between MEK and MAPK described previously (Karaiskou et al., 1999; Maton et al., 2003) is dynamic in incompetent oocyte extracts. In conclusion, in stage IV oocyte extracts, PP2A inhibition is not sufficient to trigger MPF auto-amplification and the positive feedback loop between Cdc2 and Cdc25 is not functional.

Mitotic cyclins induce the MPF auto-amplification loop in stage VI but not in stage IV oocyte extracts

It has been reported that addition of A- or B-type cyclins into a full-grown oocyte concentrated extract leads to active neo-formed complexes acting as a trigger for the MPF auto-amplification mechanism (Nebreda et al., 1995; Nebreda and Hunt, 1993). We next asked if the formation of such active neo-complexes could take place in extracts derived from incompetent oocytes.

Cyclin B1 or cyclin A was added in high-speed oocyte

extracts prepared from incompetent stage IV or stage VI oocytes. After 3 hours of incubation in the presence of ATP, the extracts were assayed for Cdc25 and Tyr15-Cdc2 phosphorylation levels as well as H1 kinase activity (Fig. 5A,B). Cyclin B or A addition in a stage VI oocyte extract led to MPF auto-amplification, as judged by the Cdc25 electrophoretic hypershift and Tyr15 dephosphorylation of Cdc2 (Fig. 5A). Interestingly, cyclin addition in stage IV oocyte extract did not lead to Cdc25 hyperphosphorylation but resulted in an increased level of the Tyr15-phosphorylated Cdc2 form (Fig. 5A). This clearly indicates that added cyclins B1 and A bind endogenous Cdc2 in a stage IV oocyte extract. In strong contrast to the full-grown oocyte extracts, these neo-formed complexes are inactivated by Tyr15 phosphorylation. However, addition of cyclin B1 in incompetent oocyte extracts led to a modest but significant activation of the Cdc2 kinase activity (Fig. 5B), showing that the increased population of inactive Tyr15-phosphorylated Cdc2-cyclin complexes co-exists with a minor fraction of active complexes. These results indicate that, in contrast to stage VI oocytes, Myt1 remains functional even after cyclin overexpression in small oocytes.

The absence of Mos and active Rsk, two negative regulators of Myt1 kinase in *Xenopus* oocytes (Palmer et al., 1998; Peter et al., 2002a) could explain why neoformed complexes between Cdc2 and cyclins are inactivated by Tyr15 phosphorylation of Cdc2. To address this question, cyclin B1 was injected in stage IV oocytes in the presence of recombinant Mos. This led to the activation of MAPK and Rsk (Fig. 6). However, despite this activation, the new complexes formed between Cdc2 and injected cyclin B1 were inactivated by Tyr15 phosphorylation and cyclin B2 electrophoretic mobility was not affected (Fig. 6). Addition of progesterone did not allow cyclin B1 and Mos to induce Cdc2 activation and Myt1 downregulation in stage IV oocytes (data not shown). Therefore, the presence of Mos and Rsk is not sufficient to downregulate Myt1 kinase activity, indicating the existence of another regulatory mechanism acting on Myt1.

In conclusion, stage IV oocytes do not support the formation of active Cdc2-cyclin B complexes, compromising the initiation of the MPF auto-amplification loop. This could imply a mechanism that 'protects' pre-MPF from dephosphorylation and activation. This hypothesis was tested by analyzing the effects of added recombinant Cdc25 phosphatase in stage IV and stage VI oocyte extracts. Indeed, the amount of Cdc25 protein requested to achieve the activation of a constant amount of cyclin B-Cdc2 complexes is 120 times higher in stage IV oocyte extracts than in stage VI oocyte extracts (data not shown), indicating in stage IV oocytes the presence of a mechanism that renders pre-MPF resistant to dephosphorylation and activation by Cdc25.

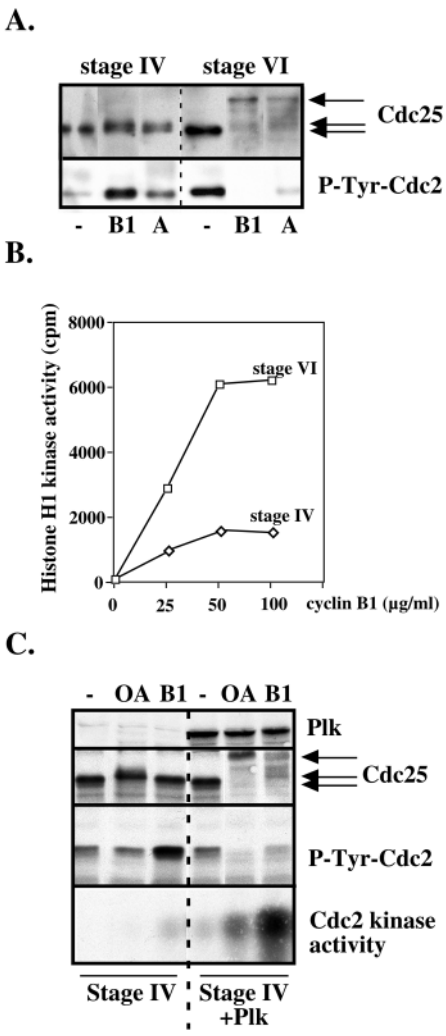

Fig. 5. Presence of Plk1 restores MPF auto-amplification induced by cyclins or okadaic acid in stage IV oocyte extracts. (A) Stage IV and stage VI oocyte extracts were incubated in the presence or in the absence of either His-cyclin B1 (B1) or GST-cyclin A (A) and ATP for 3 hours. They were western blotted with antibodies against Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2). (B) Stage IV and stage VI oocyte extracts were incubated for 3 hours in the presence of increasing amounts of human His-cyclin B1 and were then assayed for H1 kinase activity of Cdc2. (C) Stage IV oocytes were injected with human Plk1 mRNA. After overnight incubation, oocyte extracts were prepared and supplemented with 1 µM okadaic acid (OA) or His-cyclin B1 (B1) and ATP. Three hours later, extracts were western blotted with antibodies directed against Myc (indicating the presence of the Myc-tagged Plk protein), Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2). They were also assayed for H1 kinase activity of Cdc2 (lower panel shows autoradiograph of [^{32}P]histone H1).

Plk1 addition restores MPF auto-amplification loop in stage IV oocyte

As shown in Fig. 1B, Plx1, which is required for the MPF auto-amplification loop, is absent in stage IV oocytes. A likely explanation of the inability of stage VI oocyte cytoplasm to support MPF auto-amplification is the absence of Plx1 expression. To verify this hypothesis, we microinjected stage IV oocytes with mRNA encoding the human homolog of Plx1, Plk1. All the experiments were performed with either wild-type Plk1 or its constitutively active form and gave identical results. The experiments described below were performed with the constitutively active form of Plk1. Oocytes were microinjected with Plk1 mRNA and, after an overnight incubation allowing a maximum expression of the transcript, oocyte extracts were prepared and their capacity to support MPF auto-amplification was analyzed by adding okadaic acid or cyclin B1, in the presence of ATP. In response to okadaic acid, a stage IV oocyte extract, prepared from oocytes

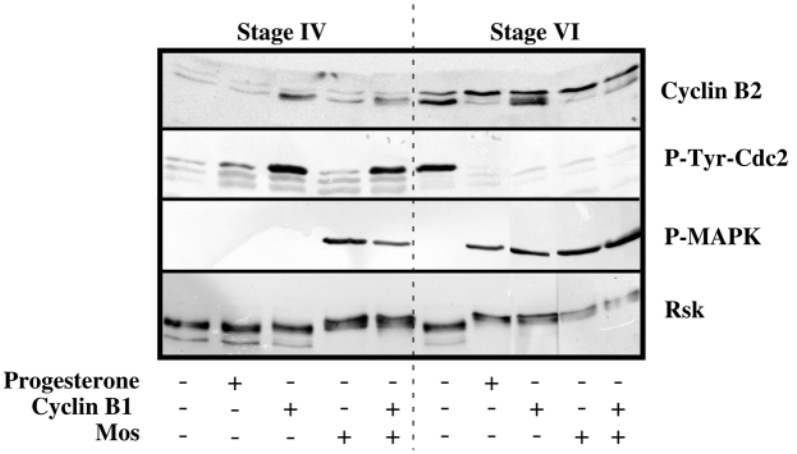

Fig. 6. Stage IV and stage VI oocytes were stimulated by progesterone or injected with either GST-cyclin B or *Xenopus* Mos protein or both GST-cyclin B and Mos. Oocytes were collected and western blotted with antibodies against cyclin B2, the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2), the active phosphorylated form of MAPK (P-MAPK) and Rsk.

expressing Plk1, becomes able to trigger the MPF auto-amplification loop, as shown by Cdc25 electrophoretic hypershift, the complete Tyr15 dephosphorylation of Cdc2 and H1 kinase activity of Cdc2 (Fig. 5C). Plk1 is therefore sufficient to restore the MPF auto-amplification loop triggered by PP2A inhibition in a stage IV oocyte extract.

We next investigated whether Plk1 expression is also sufficient to allow the initiation of MPF auto-amplification by cyclin addition. The ability of stage IV oocyte extract to activate MPF in response to cyclin addition was partially restored by the presence of Plk1 (Fig. 5C). Cdc25 electrophoretic mobility was partially retarded and a strong decrease, but not total disappearance, of Tyr15-phosphorylation of Cdc2-cyclin complexes was observed in Plk1-supplemented stage IV oocyte extracts (Fig. 5C). The high H1 kinase activity generated by cyclin B1 addition in the presence of Plk1 indicates that, despite the partial phosphorylation of Cdc25 and the incomplete Tyr15 dephosphorylation of Cdc2, the cyclin B1-Cdc2 neocomplexes are mainly active (Fig. 5C).

Therefore, the presence of Plk1 authorizes the auto-amplification mechanism to be initiated by inhibition of PP2A, which is known to antagonize Plx1 action at the level of Cdc25. By contrast, starting the mechanism by cyclin B-Cdc2 neoformed complexes is less efficient in the absence of PP2A inhibition, even though Plk1 is present.

As Plk1 was sufficient to restore in vitro MPF auto-amplification in stage IV oocyte extracts, we then asked whether Plk1 was also sufficient in vivo to restore MPF auto-amplification in response to progesterone or cyclin B injection. Stage IV oocytes were microinjected or not with Plk1 mRNA and after an overnight incubation, were incubated with progesterone. Stage VI oocytes were used as control. Constitutively active Plk1 expression did not allow stage IV oocytes to respond to progesterone, as indicated by the absence of Cdc25 and Myt1 electrophoretic shift, H1 kinase activity and the maintenance of Tyr15 phosphorylation of Cdc2 (Fig. 7A).

In full-grown oocytes, cyclin B injection triggers MPF activation by recruiting and activating Cdc2, initiating the auto-amplification mechanism. This mechanism is not functional in stage IV oocytes where injected cyclins bind monomeric Cdc2, resulting in the formation of Tyr15-phosphorylated inactive complexes (Rime et al., 1995). We observed similar effects after cyclin B microinjection in stage IV oocytes: accumulation of Tyr15-phosphorylated Cdc2-cyclin complexes, which are devoid of kinase activity, and absence of Myt1 and Cdc25 phosphorylation (Fig. 7A), as already observed in extracts (Fig. 5). By contrast, cyclin B microinjected in stage IV oocytes that express Plk1, provoked a partial electrophoretic shift of Cdc25 and Myt1, incomplete Tyr15 dephosphorylation of Cdc2 and H1 kinase activity, as well as MAPK activation (Fig. 7A). In conclusion, Plk1 kinase expression in a stage IV oocyte allows the appearance of active Cdc2-cyclin complexes in response to cyclin B microinjection, probably because some of the newly formed complexes escape the inhibitory phosphorylation by Myt1. However, the auto-amplification mechanism acting on endogenous pre-MPF is not fully functional, as shown by the partial electrophoretic shift of Cdc25 and Myt1, as well as the incomplete Tyr15 dephosphorylation of Cdc2.

Injection of okadaic acid in full-grown oocytes triggers MPF activation by directly activating the positive feedback loop of MPF, independently of the formation of a starter amount of active MPF (Felix et al., 1990; Goris et al., 1989; Izumi et al., 1992). However, it has no effect in stage IV oocytes (Rime et al., 1995), probably owing to the absence of Plx1. To ascertain this hypothesis, Plk1 mRNA was injected in stage IV oocytes, and after an overnight incubation, oocytes were injected with okadaic acid. One to 2 hours later, the pigmentation of the animal hemisphere of the injected oocytes underwent strong rearrangements. Western blot analysis revealed that Cdc2 was dephosphorylated on Tyr15 and Cdc25 was hyperphosphorylated (Fig. 7B), showing that Plk1 expression is sufficient to allow the initiation of the positive feedback loop by PP2A inhibition.

Although the Mos/MAPK pathway is not necessary to activate Cdc2 in stage VI oocytes (Dupre et al., 2002; Fisher et al., 1999; Gross et al., 2000), injection of Mos is able to trigger MPF automplification in full grown oocytes (Sagata et al., 1988). This is not the case in stage IV oocytes (Figs 3, 6). We then addressed the question whether providing Plk1 together with Mos could allow MPF auto-amplification through Myt1 downregulation. Plk1 mRNA and mouse Mos mRNA were injected in stage IV oocytes, and after an overnight incubation, oocytes were stimulated or not with progesterone. Western blot analysis revealed that MAPK was activated in response to Mos injection (Fig. 7C). However, none of these treatments was able to activate Cdc2, as ascertained by the Tyr15 phosphorylation level of Cdc2 and the electrophoretic mobility of cyclin B2 and Cdc25 (Fig. 7C). Therefore, activation of MAPK is not sufficient to restore the ability to activate MPF in stage IV oocyte, even in the presence of Plk1 and progesterone.

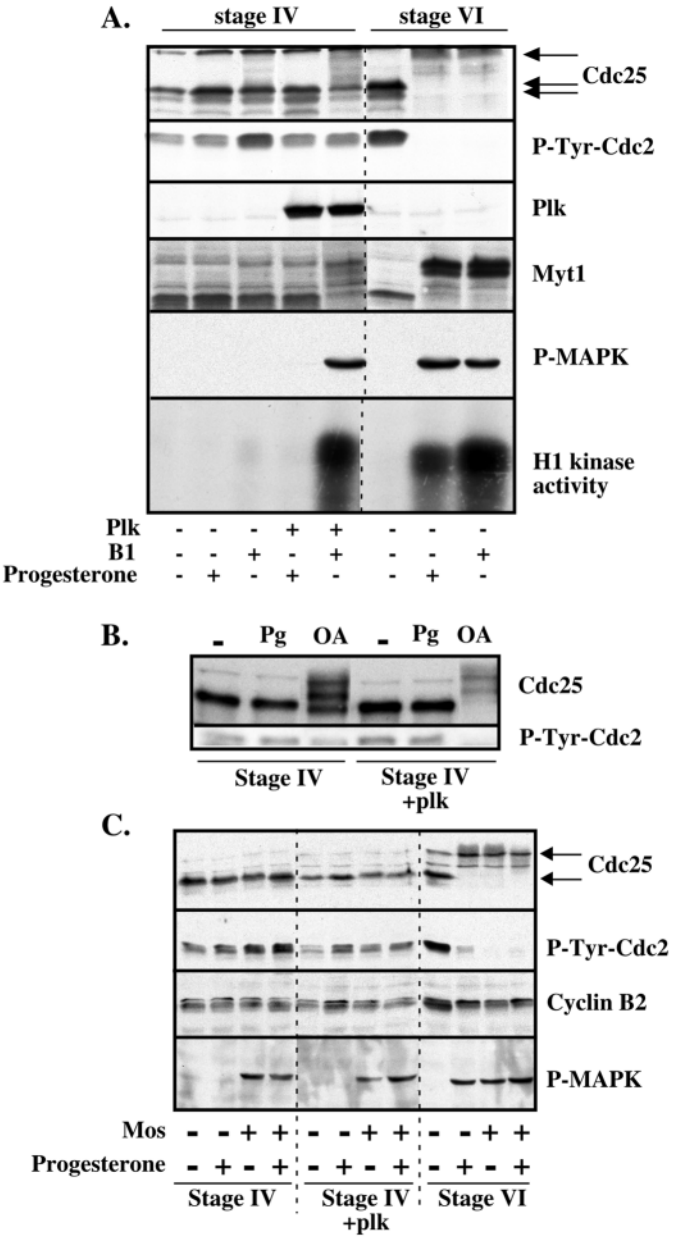

Fig. 7. In vivo rescue of MPF auto-amplification by Plk1 in stage IV oocytes. (A) Stage IV oocytes were injected or not with Plk1 mRNA. After overnight incubation, they were either incubated in the presence or not of progesterone, or injected with His-cyclin B1. Stage VI oocytes were used as control. Extracts were western blotted with antibodies against Cdc25, the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2), Myc (indicating the presence of the Myc-tagged Plk protein), Myt1 and the active phosphorylated form of MAPK (P-MAPK). They were also assayed for H1 kinase activity of Cdc2 (lower panel shows autoradiograph of [^{32}P]histone H1). (B) Stage IV oocytes were injected or not with Plk1 mRNA. After overnight incubation, they were either incubated in the presence or not of progesterone (Pg), or injected with okadaic acid (OA). Extracts were western blotted with antibodies against Cdc25 and the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2). (C) Stage IV oocytes were injected with mouse Mos mRNA and human Plk1 mRNA. After overnight incubation, they were incubated or not in the presence of progesterone. Oocytes were collected 4 hours after GVBD occurred in stage VI oocytes. Oocyte extracts were western blotted with antibodies against the active phosphorylated form of MAPK (P-MAPK), Cdc25, the Tyr15-phosphorylated form of Cdc2 (P-Tyr-Cdc2) and cyclin B2.

Discussion

It is known that, although pre-MPF and its direct activator, Cdc25, are present in small oocytes (Furuno et al., 2003; Rime et al., 1995), MPF activation does not occur in response to progesterone. First, the transduction pathway induced by progesterone is unable to connect to MPF regulators. Second, cyclin injection is unable to generate a starter amount of active MPF to induce the MPF auto-amplification mechanism. Third, the positive feedback loop operating between Cdc2 and Cdc25 cannot be initiated by PP2A inhibition.

We have shown that cyclin B1 synthesis, which occurs in full-grown oocytes in response to progesterone and independently of MPF (Frank-Vaillant et al., 1999), is not inducible by progesterone in stage IV oocytes. Although cyclin B synthesis is not required for MPF activation in stage VI oocytes (Hochegger et al., 2001), the absence of such a translational regulation of cyclin B could participate in the meiotic incompetence. To further investigate the ability of a small oocyte to initiate the MPF auto-amplification mechanism in response to a MPF primer, A- and B-type cyclins were provided, either in extracts, or in vivo by microinjection. In both cases, cyclins bind free monomeric Cdc2. However, such a binding does not result in Cdc2 activation, as it is the case in a full-grown oocyte. On the contrary, the neoformed complexes are inactivated by Tyr15 phosphorylation of Cdc2, indicating that Myt1 kinase is maintained under an active state in stage IV oocytes, even in the presence of overexpressed cyclins. Therefore, even if progesterone were able to generate a small amount of starter MPF by cyclin synthesis in growing oocytes, the new complexes would be directly inactivated by Tyr15 phosphorylation, preventing the auto-amplification loop to be initiated.

How to explain the sustained activity of Myt1 towards Cdc2 in stage IV oocytes compared with stage VI oocytes? A first possibility is that an adaptor protein is associated with cyclin B-Cdc2 complexes in small oocytes, rendering them with a higher affinity Myt1 or less affinity for Cdc25. A second explanation resides at the level of the Mos/MAPK/Rsk pathway. It has been shown that in full-grown oocytes, Mos and Rsk are able to negatively regulate Myt1 (Palmer et al., 1998; Peter et al., 2002a). As progesterone is not able to induce Mos synthesis and to turn on the MAPK pathway in incompetent oocytes, this could explain why Myt1 is maintained under a very active state. However, we show here that providing Mos and activating Rsk in small oocytes does not allow progesterone to activate MPF. Moreover, injection of Mos together with cyclin B does not prevent the inactivation of the new complexes by Myt1. Therefore, absence of Mos and of an activatable MAPK pathway is not the only event accounting for the sustained Myt1 activity in incompetent oocytes.

We show here that Plx1 protein that is crucial for the function of the auto-amplification feedback loop in full-grown oocytes is not expressed in small oocytes. Both Cdc25 and Myt1 are direct substrates of Plk1 during M phase (Nakajima

et al., 2003; Kumagai and Dunphy, 1996). Our results indicate that overexpression of Plk1 in stage IV oocytes authorizes cyclin B1 to form active complexes with Cdc2. This observation shows that in oocytes, Plk1 participates in the formation of an active MPF trigger by downregulating Myt1. Moreover, it indicates that progesterone unresponsiveness of small oocytes depends on a stable activity of Myt1 kinase, because of Plx1 absence. Although Plk1 expression prevents Tyr15 phosphorylation of Cdc2 after cyclin B overexpression, Cdc25 is not fully activated. This shows that full activation of Cdc25 requires a further regulatory mechanism. Indeed, *Xenopus* Cdc25 can be negatively regulated through Ser287 phosphorylation by several kinases, including Chk1 (Kumagai et al., 1998) and PKA (Duckworth et al., 2002). In a recent report, Margolis et al. (Margolis et al., 2003) showed that Cdc25C, which is phosphorylated on Ser287 in *Xenopus* prophase oocytes, is dephosphorylated by type 1 phosphatase (PP1) at GVBD. Since the PP1 inhibitor I prevents meiotic maturation (Huchon et al., 1981), PP1 could participate to the regulation of the MPF autoamplification loop by catalyzing the removal of the inhibitory Ser287 phosphate, and could therefore be involved in the regulation of Cdc25 during oogenesis.

In competent oocytes, Plx1 action on Cdc25 is antagonized by an okadaic acid-sensitive phosphatase, involving PP2A activity (Brassac et al., 2000; Karaiskou et al., 1999). This explains why the auto-amplification mechanism can be artificially activated by okadaic acid. However, we have shown that okadaic acid is unable to promote Cdc2 activation in small incompetent oocytes, showing that the loop implying Cdc2, Cdc25, Plx1 and PP2A is not functional in growing oocytes. The most probable explanation for this defect is the absence of Plx1 in stage IV oocytes. Indeed, we show that both in vivo and in vitro, expression of Plk1 is sufficient to restore the activation of MPF in response to okadaic acid in incompetent oocytes. Plx1 is therefore the missing factor explaining why the auto-amplification of MPF is defective in small oocytes.

Altogether, our experiments show that the incompetence of small oocytes to resume meiosis is ensured by the absence of Plx1 resulting in a double negative control on MPF activation. First, the formation of active complexes between Cdc2 and newly synthesized cyclins is prevented by a sustained activity of Myt1 that escapes downregulation by Plx1. Second, Cdc25 activation that is normally achieved through a feedback loop involving Plx1 is also prevented. Further investigation will be necessary to discover first, how Plx1 expression is controlled by cell size at the end of oogenesis; second, how PP2A controls Cdc25 activity in small oocytes; and third, how the initial steps of the progesterone transduction pathway connect to MPF regulators, allowing the female germ cell to resume meiosis when oocyte growth is completed.

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