



HAL
open science

Cdc2-cyclin B triggers H3 kinase activation of aurora-A in *Xenopus* oocytes

G. Maton, C. Thibier, A. Castro, Thierry Lorca, Cybill Prigent, Catherine
Jesus

► **To cite this version:**

G. Maton, C. Thibier, A. Castro, Thierry Lorca, Cybill Prigent, et al.. Cdc2-cyclin B triggers H3 kinase activation of aurora-A in *Xenopus* oocytes. *Journal of Biological Chemistry*, 2003, 278 (24), pp.21439-21449. hal-02677177

HAL Id: hal-02677177

<https://hal.inrae.fr/hal-02677177>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Cdc2-Cyclin B Triggers H3 Kinase Activation of Aurora-A in *Xenopus* Oocytes*

Received for publication, January 24, 2003, and in revised form, March 25, 2003
Published, JBC Papers in Press, April 1, 2003, DOI 10.1074/jbc.M300811200

Gilliane Maton‡, Catherine Thibier‡, Anna Castro§¶, Thierry Lorca§, Claude Prigent||,
and Catherine Jessus‡**

From the ‡Laboratoire de Biologie du Développement, Unite Mixte de Recherche-CNRS 7622, Université Pierre et Marie Curie, Boîte 24, 4 Place Jussieu, Paris 75252 cedex 05, the §Centre de Recherche de Biochimie Macromoléculaire, CNRS Unite Propre de Recherche 1086, 1919 Route de Mende, Montpellier 34293 cedex 5, and the ||Groupe Cycle Cellulaire, Unite Mixte de Recherche 6061 Génétique et Développement, CNRS-Université de Rennes I, Institut Federatif de Recherche 97, 2 Avenue du Pr. Léon Bernard, CS 34317, Rennes 35043 cedex, France

***Xenopus* oocytes are arrested in meiotic prophase I and resume meiotic divisions in response to progesterone. Progesterone triggers activation of M-phase promoting factor (MPF) or Cdc2-cyclin B complex and neosynthesis of Mos kinase, responsible for MAPK activation. Both Cdc2 and MAPK activities are required for the success of meiotic maturation. However, the signaling pathway induced by progesterone and leading to MPF activation is poorly understood, and most of the targets of both Cdc2 and MAPK in the oocyte remain to be determined. Aurora-A is a Ser/Thr kinase involved in separation of centrosomes and in spindle assembly during mitosis. It has been proposed that in *Xenopus* oocytes Aurora-A could be an early component of the progesterone-transduction pathway, acting through the regulation of Mos synthesis upstream Cdc2 activation. We addressed here the question of Aurora-A regulation during meiotic maturation by using new *in vitro* and *in vivo* experimental approaches. We demonstrate that Cdc2 kinase activity is necessary and sufficient to trigger both Aurora-A phosphorylation and kinase activation in *Xenopus* oocyte. In contrast, these events are independent of the Mos/MAPK pathway. Aurora-A is phosphorylated *in vivo* at least on three residues that regulate differentially its kinase activity. Therefore, Aurora-A is under the control of Cdc2 in the *Xenopus* oocyte and could be involved in meiotic spindle establishment.**

Amphibian oocyte meiotic maturation is a pioneer model system to study an unconventional post-transcriptional pathway induced by a steroid hormone, progesterone, and the regulation of M-phase promoting factor (MPF)¹ or Cdc2-cyclin B complex, the universal inducer of mitotic and meiotic divisions

(1). *Xenopus* oocytes are arrested in prophase of the first meiotic division, assimilated to the G₂ arrest of the cell cycle. When stimulated by progesterone, they resume meiotic division through the activation of MPF. The signaling pathway induced by progesterone and leading to MPF activation is not well understood (1). It involves a drop in cAMP, induced within minutes after progesterone addition, leading to the inhibition of the cAMP-dependent protein kinase, protein kinase A (2). Protein kinase A down-regulation could stimulate the synthesis of new proteins, a process required to activate Cdc2-cyclin B complex (3). The identification of the one or more proteins whose synthesis is needed for Cdc2 activation represents a major challenge. Mos kinase is synthesized and activated simultaneously with that of MPF activation and indirectly induces MAPK activation (4). It has been recently proposed that Mos synthesis and MAPK activation are not required to induce Cdc2-cyclin B activation in *Xenopus* oocyte; however, they most probably facilitate this process (5–7). Thus, one or more other proteins, not identified yet, must be synthesized in response to progesterone to allow Cdc2-cyclin B activation.

Recently, the Aurora kinases emerged as a new family of mitotic Ser/Thr kinases conserved from yeast to humans (8–13). The vertebrate Aurora family is composed of three members, termed Aurora-A, -B, and -C (14). Aurora-A localizes to the centrosomes and the spindle poles during mitosis (11). Ablation or inactivation of Aurora-A in *Caenorhabditis elegans* embryos and mammalian culture cells prevents centrosome maturation and spindle assembly, showing a role of Aurora-A in centrosome separation and bipolar establishment of the spindle (9, 15, 16). It has also been demonstrated that, in *Xenopus*, one substrate of Aurora-A could be the kinesin-related motor Eg5, involved in centrosome separation (17).

Given the regulation of spindle function by Aurora-A (11, 18), it could be proposed that this kinase plays a role downstream MPF activation in the oocyte, as described in human mitotic cell lines (19). *Xenopus* Aurora-A has been cloned and originally termed “Eg2” in a screen designed to identify mRNAs that are polyadenylated during meiotic maturation and deadenylated at fertilization (11, 20, 21). Aurora-A protein accumulates during meiotic maturation, in response to progesterone independently of MPF activation, but its kinase activity would be under the control of MPF (22). The inhibition of Aurora-A by microinjection of a specific antibody in *Xenopus* oocytes neither affects nuclear envelope breakdown (GVBD, for germinal vesicle breakdown) nor Cdc2 activation. However, oocytes arrest in metaphase I without extruding the first polar body (23). This suggests again that Aurora-A functions downstream of MPF activation and controls the meiotic spindle. However,

* This work was supported in part by the Institut de la Recherche Agronomique, CNRS, University of Paris VI and Paris XI, Association pour la Recherche Contre le Cancer (Grant 4771) and by the Ligue Nationale Contre le Cancer (to C. J. and T. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ A fellow of the Ligue Nationale Contre le Cancer.

** To whom correspondence should be addressed. Tel.: 33-1-44-27-26-42; Fax: 33-1-44-27-34-72; E-mail: jessus@ccr.jussieu.fr.

¹ The abbreviations used are: MPF, M-phase promoting factor; Cdc, cell division cycle; GVBD, germinal vesicle breakdown; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; PP, protein phosphatase; GST, glutathione S-transferase; CHX, cycloheximide; IBMX, 3-isobutyl-1-methylxanthine; OA, okadaic acid; WT, wild type.

Andrésson and Ruderman (24) cloned Aurora-A cDNA using a screen designed to identify oocyte proteins whose electrophoretic migration is retarded within 30 min after progesterone stimulation. Moreover, they reported that Aurora-A overexpression accelerates GVBD in response to progesterone (24). It was then shown in *Xenopus* oocyte that Aurora-A phosphorylates and regulates CPEB, a protein that binds the CPE sequence present in the 3' non-coding region of several mRNAs, including *Mos* mRNA, and allows their transduction (25). Therefore, Aurora-A could be an early component of the progesterone pathway stimulating *Mos* synthesis upstream of Cdc2 activation. Because the *Mos*/MAPK pathway appears to be dispensable for Cdc2 activation (5–7), it is difficult to assume that the physiological function of Aurora-A would be restricted to Cdc2 activation.

Whether Aurora-A functions upstream or downstream of Cdc2 is important to further understand the regulation of the meiotic process. In this study, we addressed two main questions. First, is Aurora-A kinase activation physiologically controlled by MPF, or by the *Mos*/MAPK pathway, or by an upstream regulator of MPF? Second, what is the molecular basis of this activation? Several reports have shown that Aurora-A activation correlates with the phosphorylation of several of its residues (26). However, the exact role of these phosphorylations remains unclear. Some of them could target the protein to the degradation pathway, others could play a docking function, while a third category could function as activators (19, 26, 27). To answer these questions, we took advantage of a new *in vitro* substrate, histone H3, that allowed us to assay Aurora-A kinase activity in a reliable manner. We demonstrate that, *in vivo* during oocyte maturation as well as *in vitro* using prophase cytosolic extracts, active MPF is necessary and sufficient to induce Aurora-A phosphorylation and activation, independently of the *Mos*/MAPK pathway.

EXPERIMENTAL PROCEDURES

Material—*Xenopus laevis* adult females (Horst Kähler, Germany) were bred and maintained under laboratory conditions. Reagents, unless otherwise specified, were from Sigma.

Purification of Recombinant Proteins—The 21 N-terminal amino acids of either wild type histone H3 (WT H3) or histone H3 mutant where Ser¹⁰ was replaced by Ala (S10A H3) fused with GST were cloned into pGEX vector (28). WT H3 and S10A H3 fusion proteins were expressed in bacteria and purified using glutathione-agarose beads (Amersham Biosciences). Recombinant GST-p21^{cip1}, sea urchin cyclin B, and GST-Cdc25A were expressed in bacteria and purified as described, respectively, in Refs. 29–31.

***Xenopus* Oocyte Treatments and Homogenization**—Fully grown *Xenopus* oocytes were isolated and prepared as described before (32). Oocytes were injected with various proteins: recombinant GST-p21^{cip1} at 40 ng per oocyte, recombinant Cdc25A at 25 ng per oocyte, sea urchin cyclin B at 5 ng per oocyte, okadaic acid at 5×10^{-6} M intracellular concentration, and morpholino antisense oligonucleotides (Gene Tools LLC) raised against *c-Mos* mRNA at 100 ng per oocyte (5). Oocytes were also incubated in the presence of various reagents: 1 μ M progesterone, 100 μ g/ml cycloheximide (CHX), 1 mM 3-isobutyl-1-methylxanthine (IBMX), or 50 μ M U0126 (Promega). GVBD was monitored by the appearance of a white spot at the animal pole. Oocytes were referred as to “metaphase II-arrested oocytes” when they were collected at least 2 h after GVBD. Activation of metaphase II-arrested oocytes was induced by electric shock (33).

Oocytes were collected by groups of 10 or 20, and lysed at 4 °C in 4 volumes of EB (80 mM β -glycerophosphate, pH 7.3, 20 mM EGTA, 15 mM MgCl₂, 1 mM dithiothreitol), supplemented with protease inhibitor mixture (Sigma, P8340) and 1 μ M okadaic acid (ICN). Lysates were centrifuged at 15,000 $\times g$ at 4 °C for 15 min and frozen at –80 °C. Western blot analysis and kinase activity assays were then performed by using the same lysate.

In Vitro Extracts—*Xenopus* prophase oocyte lysates prepared in the absence of okadaic acid were incubated at 30 °C in the presence of an ATP-regenerating system (10 mM creatine phosphate, 80 μ g/ml creatine phosphokinase, 1 mM ATP, 1 mM MgCl₂) and either 1 μ M okadaic acid

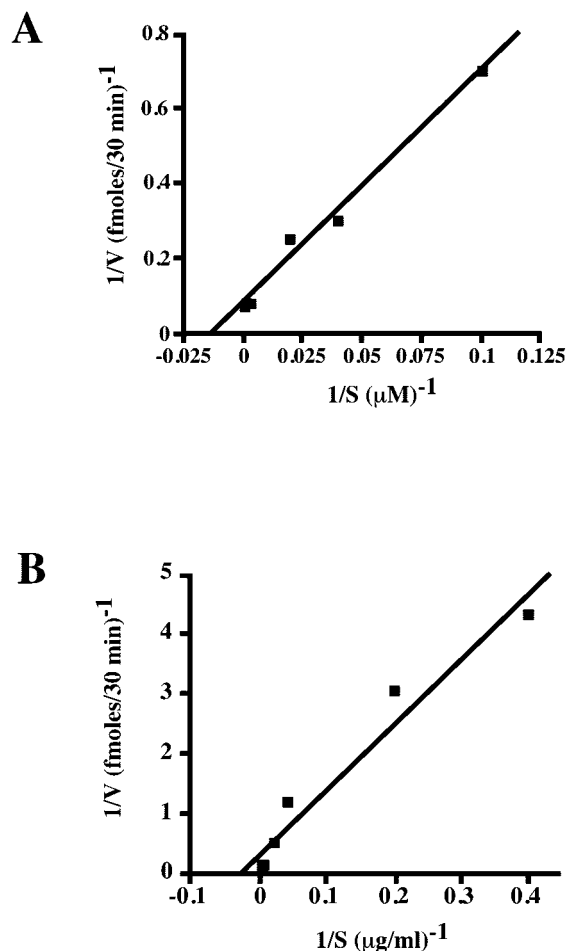


FIG. 1. Kinetic parameters of the *Xenopus* Aurora-A kinase for ATP and histone H3-peptide. Aurora-A was immunoprecipitated from metaphase II-arrested extracts with the anti-Aurora-A antibody and assayed for Aurora-A kinase activity, using histone WT H3-peptide as substrate. Kinase assays were carried out as described under “Experimental Procedures,” except that various ATP concentrations (10 μ M to 1 mM, A) or various histone H3-peptide concentrations (2.5–500 μ g/ml, B) were used.

(ICN) or 4 μ g/ml recombinant Cdc25A. Samples were collected at indicated times for Western blot analysis and kinase assays.

Western Blotting—Samples equivalent to two oocytes were electrophoresed on 12.5% SDS-PAGE Anderson system (34) and transferred to nitrocellulose filters (Schleicher and Schuell) using a semi-dry blotting system (Millipore). The rabbit anti-*Xenopus* Aurora-A polyclonal antibody and the polyclonal antibody raised against *Xenopus* cyclin B2 were described previously in Refs. 35 and 36, respectively. The mouse monoclonal antibody directed against the active phosphorylated form of MAPK, P-MAPK, was purchased from New England BioLabs. The rabbit polyclonal antibodies raised against ERK1 and *Xenopus* *Mos* protein were purchased from Santa Cruz Biotechnologies. The primary antibodies were detected with appropriated horseradish peroxidase-conjugated second antibodies (Jackson ImmunoResearch Laboratories) and the Western blot Chemiluminescence Renaissance kit from PerkinElmer Life Sciences.

Kinase Assays and Phosphatase Treatment—To measure Cdc2 kinase activity, histone H1 assays were performed on p13^{suc1} Sepharose pull-down extracts (three oocytes equivalent) in the presence of 1 μ Ci of [γ -³²P]ATP (ICN), 100 μ M ATP and 0.2 mg/ml histone H1 (Roche Applied Science) in kinase buffer (50 mM Tris-HCl, pH 7.2, 15 mM MgCl₂, 5 mM EGTA, 1 mM dithiothreitol).

Histone H3 kinase activity of Aurora-A was assayed in immunoprecipitates, incubated for 30 min at 30 °C in the presence of kinase buffer containing 3 μ Ci of [γ -³²P]ATP (ICN), 50 μ M ATP, and 0.5 mg/ml of either WT H3 or S10A H3. Kinase reactions were stopped by adding Laemmli buffer (37) and boiling. After electrophoresis and autoradiography, the bands corresponding to histone H1 and histone H3 were excised, and the associated radioactivity was measured in a Wallac

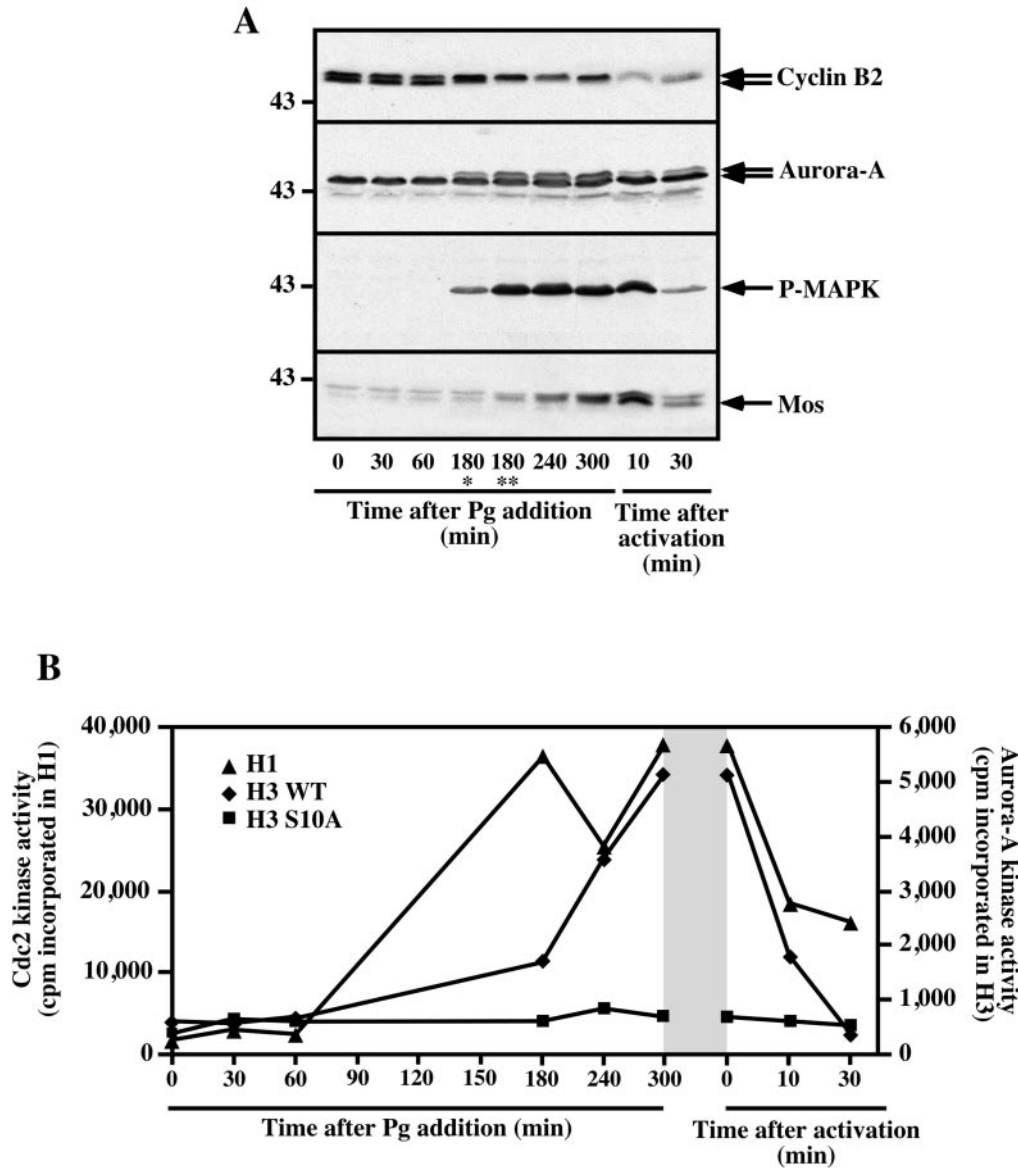


FIG. 2. H3 kinase activity of Aurora-A is up-regulated at time of GVBD during *Xenopus* oocyte maturation. Prophase oocytes were incubated in the presence of progesterone and collected at different times after progesterone (*Pg*) addition. Metaphase II-arrested oocytes were activated by electrical shock and collected at the indicated times. GVBD occurred 180 min after progesterone. *A*, lysates were subjected to Western blot with the anti-cyclin B2 antibody, the anti-Aurora-A antibody, the anti-phospho-MAPK (*P-MAPK*) antibody, or the anti-Mos antibody, as indicated. The positions of the molecular mass markers (kDa) are indicated on the *left*. At 180 min, two types of oocytes were selected: oocytes just before GVBD exhibiting the very first pigment re-arrangement (*) and oocytes at GVBD with a well formed *white spot* (**). *B*, the same lysates as in *A* were immunoprecipitated with the anti-Aurora-A antibody and assayed for Aurora-A kinase activity, using histone WT H3-peptide or S10A H3-peptide as substrate, or pulled-down on p13 beads and assayed for Cdc2 kinase activity, using histone H1 as substrate. Aurora-A and Cdc2 kinase activities are expressed as cpm incorporated in the substrate.

1409 scintillation counter. Phosphatase treatments were carried out in Aurora-A immunoprecipitates, incubated for 1 h at 30 °C in the presence of 2000 units of λ phosphatase (New England BioLabs).

RESULTS

Biochemical Characterization of Aurora-A Kinase Activity—To study the regulation of Aurora-A kinase activity, a reproducible kinase assay was developed. MBP and casein are often used to assay Aurora-A kinase activity *in vitro* (12, 22, 24, 26, 27, 38, 39). However, the activity of *Xenopus* oocyte Aurora-A kinase toward both substrates is low, rendering the quantification of the kinase activity difficult. It is established that Aurora-B phosphorylates *in vivo* histone H3 on a single residue, Ser¹⁰ (40). Given the high homology between the kinase domains of Aurora-A and Aurora-B, we checked whether histone H3 could be an *in vitro* substrate of *Xenopus* Aurora-A. Aurora-A was immunoprecipitated from metaphase II-arrested

oocytes that have been reported to contain active Aurora-A kinase (22, 24). The kinase activity was measured in immunoprecipitates using the 21 N-terminal amino acids of histone H3 (ARTKQTARKS¹⁰TGGKAPRKQLC) fused with a GST tag, as substrate. As a control, we used a mutated histone H3, where Ser¹⁰, the unique residue in H3 phosphorylated by Aurora-B, was replaced by Ala (S10A H3). This S10A H3-peptide was never phosphorylated under our conditions. To determine the optimal conditions for the kinase assay, H3-peptide phosphorylation was measured in the presence of various ATP concentrations (from 10 μ M to 1 mM) or various histone H3-peptide concentrations (from 2.5 to 500 μ g/ml) at 30 °C for 30 min (Fig. 1). The K_m for ATP and histone H3-peptide were, respectively, 80 μ M and 35 μ g/ml (Fig. 1). These values are comparable to those reported for the Cdc2-cyclin B kinase (75 μ M for ATP and 40 μ g/ml for histone H1 (41)).

Aurora-A Is Activated at GVBD Time during Meiotic Maturation—To investigate how Aurora-A is regulated during meiotic maturation, the activity of the protein was estimated, in parallel with its electrophoretic migration. Oocytes were collected at different times following progesterone stimulation (Fig. 2). As expected, MPF activity, measured by histone H1 kinase assay, increased just before GVBD, in correlation with cyclin B2 electrophoretic mobility retardation. Its activity decreased at the metaphase I-metaphase II transition and increased again, inducing entry into metaphase II. MAPK activity was estimated by Western blot, using an antibody that specifically recognizes the phosphorylated active form of the protein. As already reported, MAPK was stably activated at time of GVBD, due to Mos synthesis, until the end of the maturation process (Fig. 2A). Aurora-A kinase activity was similarly measured during meiotic maturation using histone H3-peptide as substrate (Fig. 2B). In prophase-arrested oocytes and until GVBD, Aurora-A was inactive and migrated as a single band (Fig. 2). At GVBD time, Aurora-A accumulated, as previously reported (22) and underwent an electrophoretic shift, leading to a doublet, in correlation with its kinase activation (Fig. 2). In this experiment, the level of Aurora-A activity increased continuously from GVBD until the arrest in metaphase II. In some cases, Aurora-A kinase activity is maximal at GVBD time and is maintained at a high level until metaphase II (see Figs. 4, 8, and 10 below).

Metaphase II oocytes were parthenogenetically activated by electric shock. This treatment inactivated MPF within 10 min, due to cyclin B2 degradation (Fig. 2). Mos was degraded 30 min after activation, and consequently, MAPK was inactivated at the same time (Fig. 2A). When oocytes re-entered interphase in response to activation, the *upper band* of Aurora-A partially disappeared, in correlation with a drop in its kinase activity (Fig. 2).

The H3 Kinase Activity of Aurora-A Depends on Its Phosphorylation—We next addressed the question whether Aurora-A activation results from its phosphorylation, as suggested by the electrophoretic retardation that correlates with the active form of the H3 kinase activity. Active Aurora-A kinase was immunoprecipitated from metaphase II-arrested oocytes and incubated in the presence of phosphatase λ (PP λ), that exhibits a broad range of specificity. PP λ treatment abolished Aurora-A activity, whereas heat-inactivated PP λ had no effect (Fig. 3A). The electrophoretic migration of Aurora-A was further analyzed in immunoprecipitates that allow a better resolution than crude extracts. Active Aurora-A migrated as a doublet when metaphase II oocyte extracts were loaded on a gel (see Fig. 2A). The active kinase isolated by immunoprecipitation migrated under three bands (Fig. 3B). The *lower band* migrated at the same level as the inactive prophase form (Fig. 3B). PP λ treatment induced the disappearance of the *upper band*, a decrease in the *middle band*, and the enrichment of the *lower band* (Fig. 3B). These observations indicate that Aurora-A is activated by phosphorylation and that at least two residues of Aurora-A are phosphorylated in metaphase II-arrested oocytes, resulting in the electrophoretic retardation of the protein.

Aurora-A Phosphorylation and Activation Are under the Control of cAMP and Protein Synthesis—Aurora-A phosphorylation and H3 kinase activation take place in response to progesterone at GVBD time. It is well established that progesterone induces an early drop in cAMP and the synthesis of new proteins, both events being required for Cdc2 activation and GVBD (42, 43). To ascertain whether both events are necessary for Aurora-A activation, prophase-arrested oocytes were incubated in the presence of either cycloheximide (CHX), a protein synthesis inhibitor, or IBMX, a phosphodiesterase inhibitor

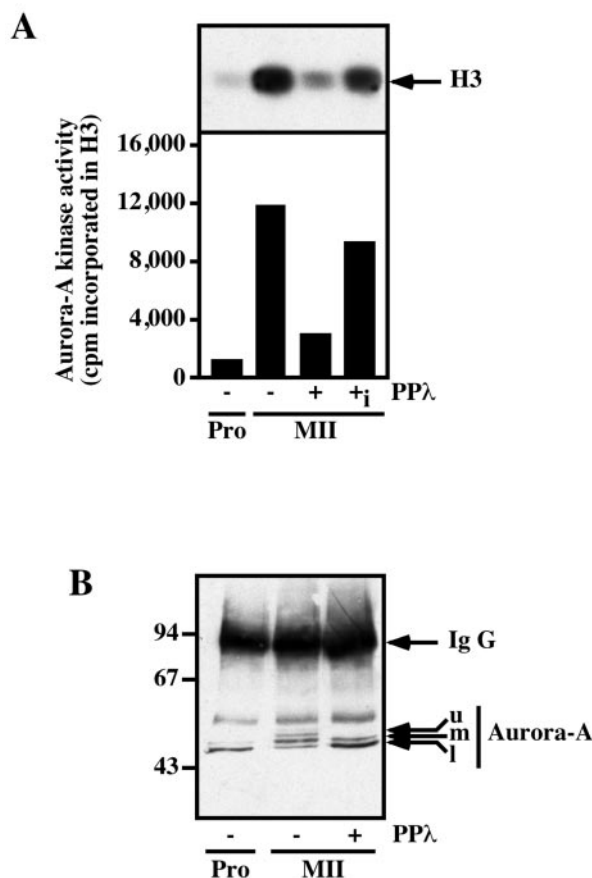
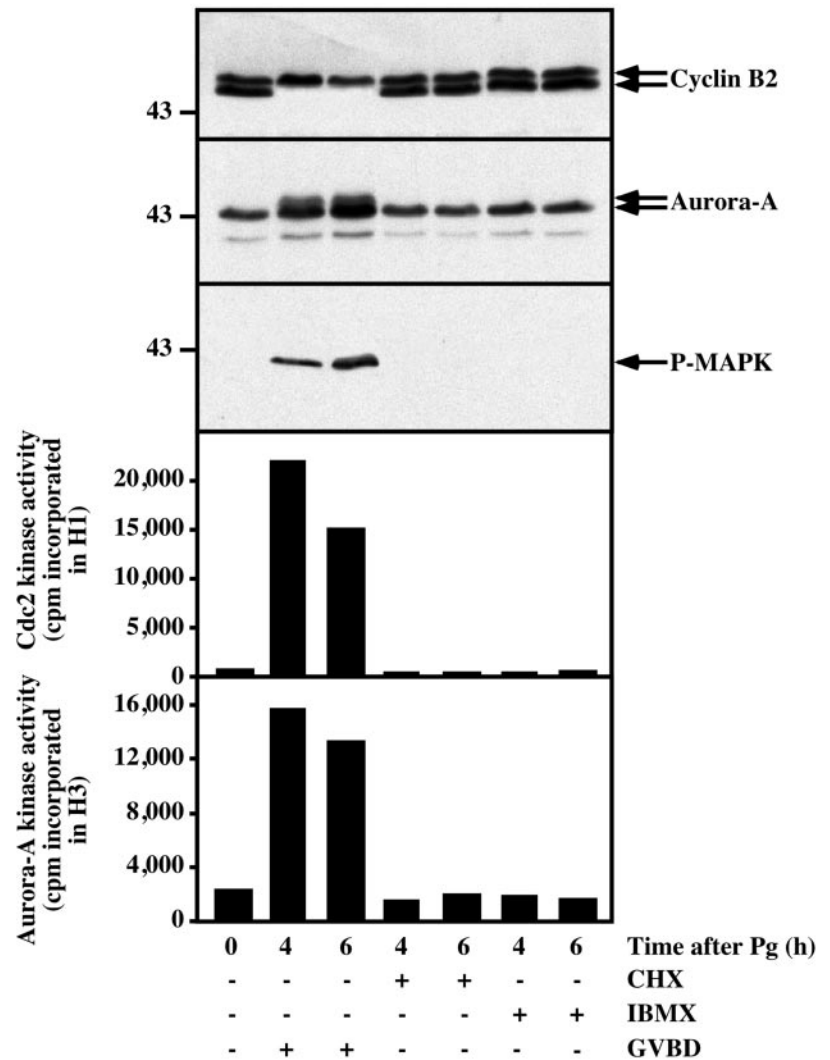


FIG. 3. Aurora-A kinase is activated by phosphorylation. Aurora-A was immunoprecipitated with the anti-Aurora-A antibody from prophase oocytes (*Pro*) or metaphase II-arrested oocytes (*MI*). Immunoprecipitates were incubated with (+) or without (-) PP λ for 1 h at 30 °C. **A**, half the immunoprecipitates were assayed for Aurora-A kinase activity using histone H3-peptide as substrate. +*i*, heat-inactivated PP λ . **Upper panel**, autoradiogram of phosphorylated H3-peptide; **lower panel**, quantification of radioactive phosphorylated H3-peptide. **B**, the other half of the immunoprecipitates was subjected to Western blot, with the anti-Aurora-A antibody. The positions of the molecular mass markers (kDa) are indicated on the left. IgG, position of the heavy chain of the antibody. Aurora-A migrates at three positions: *u*, upper band; *m*, middle band; *l*, lower band.

that maintains a high level of cAMP in the cell. The oocytes were then stimulated by progesterone. In control oocytes, Cdc2 activation and GVBD occurred 4 h after progesterone addition (Fig. 4). As expected, CHX totally prevented Cdc2 activation, as ascertained by H1 kinase assay and cyclin B2 electrophoretic migration (Fig. 4). MAPK was not activated (Fig. 4), due to the inhibition of Mos synthesis. Under these conditions, Aurora-A mobility was not shifted and no kinase activity could be detected (Fig. 4). Identical results were obtained when oocytes were first incubated in the presence of IBMX (Fig. 4). Taken together, these results show that Aurora-A phosphorylation and activation are under the control of the drop of cAMP and protein synthesis induced by progesterone.

Aurora-A Phosphorylation and Activation Are Independent of the Mos/MAPK Cascade—As shown in Fig. 2, H3 kinase activity of Aurora-A switched on at the same time as Cdc2 kinase and MAPK, the major kinases activated during meiotic maturation. We then asked whether Aurora-A activation could be dependent on one of these kinases. We first investigated the potential implication of the Mos/MAPK pathway in Aurora-A activation. Oocytes were incubated in the presence of U0126, a pharmacological MEK inhibitor (44), and progesterone was then added. GVBD occurred with 1-h delay in comparison to

FIG. 4. Aurora-A kinase activation depends on cAMP and on protein synthesis during *Xenopus* oocyte meiotic maturation. Prophase oocytes were incubated in the presence (+) or in the absence (-) of cycloheximide (CHX) or 3-isobutyl-1-methylxanthine (IBMX) for 1 h and then stimulated by progesterone (Pg). Oocytes were collected at the indicated times after progesterone addition and homogenized. In control oocytes, GVBD started 4 h after progesterone addition. Lysates were subjected to Western blot with anti-cyclin B2 antibody, anti-Aurora-A antibody, or anti-phospho-MAPK (P-MAPK) antibody, as indicated. Immunoprecipitates were performed in the same lysates with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide as substrate. A pull-down assay on p13 beads was used to measure Cdc2 kinase activity using histone H1 as substrate. Aurora-A and Cdc2 kinase activities are expressed in cpm incorporated in the substrate. +, presence of GVBD; -, absence of GVBD.



control progesterone-treated oocytes. Mos was synthesized, but MAPK was not activated (Fig. 5). In the absence of active MAPK, Cdc2 was activated at GVBD as shown by the mobility shift of cyclin B2 and H1 kinase assay. Under these conditions, Aurora-A was phosphorylated and its H3 kinase activity was increased (Fig. 5). This result shows that Aurora-A activation and phosphorylation do not depend on MAPK activation. However, because Mos is synthesized in response to progesterone in the presence of U0126, Mos could account for Aurora-A kinase activation independently of MAPK activity. To investigate this possibility, we adopted an antisense strategy to ablate Mos in the oocytes. Morpholino antisense oligonucleotides directed against Mos mRNA have been shown to prevent Mos synthesis, and consequently MAPK activation, induced by progesterone (5). In control oocytes, GVBD occurred 4 h after progesterone addition, whereas oocytes microinjected with morpholino Mos antisense underwent GVBD with a 1-h delay. As shown by Western blot, Mos was not synthesized under these conditions and consequently MAPK was not activated (Fig. 5). Cdc2 kinase was activated at GVBD (Fig. 5), as previously described (5). In the absence of Mos and MAPK activation, Aurora-A was still phosphorylated and activated at GVBD (Fig. 5). Taken together, these results show that neither Mos synthesis nor MAPK activation are required for Aurora-A phosphorylation and activation.

Activation of Cdc2 by Injection of Cdc25, OA, or Cyclin B Triggers Aurora-A Kinase Activation—We then addressed the

question whether Aurora-A activation could be under the control of Cdc2 activity. To bypass the upstream signaling pathway induced by progesterone, Cdc2 was directly activated by injection into the oocytes of either Cdc25 or cyclin B, in the absence of progesterone. The injection of Cdc25, the phosphatase directly responsible for the dephosphorylation of Cdc2 (45), induced GVBD and Cdc2 activation in the absence of progesterone (Fig. 6A). As expected, MAPK was also activated, as judged by electrophoretic retardation of the protein visualized on Western blot with an antibody recognizing all the forms of MAPK (Fig. 6A). Aurora-A was phosphorylated and activated at GVBD, as in progesterone-treated oocytes (Fig. 6A). Oocytes were also injected with cyclin B, which recruits and activates monomeric Cdc2 molecules and consequently leads to the activation of endogenous Cdc2. Aurora-A was phosphorylated and activated in response to the direct activation of Cdc2 by injected cyclin (Fig. 6B).

Okadaic acid, an inhibitor of types 1 and 2A Ser/Thr phosphatases, is a potent Cdc2 activator in the oocyte (46), although its precise targets are not well defined yet. Okadaic acid injection induced GVBD within 90 min in the absence of progesterone, leading to Cdc2 kinase activation and partial MAPK activation (Fig. 6B), as previously reported (47). Okadaic acid injection induced the activation of Aurora-A and a supershift of the doublet usually observed in progesterone-treated oocytes (Fig. 6B). Therefore, the *in vivo* inhibition of okadaic acid-sensitive phosphatases allows the phosphorylation of Aurora-A

on residues that are not phosphorylated under physiological conditions. These additional phosphorylations did not significantly increase the kinase activity (Fig. 6B). These results show that Cdc2 activation by three different agents, Cdc25, cyclin B, and okadaic acid, is sufficient to induce Aurora-A phosphorylation and activation in the absence of progesterone.

To determine whether Cdc2 induces Aurora-A activation in a protein synthesis-dependent manner, oocytes were injected with okadaic acid or cyclin B in the presence of cycloheximide. As expected, Cdc2 was still activated, whereas MAPK was not (Fig. 6B). Interestingly, Aurora-A phosphorylation and activation were still observed under these conditions, demonstrating that Aurora-A activation is induced *in vivo* by Cdc2 activation but requires neither the synthesis of new proteins nor the Mos/MAPK pathway. We have earlier shown that Aurora-A phosphorylation and activation induced by progesterone depend on protein synthesis (Fig. 4). This is obviously because of the protein synthesis dependence of the progesterone pathway leading to Cdc2 activation. Once Cdc2 is activated, then Aurora-A activation does not require any synthesis of new proteins.

To better analyze the regulation of Aurora-A kinase activation, an *in vitro* approach was adopted based on prophase cytosolic extracts that do not support protein synthesis (31, 48). Prophase cytosolic extracts were incubated in the presence of either okadaic acid or Cdc25, in the presence of an ATP-regenerating system (Fig. 7). In the presence of okadaic acid, Cdc2 was rapidly activated within 30 min (Fig. 7, A and B). As previously reported (31), MAPK was activated 60 min after okadaic acid addition, despite the absence of Mos (Fig. 7A), suggesting that MAPK activity results *in vitro* from a balance between kinase activities and okadaic acid-sensitive phosphatases. Aurora-A was activated between 30 and 60 min after okadaic acid addition (Fig. 7C). Kinase assays of Aurora-A performed with S10A H3-peptide as substrate remained at a basal level throughout the time course of the experiment (data not shown). Okadaic acid induced *in vitro* (Fig. 7A) as well as *in vivo* (Fig. 6B) a supershift of Aurora-A mobility, suggesting that, under both conditions, inhibition of okadaic acid-sensitive phosphatases leads to additional phosphorylations of the protein.

When extracts were supplemented with Cdc25, Cdc2 was activated after a lag period of 60 min, and MAPK was not activated (Fig. 7, A and B), as previously reported (31), arguing that *in vitro* activation of MAPK cannot occur in the absence of Mos, unless okadaic acid-sensitive phosphatases are inhibited. Aurora-A phosphorylation and activation occurred shortly after Cdc2 activation in response to Cdc25 addition (Fig. 7, A and C). The electrophoretic shift of Aurora-A induced by Cdc25 addition in the extracts was similar to its migration observed *in vivo* in maturing oocytes. Taken together, these results show that Cdc2 activation is sufficient to trigger Aurora-A kinase activation and phosphorylation, in the absence of progesterone, independently of protein synthesis and of the Mos-MAPK pathway.

Inhibition of Cdc2 Kinase Activation by p21^{cip1} Prevents Aurora-A Kinase Activation by Progesterone—The next question was to determine whether Cdc2 activity is not only sufficient but also necessary for physiological Aurora-A activation during meiotic maturation induced by progesterone. We used p21^{cip1}, a protein known to specifically inhibit the cyclin-dependent kinase-cyclin complexes (49) and to prevent MPF activation normally induced by progesterone by direct binding to Cdc2-cyclin B complexes (29). As expected, oocytes injected with p21^{cip1} and incubated with progesterone did not achieve GVBD, and neither Cdc2 nor MAPK was activated (Fig. 8). Interestingly, Aurora-A was neither phosphorylated nor acti-

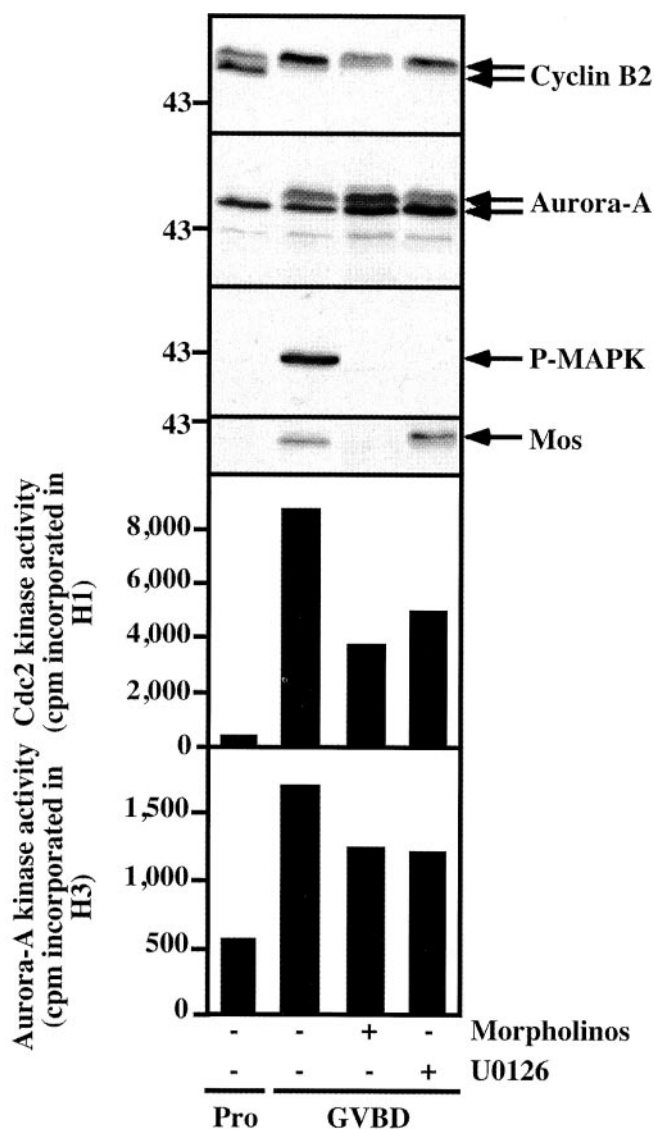


FIG. 5. Activation of Aurora-A is independent of Mos expression and MAPK activation during *Xenopus* oocyte meiotic maturation. Prophase oocytes were injected (+) or not (-) with morpholino antisense oligonucleotides directed against the mRNA of Mos, or incubated with (+) or without (-) U0126. One hour after U0126 addition or 18 h after Mos antisense injection, progesterone was added. Oocytes were collected in prophase (Pro) or at GVBD. Lysates were subjected to Western blot with anti-cyclin B2 antibody, anti-Aurora-A antibody, anti-phospho-MAPK (P-MAPK) antibody, or anti-Mos antibody, as indicated, or immunoprecipitated with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide as substrate, or pulled down on p13 beads and assayed for Cdc2 kinase activity using histone H1 as substrate. Aurora-A and Cdc2 kinase activities are expressed in cpm incorporated in the substrate.

vated (Fig. 8). These results were confirmed *in vitro* (Fig. 9). Prophase oocytes extracts were activated by okadaic acid, in the presence or not of p21^{cip1}. In the presence of p21^{cip1}, Cdc2 activation was strongly delayed (Fig. 9, A and B). MAPK was activated 60 min after OA addition. As previously reported (31), MAPK activation in these extracts is independent of Cdc2 activity. In contrast, Aurora-A phosphorylation and its kinase activation were inhibited (Fig. 9, A and C). In conclusion, Cdc2 activity is necessary and sufficient to trigger the phosphorylation and the consequent activation of Aurora-A both *in vivo* during oocyte meiotic maturation and *in vitro* in extracts reproducing the Cdc2 auto-amplification feedback loop.

Because Aurora-A activation occurs downstream of Cdc2 activation, our next goal was to determine whether MPF directly

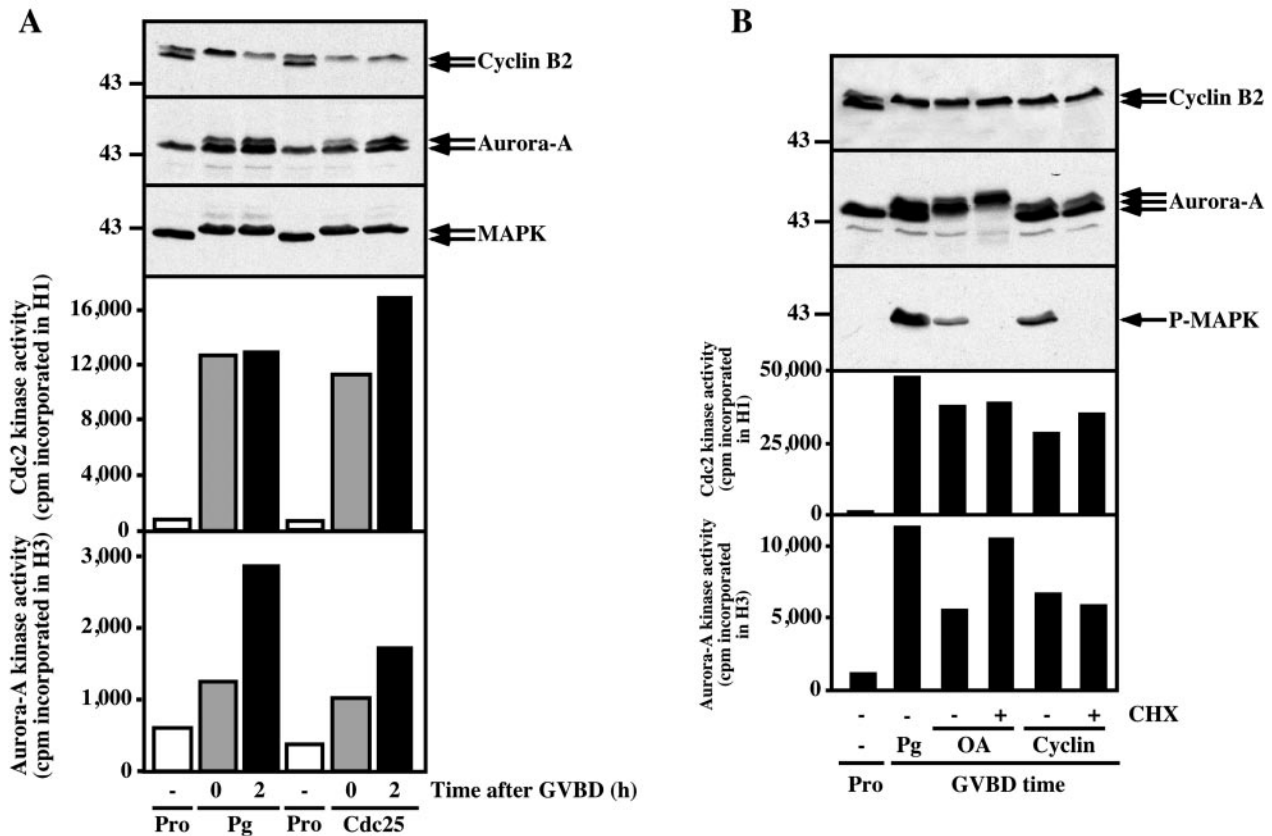


FIG. 6. Active Cdc2-cyclin B is sufficient to trigger Aurora-A kinase activation in *Xenopus* oocyte. *A*, prophase oocytes were injected with Cdc25 or stimulated by progesterone (Pg). Oocytes were collected in prophase (Pro), at GVBD stage (0) or 2 h after GVBD (2). *B*, prophase oocytes were injected with okadaic acid (OA) or cyclin B, with (+) or without (–) cycloheximide (CHX). Control oocytes were incubated in the presence of progesterone. Oocytes were collected in prophase (Pro) or at GVBD time. In *A* and *B*, lysates were subjected to Western blot with anti-cyclin B antibody, anti-Aurora-A antibody, anti-MAPK antibody, or anti-phospho MAPK (P-MAPK) antibody or immunoprecipitated with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide as substrate. A pull-down assay on p13 beads was used to measure Cdc2 kinase activity using histone H1 as substrate. Aurora-A and Cdc2 kinase activity are expressed in cpm incorporated in the substrate.

activates Aurora-A. To test this possibility, an *in vitro* kinase assay was performed using Cdc2-cyclin B kinase purified from metaphase II-arrested oocytes, either by p13^{suc1} affinity or by chromatography (36), as an enzyme source, and the bacterially expressed Aurora-A or inactive immunoprecipitated Aurora-A from prophase oocytes as a substrate. Aurora-A was not phosphorylated and not activated by purified Cdc2-cyclin B (data not shown). These results demonstrate that Aurora-A is not a direct substrate of MPF and that Cdc2-cyclin B indirectly regulates the phosphorylation and activation of Aurora-A kinase during meiotic maturation.

Cdc2 Kinase Inhibition at GVBD Does Not Inhibit Aurora-A Kinase Activity—Although Aurora-A activation is clearly Cdc2-dependent, it was important to know whether the kinase further requires Cdc2 activity to remain active. To answer this question, Cdc2 kinase activity was inhibited at GVBD stage by either inhibiting protein synthesis (incubation in the presence of CHX) or by direct Cdc2 inhibition (p21^{cip1} injection). Both treatments are known to induce inactivation of Cdc2 within 2 h (50–52) (Fig. 10A). When oocytes are incubated in the presence of CHX at GVBD, cyclin B2 completely disappears, as a consequence of its degradation occurring normally at this period and of the prevention of its synthesis (52–54). After p21^{cip1} injection, cyclin B2 was not degraded and pre-MPF was reformed as previously reported (50): cyclin B2 migrated as a doublet, as in prophase oocytes (Fig. 10A), in parallel to tyrosine phosphorylation of Cdc2 (data not shown). Under both conditions, MAPK was totally inactivated. Interestingly, Aurora-A remained active during the 2-h period following Cdc2 kinase inhibition (Fig.

10A). However, analysis of Aurora-A electrophoretic migration showed that the *upper band* disappeared under these conditions (Fig. 10A) suggesting that the protein was at least partially dephosphorylated. To determine whether the activity of this non-shifted form of the kinase was dependent on phosphorylation, it was treated by PPL. PPL totally abolished the kinase activity of Aurora-A (Fig. 10B). Therefore, when Cdc2 is inactivated after GVBD, the migration of Aurora-A is accelerated, but the protein is still phosphorylated on sites required for its activity. It can be concluded that the two phosphorylated sites inducing an electrophoretic shift require Cdc2 activity to be stabilized but are not necessary for the maintenance of the kinase activity. This experiment also reveals the existence of an initially phosphorylated site in response to Cdc2 activation that does not modify the migration of the protein in SDS-PAGE, does not require Cdc2 to be maintained under a phosphorylated state, and is crucial for the H3 kinase activity of Aurora-A. Cdc2 kinase activity is therefore no longer necessary to maintain Aurora-A kinase activity after GVBD.

DISCUSSION

The major aim of this study was to determine how Aurora-A activation is controlled during *Xenopus* oocyte meiotic maturation. To answer this question, we undertook a dual *in vitro* and *in vivo* approach, using a polyclonal anti-Aurora-A antibody and histone H3 as an Aurora-A substrate, used in the *in vitro* kinase assays. We demonstrate that Aurora-A is phosphorylated and activated at GVBD time and that this activity is regulated by phosphorylation. Because Aurora-A is activated at

the same period as Cdc2 and MAPK, the implication of both kinases in Aurora-A activation was investigated. Preventing Mos synthesis by morpholino antisense oligonucleotides, or MAPK activation by U0126, did not inhibit GVBD and Cdc2 activation as previously reported (5–7), nor did it inhibit Aurora-A phosphorylation or its activation. In contrast, our results show that 1) Aurora-A phosphorylation and activation can be induced by Cdc2 kinase activation, and 2) inhibition of Cdc2 kinase activation by injection of p21^{cip1} prevents Aurora-A phosphorylation and activation normally induced by progesterone. This means that the Mos/MAPK pathway is not involved in Aurora-A regulation and that Cdc2 activation is necessary and sufficient for Aurora-A activation. Therefore, Aurora-A is a physiological target of Cdc2 in *Xenopus* oocyte. However, Cdc2 is unable to directly phosphorylate and activate Aurora-A. These results suggest that other kinases under the control of Cdc2 kinase phosphorylate Aurora-A. It is also not excluded that active Cdc2 activates an adaptor of Aurora-A or inhibits a specific phosphatase rendering the kinase able to auto-phosphorylate and to auto-activate. Recently, it has been shown that human Aurora-A is activated during mitosis by phosphorylation, under the indirect control of Cdc2-cyclin B (19). Taken together, these results show that Aurora-A is phosphorylated and activated downstream of Cdc2-cyclin B kinase, either in mitosis or in meiosis, in different species.

In apparent contradiction with the present study and others (22, 23), it has been shown that Aurora-A is phosphorylated soon after progesterone addition, using as a criterion its electrophoretic mobility in SDS-PAGE, and that injection of the protein facilitates GVBD in response to progesterone (24). However, inactivation of Aurora-A by the microinjection of a blocking antibody does not affect the timing of GVBD (23). Because CPEB was further shown to be an Aurora-A substrate, it was proposed that Aurora-A could participate to the polyadenylation of Mos mRNA leading to Mos synthesis (25). However, Mos synthesis and MAPK activation do not appear to be required for Cdc2 activation (5–7); it is therefore not probable that the putative function of Aurora-A in Cdc2 activation could be exerted through the recruitment of the Mos/MAPK pathway. One possible way to reconcile our results and those of others (24, 25) is to assume that an active phosphorylated form of Aurora-A is generated soon after progesterone addition but that its phosphorylation cannot be detected by SDS-PAGE under our conditions and that this active form does not use histone H3 as an *in vitro* substrate. Then later, a hyperphosphorylated form could be generated under Cdc2 control at GVBD, leading to an active H3 kinase activity. The biological significance of both active forms, the first one controlling Mos synthesis and the latest one the spindle assembly, remain to be explored. Using a broad range of *in vitro* substrates, we were not able to assay the kinase activity of the putative first form of Aurora-A. We therefore studied the regulation of the active H3 kinase activity of Aurora-A, generated by phosphorylation during GVBD in response to Cdc2 activation.

The second task of our study was to approach the molecular basis of Aurora-A activation. It has been shown that *Xenopus* Aurora-A activation correlates with the phosphorylation of three residues of the protein (26). However, the exact role of these phosphorylations is still unclear. Some of them could target the protein to the degradation pathway (as Ser⁵³ (27, 35)), others could play a docking or structural function (as Ser³⁴⁹), whereas a third category could function as activators (as Thr²⁹⁵) (19, 27). We have demonstrated by PPL treatment that Aurora-A kinase activation during meiotic maturation depends on its phosphorylation. Our data establish that the phosphorylation of at least two residues of Aurora-A induce a

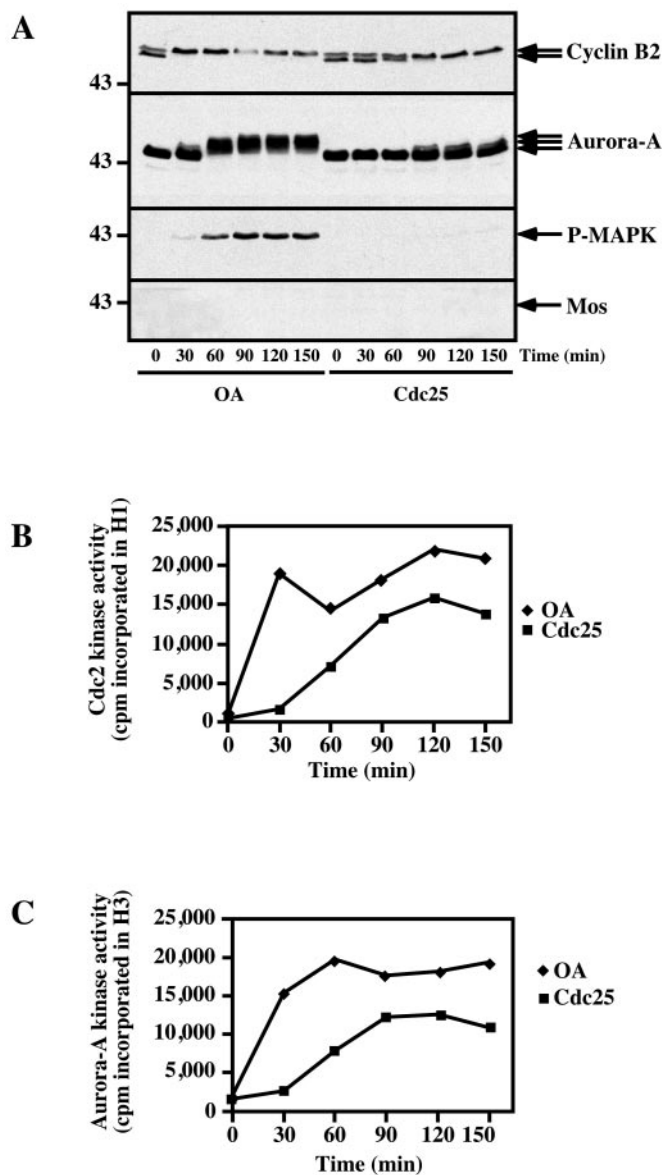


FIG. 7. Direct activation of Cdc2-cyclin B is sufficient to induce Aurora-A activation independently of Mos and MAPK in *in vitro* cell extracts. Prophase cytosolic extracts were incubated in the presence of 1 μ M okadaic acid (OA) or 10 μ g Cdc25 (Cdc25) and an ATP-regenerating system at 30 °C. Samples were collected at various times, either for Western blotting (A), histone H1 kinase assay (B), or histone H3 kinase assay (C). A, Western blots were analyzed with the anti-cyclin B2 antibody, the anti-Aurora-A antibody, the anti-phospho-MAPK (P-MAPK) antibody, or the anti-Mos antibody. The position of the molecular mass markers (kDa) is indicated on the left. B, samples were pulled-down on p13 beads and assayed for Cdc2 kinase activity using histone H1 as substrate. C, samples were immunoprecipitated with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide.

retardation of the electrophoretic mobility of the protein. The phosphorylation as well as the maintenance of the phosphorylated state of both residues depends on Cdc2 activity. This result suggests that the phosphates on both residues are subjected to an active turnover, involving the activity of one or more Cdc2-dependent kinases and opposing phosphatase(s). Because the presence of okadaic acid in the lysis buffer is required to preserve the electrophoretic shift of Aurora-A (22), it can be concluded that the phosphatases regulating both phosphorylations are sensitive to okadaic acid. They most probably correspond to a type 1 phosphatase, which is assumed to regulate the Aurora-A function in various species (55, 56).

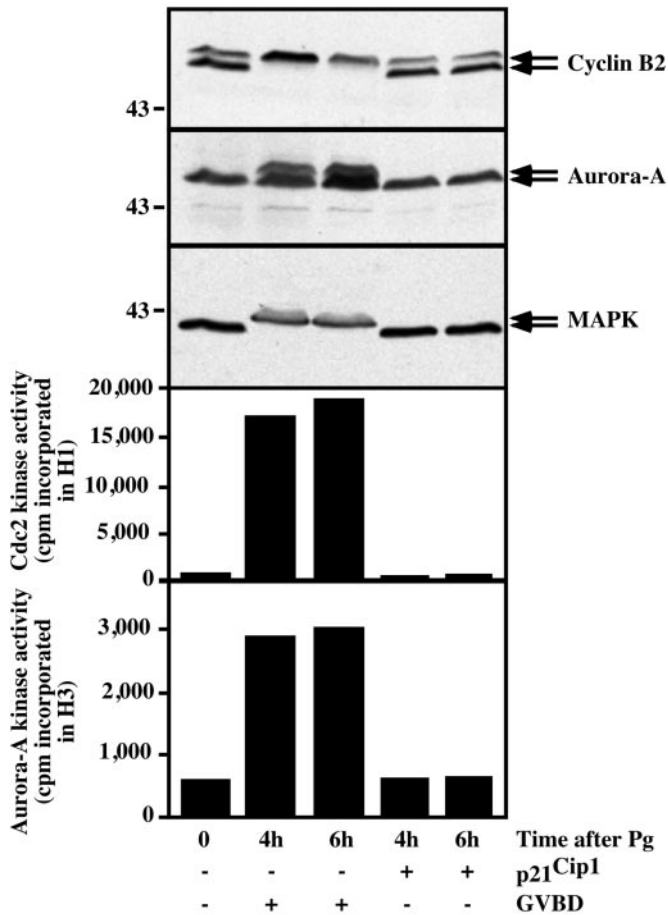


FIG. 8. Cdc2-cyclin B activation is necessary for Aurora-A kinase activation during *Xenopus* oocyte meiotic maturation. Prophase oocytes were injected (+) or not (-) with p21^{Cip1}. One hour after, oocytes were incubated in the presence of progesterone (Pg). Oocytes were collected at the indicated times after progesterone addition. In control oocytes, GVBD started 4 h after progesterone addition. Lysates were subjected to Western blot with anti-cyclin B2 antibody, anti-Aurora-A antibody, or anti-MAPK antibody, as indicated, or were immunoprecipitated with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide as substrate. A pull-down on p13 beads was assayed for Cdc2 kinase activity using histone H1 as substrate. Aurora-A and Cdc2 kinase activities are expressed in cpm incorporated in the substrate. +, presence of GVBD; -, absence of GVBD.

Interestingly, the phosphorylations of both residues, responsible for the appearance of the two retarded bands at GVBD, are not required for the stabilization of Aurora-A activity. Indeed, when Cdc2 kinase activity is inhibited at GVBD time, Aurora-A kinase activity remains high, showing that Cdc2 is not necessary to stabilize Aurora-A kinase activity. Under these conditions, Aurora-A electrophoretic migration is not retarded anymore, showing that it was dephosphorylated on the two "shifting" residues, although the kinase remains active. This finding led us to propose that Aurora-A could be subjected to three phosphorylations, in agreement with a previous report (26). At GVBD time, active Cdc2 could indirectly induce Aurora-A phosphorylation on both residues that promote an electrophoretic retardation of the protein. Both phosphorylations are not sufficient to activate Aurora-A kinase. They could correspond to a permissive event that allows the phosphorylation of a third residue responsible for Aurora-A kinase activation. This third phosphorylation does not induce any change in the electrophoretic migration of Aurora-A. When Cdc2 kinase activity is inhibited at GVBD, *i.e.* just after Aurora-A activation, the protein returns to its faster migration form but remains active,

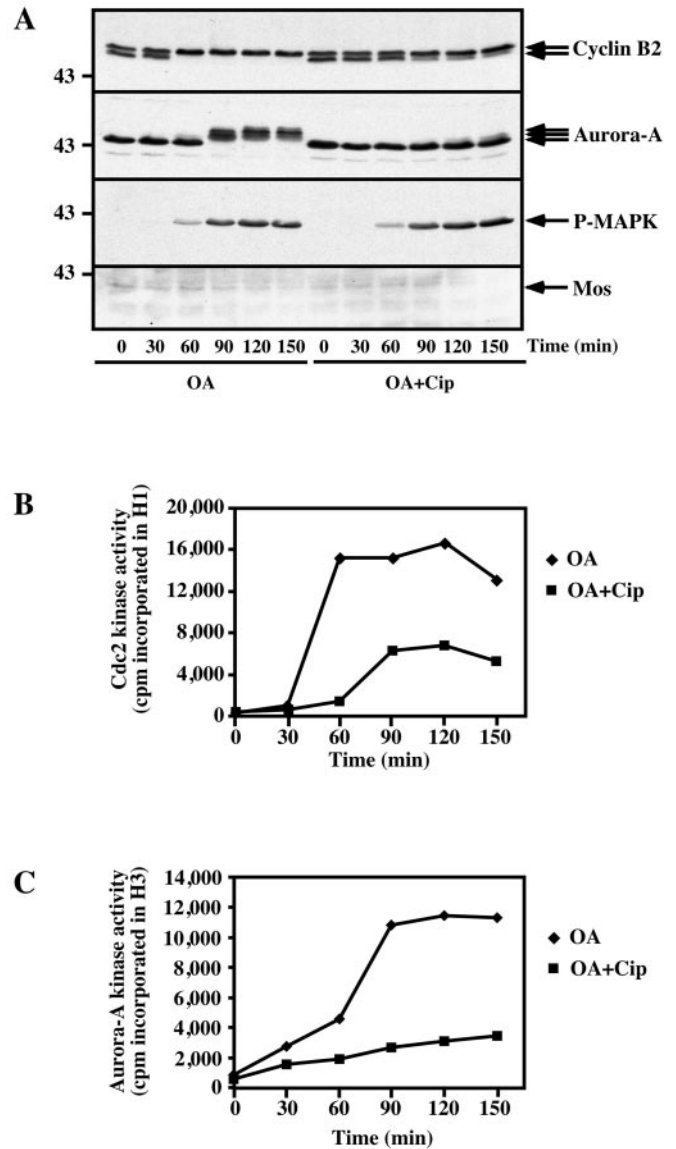


FIG. 9. Cdc2-cyclin B activity is required for Aurora-A kinase *in vitro* activation in cell extracts. Prophase cytosolic extracts were incubated in the presence of 1 μ M okadaic acid (OA) and an ATP-regenerating system, with (OA+Cip) or without (OA) 40 μ g/ml of p21^{Cip1}. Samples were collected at various times, either for Western blotting (A), histone H1 kinase assay (B), or histone H3 kinase assay (C). A, Western blots were analyzed with the anti-cyclin B2 antibody, the anti-Aurora-A antibody, the anti-phospho-MAPK (P-MAPK) antibody, or the anti-Mos antibody. The positions of the molecular mass markers (kDa) are indicated on the left. B, samples were pulled-down on p13 beads and assayed for Cdc2 kinase activity using histone H1 as substrate. C, samples were immunoprecipitated with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide. Aurora-A and Cdc2 kinase activities are expressed in cpm incorporated in the substrate.

because the phosphorylation of the third residue is preserved. Because stabilization of Aurora-A kinase activity requires the presence of okadaic acid in the lysis buffer (22), it can be concluded that the phosphorylation of this third activation site is also dependent on an okadaic acid-sensitive phosphatase, as PP1.

Interestingly, Aurora-A kinase activity is down-regulated when oocytes re-enter interphase after activation, presumably by dephosphorylation. A previous study has reported that the protein was degraded at this period (22). Indeed, we observed that Aurora-A is proteolyzed in an anaphase promoting complex-independent manner after extended periods after electric

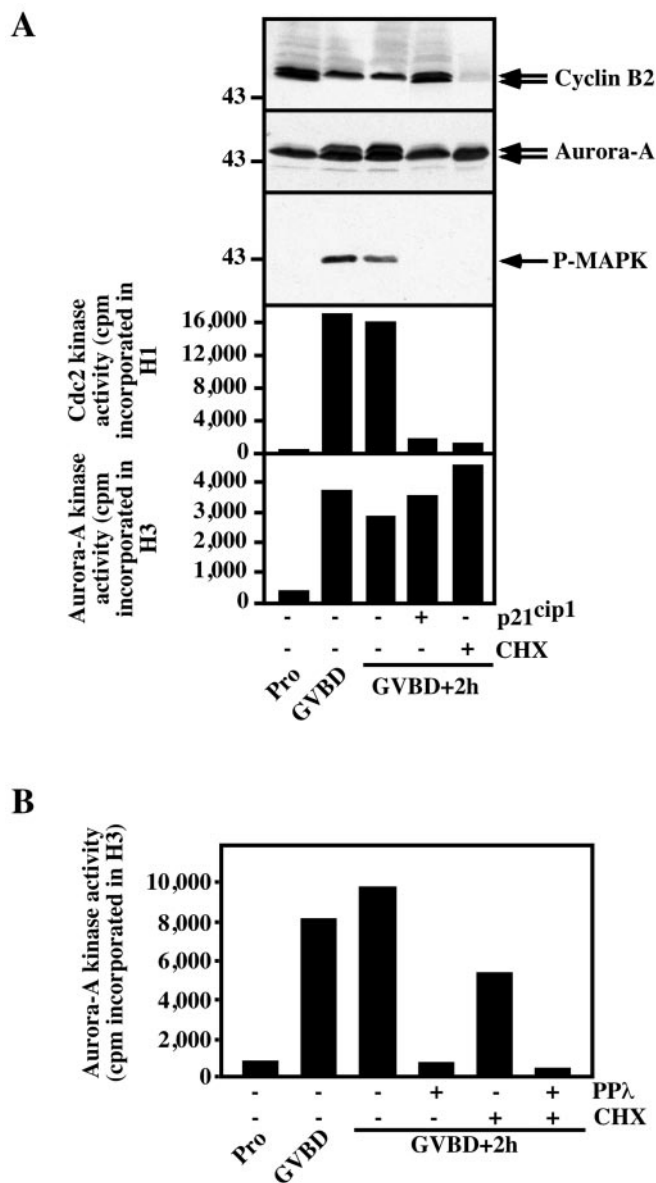


FIG. 10. Aurora-A kinase activity is stabilized independently of Cdc2-cyclin B and protein synthesis after GVBD. Prophase oocytes were incubated in the presence of progesterone, and oocytes were collected either in prophase (*Pro*), at GVBD, or 2 h after GVBD (*GVBD+2h*). **A**, GVBD oocytes were either immediately homogenized, incubated in the presence of cycloheximide (*CHX*), or injected with p21^{cip1} and collected 2 h later. Lysates were subjected to Western blot with anti-cyclin B2 antibody, anti-Aurora-A antibody, or anti-phospho-MAPK (*P-MAPK*) antibody, as indicated, or immunoprecipitated with anti-Aurora-A antibody and assayed for Aurora-A kinase activity using histone H3-peptide as substrate. A pull-down on p13 beads was assayed for Cdc2 kinase activity using histone H1 as substrate. Aurora-A and Cdc2 kinase activities are expressed in cpm incorporated in the substrate. **B**, GVBD oocytes were either immediately homogenized or incubated for 2 h in the presence (+) or in the absence (-) of cycloheximide (*CHX*). Lysates were immunoprecipitated with anti-Aurora-A antibody and incubated (+) or not (-) in the presence of λ phosphatase (*PP λ*) for 1 h at 30 °C. Immunoprecipitates were then assayed for Aurora-A kinase activity using histone H3-peptide as substrate.

shock. It is probable that, under non-physiological conditions (long periods after activation by electric shock), Aurora-A protein is especially sensitive to proteolysis and could represent an indicator of senescence or early apoptosis.

Aurora-A is hyperphosphorylated when okadaic acid is either injected into oocytes or added in cytosolic extracts that reproduce *in vitro* Cdc2 and Aurora-A activation. However, the

additional phosphorylation level generated by inhibition of okadaic acid-sensitive phosphatases does not increase the kinase activity level of Aurora-A. Therefore, because the phosphorylation on these additional sites is never observed under physiological conditions and does not control Aurora-A kinase activity, okadaic acid addition could have simply unmasked a kinase activity that does not control Aurora-A under normal conditions.

In somatic cells, Aurora-A plays a role in the mitotic spindle organization (18, 57). This function could also exist in meiosis, although no regular centrosomes are present in vertebrate acentriolar oocytes. During GVBD in *Xenopus* oocyte, a giant monopolar spindle is organized at the basal part of the germinal vesicle, associated to chromosomes. It migrates toward the animal pole, where it is reorganized in the two successive bipolar metaphase spindles (58–60). These two metaphase spindles could be regulated by Aurora-A. Supporting this idea, inhibition of Aurora-A association by antibody injection in prophase oocytes induces an arrest in metaphase I, because oocytes are unable to extrude the first polar body (23). It has been published that Eg5, a kinesin-related motor protein is a substrate of *Xenopus* Aurora-A (17). Moreover, it has recently been shown that Aurora-A binds to and phosphorylates TPX2, a prominent component of the spindle, inducing the recruitment of Aurora-A to the spindle microtubules (61). All together, these observations demonstrate that Aurora-A could be a crucial element of spindle organization in mitosis and in meiosis, under the control of Cdc2 kinase. It is now of high interest to undertake a structural analysis of Aurora-A localization in meiotic spindles and to study the consequences of its functional ablation on the microtubular organization of such spindles.

Acknowledgments—We thank all members of our laboratories, especially Prof. René Ozon and Dr. O. Haccard, for helpful discussions and advice.

REFERENCES

- Karaïskou, A., Dupre, A., Haccard, O., and Jessus, C. (2001) *Biol. Cell* **93**, 35–46
- Maller, J. L., and Krebs, E. G. (1977) *J. Biol. Chem.* **252**, 1712–1718
- Jessus, C., and Ozon, R. (1993) *Comp. Biochem. Physiol.* **106A**, 431–448
- Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J., and Vande Woude, G. F. (1988) *Nature* **335**, 519–525
- Dupre, A., Jessus, C., Ozon, R., and Haccard, O. (2002) *EMBO J.* **21**, 4026–4036
- Fisher, D. L., Brassac, T., Galas, S., and Doree, M. (1999) *Development* **126**, 4537–4546
- Gross, S. D., Schwab, M. S., Taieb, F. E., Lewellyn, A. L., Qian, Y. W., and Maller, J. L. (2000) *Curr. Biol.* **10**, 430–438
- Chan, C. S., and Botstein, D. (1993) *Genetics* **135**, 677–691
- Glover, D. M., Leibowitz, M. H., McLean, D. A., and Parry, H. (1995) *Cell* **81**, 95–105
- Schumacher, J. M., Ashcroft, N., Donovan, P. J., and Golden, A. (1998) *Development* **125**, 4391–4402
- Roghi, C., Giet, R., Uzbekov, R., Morin, N., Chartrain, I., LeGuellec, R., Couturier, A., Doree, M., Philippe, M., and Prigent, C. (1998) *J. Cell Sci.* **111**, 557–572
- Gopalan, G., Chan, C. S., and Donovan, P. J. (1997) *J. Cell Biol.* **138**, 643–656
- Kimura, M., Kotani, S., Hattori, T., Sumi, N., Yoshioka, T., Todokoro, K., and Okano, Y. (1997) *J. Biol. Chem.* **272**, 13766–13771
- Nigg, E. A. (2001) *Nat. Rev. Mol. Cell Biol.* **2**, 21–32
- Dutertre, S., Descamps, S., and Prigent, C. (2002) *Oncogene* **21**, 6175–6183
- Hannak, E., Kirkham, M., Hyman, A. A., and Oegema, K. (2001) *J. Cell Biol.* **155**, 1109–1116
- Giet, R., Uzbekov, R., Cubizolles, F., Le Guellec, K., and Prigent, C. (1999) *J. Biol. Chem.* **274**, 15005–15013
- Giet, R., and Prigent, C. (1999) *J. Cell Sci.* **112**, 3591–3601
- Marumoto, T., Hirota, T., Morisaki, T., Kunitoku, N., Zhang, D., Ichikawa, Y., Sasayama, T., Kuninaka, S., Mimori, T., Tamaki, N., Kimura, M., Okano, Y., and Sava, H. (2002) *Genes Cells* **7**, 1173–1182
- Paris, J., Le Guellec, R., Couturier, A., Le Guellec, K., Omilli, F., Camonis, J., Macneill, S., and Philippe, M. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 1039–1043
- Bouvet, P., Omilli, F., Arlot-Bonnemains, Y., Legagneux, V., Roghi, C., Bassez, T., and Osborne, H. B. (1994) *Mol. Cell Biol.* **14**, 1893–1900
- Frank-Vaillant, M., Haccard, O., Thibier, C., Ozon, R., Arlot-Bonnemains, Y., Prigent, C., and Jessus, C. (2000) *J. Cell Sci.* **113**, 1127–1138
- Castro, A., Mandart, E., Lorca, T., and Galas, S. (2003) *J. Biol. Chem.* **278**, 2236–2241
- Andresson, T., and Ruderman, J. V. (1998) *EMBO J.* **17**, 5627–5637

25. Mendez, R., Hake, L. E., Andresson, T., Littlepage, L. E., Ruderman, J. V., and Richter, J. D. (2000) *Nature* **404**, 302–307
26. Littlepage, L. E., Wu, H., Andresson, T., Deanehan, J. K., Amundadottir, L. T., and Ruderman, J. V. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 15440–15445
27. Littlepage, L. E., and Ruderman, J. V. (2002) *Genes Dev.* **16**, 2274–2285
28. Scrittore, L., Hans, F., Angelov, D., Charra, M., Prigent, C., and Dimitrov, S. (2001) *J. Biol. Chem.* **276**, 30002–30010
29. Frank-Vaillant, M., Jessus, C., Ozon, R., Maller, J. L., and Haccard, O. (1999) *Mol. Biol. Cell* **10**, 3279–3288
30. Lorca, T., Devault, A., Colas, P., Van, L. A., Fesquet, D., Lazaro, J. B., and Doree, M. (1992) *FEBS Lett.* **306**, 90–93
31. Karaiskou, A., Cayla, X., Haccard, O., Jessus, C., and Ozon, R. (1998) *Exp. Cell Res.* **244**, 491–500
32. Jessus, C., Thibier, C., and Ozon, R. (1987) *J. Cell Sci.* **87**, 705–712
33. Karsenti, E., Newport, J., Hubble, R., and Kirschner, M. (1984) *J. Cell Biol.* **98**, 1730–1745
34. Anderson, C. W., Baum, P. R., and Gesteland, R. F. (1973) *J. Virol.* **12**, 241–252
35. Castro, A., Arlot-Bonnemains, Y., Vigneron, S., Labbe, J. C., Prigent, C., and Lorca, T. (2002) *EMBO Rep.* **3**, 457–462
36. De Smedt, V., Poulhe, R., Cayla, X., Dessauge, F., Karaiskou, A., Jessus, C., and Ozon, R. (2002) *J. Biol. Chem.* **277**, 28592–28600
37. Laemmli, U. K. (1970) *Nature* **227**, 680–685
38. Walter, A. O., Seghezzi, W., Korver, W., Sheung, J., and Lees, E. (2000) *Oncogene* **19**, 4906–4916
39. Meraldi, P., Honda, R., and Nigg, E. A. (2002) *EMBO J.* **21**, 483–492
40. Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F., Lin, R., Smith, M. M., and Allis, C. D. (2000) *Cell* **102**, 279–291
41. Erikson, E., and Maller, J. L. (1989) *J. Biol. Chem.* **264**, 19577–19582
42. Maller, J., Wu, M., and Gerhart, J. C. (1977) *Dev. Biol.* **58**, 295–312
43. Maller, J. L. (1990) *Biochemistry* **29**, 3157–3166
44. Favata, M. F., Horiuchi, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feeser, W. S., Van Dyk, D. E., Pitts, W. J., Earl, R. A., Hobbs, F., Copeland, R. A., Magolda, R. L., Scherle, P. A., and Trzaskos, J. M. (1998) *J. Biol. Chem.* **273**, 18623–18632
45. Jessus, C., and Ozon, R. (1995) *Prog. Cell Cycle Res.* **1**, 215–228
46. Goris, J., Hermann, J., Hendrix, P., Ozon, R., and Merlevede, W. (1989) *FEBS Lett.* **245**, 91–94
47. Haccard, O., Jessus, C., Rime, H., Goris, J., Merlevede, W., and Ozon, R. (1993) *Mol. Reprod. Dev.* **36**, 96–105
48. Karaiskou, A., Jessus, C., Brassac, T., and Ozon, R. (1999) *J. Cell Sci.* **112**, 3747–3756
49. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, R., and Beach, D. (1993) *Nature* **366**, 701–704
50. Frank-Vaillant, M., Haccard, O., Ozon, R., and Jessus, C. (2001) *Dev. Biol.* **231**, 279–288
51. Furuno, N., Nishizawa, M., Okazaki, K., Tanaka, H., Iwashita, J., Nakajo, N., Ogawa, Y., and Sagata, N. (1994) *EMBO J.* **13**, 2399–2410
52. Huchon, D., Rime, H., Jessus, C., and Ozon, R. (1993) *Biol. Cell* **77**, 133–141
53. Maller, J. L. (1993) *Mol. Cell. Biochem.* **127–128**, 267–281
54. Hohegger, H., Klotzbucher, A., Kirk, J., Howell, M., le Guellec, K., Fletcher, K., Duncan, T., Sohail, M., and Hunt, T. (2001) *Development* **128**, 3795–3807
55. Francisco, L., Wang, W., and Chan, C. S. (1994) *Mol. Cell. Biol.* **14**, 4731–4740
56. Katayama, H., Zhou, H., Li, Q., Tatsuka, M., and Sen, S. (2001) *J. Biol. Chem.* **276**, 46219–46224
57. Bischoff, J. R., and Plowman, G. D. (1999) *Trends Cell Biol.* **9**, 454–459
58. Gard, D. L. (1992) *Dev. Biol.* **151**, 516–530
59. Huchon, D., Crozet, N., Cantenot, N., and Ozon, R. (1981) *Reprod. Nutr. Develop.* **21**, 135–148
60. Jessus, C., Huchon, D., and Ozon, R. (1986) *Biol. Cell* **56**, 113–120
61. Kufer, T. A., Sillje, H. H., Korner, R., Gruss, O. J., Meraldi, P., and Nigg, E. A. (2002) *J. Cell Biol.* **158**, 617–623

Cdc2-Cyclin B Triggers H3 Kinase Activation of Aurora-A in *Xenopus* Oocytes
Gilliane Maton, Catherine Thibier, Anna Castro, Thierry Lorca, Claude Prigent and
Catherine Jessus

J. Biol. Chem. 2003, 278:21439-21449.

doi: 10.1074/jbc.M300811200 originally published online April 1, 2003

Access the most updated version of this article at doi: [10.1074/jbc.M300811200](https://doi.org/10.1074/jbc.M300811200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 61 references, 33 of which can be accessed free at
<http://www.jbc.org/content/278/24/21439.full.html#ref-list-1>