The Polo-like kinase Plx1 is a component of the MPF amplification loop at the G2/M-phase transition of the cell cycle in Xenopus eggs

Ariane Abrieu*, Thierry Brassac*, Simon Galas, Daniel Fisher, Jean-Claude Labbé and Marcel Dorée‡

Centre de Recherches de Biochimie Macromoléculaire, CNRSUPR 1086, 1919 route de Mende, 34293 Montpellier cedex 5, France

*The two first authors contributed equally to this work
‡Author for correspondence (e-mail: Dorée@crbm.cnrs-mop.fr)

Accepted 9 April; published on WWW 27 May 1998

SUMMARY

We have investigated whether Plx1, a kinase recently shown to phosphorylate cdc25c in vitro, is required for activation of cdc25c at the G2/M-phase transition of the cell cycle in Xenopus. Using immunodepletion or the mere addition of an antibody against the C terminus of Plx1, which suppressed its activation (not its activity) at G2/M, we show that Plx1 activity is required for activation of cyclin B-cdc2 kinase in both interphase egg extracts receiving recombinant cyclin B, and cycling extracts that spontaneously oscillate between interphase and mitosis. Furthermore, a positive feedback loop allows cyclin B-cdc2 kinase to activate Plx1 at the G2/M-phase transition. In contrast, activation of cyclin A-cdc2 kinase does not require Plx1 activity, and cyclin A-cdc2 kinase fails to activate Plx1 and its consequence, cdc25c activation in cycling extracts.

Key words: Mitosis, Polo kinase, MPF amplification, Xenopus egg

INTRODUCTION

The G2- to M-phase of the cell cycle requires activation of MPF, a stoichiometric complex between cdc2 and cyclin B (for reviews, see Nurse, 1990; Dorée, 1990). MPF accumulates as an inactive complex (pre-MPF) at G2 (Solomon et al., 1990; Gautier et al., 1991; Strausfeld et al., 1991) due to phosphorylation of cdc2 in the ATP binding domain by inhibitory kinases. Xenopus eggs for example contain at least two kinases responsible for these inhibitory phosphorylations: a soluble wee1 protein kinase which phosphorylates Tyr15 exclusively (Mueller et al., 1995a), and a membrane-associated MYT1 protein kinase which phosphorylates both Thr14 and Tyr15 (Mueller et al., 1995b). MPF activation at G2/M requires dephosphorylation of both residues, which is catalyzed by the mitotic inducer cdc25c (Russell and Nurse, 1986; Strausfeld et al., 1991; Dunphy and Kumagai, 1991; Gautier et al., 1991). The activity of cdc25c is itself regulated during the cell cycle, peaking at mitosis and decreasing at interphase (Izumi et al., 1993; Jessus and Beach, 1992; Kumagai and Dunphy, 1992).

Recently, Plx1, a Xenopus member of the evolutionarily conserved family of Polo-like kinases (reviewed by Glover et al., 1996; Golsteyn et al., 1996; Lane and Nigg, 1997) was shown to phosphorylate and enhance the phosphatase activity of cdc25c (Kumagai and Dunphy, 1996). It was thus proposed that Plx1 may be the ‘trigger kinase’ for the activation of cdc25c and play an essential role in controlling the onset of mitosis, even though genetics in Drosophila and yeast had not suggested such a role for other Polo-like kinases but rather emphasized their role later in mitosis (Sunkel and Glover, 1988; Llamazares et al., 1991; Fenton and Glover, 1993; Kitada et al., 1993; Ohkura et al., 1995).

However, cyclin B-cdc2 kinase can directly phosphorylate cdc25c in vitro, and this results in cdc25c activation (Strausfeld et al., 1994; Izumi and Maller, 1993). It is not known if cdc2 kinase acts in this way in intact cells, but if this is the case, the ability of cyclin B-cdc2 to directly phosphorylate and activate cdc25c could form the basis of a minimal positive feedback loop between the two components, the so-called ‘MPF amplification’ process (Masui and Markert, 1971), which consists of a regulatory loop of post-translational events by which a small amount of active cdc2 kinase acts as a primer, resulting in production of more cdc2 kinase from an endogenous pool of inactive cdc2 kinase.

Recombinant cyclin A-cdc2 kinase has also been reported to directly phosphorylate a cdc25c fusion protein in vitro on sites identical to those phosphorylated by cyclin B-cdc2 kinase, resulting in enhancement of cdc25c activity (Izumi and Maller, 1993). However, this result has been challenged by Hoffmann et al. (1993). Using active cyclin A-cdc2 kinase complexes isolated from HeLa cells or assembled in Xenopus egg extracts, these authors reported that, at variance with cyclin B-cdc2 kinase, immunoprecipitated cyclin A-cdc2 complexes failed to phosphorylate full-length recombinant cdc25c.

In the present work, we investigated, using Xenopus egg extracts, the role that Plx1 plays at the G2- to M-phase transition in the first embryonic mitotic cell cycles. We find that
Plx1 is a component of an amplification loop absolutely required for MPF activation. Cycling egg extracts to which an antibody that prevents Plx1 activation has been added still support cyclin A-cdc2 kinase activation, but do not activate cyclin B-cdc2 kinase and do not enter mitosis. These results are discussed in connection with previous reports that cyclin A does not require cdc25c to form an active kinase complex with cdc2 (Clarke et al., 1992), and acts synergistically with cyclin B during the G2/M-phase transition (Westendorf et al., 1989; Lehner et al., 1991; Knoblich and Lehner, 1993; Devault et al., 1991, 1992).

MATERIALS AND METHODS

**Xenopus egg extracts**

Cycling and CSF-arrested extracts were prepared exactly as described by Morin et al. (1994), according to minor modifications of procedures described by Murray and Kirschner (1989). Interphase extracts were prepared according to three different procedures, as indicated in the text. In the first procedure, 0.4 mM CaCl2 was added to a CSF extract, which was further incubated for 40 minutes at room temperature, at which time experiments were initiated. In the second procedure, eggs were parthenogenetically activated, then further incubated for 40 minutes at room temperature, at which time homogenates were prepared. The last procedure is a variant of the preceding one in which parthenogenetically activated eggs were incubated for 2 hours in the presence of 100 μg/ml cycloheximide before homogenisation. As both CSF and interphase extracts were kept frozen at −70°C until use, they hardly translate cyclins and do not ‘cycle’. In contrast, cycling extracts were not frozen, and directly used after preparation from parthenogenetically activated eggs.

**Immunological procedures**

The polyclonal antibody directed against Plx1 (Kumagai and Dunphy, 1996) was raised by immunizing rabbits with a peptide encoding the 17 last amino acids of Plx1 C terminus (plus a cysteine residue at the N terminus) cross-linked to thyroglobulin by the MBS procedure. Antibodies were purified by affinity on the same peptide cross-linked to albumin. The affinity-purified antibodies were concentrated to 2 mg/ml and kept frozen until use. The rabbit immunoglobulins used as control were obtained from Sigma. Polyclonal antibodies against Xenopus cyclin B1, Xenopus cyclin A, Xenopus cdc25 and sea urchin cyclin B were raised against recombinant proteins and have been described previously (Abrieu et al., 1996, 1997). Immunoprecipitations were performed after dilution in RIPA buffer and immunoprecipitates collected on Protein A-Sepharose, washed with 50 mM Tris-HCl, pH 7.5, and used for determination of kinase activities or western blotting. Immunoblots were analyzed by ECL.

**RESULTS**

**Plx1 kinase is activated at the G2- to M-phase transition in the first meiotic and the first mitotic cycles of Xenopus eggs**

To monitor cell cycle-dependent changes in Plx1 activity in Xenopus (Fig. 1A) homogenates were prepared from groups of five oocytes or eggs at the following stages: arrest at the germinal vesicle (GV) stage (lane 1), metaphase I (1 hour after GV breakdown induced by either progesterone or microinjection of the c-mos proto-oncogene; lanes 2 and 3, respectively), metaphase II arrest (lane 4), G2 arrest in fertilized eggs treated for 2 hours with cycloheximide (5), first mitotic metaphase (6 and 7).

![Fig. 1. Plx1 is active at M-phase and inactive at interphase, in meiotic and the first mitotic cell cycles.](image)

(A) Homogenates prepared from 5 oocytes or eggs at the following stages: GV (1), metaphase I induced by progesterone (2) or microinjection of c-mos (3), metaphase II arrest (4) G2 arrest in fertilized eggs treated for 2 hours with cycloheximide (5), first mitotic metaphase (6 and 7).

Upper panel: Plx1 kinase activities were measured in immunoprecipitates using casein as a substrate (lane 7 shows a control in which anti-Plx1 antibodies were first saturated with the antigenic peptide before immunoprecipitation). Lower panel: determination of H1 kinase activities in the same homogenates. (B) Immunoblot showing Plx1 in the same homogenates, prepared from GV oocytes (1,3) or fertilized eggs arrested at G2 by cycloheximide (2,4) before (1,2) or after (3,4) immunodepletion with anti-Plx1 antibodies.
fertilized eggs treated for 2 hours with cycloheximide (lane 5), first mitotic metaphase (1 hour after fertilization, lane 6). Plx1 was immunoprecipitated from these homogenates using affinity-purified antibodies directed against its C terminus and kinase activity assayed in immunoprecipitation using α-casein as a substrate (Golsteyn et al., 1995), in parallel with determination of H1 kinase activity in whole homogenates. Plx1 kinase activity was readily detected in homogenates prepared at meiotic and mitotic metaphases, and absent or extremely reduced in homogenates prepared at meiotic or mitotic G2. As expected, no casein kinase activity was detected in homogenates prepared at M-phase when antibodies were saturated with the C-terminal peptide used as immunogen (lane 7). Absence of Plx1 kinase activity at G2 was not due to failure of the antibody to immunoprecipitate Polo at this stage of the cell cycle, as the antibody quantitatively immunodepleted Polo from homogenates (Fig. 1B).

**Prevention of Plx1 kinase activation suppresses hyperphosphorylation of cdc25c and cyclin B-cdc2 kinase activation in Xenopus egg extracts**

To investigate the role of Plx1 kinase in activation of cyclin B-cdc2 kinase, we first used non-cycling egg extracts, in which synthesis of endogenous cyclins is deficient. In a first set of experiments, the egg extract was made from eggs taken 40 minutes after parthenogenetic activation and kept frozen until use. After standing for 10 minutes at room temperature, this interphase extract was split into two parts. One was immunodepleted of Plx1 using affinity-purified antibodies directed against its C terminus, and the second was mock-depleted using the same amount of non-immune rabbit immunoglobulins. Then each part received in vitro translated 35S-labelled sea urchin cyclin B. Samples were taken as a function of time, checked for efficiency of Plx1 depletion (Fig. 2A, upper panel) and assessed for determination of H1 kinase activities of anti-sea urchin cyclin B immunoprecipitates. As shown in Fig. 2A, lower panel, active cyclin B-cdc2 kinase readily formed in mock-depleted extracts, but not in Plx1-depleted extracts. As expected, Cdc2 remained tyrosine-phosphorylated in Plx1-depleted extracts (Fig. 2B). Addition of recombinant Plx1 (0.1 mg/ml) readily restored the ability of depleted extracts to activate cyclin B-cdc2 kinase (not shown). In the course of these experiments, we observed that removal of Plx1 antibodies by Protein A-Sepharose beads was in fact not necessary. The same results could actually be obtained if the affinity-purified antibodies were added before (but not after) activation of Plx1. The ability of these antibodies to prevent, but not reverse, Plx1 activation, was used in the next series of experiments.

In this case, an interphase extract (in which Plx1 is inactive) was prepared in vitro by addition of 0.4 mM CaCl2 to a 'CSF' (metaphase II-arrested) extract prepared from unfertilized eggs, which triggered degradation of mitotic cyclins and MPF inactivation (Murray and Kirschner, 1989). After further incubation for 40 minutes at room temperature, the interphase egg extract was split into four parts. The first (B in Fig. 3) received 200 μg/ml of the affinity-purified antibodies directed against the C terminus of Plx1, the second (A) received the same amount of control rabbit immunoglobulins, the two others (C and D) remained unmodified. Three of the four extracts then received identical amounts of both in vitro translated 35S-labelled *Xenopus* cdc25c and a fusion protein of sea urchin cyclin B with GST. One of them (free of immunoglobulin) received cdc25c only (D). Aliquots were taken as a function of time and analysed after immunoprecipitation with anti-Plx1 or anti-sea urchin cyclin B for Plx1 or cdc2 kinase activities, or by autoradiography directly for changes in electrophoretic mobility of cdc25c.

As shown in Fig. 3 (upper panel, lanes C), addition of recombinant cyclin B to interphase extracts readily activated Plx1 kinase, and this was correlated with both hyperphosphorylation of cdc25c and activation of cdc2 kinase. In contrast, neither Plx1 nor cdc2 kinases were activated in the presence of anti-Plx1 antibodies, and cdc25c did not undergo hyperphosphorylation under such conditions (lanes B). This was a specific effect of adding the anti-Plx1 antibodies, as addition of the same amount of control rabbit immunoglobulins had no such effect (lanes A). In this experiment it can be seen that although H1 and Polo kinases are activated concomitantly, there appears to be a time delay with respect to the hyperphosphorylation of cdc25c. The most likely explanation is the technical difficulty in correlating an exact timing of these
events: the samples for cdc25c analysis were immediately fixed by freezing, whereas samples for H1 and casein kinase activity measurements were immunoprecipitated on ice and then kinase assays performed. It is not possible to perform cdc25 activity measurements in such a time-course. Nevertheless it is clear that in experiments where cdc25c hyperphosphorylation does not occur, H1 kinase is not activated.

Cyclin B-cdc2 kinase activates Plx1 kinase
In the above experiments, both cyclin B-cdc2 and Plx1 were activated in parallel, allowing the possibility that, although Plx1 kinase is required for cyclin B-cdc2 kinase activation, a positive feedback loop may occur whereby cyclin B-cdc2 kinase may enhance Plx1 kinase activity (in the absence of anti-Plx1 antibodies).

To directly assess this possibility, G2-arrested extracts, free of mitotic cyclins and Plx1 activity, were prepared by incubating extracts prepared from parthenogenetically activated eggs for two hours in the presence of cycloheximide. Highly purified starfish cyclin B-cdc2 kinase (Labbé et al., 1991) was then added: as shown in Fig. 4, the starfish kinase, indeed, readily activated Plx1 kinase. We did not investigate if Plx1 is directly phosphorylated by cdc2 kinase, but this seems unlikely, as Plx1 lacks cdc2 consensus sites.

Cyclin A-cdc2 kinase is not sufficient for activation of cyclin B-cdc2 kinase and for cycling egg extracts to enter M-phase: Plx1 activity is required
As the use of recombinant cyclin A has led to contradictory results in experiments aimed to investigate its possible role in cdc25c phosphorylation and activation (see Introduction), we took advantage of our recent finding (Abrieu et al., 1997) that prevention of MAPK inactivation after fertilization does not inhibit activation of endogenous cyclin A-cdc2 complexes, but prevents cdc25-dependent tyrosine dephosphorylation of cdc2 in cyclin B-cdc2 complexes, thereby suppressing both their activation and entry of cycling egg extracts in M-phase. Unlike the arrested extracts used in the previous sections, synthesis of mitotic cyclins readily occurs in cycling extracts, and this normally allows them to spontaneously progress from interphase into mitosis (Murray and Kirschner, 1989).

As shown in Fig. 5A, suppression of MAP kinase inactivation (here by early addition of recombinant c-mos) was found to suppress Plx1 activation (lower panel). As activation of cyclin A-cdc2 kinase was not suppressed (upper panel), this experiment strongly suggested that cyclin A-cdc2 kinase is not sufficient to activate Plx1, and that failure to activate Plx1 had compromised cdc25-dependent activation of cyclin B-cdc2 (middle panel). However, recombinant c-mos could possibly have prevented Plx1 from being activated in the presence of otherwise activatory cyclin A-cdc2 kinase.

To more directly assess the requirement of Plx1 for cyclin B-cdc2 kinase activation, affinity-purified antibodies against Plx1 were added to cycling extracts early in the first mitotic

Fig. 3. Prevention of Plx1 kinase activation suppresses hyperphosphorylation of cdc25 and cyclin B-cdc2 kinase activation in interphase egg extracts derived from CSF extracts and receiving at zero time a fusion protein of sea urchin cyclin B with GST. Upper panel: Plx1 kinase activities of immunoprecipitates. Middle panel: electrophoretic mobilities of Xenopus 35S-labelled cdc25c. Lower panel: H1 kinase activities of anti-urchin cyclin B immunoprecipitates. (A,B,C,D) The interphase extract was split into four parts before cyclin B addition. A (control) received non-immune gammaglobulins (200 μg/ml), 35S-labelled cdc25c and cyclin B; C received only 35S-labelled cdc25c and cyclin B; D received only 35S-labelled cdc25c. Samples were taken at 1, 5, 10, 20 and 40 minutes after cyclin B addition and analysed for Plx1 kinase activity, sea urchin cyclin B-cdc2 kinase activity, and hyperphosphorylation of cdc25c.

Fig. 4. Cyclin B-cdc2 kinase triggers Plx1 activation in interphase extracts. G2-arrested extracts were prepared free of both mitotic cyclins and Plx1 kinase activity by incubating an extract prepared from parthenogenetically activated eggs, part of which was immunodepleted of Plx1, for two hours in the presence of cycloheximide. Highly purified starfish cyclin B-cdc2 kinase was then added, or not, to both the Plx1-depleted and the non-depleted extracts. Thirty minutes later, Plx1 was immunoprecipitated and its activity measured using casein as a substrate. 1: interphase extract alone; 2: interphase extract plus cyclin B-cdc2 kinase; 3: Plx1-depleted interphase extract plus cyclin B-cdc2 kinase.
cell cycle (an identical amount of rabbit gamma-globulins was added in the control cycling extract). As expected, the antibody suppressed Plx1 kinase activation (Fig. 5B, lower panel). As a consequence, no activation of cyclin B-cdc2 kinase occurred (middle panel), and extracts did not enter M-phase, as ascertained by failure of sperm nuclei to undergo nuclear envelope breakdown and chromosome condensation (not shown). In contrast, cyclin A-cdc2 kinase readily underwent activation in the absence of Plx1 activity (upper panel).

We conclude that physiological levels of cyclin A-cdc2 kinase are not sufficient to activate Plx1, and that Plx1 activity is required for MPF to be converted from its inactive into its active form.

As observed in the case of arrested extracts, hyperphosphorylation of cdc25c was suppressed in cycling extracts prevented to activate Plx1 kinase (Fig. 6A). Activation of cyclin B-cdc2 kinase depends not only on activation of cdc25c, but also on inhibition of the WEE1/MYT1 kinases, that cooperate to maintain phosphorylation of cdc2 on the inhibitory residues Thr14 and Tyr15. Both WEE1 and MYT1 from Xenopus are active during interphase but show greatly reduced activity at mitosis as a result of extensive phosphorylation by cdc2 and another unknown kinase (Mueller et al., 1995a,b). As shown in Fig. 6B, hyperphosphorylation of at least MYT1 was strongly reduced in cycling extracts prevented from activating Plx1 kinase.

**DISCUSSION**

In the present work, we show that Plx1 kinase, which has been previously demonstrated to hyperphosphorylate and activate cdc25c in vitro (Kumagai and Dunphy, 1996), is a component of the MPF amplification loop. Indeed cyclin B-cdc2 triggers activation of this Polo-like kinase, which itself, through activation of cdc25c and possibly inactivation of MYT1, increases the rate of cyclin B-cdc2 complex conversion from its inactive (pre-MPF) into its active state (MPF). However, cyclin B-cdc2 kinase has also been reported to directly hyperphosphorylate and activate cdc25 in vitro, thus we investigated if Plx1 is actually required in Xenopus for MPF amplification at the G2/M-phase transition of the cell cycle.

Using cell-free extracts taken after degradation of mitotic cyclins at G2 of the first mitotic cell cycle, in which cdc25c is underphosphorylated and has limited activity, we show that either specific depletion of Plx1 or prevention of its activation (by blocking its C terminus with a polyclonal antibody) suppresses activation of cyclin B-cdc2 kinase upon cyclin B addition. This is because cyclin B-cdc2 complexes first accumulate with cdc2 phosphorylated on inhibitory residues, and enhanced cdc25c activity is required to counteract WEE1/MYT1 inhibitory kinases and dephosphorylate inhibitory residues of cdc2. The small proportion of cyclin B-cdc2 complexes that escapes inhibition, not sufficient to activate cdc25c directly, at least partially activates Plx1 kinase: this triggers a positive feedback loop that allows complete activation of cyclin B-cdc2 kinase. Under such conditions, Plx1 kinase is absolutely required for activation of the feedback loop and MPF activation. This implies that even if only partially activated, the cdc2/Plx1 cascade of protein kinases is much more efficient than cdc2 kinase alone to activate the feedback loop.
kinase are able to activate Plx1 in the absence of cyclin B-cdc2 in cell cycle extracts. Cyclin A-cdc2 kinase does not require cyclin B, further demonstrate that cyclin A-cdc2 kinase does not require Plx1 in extracts containing only cyclin B. These results, consistent with our findings that suppression of Plx1 activation has no effect on either the extent or the kinetics of cyclin A-cdc2 kinase activation in cycling egg extracts. In contrast, activation of Plx1 is absolutely required in Xenopus egg extracts (Abrieu et al., 1997). Even though both cyclin A and cyclin B accumulate at G2, Cyclin A- and cyclin B-dependent protein kinases have been reported to be regulated by different mechanisms in Xenopus egg extracts (Clarke et al., 1992; Devault et al., 1992). Even though both cyclin A and cyclin B associate with cdc2 exclusively in this system, cdc2 is only weakly tyrosine-phosphorylated when bound to cyclin A and activated without a lag phase, suggesting that active complexes can form without intervening phosphorylation of cdc2 on inhibitory residues. In agreement with this view, we show in the present work that suppression of Plx1 activation has no effect on the kinetics of cyclin A-cdc2 kinase activation in cycling egg extracts. In contrast, activation of cyclin B-cdc2 kinase, which requires cdc25c, did not occur in cycling extracts prevented from activating Plx1, and they arrested before nuclear envelope breakdown and chromosome condensation. These results, consistent with our finding that Plx1 is required to activate cdc25c in extracts containing only cyclin B, further demonstrate that cyclin A-cdc2 kinase does not efficiently activate cdc25c in the absence of Plx1, at least in cell cycle extracts.

To investigate whether physiological levels of cyclin A-cdc2 kinase are able to activate Plx1 in the absence of cyclin B-cdc2 kinase, we arrested cycling extracts at G2 by preventing MAP kinase inactivation, which normally occurs prior to cyclin B-cdc2 kinase activation in the first mitotic cell cycle (Abrieu et al., 1997; Walter et al., 1997). Under such conditions, we found that Plx1 was not activated, even though cyclin A-cdc2 kinase was activated to the same extent and with the same kinetics as in cycling egg extracts. It is unlikely that MAP kinase prevents Plx1 activation, as unfertilized eggs contain both high MAP kinase and Plx1 activities. Thus, our results strongly suggest that, unlike cyclin B-cdc2 kinase, endogenous cyclin A-cdc2 kinase is unable to trigger Plx1 activation and its consequence, cdc25c activation, at least under the conditions in cell cycle extracts (however, partial activation of Plx1 was observed when recombinant cyclin A added to the extracts to produce levels of H1 kinase higher to that of endogenous cyclin B-cdc2 kinase).

Suppression of Plx1 activation prevents cdc25c and reduces MYT1 hyperphosphorylation in cycling egg extracts. A cycling extract received at zero time 200 µg/ml of affinity-purified antibodies directed against the C terminus of Plx1 (Ab Plx) or 200 µg/ml non-immune rabbit immunoglobulins (control). (A) Samples were collected at 40 (2), 50 (3), 60 (4) and 85 minutes (5) after antibody addition, as well as just before (1), and processed for detection of endogenous cdc25c by western blotting. (B) Reticulocyte-translated MYT1 was added at zero time (1) and samples, collected at the same times as above, were processed by autoradiography for monitoring changes in the electrophoretic mobility of MYT1.

Taken together, our results strongly support the view that Plx1 is absolutely required in Xenopus for MPF activation and cell cycle progression from G2 to mitosis, at least in the first mitotic cell cycle. In contrast, genetics has failed to detect a role for Polo in activating MPF at onset of mitosis and rather demonstrated its requirement for later steps of mitosis (Sunkel and Glover, 1988; Llamazares et al., 1991; Fenton and Glover, 1993; Kitada et al., 1993; Ohkura et al., 1995). Cyclin A-cdc2 kinase has been suggested to play an important role in MPF amplification at the G2/M-phase transition in early Drosophila embryos (Lehner et al., 1991; Knoblich and Lehner, 1993), contrasting with our present results in Xenopus cell cycle extracts. This possibly indicates that cyclin A-cdc2 kinase may participate, or not, to the MPF amplification process, depending on species. A Polo-like kinase activity is perhaps not required for the G2/M-phase transition in systems using cyclin A-cdc2 kinase synergistically with cyclin B-cdc2 kinase to activate the MPF amplification loop.

Although cyclin B-cdc2 kinase was shown in the present work to trigger Plx1 kinase activation, we were not able to address the question as to whether it does so by directly phosphorylating Plx1. This seems, however, unlikely for at least two reasons. Firstly, Plx1 lacks typical consensus sites for phosphorylation by cdc2 kinases. Secondly, attempts by other investigators to stoichiometrically phosphorylate recombinant or immunoprecipitated Polo-like kinases in vitro with purified cyclin B-cdc2 kinase were not successful (Hamanaka et al., 1995) even though mitotic extracts readily phosphorylate and activate recombinant Polo-like kinases (Kumagai and Dunphy, 1996). Whilst the mechanism of activation of Polo kinases at the G2/M-phase transition (Golsteyn et al., 1995; Tavares et al., 1996; the present work) remains elusive, our finding that immunoglobulins interacting with the C terminus of Plx1 prevent its activation, but do not suppress its activity, strongly suggest a role for the C terminus in regulation of Plx1 kinase activity though cell cycle. Accordingly, catalytic activity of the mammalian Polo-like kinase Plk1 has been shown to strongly increase in mutants with the C-terminal tail deleted (Mundt et al., 1997; Lee and Erikson, 1997), and phosphorylation of this domain has been suggested to be involved in activation of Polo-like kinases.
REFERENCES


