Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 352–356, January 1996
Cell Biology

An inhibitor of $p34^{cdc2}$/cyclin B that regulates the G_2/M transition in *Xenopus* extracts

(mitotic cdc2 inhibitor/cell cycle/interphase/cyclin/membrane protein)

TINA H. LEE AND MARC W. KIRSCHNER

Harvard Medical School, Department of Cell Biology, 25 Shattuck Street, Boston, MA 02115

Contributed by Marc W. Kirschner, September 22, 1995

ABSTRACT **The activity of maturation-promoting factor (MPF), a protein kinase complex composed of $p34^{cdc2}$ and cyclin B, is undetectable during interphase but rises abruptly at the G_2/M transition to induce mitosis. After the synthesis of cyclin B, the suppression of MPF activity before mitosis has been attributed to the phosphorylation of $p34^{cdc2}$ on sites (threonine-14 and tyrosine-15) that inhibit its catalytic activity. We previously showed that the activity of the mitotic $p34^{cdc2}$/cyclin B complex is rapidly suppressed when added to interphase *Xenopus* extracts that lack endogenous cyclin B. Here we show that a mutant of $p34^{cdc2}$ that cannot be inhibited by phosphorylation (threonine-14 → alanine, tyrosine-15 → phenylalanine) is also susceptible to inactivation, demonstrating that inhibitory mechanisms independent of threonine-14 and tyrosine-15 phosphorylation must exist. We have partially characterized this inhibitory pathway as one involving a reversible binding inhibitor of $p34^{cdc2}$/cyclin B that is tightly associated with cell membranes. Kinetic analysis suggests that this inhibitor, in conjunction with the kinases that mediate the inhibitory phosphorylations on $p34^{cdc2}$, maintains the interphase state in *Xenopus*; it may play an important role in the exact timing of the G_2/M transition.**

Entry into mitosis depends on the activation of maturation-promoting factor (MPF) (1–4), a protein kinase complex composed of a catalytic subunit, $p34^{cdc2}$, and its essential regulatory subunit, cyclin B (5–15). The reaction pathways that govern MPF activation are complex (reviewed in refs. 16–21). Briefly, as cyclin B accumulates during interphase, it binds to $p34^{cdc2}$ and allows phosphorylation of $p34^{cdc2}$ on threonine 161, a site essential for catalytic activity of the kinase complex (17, 22–26). However, the bulk of $p34^{cdc2}$/cyclin B is held inactive because cyclin binding simultaneously allows phosphorylation of $p34^{cdc2}$ (20, 27, 28) on a pair of adjacent inhibitory sites, Thr-14 and Tyr-15 (29–33), by Wee1/Mik1 (34–39) and Myt1, a recently identified membrane-associated kinase (40–42). The activity of the Thr-14 and Tyr-15 kinases is held high during interphase, while the activity of Cdc25 (43, 44), the enzyme specific for dephosphorylating both Thr-14 and Tyr-15 (45–47), is held low. When cyclin B accumulates to a critical threshold level, it sets off a cascade of events that leads, after a defined lag, to a switch in the balance between Cdc25 and Wee1/Mik1 and Myt1 activities (20, 40–42, 48–55) such that the inhibitory phosphorylations on $p34^{cdc2}$ are suddenly removed and MPF is abruptly activated.

Although the inhibition of $p34^{cdc2}$/cyclin B by phosphorylation on Thr-14 and Tyr-15 is clearly a central mechanism for suppressing its activity during interphase, a number of studies have suggested that this mechanism cannot account entirely for the regulation of the interphase to mitosis transition. Studies in *Xenopus* extracts revealed, surprisingly, that the lag phase preceding MPF activation could be prolonged (by protein phosphatase 2A) without modulation of either Cdc25 or Wee1/Mik1 and Myt1 activities (56). In addition, studies in *Saccharomyces cerevisiae*, in which the inhibitory phosphorylation sites in $p34^{cdc2}$ were rendered unphosphorylatable by site-directed mutagenesis, demonstrated the existence of a mechanism(s), other than phosphorylation of the inhibitory sites on $p34^{cdc2}$, for delaying the onset of mitosis (57, 58). Most recently, an analysis in *Xenopus* extracts showed that the delay in the onset of $p34^{cdc2}$/cyclin activation, imposed by unreplicated DNA, occurs without modulation of Wee1/Mik1 and Myt1 or Cdc25 activities (59).

Here, we describe a pathway that is essential for the suppression of $p34^{cdc2}$/cyclin B during the lag phase. A mutant form of $p34^{cdc2}$/cyclin B, with Thr-14 and Tyr-15 altered to unphosphorylatable residues, is still susceptible to negative regulation in *Xenopus* interphase extracts. Inhibition of the mutant form of $p34^{cdc2}$/cyclin B appears to occur through the reversible binding of an inhibitor. We have partially characterized this binding inhibitor as a large (≈400–500 kDa) protein complex that is tightly associated with cell membranes. Kinetic analysis suggests that this inhibitor may play an important role in the exact timing of mitosis.

METHODS

Preparation of $p34^{cdc2}$/Cyclin B. Reticulocyte lysates (Promega), containing ≈10 ng of wild-type or mutant (thr14ala, tyr15phe) $p34^{cdc2}$ per ml (46), and supplemented with 10 mM Mg^{2+}-ATP, were mixed with 1/3 volume of 20 nM glutathione *S*-transferase (GST)-cyclin B, prepared as described (56), plus 1/3 volume of [1:100 into extract buffer (XB; ref. 20)] diluted interphase extract, prepared as described (56). The final reaction mixtures were incubated at room temperature (RT) for 1 hr. Aliquots (1.5 μl) of each reaction mixture were bound to aliquots (10 μl) of glutathione agarose beads. The bead-bound complexes were washed with 400 μl of XB/1 mM dithiothreitol (DTT) and used as substrates for subsequent inhibitor assays.

Preparation of [^{35}S]Methionine-Labeled (thr14ala, tyr15phe) $p34^{cdc2}$/Cyclin B. Four microliters of reticulocyte lysate containing [^{35}S]methionine-labeled (thr14ala, tyr15phe) $p34^{cdc2}$ (46) was incubated with 20 μl of interphase extract plus 20 μl of GST-cyclin B (40 nM in XB) and 4 μl of 12CA5 antibody (1 μg/μl; Upstate Biotechnology). After 1 hr at room temperature, aliquots (10 μl) were bound to aliquots (10 μl) of protein A-Sepharose beads. The immune complexes were washed three times with 400 μl of XB.

Inhibitory Kinase Assay and Washes. Detection of Thr-14, Tyr-15 inhibitory kinase followed a procedure communicated to us by X. F. Xheng and J. Ruderman. Membrane pellets were solubilized with the original (concentrated extract) volume of a buffer containing 0.5% Triton X-100, 50 mM Tris (pH 8), 0.5 M NaCl, 25 mM $MgCl_2$, 1 mM ATP, and 1 mM DTT. Inhibitory

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MCI, mitotic cdc2 inhibitor; OG, octyl glucoside; MPF, maturation-promoting factor; GST, glutathione *S*-transferase.

kinase activity was detected by incubating the solubilized extract (diluted 1:4 into XB) with bead-bound p34^{cdc2}/cyclin B containing the wild-type p34^{cdc2} subunit plus Mg^{2+}-ATP. After 30 min at RT, the beads were stringently washed with 2× 400 μl of XB/0.5 M NaCl/0.2% Nonidet P-40 (NP-40)/1 mM DTT [which removes mitotic cdc2 inhibitor (MCI)], followed by one wash with 400 μl of XB.

Solubilization and Fractionation of MCI. Ten micrograms of pepstatin, chymostatin, and leupeptin per ml as well as 1 mM DTT was added to all buffers. The membrane pellet (140,000 × *g*, 1 hr) from 1 ml of interphase extract was solubilized in 6 ml of XB plus 4% octyl glucoside (OG), incubated on ice for 30 min, and recentrifuged to remove the insoluble material. For the MCI assay, the solubilized preparation was diluted below the critical micelle concentration (cmc) (to 0.3% OG; cmc = 0.6%) of OG with XB/15% glycerol and the solution was concentrated using Centriprep-10 and Centricon-10 concentrators (Amicon) to a final volume of 200 μl.

Sucrose gradient. Two hundred microliters of the above preparation was adjusted to 4% OG and loaded onto a 2-ml 15–40% sucrose gradient (in XB/4% OG) and centrifuged in a Sorvall TL55 rotor at 55,000 rpm for 10 hr at 4°C. Fractions (200 μl) were diluted (to 0.3% OG) and reconcentrated (to 50 μl) for the MCI assay.

Gel filtration. Two hundred microliters of the preparation described above was adjusted to 3% OG and loaded onto a 24-ml Superose 6 FPLC column (Pharmacia) equilibrated in XB/3% OG/15% glycerol. Fractionation took place at 0.2 ml/min, and 0.8-ml fractions were collected. Each fraction was diluted into 15 ml of XB/15% glycerol and reconcentrated (above) for the MCI assay.

Heat Treatment of Membranes. The membrane pellet (140,000 × *g*, 1 hr) from 400 μl of interphase extract was resuspended in 400 μl of XB. Aliquots (200 μl) were incubated at 37°C or 4°C (control) for 15 min. One hundred microliters of each treated aliquot was diluted into 1 ml of XB, recentrifuged (4°C), and resuspended in XB for MCI assay or in 0.5% Triton X-100 and assayed for inhibitory kinase activity (above).

RESULTS AND DISCUSSION

Previously, we showed that *Xenopus* interphase extracts that lack endogenous cyclin B contain an inactivator of p34^{cdc2}/cyclin B (56). To determine whether this inhibition was mediated solely by phosphorylation of p34^{cdc2} on the inhibitory sites, we tested whether an active p34^{cdc2}/cyclin B complex containing a mutant form of p34^{cdc2}, that cannot be inhibited by phosphorylation (thr14ala, tyr15phe), was still susceptible to inactivation. Active wild-type or mutant p34^{cdc2}/cyclin B was incubated in interphase extracts, and, at various times, the kinase was reisolated and assayed for histone H1 kinase activity. As demonstrated previously (56), the interphase extract rapidly inactivates wild-type p34^{cdc2}/cyclin B (Fig. 1*A*), with $t_{1/2} < 30$ sec. Significantly, the mutant complex is also susceptible to inactivation (Fig. 1*A*), although with slower kinetics ($t_{1/2} \approx 2$–5), indicating that the inhibitory phosphorylations cannot be the sole means of inactivation.

Aside from the inhibitory phosphorylations at thr14, tyr15, the only other known modes of inhibiting cyclin-dependent kinases are by regulating the activating (Thr-161) phosphorylation site (22–26) or by the binding of stoichiometric inhibitors (reviewed in refs. 60–62). We have shown that the phosphate group on Thr-161 does not turn over in interphase extracts (56); therefore it seemed unlikely that loss of Thr-161 phosphorylation would account for the inactivation of mutant p34^{cdc2}/cyclin B. To confirm this, we compared the state of Thr-161 phosphorylation in the active complex with that of the inactivated complex. This can be seen in Fig. 1*B*. Lane 2 shows

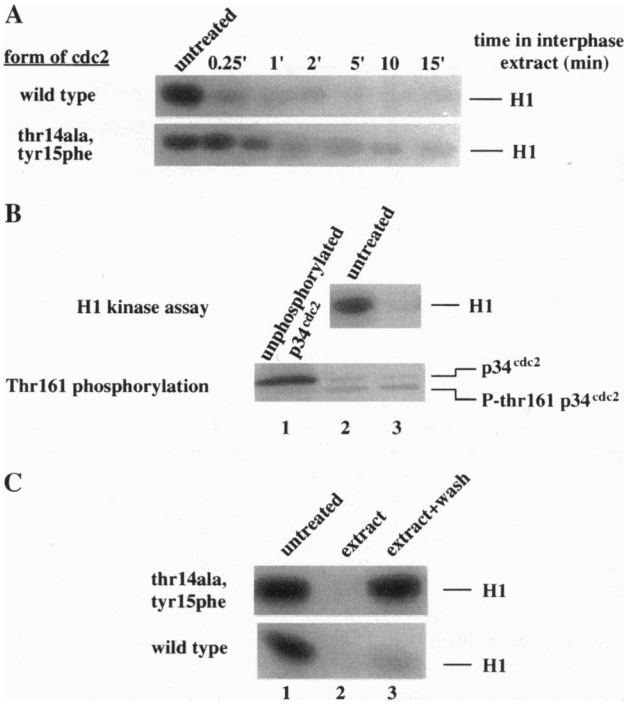

FIG. 1. (*A*) Inactivation of wild-type and mutant (thr14ala, tyr15phe) p34^{cdc2}/cyclin B in interphase extracts. Ten microliters of beads containing either wild-type or mutant p34^{cdc2}/cyclin B was incubated with 10 μl of interphase extract (diluted 1:1 with XB). At the indicated times, the beads were washed with 400 μl of XB and assayed for histone H1 kinase activity. (*B*) Thr-161 dephosphorylation does not account for the inactivation of mutant p34^{cdc2}/cyclin B. Lane 2, starting H1 kinase activity of the mutant complex as well as the gel mobility (56) of the p34^{cdc2}. Unphosphorylated p34^{cdc2} is in lane 1. Lane 3, H1 kinase activity as well as the p34^{cdc2} gel mobility of mutant p34^{cdc2}/cyclin B after incubation in an interphase extract for 15 min. (*C*) The H1 kinase activity of the mutant but not wild-type p34^{cdc2}/cyclin B is restored by washing stringently. Inactivated wild-type or mutant p34^{cdc2}/cyclin B (see *A*) was washed either with XB (lane 2) or with XB/0.5 M NaCl/0.2% NP-40 (lane 3) and assayed for H1 kinase activity.

the H1 kinase activity of the activated p34^{cdc2}/cyclin B as well as the increased mobility of the p34^{cdc2} subunit that is indicative of Thr-161 phosphorylation (56, 63). Incubation of this Thr-161 phosphorylated complex with interphase extracts resulted in the complete loss of H1 kinase activity, but the level of Thr-161 remained undiminished (Fig. 1*B*, lane 3). Therefore, dephosphorylation of Thr-161 does not account for inactivation of the mutant complex.

The recent discovery of a number of stoichiometric inhibitors of cyclin-dependent kinases (reviewed in refs. 60–62) led us to test whether mutant p34^{cdc2}/cyclin B is inactivated by the binding of an inhibitor protein. If the inactivation occurred via association with an inhibitor, then the activity might be restored by washing stringently (64). A 0.5 M NaCl/0.2% NP-40 wash completely restored H1 kinase activity of the mutant complex (Fig. 1*C*). This result suggests that interphase extracts contain a binding inhibitor of p34^{cdc2}/cyclin B. In contrast, the inactivation of the wild-type complex was not reversed by washing (Fig. 1*C*). This was expected because tyrosine phosphorylation on p34^{cdc2} coincides with its inactivation (56); reactivation of wild-type p34^{cdc2}/cyclin B would additionally require tyrosine dephosphorylation.

Any direct inhibitor of p34^{cdc2}/cyclin B must be overcome prior to entry into mitosis. To determine whether the phosphorylation-independent mechanism of p34^{cdc2} inhibition is similarly regulated, we compared the rate of inactivation of mutant p34^{cdc2} in interphase versus mitotic extracts. Mitotic

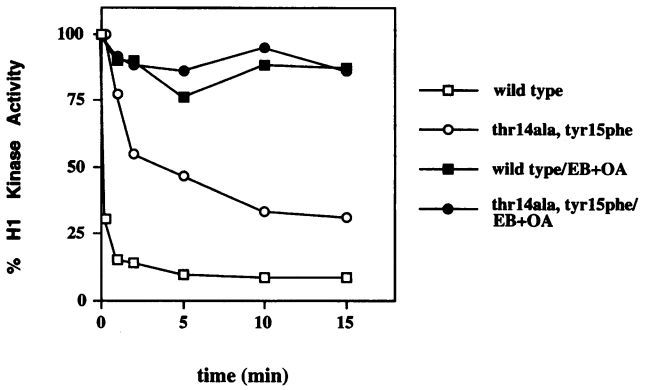

FIG. 2. Neither wild-type nor mutant (thr14ala, tyr15phe) $p34^{cdc2}$/cyclin B is inactivated in surrogate mitotic extracts. Ten microliters of beads containing wild-type (squares) or mutant (circles) $p34^{cdc2}$/cyclin B was incubated with 10 μl of an interphase extract diluted 1:1 with XB (open symbols) or an interphase extract diluted 1:1 with EB buffer plus 2 μM okadaic acid (OA) (closed symbols). At the indicated times, the beads were washed with 400 μl of XB and assayed for H1 kinase activity.

extracts exhibit high levels of endogenous H1 kinase activity, due to mitotically activated $p34^{cdc2}$/cyclin B. Because this background would obscure the loss of activity associated with exogenously added $p34^{cdc2}$/cyclin B, we generated a surrogate state of mitosis. The components of EB buffer (65), plus okadaic acid (66), can induce at least a subset of events that characterize the mitotic state (67), including the mitotic phosphorylation of Cdc25, chromosome condensation, and nuclear envelope dissolution, in the apparent absence of cyclin B and <5% of normal mitotic cdc2 kinase activity (67). Fig. 2 shows that the inactivation of wild-type and mutant $p34^{cdc2}$/cyclin B is blocked by EB plus 2 μM okadaic acid (EB buffer alone had a relatively minor effect on the kinetics of inactivation, suggesting that the effect is not simply due to the ionic strength of EB). Thus, the binding inhibitor appears to be inactivated during an M phase-like state.

To characterize the inhibitor, we separated the interphase extract into cytosolic and particulate fractions by high-speed centrifugation (140,000 × g, 1 hr). Fig. 3*A* shows that the majority of the inhibitor was recovered in the pellet. To determine whether the activity was membrane-associated, we resuspended the pellet (from Fig. 3*A*) in 50% sucrose and separated the membrane-associated material from other insoluble material by flotation through a sucrose step gradient. The inhibitory activity was recovered at the 10%/40% sucrose interface, indicating that the inhibitor is membrane-associated (data not shown). Next, we explored the nature of the membrane association, by extracting isolated membranes with agents that remove peripheral membrane proteins. As shown in Fig. 3*B*, the inhibitory activity was only released from the pellet under conditions that solubilized the membranes (4% OG). After detergent solubilization, ≈35% of the starting activity could be recovered in the supernatant (Fig. 3*B*). Taken together, these data suggest that the binding inhibitor is an integral membrane protein that remains partially active after detergent solubilization.

To estimate the complexity of the solubilized inhibitory activity, we determined its behavior after sucrose gradient sedimentation and gel filtration chromatography (Fig. 3*C*). The inhibitory activity migrates as a discrete entity with an approximate sedimentation value of 15S and fractionates as a discrete peak at a position corresponding to approximately 420 kDa (Fig. 3*D*). Heat sensitivity studies indicated that the activity is stable to a 15-min incubation at 37°C and 42°C, but increasingly labile at higher temperatures (Fig. 3*E*). Taken

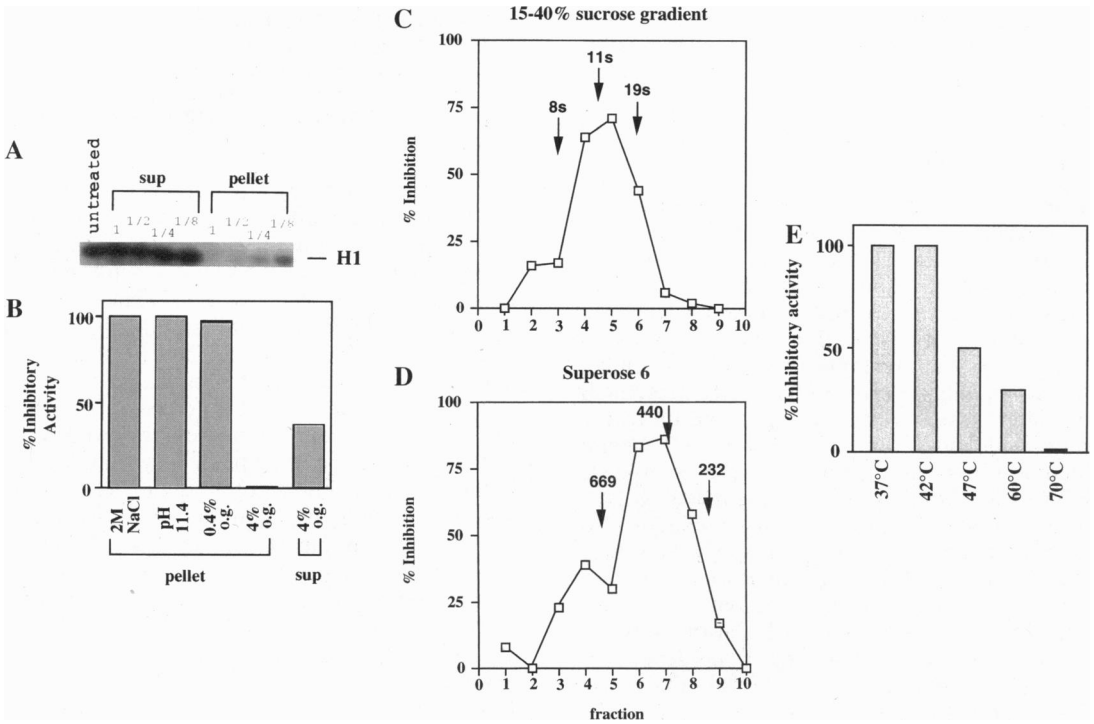

FIG. 3. Characterization of the binding inhibitor. (*A*) Separation, by high-speed centrifugation (140,000 × g, 1 hr), of an interphase extract into soluble and insoluble fractions. Cytosolic and membrane fractions were restored to one-half of the original (concentrated extract) volume. Serial dilutions of each were assayed for inhibitory activity toward the mutant (thr14ala, tyr15phe) $p34^{cdc2}$/cyclin B complex in the absence of ATP. (*B*) The membrane pellet was washed with 2 M NaCl/100 mM Na_2CO_3, pH 11.4, or 0.4% OG, to remove peripherally bound proteins. The washed membranes were restored to the original volume and assayed for the ability to inhibit mutant $p34^{cdc2}$/cyclin B. The 4% OG-solubilized material was assayed as described in the text. (*C* and *D*) Fifteen to 40% sucrose density gradient centrifugation (*C*) and Superose 6 gel filtration fractionation (*D*) of detergent-solubilized membrane protein. (*E*) Heat sensitivity of the membrane-associated inhibitor.

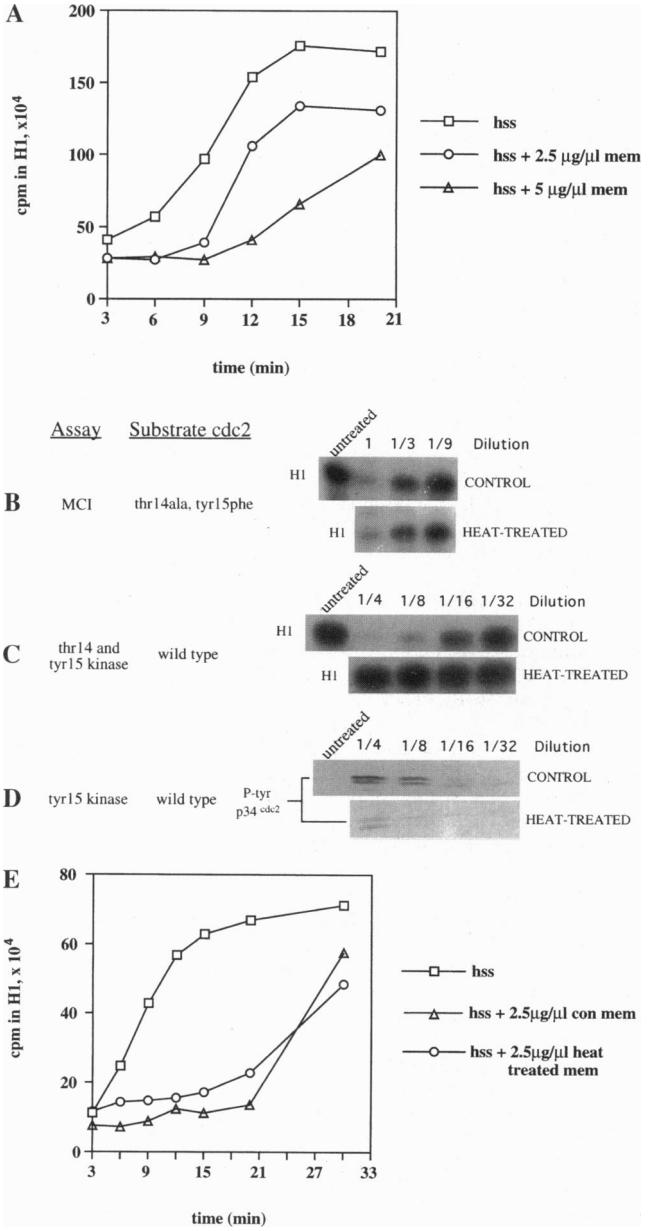

FIG. 4. An inhibitor of cdc2 activation resides in membranes and correlates with MCI activity. (*A*) An inhibitor of cdc2 kinase activation is removed from extracts by high-speed centrifugation. The membranes were depleted from an undiluted interphase extract (200,000 × *g*, 30 min). GST-cyclin B (20 nM final) was added to the high-speed supernatant (hss) diluted 1:1 either with XB (squares) or with two different concentrations of the membrane fraction to give 2.5 μg of membrane proteins per μl (circles) and 5 μg of membrane proteins per μl (triangles). At the indicated times, 0.5 μl of each reaction mixture was diluted into 12 μl of EB and assayed for H1 kinase activity. (*B–D*) Comparison of the heat sensitivity of MCI with the heat sensitivity of the membrane-associated Thr-14 and Tyr-15 inhibitory kinase(s). Control and heat-treated membranes (37°C for 15 min; see text) were restored to the original volume, diluted, and then assayed for MCI activity (*B*) or assayed for inhibitory kinase activity (*C* and *D*). Inhibitory kinase activity in *C* is measured by the inhibition of the H1 kinase activity of a wild-type substrate. Inhibitory kinase activity in *D* is measured by a direct assay for Tyr-15 kinase activity by probing the resulting complex (same as in *C*) with a phosphotyrosine antibody. (*E*) Control and heat-treated membranes are equally able to delay cdc2 activation in membrane-depleted extracts. A membrane-depleted interphase extract (hss) was diluted 1:1 with XB (squares), control membranes (triangles), or heat-treated membranes (circles) in the presence of 20 nM (final) GST-cyclin B. At the indicated times, 0.5 μl of each reaction mixture was diluted into 12 μl of EB and assayed for H1 kinase activity.

together, the biochemical characterization suggests that the mitotic cdc2 inhibitor, which we call MCI, is a large protein complex (≈400–500 kDa) that is partially heat stable and tightly associated with cell membranes.

The localization of MCI to the membrane fraction prompted us to assign a physiological role for MCI. As previously demonstrated (41), membranes contribute a significant inhibition of $p34^{cdc2}$ activation (also shown in Fig. 4*A*). Because these experiments utilize wild-type $p34^{cdc2}$, this inhibition may be mediated by previously characterized Thr-14, Tyr-15 inhibitory kinases that are membrane-associated (40–42).

To distinguish MCI from these enzymatic membrane-associated inhibitors (42), we studied the heat sensitivity of MCI and the membrane-associated Thr-14, Tyr-15 kinase(s), using specific substrates or conditions that would uniquely reveal each activity. To summarize the results, a standard incubation of the membrane fraction at 37°C for 15 min caused an ≈80% inhibition of the total Thr-14 and Tyr-15 kinase activity in the membranes but had no effect on MCI activity. This is shown in Fig. 4 *B–D*. In Fig. 4*B*, we assayed for MCI activity using mutant $p34^{cdc2}$/cyclin B that cannot be inhibited by phosphorylation. Gentle washing conditions were used to preserve the interaction between MCI and $p34^{cdc2}$/cyclin B. In Fig. 4*C*, we assayed for the total membrane-associated inhibitory kinase activity, using the wild-type $p34^{cdc2}$/cyclin B complex as a substrate and washing the treated complexes stringently to eliminate the contribution of MCI. Finally, as indicated in Fig. 4*D*, we assayed directly for inhibitory kinase activity using the same substrate and conditions as for Fig. 4*C*, but probing for the presence of phosphotyrosine in the substrate by immunoblot. In summary, the results indicate not only that MCI activity is biochemically distinguishable from the membrane-associated inhibitory (Thr-14, Tyr-15) kinase activities but furthermore provide a means for preparing a membrane fraction that contains normal levels of MCI activity but is largely inhibited (by ≈80%) in Thr-14 and Tyr-15 kinase activity.

To test which of the two general types of inhibitor in the membrane fraction (phosphorylation-dependent and/or independent) accounted for the ability of membranes to delay $p34^{cdc2}$ activation, we prepared two types of membrane fractions: a heat-treated membrane fraction that contained primarily MCI activity and a control membrane fraction that contained MCI and inhibitory kinase activities (see Fig. 4 *B–D*). Fig. 4*E* shows that the two membrane preparations were equally capable of delaying cdc2 activation. These results argue that the delay in cdc2 activation correlates more strongly with MCI activity than with the membrane-associated Thr-14 and Tyr-15 kinase activities. The most likely explanation is that MCI activity contributes an important function that, in conjunction with Thr-14 and Tyr-15 phosphorylation, is required for the normal sigmoidal-shaped kinetics of $p34^{cdc2}$/cyclin B activation (see also ref. 59).

In summary, we have identified a membrane-associated stoichiometric inhibitor of $p34^{cdc2}$/cyclin B, which may cooperate with the inhibitory kinases (Wee1/Mik1 and Myt1) to ensure the correct timing of $p34^{cdc2}$/cyclin B activation. It is therefore likely that the purification of MCI and the description of its interplay with Wee1/Mik1 and Myt1 will be necessary for formulating a realistic mechanism for MPF activation.

We thank Xiao-Feng Zheng and Joan Ruderman for sharing their unpublished method of extracting the inhibitory kinase activity from membranes. A. Linstedt, J. Kuang, and members of the Kirschner lab made helpful comments on the manuscript. This work was supported by the National Institute of General Medical Sciences (GM 39023-05) and the Program of Excellence in Molecular Biology of the National Heart, Lung and Blood Institute.

1. Gerhart, J., Wu, M. & Kirschner, M. W. (1984) *J. Cell Biol.* **98,** 1247–1255.
2. Masui, Y. & Markert, C. (1971) *J. Exp. Zool.* **177,** 129–146.
3. Miake-Lye, R., Newport, J. & Kirschner, M. W. (1983) *J. Cell Biol.* **97,** 81–91.
4. Smith, L. D. & Ecker, R. E. (1971) *Dev. Biol.* **25,** 232–247.
5. Arion, D., Meijer, L., Brizuela, L. & Beach, D. (1988) *Cell* **55,** 371–378.
6. Booher, R. N., Alfa, C. E., Hyams, J. S. & Beach, D. H. (1989) *Cell* **58,** 485–497.
7. Draetta, G. & Beach, D. (1988) *Cell* **54,** 17–26.
8. Draetta, G., Luca, F., Westendorf, J., Brizuela, L., Ruderman, J. & Beach, D. (1989) *Cell* **56,** 829–838.
9. Dunphy, W. G., Brizuela, L., Beach, D. & Newport, J. (1988) *Cell* **54,** 423–431.
10. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. & Hunt, T. (1983) *Cell* **33,** 389–396.
11. Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T. & Maller, J. L. (1990) *Cell* **60,** 487–494.
12. Gautier, J., Norbury, C., Lohka, M., Nurse, P. & Maller, J. (1988) *Cell* **54,** 433–439.
13. Labbe, J. C., Capony, J. P., Caput, D., Cavadore, J. C., Derancourt, J., Kaghad, M., Lelias, J. M., Picard, A. & Doree, M. (1989) *EMBO J.* **8,** 3053–3058.
14. Labbe, J. C., Lee, M. G., Nurse, P., Picard, A. & Doree, M. (1988) *Nature (London)* **355,** 251–254.
15. Lohka, M. J., Hayes, M. K. & Maller, J. L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3009–3013.
16. Fleig, U. N. & Gould, K. L. (1991) *Semin. Cell Biol.* **2,** 195–204.
17. Draetta, G. (1993) *Trends Cell Biol.* **3,** 287–289.
18. Dunphy, W. G. (1994) *Trends Cell Biol.* **4,** 202–207.
19. Nurse, P. (1990) *Nature (London)* **344,** 503–508.
20. Solomon, M. J., Glotzer, M., Lee, T., Philippe, M. & Kirschner, M. W. (1990) *Cell* **63,** 1013–1024.
21. Solomon, M. J. (1993) *Curr. Opin. Cell Biol.* **5,** 180–186.
22. Booher, R. & Beach, D. (1986) *Mol. Cell. Biol.* **6,** 3523–3530.
23. Ducommon, B., Brambilla, P., Felix, M. A., Franza, B. R. J., Karsenti, E. & Draetta, G. (1991) *EMBO J.* **10,** 3311–3319.
24. Gould, K. I., Moreno, S., Owen, D. J., Sazer, S. & Nurse, P. (1991) *EMBO J.* **10,** 3297–3309.
25. Krek, W. & Nigg, E. A. (1991) *EMBO J.* **10,** 305–316.
26. Solomon, M. J., Lee, T. & Kirschner, M. (1992) *Mol. Biol. Cell* **3,** 13–27.
27. Meijer, L., Azzi, L. & Wang, J. Y. (1991) *EMBO J.* **10,** 1545–1554.
28. Parker, L. L., Atherton-Fessler, S., Lee, M. S., Ogg, S., Falk, J. L., Swenson, K. J. & Piwnica-Worms, H. (1991) *EMBO J.* **10,** 1255–1263.
29. Dunphy, W. G. & Newport, J. W. (1989) *Cell* **58,** 181–191.
30. Gautier, J., Matsukawa, T., Nurse, P. & Maller, J. (1989) *Nature (London)* **339,** 626–629.
31. Gould, K. L. & Nurse, P. (1989) *Nature (London)* **342,** 39–45.
32. Labbe, J. C., Picard, A., Peaucellier, G., Cavadore, J. C., Nurse, P. & Doree, M. (1989) *Cell* **57,** 253–263.
33. Morla, A. O., Draetta, G., Beach, D. & Wang, J. Y. (1989) *Cell* **58,** 193–203.
34. Booher, R. N., Deshaies, R. J. & Kirschner, M. W. (1993) *EMBO J.* **12,** 3417–3420.
35. Featherstone, C. & Russell, P. (1991) *Nature (London)* **349,** 808–811.
36. Lundgren, K., Walworth, N., Booher, R., Kirschner, M. W. & Beach, D. (1991) *Cell* **64,** 1111–1122.
37. McGowan, C. H. & Russell, P. (1993) *EMBO J.* **12,** 75–85.
38. Parker, L. L. & Piwnica-Worms, H. (1992) *Science* **257,** 1955–1957.
39. Russell, P. & Nurse, P. (1987) *Cell* **49,** 559–567.
40. Atherton-Fessler, S., Liu, F., Gabrielli, B., Lee, M. S., Peng, C. Y. & Piwnica-Worms, H. (1994) *Mol. Biol. Cell* **5,** 989–1001.
41. Kornbluth, S., Sebastian, B., Hunter, T. & Newport, J. (1994) *Mol. Biol. Cell* **5,** 273–282.
42. Mueller, P. R., Coleman, T. R., Kumagai, A. & Dunphy, W. G. (1995) *Science* **270,** 86–89.
43. Millar, J. B. A. & Russell, P. (1992) *Cell* **68,** 407–410.
44. Russell, P. & Nurse, P. (1986) *Cell* **45,** 145–153.
45. Dunphy, W. G. & Kumagai, A. (1991) *Cell* **67,** 189–196.
46. Gautier, J., Solomon, M. J., Booher, R. N., Bazan, J. F. & Kirschner, M. W. (1991) *Cell* **67,** 197–211.
47. Strausfield, U., Labbe, J. C., Fesquet, D., Cavadore, J. C., Picard, A., Sadhu, K., Russell, P. & Doree, M. (1991) *Nature (London)* **351,** 242–248.
48. Izumi, T., Walker, D. H. & Maller, J. L. (1992) *Mol. Biol. Cell* **3,** 927–939.
49. Kumagai, A. & Dunphy, W. G. (1992) *Cell* **70,** 139–151.
50. Smythe, C. & Newport, J. W. (1992) *Cell* **68,** 787–797.
51. Coleman, T. R., Tang, Z. & Dunphy, W. G. (1993) *Cell* **72,** 919–929.
52. Mueller, P. R., Coleman, T. R. & Dunphy, W. G. (1995) *Mol. Biol. Cell* **6,** 119–134.
53. Parker, L. L., Walter, S. A. & Piwnica-Worms, H. (1993) *Nature (London)* **363,** 736–738.
54. Tang, Z., Coleman, T. R. & Dunphy, W. G. (1993) *EMBO J.* **12,** 3427–3436.
55. Wu, L. & Russell, P. (1993) *Nature (London)* **363,** 738–741.
56. Lee, T. H., Turck, C. & Kirschner, M. W. (1994) *Mol. Biol. Cell* **5,** 323–338.
57. Amon, A., Surana, U., Muroff, I. & Nasmyth, K. (1992) *Nature (London)* **355,** 368–371.
58. Sorger, P. & Murray, A. W. (1992) *Nature (London)* **355,** 365–368.
59. Kumagai, A. & Dunphy, W. G. (1995) *Mol. Biol. Cell* **6,** 199–213.
60. Nasmyth, K. & Hunt, T. (1993) *Nature (London)* **366,** 634–635.
61. Peter, M. & Herskowitz, I. (1994) *Cell* **79,** 181–184.
62. Pines, J. (1994) *Trends Biochem. Sci.* **19,** 143–145.
63. Lorca, T., Labbe, J. C., Devault, A., Fesquet, D., Capony, J. P., Cavadore, J. C., Le Bouffant, F. & Doree, M. (1992) *EMBO J.* **11,** 2381–2390.
64. Peter, M. & Herskowitz, I. (1994) *Science* **265,** 1228–1231.
65. Wu, M. & Gerhart, J. C. (1980) *Dev. Biol.* **79,** 465–477.
66. Bialojan, C. & Takai, A. (1988) *Biochem. J.* **256,** 283–290.
67. Izumi, T. & Maller, J. L. (1995) *Mol. Biol. Cell* **6,** 215–226.