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cdc2 kinase-induced destabilization of MAP2-coated microtubules in *Xenopus* egg extracts

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Summary

During the interphase to metaphase transition, microtubules are destabilized by a cdc2 kinase-dependant phosphorylation event. This destabilization is due to a dramatic increase in the rate at which each growing microtubule starts to shrink (catastrophe rate). In principle, this could be brought about by lowering the affinity of stabilizing MAPs for the microtubule wall, by activating a factor that would actively increase the catastrophe rate or by an alteration of both parameters. Here we examine the stabilizing effect of bovine brain MAP2 on microtubules assembled in interphase *Xenopus* egg extracts. We show that this MAP strongly stabilizes microtubules assembled in the extracts against nocodazole-induced depolymerization. However, it does not protect them from the cdc2 kinase-induced shortening and destabilization. Moreover, the steady-state length of centrosome-nucleated microtubules in cdc2-treated extracts containing MAP2 is similar to that found in extracts lacking exogenous MAP2. We also show that although exogenous MAP2 is phosphorylated by cdc2 kinase in the extract, this is not the cause of microtubule destabilization. These results indicate that increased microtubule dynamics during mitosis is due to the activation of a factor that can function independently of the presence of active, stabilizing factors.

Key words: MAP2, cdc2, microtubule dynamics.

Introduction

At the onset of metaphase, assembly of the mitotic spindle occurs after dissolution of the interphase network of microtubules and requires highly dynamic microtubules (Karsenti, 1991; Mitchison, 1989). In *Xenopus* egg extracts that closely mimic *in vivo* conditions of the cytoplasm, it has been shown that the mitotic regime of microtubule dynamics can be activated by addition of purified mitotic cdc2 kinase (Verde et al. 1990). Addition to the extracts of recombinant sea urchin Δ90 cyclin B produced in bacteria activates the endogenous cdc2 kinase (Solomon et al. 1990). This also results in microtubule destabilization in the extract by increasing the transition rates between the growing and shrinking phases (Belmont et al. 1990). Recently, a microtubule-severing factor specifically activated in mitotic extracts has been described (Vale, 1991). Although this factor could be responsible for the increased microtubule instability observed in mitotic extracts, this is unlikely, since centrosome-nucleated mitotic microtubules do not behave as if they were severed, but rather alternate abruptly between growing and shrinking, showing clear elongation and depolymerization phases (Belmont et al. 1990; Verde, unpublished). It is commonly thought that microtubules are destabilized during mitosis because the MAPs (microtubule-associated proteins) that stabilize them in interphase are phosphorylated and their affinity for microtubules is reduced. In *Xenopus* eggs, a MAP (X-MAP) that strongly promotes microtubule elongation specifically at the plus end, has been identified and purified (Gard and Kirschner, 1987). Since this MAP is more phosphorylated during mitosis, it is possible that this phosphorylation results in microtubule destabilization. This is supported by the finding that the affinity or stabilizing properties of brain MAPs can be decreased by phosphorylation (Murphy and Flavin, 1983; Nishida et al. 1987; Tsuyama et al. 1987; Yamamoto et al. 1985; Yamamoto et al. 1988). Also, a recent report has shown that in *Xenopus* egg extracts, a purified MAP kinase alters microtubule dynamics, suggesting that it could act downstream from cdc2 kinase by phosphorylating a MAP and inhibit its stabilization activity (Gotoh et al. 1991). However, this does not take into account the finding that the observed elongation rate of microtubules is similar in mitosis and interphase (Belmont et al. 1990; Cassimeris et al. 1988; Hayden et al. 1990) and is one order of magnitude higher than the elongation rate observed *in vitro* for pure tubulin at concentrations similar to those observed *in vivo* (Bré and Karsenti, 1990; Walker et al. 1988). This suggests that, during mitosis, molecules such as X-MAP remain active and continue to promote microtubule growth, while other

factors that increase the catastrophe rate are also activated (Karsenti, 1991; Karsenti et al. 1991). If such a factor exists it could be identified and the molecular mechanism of its action on microtubule dynamics studied.

To approach this problem, we have examined whether, in *Xenopus* egg extracts, cdc2 kinase could also destabilize microtubules extensively coated with MAP2 and whether phosphorylation by cdc2 kinase would decrease the stabilizing effect of MAP2 on microtubules. This brain MAP, which is not normally present in eggs, strongly promotes the nucleation of microtubules in pure solution of tubulin, stabilizes microtubules (Murphy and Borisy, 1975; Wiche, 1985; Wiche, 1989) and its hyperphosphorylation (more than 30 moles of phosphate per mole of protein) reduces its capacity to bind to microtubules (Tsuyama et al. 1987).

We find that MAP2 strongly stabilizes microtubules in interphase egg extracts as expected, but that cdc2 kinase can destabilize such MAP2-coated microtubules. We show that although MAP2 is phosphorylated by cdc2 kinase under the same conditions, this is not the cause of microtubule destabilization. This suggests that a mechanism other than phosphorylation of MAPs is involved in the increase in microtubule dynamics observed at the onset of mitosis.

Materials and methods

Preparation of extracts

Xenopus eggs were obtained and prepared as previously described (Félix et al. 1989). Interphasic 100,000 *g* extracts were prepared from eggs activated by an electric shock and incubated with 100 $\mu g\ ml^{-1}$ cycloheximide for 90 min in MMR/4 buffer (25 mM NaCl, 0.4 mM KCl, 0.25 mM $MgSO_4$, 0.5 mM $CaCl_2$, 1.25 mM Hepes, 25 mM EDTA, pH 7.2). The first cytoplasmic supernatant was obtained by crushing packed eggs by centrifugation at 10,000 *g* for 10 min in a minimal volume of acetate buffer (100 mM potassium acetate, 2.5 mM magnesium acetate, 60 mM EGTA, 5 $\mu g\ ml^{-1}$ cytochalasin D, and 1 mM dithiothreitol (DTT), pH 7.2). To the resulting cytoplasmic fraction, an ATP-regenerating system consisting of 1 mM ATP, 10 mM creatine phosphate and 80 $\mu g\ ml^{-1}$ creatine phosphokinase was added before further centrifugation at 100,000 *g* for 60 min. The cytoplasmic fraction was divided into aliquots and stored in liquid nitrogen before use. These extracts contained 40-50 mg ml^{-1} protein and 12-20 μM tubulin.

Centrosome purification

Centrosomes were purified from KE-37 human lymphoid cells as previously described (Bornens et al. 1987).

MAP2 purification

MAP2 was purified from two-cycle beef brain microtubules according to the method of Drubin and Kirschner (1986), using a Sephacryl S-400 (Pharmacia) column for the gel filtration step.

Microtubule polymerization in extracts and immunofluorescence

Microtubule polymerization was studied in 12 μl samples of extract in the presence of centrosomes at room temperature. MAP2 was added at a molar ratio to the endogenous tubulin of approximately 1:10. The molar ratio was determined using M_r values of 110×10^3 for tubulin and 199×10^3 for MAP2 (as determined from the sequence data; Lewis et al. 1988). Extracts were fixed in 1 ml of 0.25% glutaraldehyde in RG1 buffer (80 mM K-Pipes, pH 6.8, 1 mM EGTA, 1 mM $MgCl_2$, and 1 mM GTP) for 3 min at room temperature. The fixed extracts were layered onto a 5 ml cushion of 25% glycerol in RG2 buffer (RG1 minus GTP) in 15 ml Corex tubes containing coverslips placed on adaptors and centrifuged at 25,000 *g* for 10 min. The coverslips were post-fixed in methanol at −20°C for 5 min and quenched with freshly prepared 0.1% sodium borohydride in PBS for 10 min. After washing in PBS+0.1% Triton X-100 the coverslips were incubated with a monoclonal anti-tubulin antibody and with a Texas Red rat anti-mouse antibody for 5 min each. After each antibody incubation the coverslips were washed in PBS+0.1% Triton X-100 and finally mounted in Moewiol. Photographs were taken using a Neofluar 63× lens on a Zeiss Axiophot Photomicroscope on Kodak TMY-400 film.

Microtubule measurements

The microtubules from enlarged negatives were traced interactively on a computer screen and their lengths and length distribution were calculated using a computer program. Between 200 and 250 microtubules were measured for each experimental condition (Verde et al. 1990).

Histone kinase assay

The histone kinase assay was performed as previously described (Félix et al. 1989). Routinely, 15-20 pmol min^{-1} μl^{-1} of the purified cdc2 kinase was used in the following experiments.

Phosphorylation of MAP2

For determination of phosphate incorporation, 15 μl samples containing 4 μg MAP2, 0.1 mM ATP, 20 μCi [γ-^{32}P]ATP and cdc2 (20 pmol min^{-1} μl^{-1}; Labbé et al. 1988, or Labbé et al. 1989) were incubated in acetate buffer (100 mM potassium acetate, 2.5 mM magnesium acetate, 10 mM EGTA) for 2, 5, 15, 30 and 40 min. The reaction was stopped by addition of the reaction mixture to P81 phosphocellulose paper. The paper was washed extensively in 150 mM phosphoric acid and dried in ethanol. Samples were then counted in the tritium channel of a scintillation counter.

SDS-polyacrylamide gel electrophoresis

For PAGE 2 μg samples of MAP2 were incubated in extracts containing 1 mM thio-ATP (sigma) without or with cdc2 (20 pmol min^{-1} μl^{-1}) or without extract in modified acetate buffer (33 mM potassium acetate, 3 mM magnesium sulphate, 0.4 mM thio-ATP or ATP). The samples were incubated for 15 min at room temperature, then diluted with an equal volume of acetate buffer before heating for 5 min at 95°C. The supernatant containing the heat-stable MAP2 was removed after spinning at 10,000 *g* for 5 min at 4°C. The MAP2 samples were added to gel-dissociation buffer and run on 4% polyacrylamide gels containing 2% urea (Bloom et al. 1985). The gels were then silver stained (Heukeshoven and Dernick, 1988).

Phosphatase treament of MAP2

MAP2 was phosphorylated in extracts as above (without thio-ATP) and the MAP2-containing supernatant obtained after heat denaturation of the extract was treated with 1500 i.u. ml^{-1} of alkaline phosphatase in buffer (50 mM Tris-HCl, pH

8.0, 1 mM DTT) for 30 min at room temperature. The sample was then processed as above for PAGE.

Results

MAP2 stabilizes microtubules against nocodazole-induced depolymerization in frog egg extracts

The assembly of microtubules from purified centrosomes in interphase extracts lacking MAP2 is shown in Fig. 1 (A,B,C). Microtubule lengths were very heterogeneous and they had a typical sinuous aspect, probably reflecting their lack of rigidity. The addition of MAP2 to an interphase extract (molar ratio of 1:10 to the endogenous tubulin) in the presence of centrosomes resulted in an apparent increase in microtubule nucleation by the centrosomes (Fig. 1D), but most strikingly the microtubules were much more rigid and

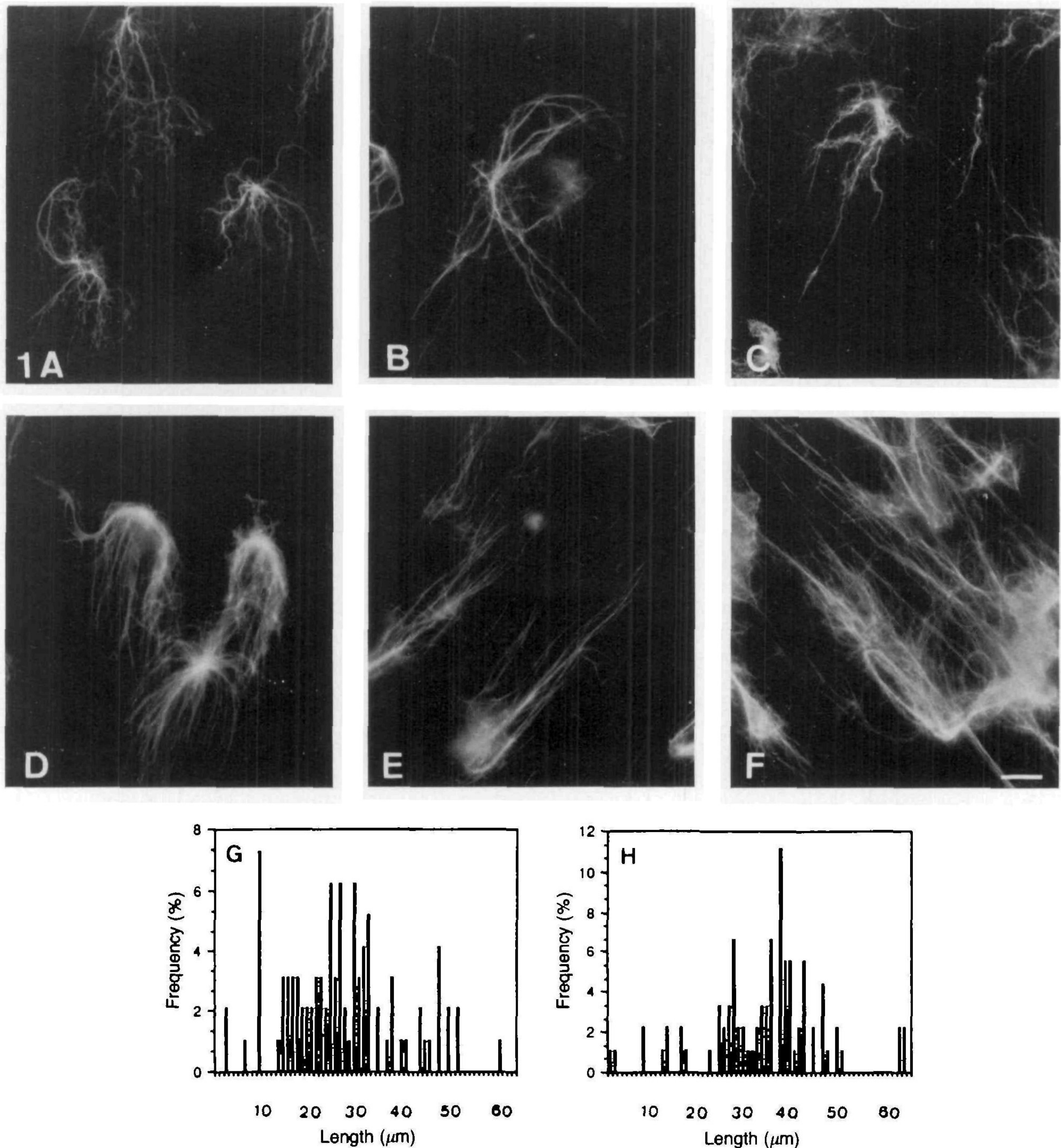

Fig. 1. Effect of MAP2 addition (about one molecule of MAP2 for 10 molecules of tubulin in the extract) on microtubule dynamics in interphase extracts. Centrosomes and an interphase extract were incubated without (A,B,C,G) and with (D,E,F,H) MAP2 and fixed at 1 min (A,D,G,H), 3 min (B,E) and 15 min (C,F). (G and H) show the microtubule length distribution after 1 min of incubation. Bar, 10 μm.

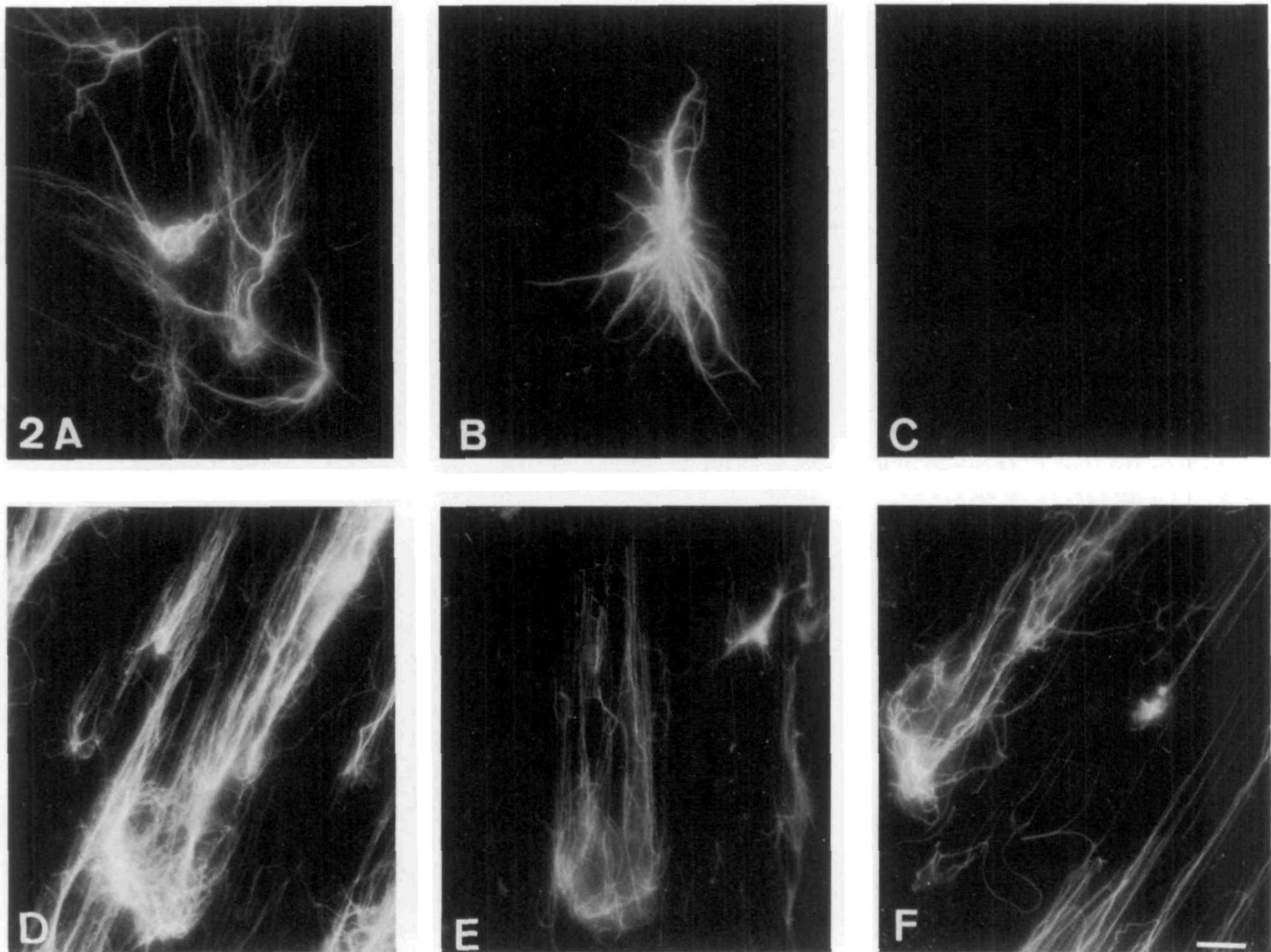

Fig. 2. Kinetics of microtubule depolymerization following addition of nocodazole. Interphase extracts and centrosomes were incubated without (A,B,C) and with (D,E,F) MAP2 for 15 min. After nocodazole (33 μM) addition, samples were fixed at 0 min (A,D), 10 min (B), 20 min (C,E) and 30 min (F). Bar, 10 μm.

their lengths were more homogeneous, as is best seen at 1 min after the beginning of the incubation (Fig. 1D, and compare G and H). After 15 min of regrowth in the presence of MAP2, there was more spontaneous polymerization and the centrosome-nucleated microtubules did not stop growing as they did in control extracts. In fact they reached great lengths, making it difficult to distinguish the asters (Fig. 1F). Since spontaneous polymerization probably decreased the free tubulin concentration in these extracts, this suggests that MAP2 reduced considerably the steady-state tubulin concentration required for microtubule elongation. This was expected from the known effects of MAP2 on pure tubulin.

To test the stability of the microtubules regrown in the presence of MAP2, we examined the rate of their depolymerization after addition of 33 μM nocodazole. After 15 min of growth with or without MAP2 nocodazole was added, and the microtubules were fixed at 0 min (A,D) 10 min (B) 20 min (C,E) and 30 min (F). In the absence of MAP2 most non-centrosomal microtubules disappeared by 10 min after addition of nocodazole, and all centrosomal microtubules had disappeared by 20 min (Fig. 2 A-C). By contrast, in the presence of MAP2, a large number of very long centrosomal and non-centrosomal microtubules were still present 30 min after nocodazole addition (Fig. 2 D-F). Although we did not determine precisely the half-life of MAP2-coated microtubules in the presence of nocodazole, it was at least 30 min, against 10 min in extracts lacking MAP2. Therefore, MAP2 greatly increased microtubule assembly competence in frog egg extracts and protected them from rapid depolymerization when nocodazole was added.

MAP2 does not prevent cdc2 kinase-induced microtubule destabilization in frog egg extracts

The typical cdc2 kinase-induced shrinkage of centrosome nucleated microtubules in interphase egg extracts (Verde et al. 1990) is shown in Fig. 3A-C. Microtubules shrink from an average length of more than 20 μm, to a new steady-state length of 7-12 μm (Fig. 3G and I). In the presence of MAP2 (Fig. 3D-F), centrosome-nucleated microtubules also started to shrink by 2 min (not shown) after addition of cdc2 kinase and spon-

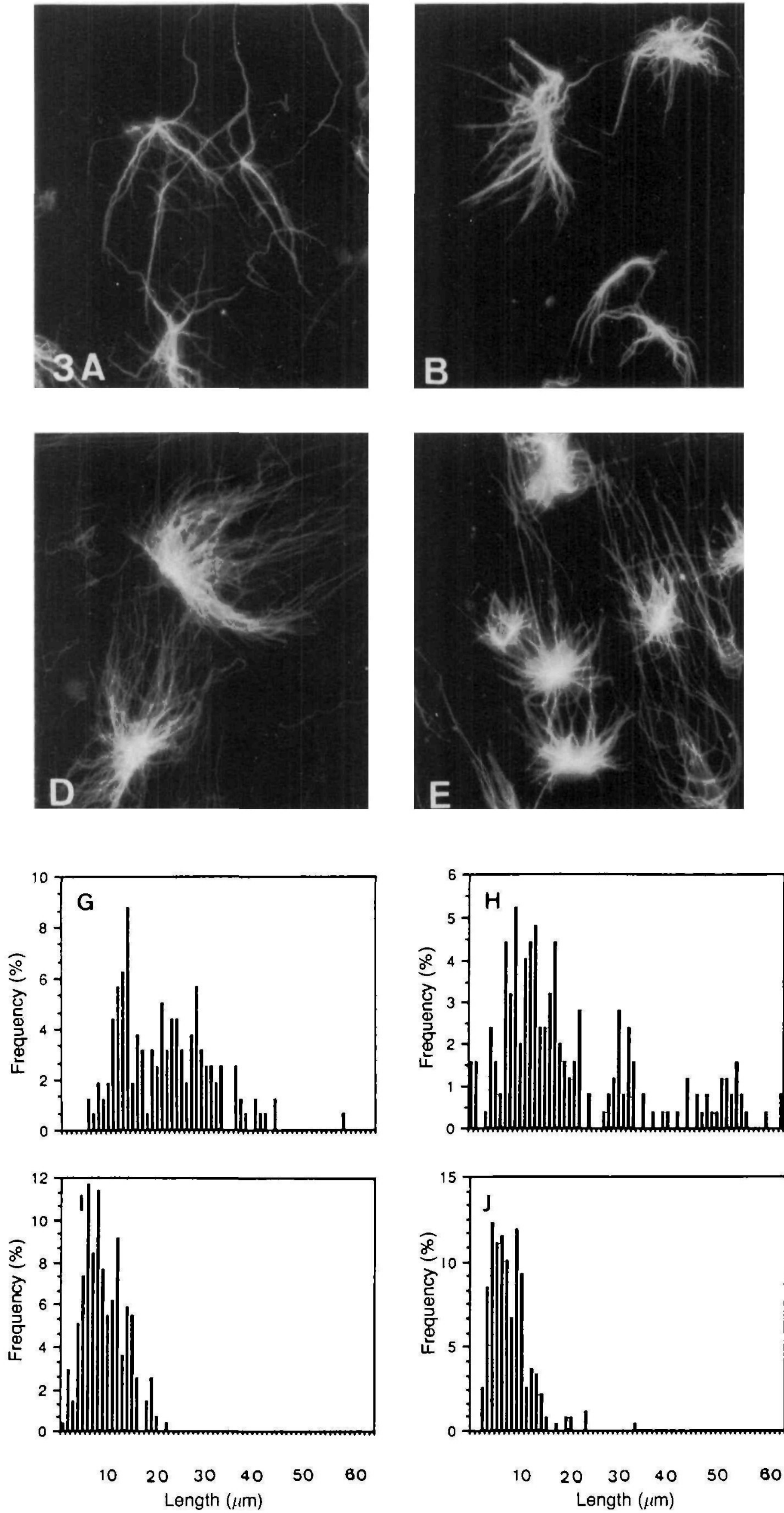

Fig. 3. Effect of cdc2 kinase on microtubule length. Interphase extracts plus centrosomes were incubated without (A,B,C,G,I) and with (D,E,F,H,J) MAP2 for 15 min. After addition of cdc2 (20 pmol $\min^{-1}$ μl^{-1}) samples were fixed at 0 min (A,D), 7 min (B,E,G,H) and 15 min (C,F I,J). G,H,I and J show microtubule length distribution at the indicated times. Bar, 10 μm.

taneously polymerized microtubules disappeared by 5 min. By 7 min, most centrosomal microtubules were short although some long ones persisted, and by 15 min they were all short, having reached a new steady-state length of 9 μm (Fig. 3H, J). At all time points, the MAP2-containing sample had asters with more microtubules than the control. In neither case did cdc2 seem to increase the apparent nucleating activity of the centrosomes. Again, in the presence of MAP2, microtubules looked stiffer than in the control. Therefore, although MAP2 greatly stabilizes microtubules against the depolymerizing effect of nocodazole it is unable to do so against the destabilizing effect of cdc2 kinase.

MAP2-coated microtubules are unstable in egg extracts containing cdc2 kinase activity

The short steady-state length of centrosome-nucleated microtubules observed in cdc2 kinase-treated egg extracts is due to their increased turnover rate as determined by nocodazole sensitivity and real-time video microscopy (Belmont et al. 1990; Verde et al. 1990). We therefore wondered whether the short microtubules present in extracts containing both MAP2 and cdc2 kinase were also very dynamic. To address this question, we compared the rate of disappearance of the asters nucleated in cdc2 kinase-treated extracts in the absence and presence of MAP2 (Fig. 4). After 15 min of growth in extracts containing cdc2 kinase (20 pmol min^{-1} μl^{-1}) in the absence (Fig. 4A-B) and presence (Fig. 4D-F) of MAP2, nocodazole (33 μM) was added and samples fixed immediately or after 1 (A,D), 5 (E) and 10 min (F). In the absence of MAP2 all microtubules disappeared within 1 min of nocodazole addition. In the presence of MAP2 some asters were still present at 5 min, but they were all gone by 10 min. From the curve shown in Fig. 4C, it is apparent that microtubule half-life was about 30 s in the absence and about 2.5 min in the presence of MAP2 after nocodazole addition. Thus, in the presence of MAP2, cdc2 kinase reduces the half-life of microtubules from 30 min in interphase extracts to 2.5 min in the cdc2-treated sample in the presence of nocodazole. This means that MAP2 does not prevent overall microtubule destabilization by cdc2 kinase.

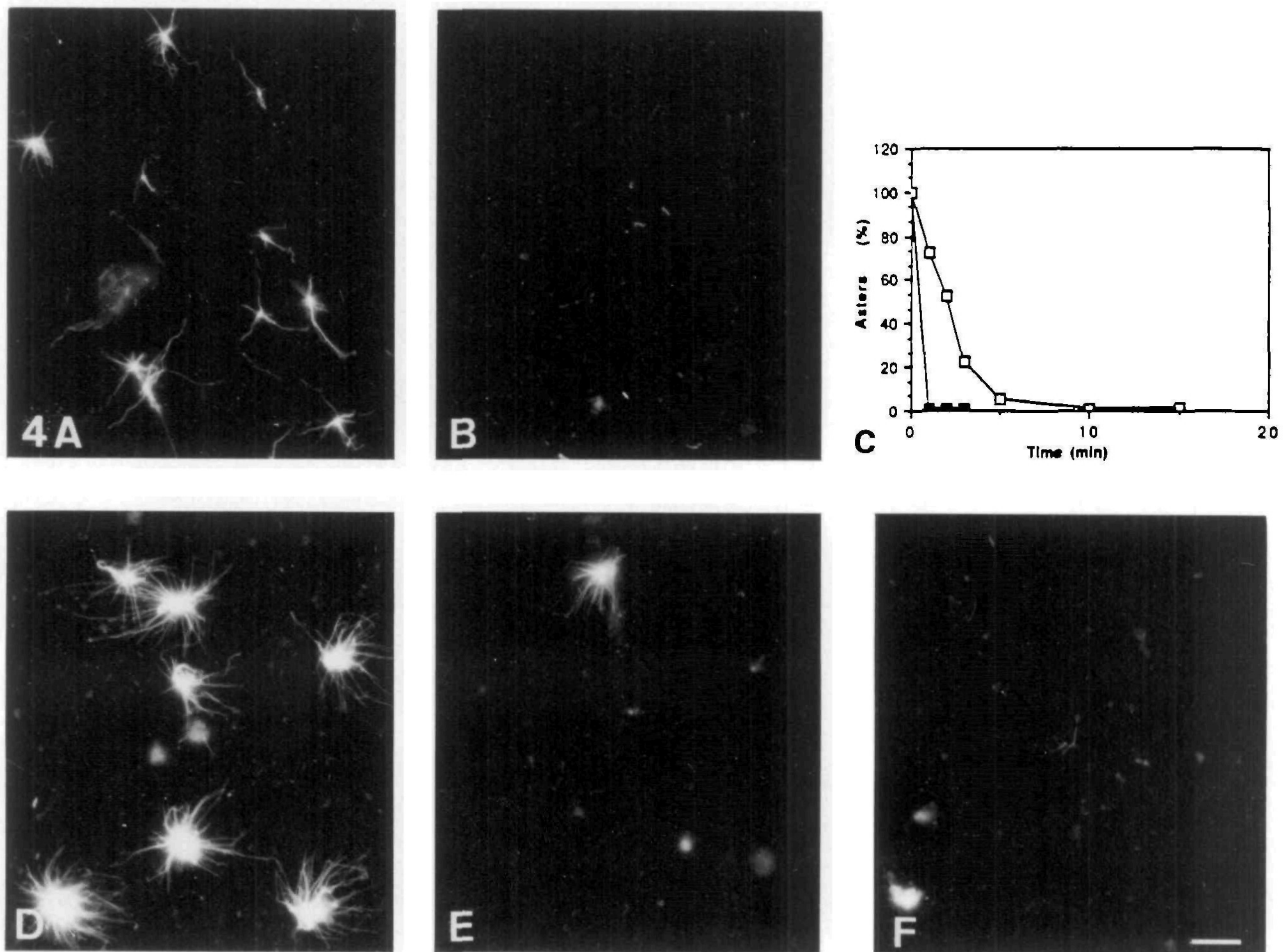

Fig. 4. Effect of cdc2 kinase on kinetics of microtubules depolymerization by nocodazole. Interphasic extracts, centrosomes and cdc2 were incubated without (A,B) and with (D,E,F) MAP2 for 15 min. After addition of nocodazole (33 μM) samples were fixed at 0 min (A,D), 1 min (B), 5 min (E) and 10 min (F). C shows the percentage of asters present as a function of time after nocodazole addition in the absence (■——■) and presence (□——□) of MAP2. Bar, 10 μm.

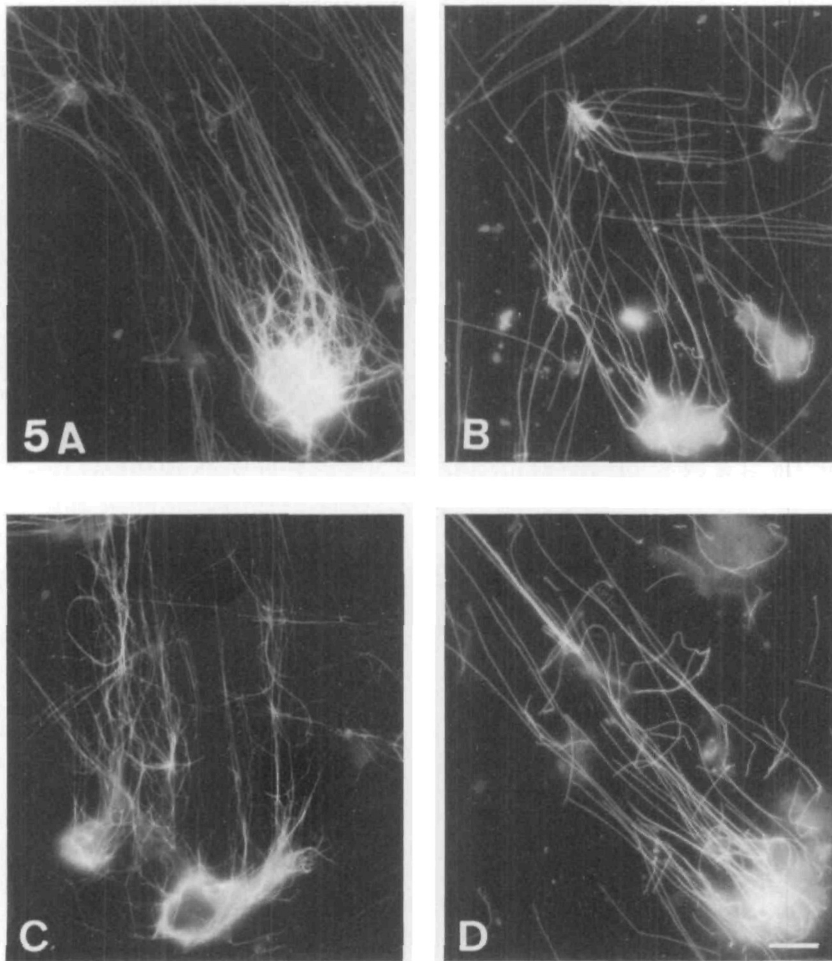

Fig. 5. Microtubule stabilization by phosphorylated MAP2. Interphase extracts containing 1 mM thio-ATP and MAP2 were incubated without and with cdc2 kinase (20 pmol min^{-1} μl^{-1}) for 15 min. The samples were heated at 95°C for 5 min to denature the kinase activity as well as most of the proteins of the extract and then spun at 10,000 ***g*** for 10 min. The heat-stable MAP2 was recovered in the supernatants. The unphosphorylated (A,B) and phosphorylated (C,D) MAP2 was added to another interphase extract with centrosomes and incubated for 15 min. After addition of nocodazole (33 μM) samples were fixed at 0 min (A,C) and 30 min (B,D). Bar, 10 μm.

Phosphorylation of MAP2 by cdc2 kinase in egg extracts does not alter its microtubule-stabilizing properties

Since MAP2 has been reported to be phosphorylated by cdc2 kinase (Erikson and Maller, 1989), it was possible that this phosphorylation was responsible for the large inhibition of the microtubule-stabilizing activity of MAP2 in cdc2 kinase-treated extracts. To test this hypothesis, we have incubated MAP2 in an interphase extract containing 1 mM thio-ATP and MAP2 in the absence and presence of cdc2 kinase (20 pmol min^{-1} μl^{-1}) for 15 min. Since MAP2 is a thermostable protein, the extracts were then heated to 95°C for 5 min, centrifuged and the supernatant containing MAP2 was dialysed for 30 min at 4°C to remove any toxic components generated by heating. The supernatant contained MAP2 and a few other thermostable proteins present in the extract (not shown). The MAP2 concentration was estimated by protein determination (Bradford, 1976) and from the ratio of MAP2 to the other proteins detected on the gel. The protein was then added to another interphase extract at a ratio of about 1:10 relative to the endogenous tubulin together with centrioles. Microtubules were allowed to grow for 15 min at room temperature under these conditions. As shown in Fig. 5A and C, this resulted in extensive microtubule assembly with both unphosphorylated (A,B) and phosphorylated (C,D) MAP2. Then, nocodazole (33 μM) was added and samples fixed immediately or after a further incubation of 30 min at room temperature. Numerous very long microtubules were still present in the extracts 30 min after nocodazole addition whether MAP2 was pre-incubated in an interphase extract (Fig. 5B) or in the same extract treated with cdc2 kinase (Fig. 5D). We have verified that after incubation in the second extract the thiophosphorylated MAP2 was not dephosphorylated, by determining its mobility properties on a 4% gel (data not shown). We show in the next section that phosphorylated MAP2 migrates with a slightly slower mobility than the unphosphorylated form and that this modification is indeed due to phosphorylation. In summary, phosphorylation of MAP2 by cdc2 kinase in egg extracts does not significantly alter its ability to stabilize microtubules.

Phosphorylation of MAP2 by cdc2

It was of interest to determine the level of phosphorylation of MAP2 achieved under the conditions used in this study. Unfortunately, due to the high level of ATP present in the extracts (2.8 mM) and high exchange between ATP and free phosphate, it proved difficult to obtain suffícent labelling of MAP2 with ^{32}P to determine the extent of its phosphorylation under exactly the

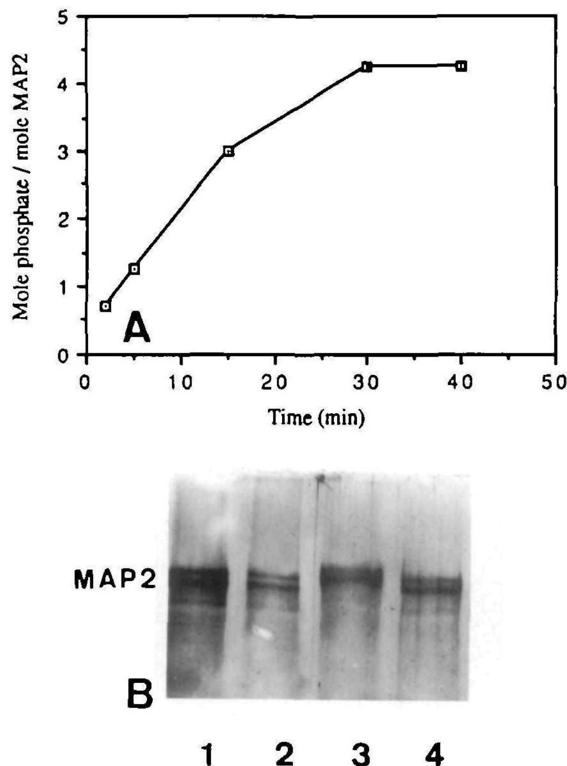

Fig. 6. (A) Phosphorylation of MAP2 by cdc2 kinase. MAP2 was incubated with cdc2 (20 pmol min^{-1} μl^{-1}), 0.1 mM ATP and 20 μCi [γ-^{32}P]ATP in buffer. The abscissa shows the time of incorporation at room temperature, and the ordinate the moles of phosphate incorporated per mole of MAP2 calculated from the specific activity of the ATP used. (B) Phosphorylation of MAP2 by cdc2 in extracts. MAP2 alone (lane 1), MAP2 in extract without cdc2 (lane 2), MAP2 in extract with cdc2 (20 pmol min^{-1} μl^{-1}) (lane 3), and MAP2 phosphorylated previously (as in lane 3) and then subsequently dephosphorylated by alkaline phosphatase (lane 4).

same conditions as used for the functional study. In the absence of extract and the presence of 0.1 mM ATP, approximately 3 moles of phosphate were incorporated per mole of MAP2 after 15 min of incubation at room temperature and 4-5 moles after 30 min in the presence of a cdc2 kinase activity of 20 pmol min^{-1} μl^{-1} (Fig. 6A).

We noticed that the phosphorylation of MAP2 by cdc2 kinase resulted in reduced mobility on 4% polyacrylamide gels. As shown in Fig. 6B, native (lane 1), and the same MAP2 incubated in an interphase extract for 15 min in the absence of cdc2 kinase and then boiled (lane 2), had the same migration properties on a 4% gel. However, when MAP2 was incubated in an extract in the presence of cdc2 kinase, its migration was clearly retarded (Fig. 6 B, lane 3). A similar shift occurred when MAP2 was incubated in the absence of extract in acetate buffer containing cold 2 mM ATP and cdc2 kinase (data not shown). However, the gel shift observed in the presence of 0.1 mM ATP (conditions as in Fig. 6A) was less clear, suggesting that the number of moles of phosphate incorporated in MAP2 calculated from the labelling experiment is an underestimate compared to what happens in the extract or with pure cdc2 at physiological ATP concentrations. To test whether the shift in electrophoretic mobility was really due to phosphorylation, MAP2 phosphorylated in a cdc2-treated extract and extracted by boiling as above, was incubated with alkaline phosphatase for 30 min. On 4% gels, the MAP2 treated in this way showed a similar mobility to that of native MAP2 (Fig. 6B, lane 4). This strongly suggests that the retardation is due to phosphorylation. The kinetics of MAP2 phosphorylation using the gel shift as a test showed that maximum phosphorylation was achieved in 30 min and the kinetics was similar in the absence (plus 2.8 mM ATP) and presence of extract (data not shown).

Discussion

Although cdc2 kinase has been shown to increase microtubule dynamics at the onset of metaphase, it is not clear how this occurs, and the *in vivo* substrates of this kinase that are involved in the regulation of microtubule dynamics have not been identified. One view is that phosphorylation of MAPs alters their ability to stabilize microtubules. In *Xenopus*, the major embryonic MAP, X-MAP, is phosphorylated during metaphase (Gard and Kirschner, 1987) and may play a role in the change in microtubule dynamics observed during mitosis. As previously mentioned, we do not favour this idea because microtubule elongation is not seriously reduced during mitosis. We prefer the possibility that the increased catastrophe rate observed during metaphase is due to the activation of a factor that actively induces this process independently of the presence of active MAPs such as X-MAP or others that maintain a high elongation rate. The results reported in this paper support this last possibility. First, we show that addition of MAP2 to egg extracts at a ratio of 1/10 relative to the endogenous tubulin (close to the *in vivo* ratio in the brain) strongly stabilizes microtubules against the effect of nocodazole. Microtubules in differentiated cells like MDCK are also more stable and resistant to this drug than those in fibroblasts (Bré et al. 1987; Bré et al. 1990; Pepperkok et al. 1990), suggesting that they contain MAP2-like stabilizing factors. However, we show here that nocodazole does not prevent microtubule destabilization by cdc2 kinase. Moreover, we show that in the presence of cdc2, destabilization is not due to MAP2 phosphorylation. This strongly suggests that the activation of other factor(s) by cdc2 is responsible for the increased microtubule dynamics during mitosis and that this factor(s) can overcome the strong stabilizing property of MAP2. Recently, a factor has been described in meiotic and mitotic *Xenopus* egg extracts that severs taxol-stabilized microtubules (Vale, 1991). Such an activity has been proposed to aid in the reorganization of interphasic to mitotic microtubule arrays. It was also reported that coating taxol-stabilized microtubules with saturating amounts of MAP2 could protect them against the severing activity. At first sight,

this seems contradictory to our present results, since we find that cdc2 kinase can destabilize MAP2-coated microtubules.

However, there are two important differences between the two experiments. First, we do not use taxol-stabilized microtubules; and second, we do not use saturating amounts of MAP2. We have noticed, however, that when MAP2 is added at a ratio of 1/2 relative to endogenous tubulin, microtubules start to shrink 15 min after cdc2 kinase addition. Therefore, this delays but does not inhibit cdc2 kinase-induced microtubule shortening. Overall, our results are compatible with the idea that the severing factor may be involved in disassembly of interphasic microtubules but not in regulating the steady-state regime of metaphase microtubule dynamics.

There are many studies showing an alteration of MAP2 function with its degree of phosphorylation (Nishida et al. 1987; Yamamoto et al. 1985; Yamamoto et al. 1988). One study shows the occurrence of two types of MAP2 in living rat brain. One type contains about 46 moles of phosphate/mole of MAP2 and does not bind to microtubules and the other type contains about 10-16 moles and binds to microtubules (Tsuyama et al. 1987). Our results show that phosphorylation of MAP2 by cdc2 kinase does not alter its ability to stabilize microtubules. Moreover, we did these experiments with the pure kinase, in the absence and presence of extracts and find the same result in both cases. In the experiment carried out in egg extracts, MAP2 could be phosphorylated also by another kinase like the MAP kinase, which may be activated by cdc2 (Gotoh et al. 1991). These results strongly suggest that the effect of MAP2 on microtubules is not regulated by cdc2 kinase. This is not an unexpected result, since this MAP is present in brain cells that normally do not divide and do not contain cdc2 (Hayes et al. 1991). We have ruled out the possibility that MAP2 was dephosphorylated during incubation in the interphase extracts in which its effect on microtubule dynamics was assayed by doing the phosphorylation in the first extract in the presence of thio-ATP (Fig. 5A). Also MAP2 phosphorylated in this way could not be easily dephosphorlated by alkaline phosphatase. This confirms that high microtubule turnover in mitosis is due to the activity of a destabilizing factor that is dominant over the stabilizing action of MAP2 (in our experiments). In order to clarify the role of MAP phosphorylation in the reorganization of microtubule arrays during mitosis and in *Xenopus* egg extracts in particular, it is necessary to identify and characterize the endogenous MAPs. One interesting MAP in this respect, is MAP4 (which may be equivalent to X-MAP). Although MAP4 becomes phosphorylated during mitosis, it still interacts with microtubules, since it is present in the mitotic spindle (Vandré et al. 1991). It is possible that the affinity of MAP4 for microtubules is affected in a subtle way by phosphorylation during mitosis. For example, the on-rate of this kind of MAPs on the microtubule wall may not be affected by phosphorylation; however, the off-rate could be increased. This would allow rapid microtubule growth, but still permit the action of factors increasing the catastrophe rate. In fact, the rate of exchange between MAPs and the microtubule wall has been shown to increase during mitosis (Olmsted et al. 1989). Our experiments do not rule out this possibility for MAP2. In fact, it seems that MAP2 does bind to microtubules even in the presence of cdc2, since in extracts containing both cdc2 and MAP2 microtubules are slightly more resistant to nocodazole. The regulation of microtubule dynamics through the phosphorylation of MAPs will certainly prove to be subtle and interesting to study in detail, particularly by video microscopy. Until now this has been studied mainly by following microtubule assembly using turbidimetric methods, which are not informative enough for us to understand precisely how interactions between MAPs and microtubules are regulated.

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