

# The *Xenopus cdc2* Protein Is a Component of MPF, a Cytoplasmic Regulator of Mitosis

William G. Dunphy,\* Leonardo Brizuela,†  
David Beach,† and John Newport\*

\* Department of Biology  
University of California, San Diego  
La Jolla, California 92093

† Cold Spring Harbor Laboratory  
Cold Spring Harbor, New York 11724

## Summary

In *Xenopus*, a cytoplasmic agent known as MPF induces entry into mitosis. In fission yeast, genetic studies have shown that the *cdc2* kinase regulates mitotic initiation. The 13 kd product of the *suc1* gene interacts with the *cdc2* kinase in yeast cells. We show that the yeast *suc1* gene product (p13) is a potent inhibitor of MPF in cell-free extracts from *Xenopus* eggs. p13 appears to exert its antagonistic effect by binding directly to MPF. MPF activity is quantitatively depleted by chromatography on a p13 affinity column. Concomitantly, the *Xenopus* counterpart of the yeast *cdc2* protein is adsorbed to the column. A 42 kd protein also binds specifically to the p13 affinity matrix. These findings suggest that the *Xenopus cdc2* protein and the 42 kd protein are components of MPF.

## Introduction

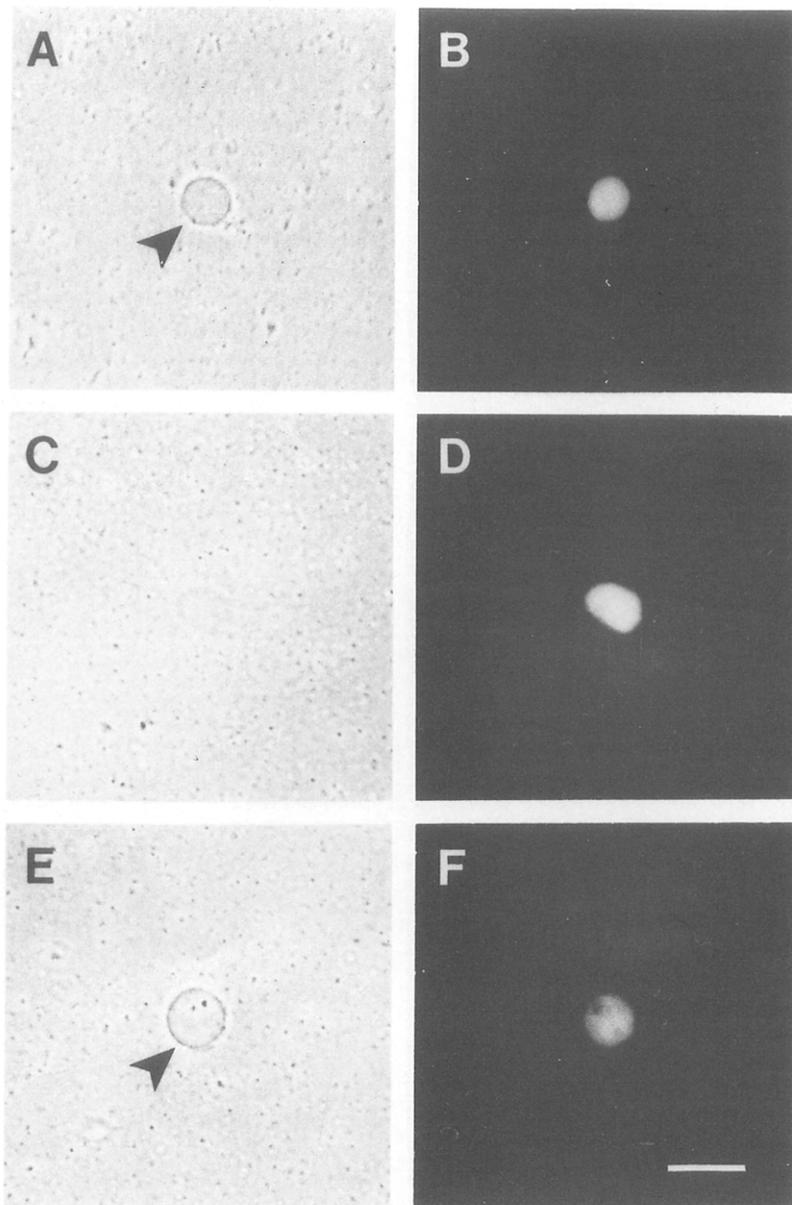
During the cell cycle, most eukaryotic cells double in mass, replicate their DNA, and then distribute identical copies of their genome to progeny cells at mitosis. It is widely assumed that an internal biochemical clock ensures that the diverse events of the cell cycle occur in the proper sequence and at the appropriate time. A critical component of this regulatory clock is maturation, or M phase promoting factor (MPF). As cells enter mitosis, MPF sets in motion the widespread enzymatic and ultrastructural changes that are necessary for cell division. In higher eukaryotes, these mitosis-specific alterations include the disassembly of the nuclear envelope, the packaging of the genome into chromosomes, and the construction of a cytoskeletal spindle that will faithfully distribute the replicated genomes to each daughter cell. MPF was originally described as a cytoplasmic activity from metaphase-arrested frog eggs that would induce oocytes to resume meiosis prematurely, and in the absence of protein synthesis (Masui and Markert, 1971; Smith and Ecker, 1971). Subsequently, it has become clear that MPF plays a general role in mitotic induction in somatic cells ranging from yeast to man (Gerhart et al., 1984; Newport and Kirschner, 1984; Sunkara et al., 1979; Weintraub et al., 1982; Kishimoto et al., 1982; Tachibana et al., 1987).

Over the past several years, it has become possible to reconstitute many of the events of the cell cycle in cell-free extracts, mainly from amphibian eggs. In interphase extracts, nuclear reassembly around chromatin or DNA

(Lohka and Masui, 1983, 1984; Forbes et al., 1983; Burke and Gerace, 1986; Newport, 1987; Wilson and Newport, 1988), DNA replication (Blow and Laskey, 1986; Newport, 1987), and nuclear transport (Newmeyer et al., 1986) proceed efficiently. In mitotic extracts, nuclear envelope disassembly, chromosome condensation, and spindle formation occur faithfully (Lohka and Maller, 1985; Miake-Lye and Kirschner, 1985; Suprynowicz and Gerace, 1986; Newport and Spann, 1987; Dunphy and Newport, 1988). The conversion from interphase to mitosis follows the addition of MPF-containing fractions to interphase extracts (Miake-Lye and Kirschner, 1985; Lohka and Maller, 1985; Lohka et al., 1988; Dunphy and Newport, 1988). These cell-free systems have greatly facilitated the study of the dynamics of MPF and its molecular nature. MPF is very unstable, and its purification has proven difficult (Wu and Gerhart, 1980; Gerhart et al., 1985; Nguyen-Gia et al., 1986; Adlakha et al., 1985). However, Lohka et al. (1988) have recently purified MPF extensively, and found that two polypeptides (32 kd and 45 kd) cofractionate with the activity.

Genetically tractable organisms such as the fungi have provided a different avenue for the study of the mechanisms of cell-cycle progression. In *Saccharomyces cerevisiae* (Hartwell, 1974), *Schizosaccharomyces pombe* (Nurse et al., 1976), and *Aspergillus nidulans* (Morris, 1976), a variety of mutations that perturb the regulation of cell division have been isolated. For mitotic control in the fission yeast *S. pombe*, a network of interacting genes regulates the onset of mitosis. A key component in this network is the *cdc2* protein kinase (Nurse and Bisset, 1981; Beach et al., 1982; Hindley and Phear, 1984; Simanis and Nurse, 1986), which is required for both the G2-M and G1-S transitions of the cell cycle. The homolog of *cdc2* in *S. cerevisiae* (*CDC28*) is likewise a protein kinase (Reed et al., 1985) that is required for "start" (Hartwell, 1974) and possibly mitosis (Piggott et al., 1982). In human cells, the *cdc2* homolog (Draetta et al., 1987; Lee and Nurse, 1987) is present in a complex, and this complex possesses elevated kinase activity during mitosis (Draetta and Beach, 1988).

A number of genes that interact with *cdc2* in the regulation of mitosis have been identified. These include *cdc25* (Russell and Nurse, 1986; Fantes, 1979), *wee1* (Russell and Nurse, 1987), *cdc13* (Booher and Beach, 1987), and *suc1* (Hayles et al., 1986a). Among these, only the interaction between *cdc2* and *suc1* has been elaborated in molecular detail (Brizuela et al., 1987). *suc1* was isolated as a plasmid-borne, allele-specific suppressor of *cdc2* mutants (Hayles et al., 1986a). It has been found that the *suc1* gene encodes a 13 kd protein (p13; Hindley et al., 1987) that exists in a complex with the *cdc2* protein (Brizuela et al., 1987). The precise function of *suc1* has been unknown. Its product is not, for example, a substrate of the *cdc2* kinase (Brizuela et al., 1987), but interaction between *suc1* and *cdc2* is essential for the regulation of mitotic initiation (Hayles et al., 1986b; Hindley et al., 1987).



**Figure 1. Fission Yeast p13 Antagonizes MPF-Induced Nuclear Disassembly in a Cell-Free System**

Reconstituted nuclei were formed by the addition of sperm chromatin to interphase egg extracts (Lohka and Masui, 1983). Interphase extracts containing the *in vitro*-assembled nuclei were incubated in the absence (A, B) or presence (C-F) of 2 U of MPF per assay. In E and F, p13 (50  $\mu$ g/ml) was included. After a 90 min incubation, nuclear disassembly was ascertained by the loss of the nuclear envelope (arrowhead). Phase-contrast microscopy (A, C, E). Hoechst staining of nuclear DNA (B, D, F). Bar = 20  $\mu$ m.

Cell-free assays for mitotic induction have not yet been developed in fission yeast or the other fungal systems. Thus, it has not been possible to approach biochemically the role of the known *cdc* gene products in mitotic regulation. In particular, the relationship of the *cdc* proteins to MPF has been unclear. We show that it is possible to combine the respective technical advantages of *Xenopus* and yeast for analysis of the cell cycle. We demonstrate that a purified fission yeast *cdc* gene product can function *in vitro* to regulate the entry into mitosis in *Xenopus* egg extracts. Specifically, the product of the *suc1* gene (p13) blocks the action of MPF by direct physical association. We have been able to take advantage of this interaction to identify and isolate polypeptide components of MPF. One such component is the frog homolog of *cdc2*.

## Results

### Yeast p13 Blocks Mitotic Conversion in *Xenopus* Egg Extracts

Several groups (Lohka and Maller, 1985; Miake-Lye and Kirschner, 1985; Dunphy and Newport, 1988) have described cell-free systems from *Xenopus* eggs that recreate the MPF-dependent entry into mitosis *in vitro*. In brief, reconstituted nuclei are formed by the addition of sperm chromatin, metaphase chromosomes, or protein-free DNA to interphase cytoplasmic extracts from activated *Xenopus* eggs (Lohka and Masui, 1983; Blow and Laskey, 1986; Newport, 1987). Upon the addition of crude MPF, the interphase cytoplasm is converted to the mitotic state. As a result of this mitotic conversion, the nuclear envelope sur-

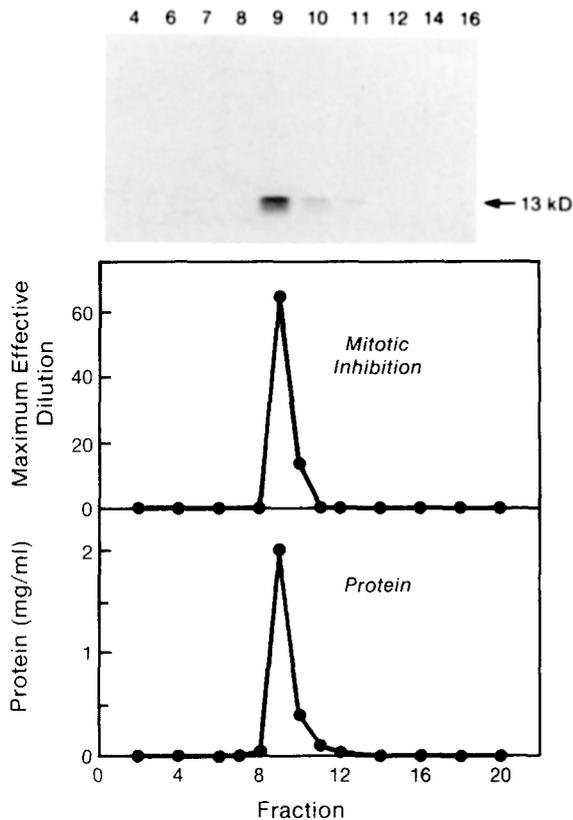


Figure 2. Mitotic Inhibitory Activity Cofractionates with Homogeneous p13

For FPLC chromatography, p13 (4 mg protein) was applied to a Mono Q column (HR5/5; Pharmacia) equilibrated in 50 mM Tris (pH 8.0). The column was eluted with a gradient of 0–400 mM NaCl in the same buffer. Fractions (1 ml) were collected and assayed for protein (bottom panel) and for the maximum dilution at which inhibition of MPF-induced nuclear disassembly occurred (middle panel). p13 eluted at 140 mM NaCl. Each fraction was also electrophoresed in a 12.5% polyacrylamide gel and stained with Coomassie blue (top panel).

rounding the reconstituted nuclei is disassembled. Thus, one can assess conveniently and rapidly the MPF-dependent entry into the mitotic state by observing the dissolution of the nuclear envelope (see Figure 1).

To define the molecular components that are responsible for the MPF-induced nuclear disassembly, we have attempted to develop a heterologous system by including known yeast *cdc* proteins in the *Xenopus* cell-free mitotic conversion assay. We found that one protein—the product of the fission yeast *suc1* gene (p13)—inhibits MPF-induced nuclear disassembly. The p13 used in these experiments was derived from a strain of *Escherichia coli* that expresses the protein at high levels (Brizuela et al., 1987). Since p13 remains soluble in the bacteria, the protein can be readily purified. In the presence of p13, the MPF-induced loss of the nuclear envelope was completely neutralized (Figure 1). Under these MPF assay conditions, chromosome condensation occurs poorly (see Dunphy and Newport, 1988). Under conditions favorable for chromosome formation, p13 also inhibited MPF-induced DNA condensation (not shown).

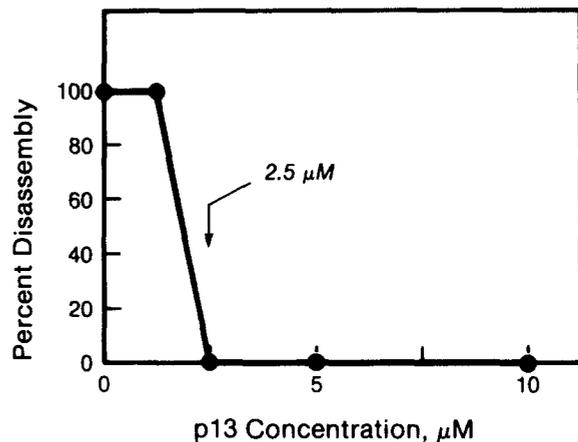


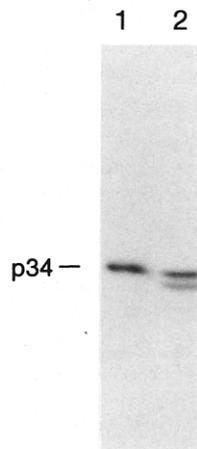
Figure 3. Dose Response of Mitotic Inhibition by p13  
MPF-induced nuclear disassembly was quantitated in the presence of 2 U of MPF per assay and the indicated concentrations of p13. Fifty randomly selected nuclei were examined for each data point.

To confirm that p13 was the active agent responsible for the inhibition, we rechromatographed highly purified p13 on a Mono Q ion-exchange column. The observed inhibitory activity co-chromatographed precisely with the p13 protein (Figure 2). No other detectable polypeptides were present in the inhibitory fractions as determined by SDS gel electrophoresis. We found that the block in nuclear disassembly caused by p13 required an intact polypeptide chain. Digestion of p13 with trypsin (5% by weight of p13) for 15 min at 37°C destroyed the ability of p13 to antagonize mitotic conversion. On the other hand, p13 is a thermostable protein. Treatment at 100°C for 10 min had no effect on the inhibitory activity of p13.

A variety of observations suggest that the inhibitory effect of p13 is physiologically significant. First, p13 acts at low concentrations. In the standard MPF assay, maximal inhibition occurred at a concentration of 2.5 μM or approximately 30 μg/ml (Figure 3). This represents approximately 1 part in 1000 of the total protein in the *in vitro* incubation (~25 mg/ml). We also found that p13 blocked MPF-induced oocyte maturation (Wu and Gerhart, 1980) when injected into oocytes at comparable concentrations (not shown).

Second, p13 is not a general inhibitor of nuclear events in the egg extract. Even at ~30-fold higher concentrations (1 mg/ml; the highest concentration tested), p13 had no effect on either the rate or extent of *in vitro* nuclear assembly around sperm chromatin (not shown). This suggests that p13 does not block MPF-induced mitotic conversion by a nonspecific mechanism such as protein denaturation or ATP depletion, since the very complex events of nuclear assembly proceed normally.

A further demonstration that the effect of p13 is specific is that the inhibition of mitotic conversion is reversible. At an MPF concentration of 2 U per assay, 2.5 μM p13 was sufficient for complete inhibition. However, at 10 U of MPF per assay, 5- to 10-fold higher concentrations of p13 were required for the block in disassembly. This observation enabled us to perform the following experiment. We in-



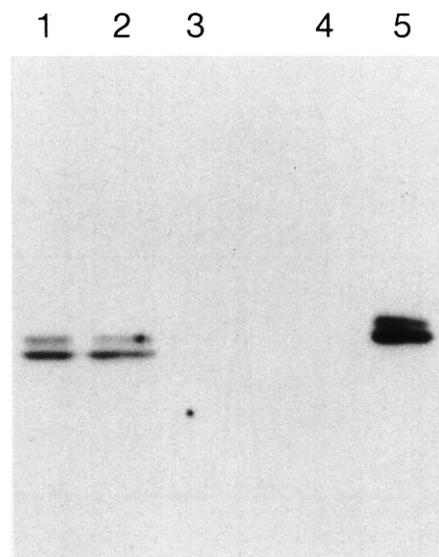
**Figure 4. Xenopus Eggs Contain Yeast *cdc2* Homologs**  
Lane 1, 50 ng of purified yeast *cdc2* protein. Lane 2, 100  $\mu$ g of mitotic extract from *Xenopus* eggs. The immunoblot was probed with an affinity-purified antibody against the yeast *cdc2* protein as described in Experimental Procedures.

cubated interphase extracts with 2 U of MPF in the presence of 2.5  $\mu$ M p13. No nuclear disassembly occurred. After a 1 hr incubation, we added an extra 10 U of MPF. With this added excess of MPF, nuclear disassembly now occurred normally in approximately 1 hr. Thus, the interphase extract is not irreversibly inactivated by p13. These findings are also consistent with a direct interaction between p13 and MPF (see below).

Finally, there was a very steep threshold for the concentration of p13 required for mitotic inhibition (Figure 3). Although total inhibition occurred at 2.5  $\mu$ M, p13 had no effect on MPF-induced nuclear envelope disassembly at only 2-fold lower concentrations. This phenomenon is reminiscent of the high-order dependence that MPF has for mitotic induction in vivo (Wu and Gerhart, 1980) and in vitro (Dunphy and Newport, 1988).

#### Xenopus Eggs Contain Yeast *cdc2* Homologs

From genetic (Hayles et al., 1986a, 1986b; Hindley et al., 1987) and biochemical studies (Brizuela et al., 1987) in yeast, p13 is known to interact physically with the *cdc2* protein kinase. Moreover, the *cdc2* kinase is thought to play a direct role in mitotic induction in yeast (Nurse and Bisset, 1981; Beach et al., 1982; Simanis and Nurse, 1986). We have now found that *Xenopus* eggs contain polypeptides that are immunologically cross-reactive with the yeast *cdc2* product. We observed that affinity-purified rabbit antibodies directed against the fission yeast *cdc2* protein specifically recognize two polypeptides in *Xenopus* egg extracts with molecular weights of 34 kd and 33 kd (Figure 4). The 34 kd species comigrated precisely with the purified yeast *cdc2* protein (known to be 34 kd in molecular mass). The presence of *cdc2* protein in *Xenopus* is consistent with the high degree of conservation of this polypeptide from yeast to humans (Lőrincz and Reed, 1984; Draetta et al., 1987; Lee and Nurse, 1987).



**Figure 5. A p13 Affinity Column Binds the *Xenopus cdc2* Homologs**  
Crude MPF (enriched by ammonium sulfate precipitation; see Experimental Procedures) was applied to a 0.3 ml column of either control agarose or p13 agarose equilibrated in EB (see Experimental Procedures). For this experiment, 0.9 mg of crude MPF was used. The flow-through fraction was collected, and the columns were washed with 3 ml of EB. Then, each column was eluted with SDS gel sample buffer (Laemmli, 1970). All manipulations were at 22° C. Lane 1, crude MPF (150  $\mu$ g). One-sixth of the flowthrough fraction from either control agarose (lane 2) or p13 agarose (lane 3). One-half of the eluate from control agarose (lane 4) or p13 agarose (lane 5). The immunoblot was stained with anti-yeast *cdc2* antibodies. The molecular masses of the stained doublet of proteins are 34 kd and 33 kd.

#### Yeast p13 Binds *Xenopus cdc2*

We set out to determine whether yeast p13 might inhibit MPF-induced nuclear disassembly by binding to the *Xenopus cdc2* homologs in egg extracts. For this purpose, we covalently coupled p13 to CNBr-activated agarose (see Experimental Procedures). As a control, we subjected agarose to the same chemical procedure, but in the absence of p13. *Xenopus* extracts (crude MPF) were applied to either the p13 agarose column or to the control column (Figure 5). Immunoblotting revealed that virtually all of the *cdc2* protein (98%) was retained on the p13 column. However, there was negligible retention of the *cdc2* products on the control column, which demonstrated that the retention of the *cdc2* protein was p13-dependent. Typically, 95%–99% of the total loaded protein was recovered in the flowthrough from either type of column (see Figure 6). About 0.3%–1% of the total protein (depending on the initial column washing conditions) was recovered by elution of the columns with SDS gel sample buffer. Thus, the interaction between p13 and the *cdc2* protein is quite selective in that little of the total extract protein binds to the affinity column.

The *Xenopus cdc2* proteins were very tightly bound to the p13 affinity column. Treatment of the p13 column with either 2 M NaCl, 50% ethylene glycol, 0.1 M sodium acetate (pH 4.0), 0.1 M Tris (pH 9.0), 10 mM EDTA, 1 mM ATP,

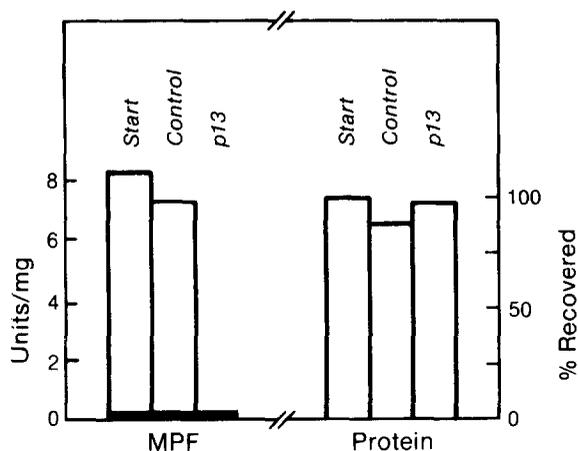


Figure 6. Affinity Chromatography on p13-Agarose Depletes MPF. The data are from the same experiment as in Figure 5. The flow-through fractions from either a control column or a p13 agarose column were assayed for MPF activity (left) or protein (right). The levels of MPF activity and protein in the initial MPF preparation (designated "Start") are also indicated. The recovery of MPF activity from the control column was 86%. There was no detectable MPF activity in the flow-through from the p13 column. The limit of detection for MPF is indicated by the darkened bar. According to this value, greater than 96% of the MPF activity was removed by this step.

or 1 M urea released negligible amounts of the protein. However, the *cdc2* protein was not covalently bound to the column since it could be recovered (yield = 89% for the experiment in Figure 5) in SDS gel sample buffer. The tight interaction between p13 and the *cdc2* products also suggests that this binding is physiologically relevant.

These experiments demonstrate conclusively that yeast p13 can physically associate with the frog *cdc2* product. This underscores the extent to which *cdc2* protein has been conserved structurally and functionally during evolution. The *suc1* product (p13) also exists in human cells (Draetta et al., 1987), but it is not yet known whether *Xenopus* contains a p13 homolog. If there is an endogenous p13 in the *Xenopus* egg extracts, there are two explanations for the retention of such a large percentage of *Xenopus cdc2* protein on immobilized yeast p13. Yeast p13 might displace the *Xenopus* protein during chromatography or, alternatively, only a small fraction of the *Xenopus cdc2* protein might be associated with the hypothetical frog p13 homolog.

#### Chromatography on p13-Agarose Depletes MPF Activity

The most straightforward interpretation of the above findings is that yeast p13 blocks MPF-induced nuclear envelope disassembly by binding to the *Xenopus cdc2* protein. In principle, p13 could block MPF-dependent nuclear breakdown by physically binding to MPF. To determine whether there is such a physical interaction, we asked whether MPF activity binds to a p13 column. As in the previous experiment (Figure 5), we chromatographed crude MPF on p13 agarose or mock-coupled agarose (Figure 6). MPF activity was quantitatively depleted (>96%;

the limit of detection) from the flowthrough of the p13 affinity column. MPF did not bind to the control column, which lacked p13 (recovery = 86%). Furthermore, under identical chromatographic conditions, MPF did not bind to agarose coupled with a number of control (irrelevant) polypeptides, including calmodulin, casein, *Staphylococcus aureus* protein A, or rabbit IgG. The binding of MPF to p13 agarose occurred rapidly (within 5 min) at either 4°C or 22°C. It appears that p13 binds to MPF rapidly and specifically, and by implication this binding would block the conversion of the egg extracts to the mitotic state.

We found that there was a close correspondence between the retention of MPF activity on p13 agarose and the binding of the *Xenopus cdc2* protein to the resin. Complete retention (95% or greater) of MPF activity and the *cdc2* products occurred when the concentration of p13 on the agarose was 5 mg/ml (the standard concentration in this report). At 3 mg of p13 per ml of resin, 75%–85% of both MPF activity and *cdc2* was bound. With 1 mg/ml of ligand, approximately 50% of MPF and *cdc2* was retained (data not shown). The identical binding properties of MPF activity and the *cdc2* protein to p13 columns argue strongly that the *cdc2* protein is MPF or is a component of MPF necessary for its mitosis-inducing activity.

Control experiments indicated that the depletion of MPF activity from the flowthrough of the p13 column was not due to the activation (or introduction) of an inhibitor of MPF. In mixing experiments, the flowthrough from the p13 column did not inhibit active MPF. Also, we demonstrated by immunoblotting with antibodies against yeast p13 (Brizuela et al., 1987) that no detectable p13 had leached from the affinity column during the chromatography of crude, MPF-containing extracts.

To date, it has not been possible to find conditions which cause the elution of MPF in an active form from the affinity column. This appears to be due to the fact that the binding of the *cdc2* protein to the p13 column is so strong that only denaturants are effective for elution. In addition to SDS gel sample buffer, only 0.1 M diethylamine (pH 10), 0.1 M glycine (pH 2.0), heating to 45°C for 15 min, and 1% Mega-10, a detergent, eluted significant amounts of *cdc2* protein, as monitored by immunoblotting. Each of these treatments inactivates MPF (unpublished data). It is also possible that the true function of p13 is related to the inactivation of MPF during the exit from mitosis. In this event, it would be necessary to establish conditions for the reversal of the inactivation process.

#### p13 Affinity Chromatography Extensively Enriches *Xenopus cdc2* and a 42 kd Protein

The coordinate and specific retention of MPF and *Xenopus cdc2* on p13 agarose suggests that the *cdc2* protein is MPF or is associated with MPF. Recently, Lohka et al. (1988) have extensively purified MPF in a seven-step procedure with ~1% yield. MPF activity cofractionated with two polypeptides of molecular mass 32 kd and 45 kd. Based on the results presented here, it is very likely that the 32 kd species is the *Xenopus cdc2* product (~33–34 kd under our electrophoretic conditions).

Since 1% or less of the total applied protein bound to

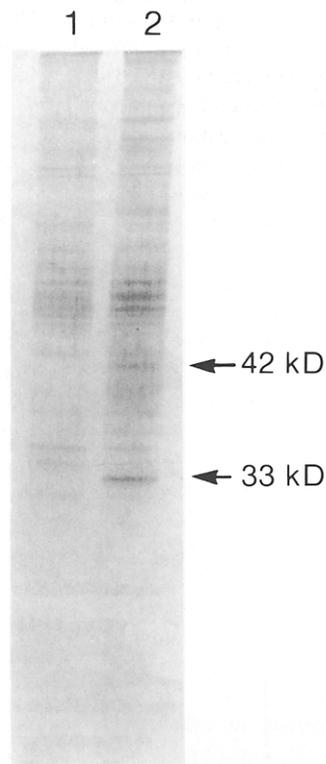


Figure 7. The p13 Affinity Column Enriches Extensively the *Xenopus cdc2* Protein and a 42 kd Polypeptide

The protocol was similar to that described in Figure 5, except that the SDS-containing eluates from either a control column (lane 1) or a p13 agarose column (lane 2) were silver-stained after electrophoresis. Proteins (33 kd and 42 kd) that reproducibly bound to p13 agarose in a specific manner are indicated. Only the 33 kd form of *Xenopus cdc2* was evident in this MPF preparation, as noted by immunoblotting. Molecular size standards (not shown) were: phosphorylase b (97 kd); bovine serum albumin (66 kd); ovalbumin (43 kd); carbonic anhydrase (31 kd); soybean trypsin inhibitor (21 kd); and lysozyme (14 kd).

either p13 agarose or control agarose, we investigated whether p13 affinity chromatography might enrich the polypeptide components of MPF extensively. *Xenopus* extract proteins were applied to a control column and a p13 agarose column, and the columns were washed extensively with buffer. The columns were then eluted with SDS gel sample buffer, and the eluted proteins were fractionated by gel electrophoresis and silver-stained (Figure 7). We observed a background of proteins that bound nonspecifically to both control and p13 agarose. For the most part, these proteins were abundant silver-staining species in the crude MPF extract. However, we found a protein (33 kd) bound to p13 agarose but not to the control column (Figure 7, lane 2). The silver-stained 33 kd protein corresponded exactly to a band that reacted with anti-*cdc2* antibodies. In comparison with the silver-staining intensities of known amounts of purified yeast *cdc2* protein, we estimate that there is ~0.4  $\mu$ g of *Xenopus cdc2* protein

per mg of crude MPF (or 0.04% of the total protein). The *Xenopus cdc2* protein did not correspond to a major silver-staining species in the extract that we applied to the column.

In addition to the *cdc2* protein, we consistently observed a 42 kd protein that also was specifically retained on the p13 agarose column (Figure 7). This was the only additional specifically bound protein that we observed in ten similar experiments. The 42 kd protein did not comigrate with a major polypeptide in the crude MPF extract. This polypeptide might bind directly to p13 or, alternatively, it might exist in a complex with the *Xenopus cdc2* protein that is bound to the column. The 42 kd protein did not react, even weakly, with anti-*cdc2* antibodies. There are precedents in yeast and human cells for proteins that bind to *cdc2*. In *S. cerevisiae*, the *cdc2* homolog (*CDC28*) binds to a 40 kd protein (Mendenhall et al., 1987), whereas in human cells (Draetta and Beach, 1988) and *S. pombe* (Booher and Beach, unpublished data), *cdc2* complexes with a 62 kd protein. Recently, Lohka et al. (1988) have observed a 45 kd *Xenopus* polypeptide in highly enriched MPF. Based on the data presented above, it seems quite likely that the 42 kd protein that binds to p13 agarose and the 45 kd protein reported by Lohka et al. (1988) are related.

## Discussion

In the experiments reported here, we found that the isolated 13 kd product (p13) of the yeast *suc1* gene blocks the ability of MPF to convert interphase extracts from *Xenopus* eggs to the mitotic state. In yeast it has been established that p13 binds to the *cdc2* kinase (Brizuela et al., 1987). We found that *Xenopus* contains homologs of the *cdc2* protein, and that yeast p13 also binds to these homologs. We also showed that the *Xenopus cdc2* homologs are efficiently retained on a p13 affinity column. Simultaneously, MPF is quantitatively retained on the same p13 affinity support. The *Xenopus cdc2* homologs (~33–34 kd) and a 42 kd polypeptide are the predominant proteins that bind specifically to p13 agarose. This polypeptide composition is very similar to that reported by Lohka et al. (1988) for MPF that was purified by conventional chromatographic steps (32 kd and 45 kd). Taken together, these findings suggest that MPF is a mitotic regulator composed of two proteins: the *Xenopus cdc2* homolog and a 42 kd protein. In addition, our results suggest that p13 regulates the function of this complex.

A body of circumstantial evidence suggests that protein phosphorylation is an important aspect of mitotic control. Many proteins (e.g., the nuclear lamins and histones) are hyperphosphorylated during mitosis (Gerace and Blobel, 1980; Davis et al., 1983; Karsenti et al., 1987; Lohka et al., 1987). Also, many of the fungal *cdc* gene products are kinases or possess sequence motifs typical of kinases (Lőrincz and Reed, 1984; Hindley and Phear, 1984; Simanis and Nurse, 1986; Russell and Nurse, 1987; Osmani et al., 1988). Similarly, indirect evidence has suggested that MPF might be a phosphoprotein (Wu and Gerhart, 1980; Gerhart et al., 1985). In yeast and humans,

the kinase activity of *cdc2* is documented (Simanis and Nurse, 1986; Mendenhall et al., 1987; Draetta and Beach 1988). Although it is not known whether *Xenopus cdc2* is a kinase, this is a reasonable expectation. Consistently, Lohka et al. (1988) have demonstrated that highly purified MPF contains kinase activity. Thus, the *cdc2* kinase (or perhaps some other activity associated with MPF) might represent the widely surmised pleiotropic stimulator of a protein kinase cascade that would effect the morphological and ultrastructural alterations at the onset of mitosis.

Our results show that yeast p13 inhibits the action of MPF in an in vitro system. In line with this observation, mild overexpression of p13 in wild-type yeast partially delays cell division, whereas extreme overproduction leads to mitotic arrest (Hayles et al., 1986b; Hindley et al., 1987). Endogenous inhibitors of MPF in *Xenopus* eggs and oocytes have been described (Gerhart et al., 1984; Cyert and Kirschner, 1988; Dunphy and Newport, 1988). However, the relationship of these inhibitors to p13 is currently unknown. Draetta et al. (1987) have identified a homolog of p13 in human cells, indicating that a *Xenopus* counterpart is likely to exist. On the basis of the present results and the observed effect of the overexpression of p13 on the cell cycle in yeast cells, it is probable that p13 plays a crucial regulatory role in mitosis through direct interaction with MPF in vivo.

An expected property of a mitosis-inducing factor is that it would be synthesized or activated in concert with the mitotic cycle. In fission yeast, the amounts of *cdc2* protein and its kinase activity have not been reported to oscillate in any discernible manner during the progression of the cell cycle (Simanis and Nurse, 1986). Also, overproduction of the *cdc2* protein does not perturb cell division (Booher and Beach, 1986; Durkacz et al., 1986). One explanation for this apparent paradox would be that another polypeptide(s) is required for the proper functioning of the *cdc2* protein. This auxiliary protein(s) might stimulate the *cdc2* kinase or allow the kinase to recognize its physiological target(s). One clear candidate for such an auxiliary protein in *Xenopus* would be the 42 kd polypeptide that binds to the p13 agarose column. Thus, active MPF might consist of a complex between the *cdc2* protein and the 42 kd polypeptide. In budding yeast, the level of the *CDC28* product is also invariant during the cell cycle, but the activity of the protein kinase against a 40 kd endogenous substrate is maximal in G1 (Mendenhall et al., 1987). In HeLa cells the situation is different. The *cdc2* homolog is also present throughout the cell cycle, but is inactive (as determined by an in vitro kinase assay) during G1 and is maximally active during mitotic metaphase (Draetta and Beach, 1988). Mitotic activation of human *cdc2* is associated both with cell-cycle dependent phosphorylation of *cdc2*, and complex formation with a 62 kd protein that appears to act as a substrate of the kinase in vitro. Thus, the complex of *cdc2* and p62 in human cells possesses exactly the biochemical properties of a mitotic inducer. However, MPF activity of the *cdc2*-p62 complex has not been investigated, nor is it known whether the *Xenopus* 42 kd polypeptide is related to the human 62 kd *cdc2*-associated protein.

Recent indirect studies have indicated that MPF, although active only during mitosis, is present in a latent form during interphase in the cleaving *Xenopus* embryo (Dunphy and Newport, 1988). These findings are consistent with the fact that the *cdc2* polypeptide is present throughout the cell cycle in yeast and human cells (Simanis and Nurse, 1986; Mendenhall et al., 1987; Draetta and Beach, 1988). A key question therefore is how the latent, interphase-form of MPF is activated for the initiation of mitosis. In the early *Xenopus* embryo, protein synthesis is required for each successive mitosis (see Miake-Lye et al., 1983). This protein synthesis requirement is intriguing, since most (if not all) of the structural components for the assembly of thousands of cells are stored in the egg. Similarly, MPF is present constitutively during this early embryonic period (Dunphy and Newport, 1988). It seems likely that an activator of MPF must be manufactured in each cell cycle for mitotic progression (Swenson et al., 1986; Pines and Hunt, 1987; Murray, 1987; Dunphy and Newport, 1988). Good candidates for this activator are the known cyclin proteins, whose concentrations rise and fall in synchrony with each mitosis (Swenson et al., 1986; Pines and Hunt, 1987). Alternatively, an unidentified cyclin-like molecule might be involved. It is not known whether the level of the 42 kd component of MPF oscillates with the cell cycle.

Specific models for the function of the putative activator of MPF can now be envisioned. Perhaps this activator is a positive effector that binds directly to *cdc2* or to a *cdc2*-associated protein. Alternatively, the activator could mediate the formation of a complex between the *cdc2* product and other factors. Finally, the activator could neutralize an inhibitor that suppresses MPF during interphase. The comparison of active MPF with latent MPF from interphase cytoplasm (Dunphy and Newport, 1988) will help distinguish these possibilities, now that molecular probes for MPF are available.

The findings described here provide a link between two diverse areas of research of the cell cycle, namely the genetics of division control in unicellular eukaryotes and the cellular biochemistry of the cell cycle in early animal embryos. In the early *Xenopus* embryo, for example, the phenomenology of cell-cycle progression is well characterized, and many of the biochemical events of the cell cycle can be recreated in vitro. However, regulatory enzymes involved in cell-cycle control have proven difficult to isolate in convenient quantities (Wu and Gerhart, 1980; Gerhart et al., 1985; Lohka et al., 1988). In the fungi, the opposite situation largely exists. A large number of mutants in cell-cycle control are available, but other than the important insight that many of the known gene products are kinases or are related to known kinases, little is understood about the biochemistry of these products. The examination of the function of the frog homologs to the *cdc* proteins (or the isolated yeast *cdc* proteins) in the *Xenopus* in vitro systems should circumvent the lack of comparable cell-free systems in yeast, and allow the characterization of the biochemical properties of these regulatory gene products.

In summary, we have shown that the *Xenopus cdc2* protein is a component of MPF. We have established a heterologous system in which a purified yeast protein (p13) regulates the function of *Xenopus* MPF in vitro. Moreover, the polypeptide components of MPF now can be isolated conveniently and in high yield by affinity chromatography on p13 agarose. These findings make it technically feasible to address long-standing questions about the enzymology of MPF and the cell-cycle clock to which it belongs.

### Experimental Procedures

#### Cell-Free Assay for MPF

Cell-free assays for MPF (Lohka and Maller, 1985; Miake-Lye and Kirschner, 1985; Dunphy and Newport, 1988) were performed exactly with the modifications described previously (Dunphy and Newport, 1988). Preparation of mitotic cytoplasm and crude MPF (enriched and concentrated by ammonium sulfate precipitation) from unfertilized *Xenopus* eggs, based largely on the method of Wu and Gerhart (1980), was conducted exactly as published before (Dunphy and Newport, 1988). The crude MPF extract (6 mg/ml protein) used in this paper was enriched 8-fold (yield = 80%) for mitotic induction relative to unfractionated mitotic cytoplasm. MPF-extraction buffer (EB; Wu and Gerhart, 1980) contained 80 mM glycerol phosphate (pH 7.3), 20 mM EGTA, 15 mM MgCl<sub>2</sub>, and 1 mM dithiothreitol. The definition of a unit of MPF activity in the cell-free assay was described in Dunphy and Newport (1988). In brief, U of MPF were defined as the inverse of the maximum dilution at which an MPF-containing extract would induce mitotic conversion. The specific activity of the crude MPF extract used in this paper was 8.3 U per mg protein.

#### Preparation of Fission Yeast p13

p13 was purified from an overproducing strain of *E. coli* by gel filtration on Sepharose CL-6B (Pharmacia) as described previously (Brizuela et al., 1987). For some experiments, p13 was further purified by chromatography on a Mono Q column (see legend to Figure 2 for details) or a Biogel P-100 column (equilibrated with 0.1 M sodium borate; pH 8.0). Prior to addition to the cell-free *Xenopus* extracts, p13 was equilibrated in EB by dialysis.

#### Preparation of p13 Affinity Columns

p13 was conjugated to either CNBr-activated Sepharose 4B (Pharmacia) or Affigel-10 (Bio-Rad) according to the instructions of the manufacturer. Unreacted groups on the gels were quenched with 1 M ethanolamine (pH 8.0). The concentration of coupled p13 was 5 mg protein per ml of gel unless indicated otherwise.

#### Electrophoretic Techniques

Typically, samples were electrophoresed in 10% polyacrylamide gels (Laemmli, 1970). For visualization of p13, a 12.5% gel was used. Electrophoresed proteins were transferred to Immobilon membranes (Millipore) with the Polyblot apparatus (American Bionetics). Before incubation with antibodies, immunoblots were treated with Tris-buffered saline (10 mM Tris-Cl, 150 mM NaCl; pH 8.0) containing 5% powdered milk and 0.1% Tween-20. After antibody binding, the blots were exposed to <sup>125</sup>I-protein A (ICN). Affinity-purified antibodies against the fission yeast *cdc2* and *suc1* proteins were described previously (Draetta et al., 1987; Brizuela et al., 1987). The batch of *cdc2* antibody used in this paper is the same one used to characterize the human homolog of *cdc2* (Draetta et al., 1987). The human *cdc2* polypeptide was shown to be related to the fission yeast *cdc2* protein by peptide mapping.

#### Miscellaneous

Protein concentration was determined with the Bio-Rad protein assay kit with rabbit immunoglobulin as the standard. Silver staining was performed with the kit from Bio-Rad.

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