COMMENTARY

Mitotic control by metaphase-promoting factor and *cdc* proteins

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Introduction

Entry into mitosis brings about a dramatic reorganization of both nuclear and cytoplasmic structures in preparation for cell division. In general, three approaches have been taken to study the mechanisms controlling the onset of this reorganization: (1) genetic analysis of mutants, mostly of yeast and other fungi, that are defective in the cell division cycle (*cdc* mutants); (2) biochemical assays of protein kinases and other enzymes whose activities oscillate with the cell cycle and peak during mitosis; (3) the use of biological assays to test for mitotic inducers in dividing cells. The underlying premise of these approaches, which utilize a variety of different cell types, is that at least some of the mechanisms regulating the transition from G_2 to mitosis will prove to be similar in all eukaryotes. Indeed, this premise seems to be correct. Recently published results from each of the approaches have implicated the same proteins, the products of the yeast *cdc2/CDC28* genes and their homologues, in the regulation of early mitotic events.

Genetic analysis of *cdc2/CDC28* mutants

In *Schizosaccharomyces pombe* the *cdc2* gene (*cdc2*(Sp)) encodes a $34\times10^3 M_r$ protein kinase (p34) that is required at two points in the cell cycle, at the transitions from G_1 to S and from G_2 to mitosis (for more detailed reviews, see Nurse, 1985; Hayles & Nurse, 1986*a*; Lee & Nurse, 1988). Some temperature-sensitive mutants of *cdc2* arrest in either G_1 or G_2 when cells are incubated at non-permissive temperatures. Others are dominant and cause cells to advance into mitosis precociously, with a reduced cell size and shortened G_2 period. These phenotypes indicate that the *cdc2* gene product is involved in the mechanisms that control the timing of cell division.

The *CDC28* gene of the budding yeast, *Saccharomyces cerevisiae*, also encodes a $34\times10^3 M_r$ protein kinase required during G_1 (Reed *et al.* 1985) and possibly again during G_2 (Piggott *et al.* 1982). *CDC28* is functionally equivalent to *cdc2* in that it can complement mutants of *S. pombe* deficient in *cdc2* function (Beach *et al.* 1982), permitting growth at restrictive temperatures. A similar complementation assay has been used to isolate a homologue of *cdc2*, *cdc2*(Hs), from a human cDNA library (Lee & Nurse, 1987). The proteins encoded by *cdc2*(Sp), *cdc2*(Hs) and *CDC28* share approximately 60% amino acid sequence identity between any two of them, including a stretch of 14 amino acids (EGVPSTAIRELLKE), referred to as the PSTAIR sequence, that is perfectly conserved in all. Antisera raised against *cdc2*(Sp) (Draetta *et al.* 1987) or against peptides containing the PSTAIR sequence (Lee & Nurse, 1987), or the amino-terminal sequence of *CDC28* (Mendenhall *et al.* 1987), recognize $34\times10^3 M_r$ proteins in cells of yeast, humans and a variety of other eukaryotes (see below). Since genes that are functionally equivalent to *cdc2* are conserved from yeast to man, their products are likely to play a fundamental role in the regulation of the eukaryotic cell cycle.

The exact role of p34 in the initiation of cell division is not yet understood. Entry into mitosis is not triggered by an accumulation of p34 during G_2, since its levels do not change appreciably during the cell cycle of *S. pombe* (Simanis & Nurse, 1986), *S. cerevisiae* (Wittenberg & Reed, 1988) or HeLa cells (Draetta & Beach, 1988). In contrast, the protein kinase activity of $p34^{CDC28}$ and $p34^{cdc2(Hs)}$ oscillates with the cell cycle, reaching highest levels in G_1 for *S. cerevisiae* (Wittenberg & Reed, 1988) and in late G_2 and mitosis for HeLa cells (Draetta & Beach, 1988). Coincident with the increase in protein kinase activity in both species is the formation of a high molecular weight complex of p34 and other proteins (see below). Similar cell cycle-dependent changes are not observed in *S. pombe* but, instead, the p34 protein kinase activity changes in response to nutritional conditions (Simanis & Nurse, 1986).

Biochemical analysis of mitotic protein kinases

The onset of mitosis is marked by an increase in the phosphorylation of a large number of intracellular proteins. Although most of the phosphoproteins remain unidentified, lamins (Ottaviano & Gerace, 1985), vimentin (Evans & Fink, 1982), histone H1 (Bradbury *et al.* 1973, 1974) and histone H3 (Gurley *et al.* 1974) are known to become highly phosphorylated. The phosphorylation of histone H1 has long been known to

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accompany cell proliferation, and the protein kinase responsible is called the 'growth-associated H1 kinase' (GAK) (Lake & Salzman, 1972). Specific sites in the carboxyl- and amino-terminal regions of the H1 molecule, but not in the globular central region, are phosphorylated by GAK (Langan, 1978). H1 phosphorylation begins in G_1, continues through S and G_2, and rises sharply in mitosis (Lake & Salzman, 1972; Bradbury *et al.* 1974). The rapid increase in H1 phosphorylation during mitosis has led to the suggestion that GAK activity might be involved in mitotic chromosome condensation (Bradbury *et al.* 1973), although no conclusive evidence for such a role has been obtained.

An H1 kinase, which has peak levels of activity in mitosis, has been purified from starfish eggs (Labbe *et al.* 1988; Arion *et al.* 1988). This H1 kinase activity co-purifies with a $34\times10^3 M_r$ protein and is both immunoprecipitated and recognized on immunoblots by antibodies to $p34^{cdc2}$, indicating that the mitotic kinase is a starfish homologue of $p34^{cdc2}$. Conclusive evidence that $p34^{CDC28}$ is functionally identical to mammalian GAK has recently been obtained (Langan *et al.* 1988). Lysates of wild-type *S. cerevisiae* have an H1 kinase activity whose levels are elevated in mitosis, and which phosphorylates histone H1 on the same sites as GAK purified from Novikoff rat hepatoma cells. This H1 kinase is temperature-sensitive in extracts prepared from *CDC28* mutants. Moreover, the PSTAIR antibody reacts with a 32–34 $(\times10^3) M_r$ protein in the purified mammalian GAK preparation. Taken together these results indicate that the GAK from starfish, mammals and probably a variety of other cells are homologues of the yeast *cdc2* gene product, and are likely to be a part of a conserved mechanism that is involved in the regulation of cell division.

Biological assays for mitotic inducers

Maturation-promoting factor

Proteins that induce entry into mitosis can be assayed by microinjection into frog or starfish oocytes. Fully grown oocytes of these species are physiologically arrested in prophase I of meiosis (G_2) and when treated with the appropriate hormone, such as progesterone for frogs or 1-methyladenine for starfish, resume cell division as they mature into unfertilized eggs (for reviews, see Masui & Clarke, 1979; Meijer & Guerrier, 1984). Resumption of cell division can be easily monitored by the breakdown of the oocyte nuclear envelope (the process known as germinal vesicle breakdown (GVBD)), chromosome condensation and completion of meiosis I. When the cytoplasm from maturing oocytes is transferred to immature oocytes, the recipients enter metaphase and, without hormonal stimulation, undergo all of the changes associated with oocyte maturation (Masui & Markert, 1971; Smith & Ecker, 1971; Kishimoto & Kanatani, 1976). The component transferred between oocytes, which is responsible for inducing metaphase, was named 'maturation-promoting' or 'metaphase-promoting' factor (MPF). MPF activity has been detected in maturing oocytes from a large number of invertebrate and vertebrate species, and in mitotically dividing sea urchin and amphibian embryos, mammalian tissue culture cells and budding yeast (for recent reviews, see Maller, 1985; Kishimoto, 1988). In all of these cells, activity is highest during metaphase and decreases precipitously to undetectable levels at other times. MPF can induce GVBD in the absence of new protein synthesis, suggesting that it is a fundamental component of the mechanism that regulates metaphase in both meiotic and mitotic cells.

MPF activity can also be detected by another recently developed method, which utilizes two types of extracts from *Xenopus laevis* eggs that support *in vitro* the same nuclear behaviour that occurs in cells from which the extracts are prepared (for review, see Lohka & Maller, 1987). G_1/S-phase extracts can assemble a nuclear envelope around sperm chromatin and decondense the chromatin, forming interphase nuclei capable of DNA replication. On the other hand, M-phase extracts induce interphase nuclei to undergo nuclear envelope breakdown, chromosome condensation, and spindle formation. The addition of a soluble fraction from M-phase extracts to G_1/S-phase extracts induces the newly formed nuclei to enter metaphase (Lohka & Maller, 1985; Miake-Lye & Kirschner, 1985). Since partially purified MPF causes the same changes, this cell-free system offers a novel alternative to oocyte microinjection for the detection of MPF activity.

Purification of maturation-promoting factor

MPF activity is very unstable and has been difficult to purifiy. Despite attempts by several laboratories, MPF from a number of different meiotic and mitotic cells could only be purified 20- to 200-fold when oocyte microinjection was used to monitor activity (Wu & Gerhart, 1980; Adlakha *et al.* 1985; Nguyen-Gia *et al.* 1986; Kishimoto & Kondo, 1986). Recently, the response of nuclei incubated in cell-free extracts was used to monitor MPF during its purification from *Xenopus* eggs (Lohka *et al.* 1988). Ammonium sulphate precipitation and six chromatographic steps resulted in a greater than 3000-fold purification of a protein fraction that could induce metaphase *in vitro*. The most active fractions in the cell-free assay also induced GVBD when microinjected into cycloheximide-treated oocytes, indicating that MPF activity had been purified. Proteins of $45\times10^3 M_r$ (p45) and $32\times10^3 M_r$ (p32) were enriched in these fractions, although some side fractions that were active only in the cell-free system contained p45 without detectable p32. In the most active fractions p32 correlated with a protein, serine–threonine kinase, that could phosphorylate the endogenous p45, histone H1, casein and phosphatase inhibitor-1. Conclusive evidence that p32 was a protein kinase came from the observations that antibodies to the PSTAIR sequence of *cdc2* recognized p32 both in immunoblots of purified MPF and by immunoprecipitation (Gautier *et al.* 1988). The immune complexes not only retained protein kinase activity, with the same substrate specificity as the MPF-associated kinase, but also phosphorylated histone H1 at the same sites as the mammalian growth-associated kinase and $p34^{cdc28}$ (Lan-

gan *et al.* 1988). Therefore, the MPF-associated p32 is a member of a conserved family of protein kinases encoded by *cdc2/CDC28* genes and their homologues. Yeast, amphibian and mammalian members of this family phosphorylate histone H1 at the same sites that are extensively phosphorylated during mitosis in mammalian cells. These kinases also phosphorylate other substrates, which may have important roles during the onset of mitosis (see below).

The co-purification of MPF and a protein encoded by a *Xenopus* homologue of the *cdc2/CDC28* genes strongly suggests that at least some of the components that regulate mitotic entry are conserved among eukaryotes. However, despite the strong genetic evidence that *cdc2* is essential for mitosis in yeast and direct biochemical evidence that $p34^{cdc2}$, or its equivalent, corresponds to the GAK, evidence that p32 is a component of MPF is still circumstantial. A requirement for p32 in MPF activity is uncertain because some fractions that contained p45 induced metaphase in the cell-free system despite the apparent absence of p32, and little or no protein kinase activity (Lohka *et al.* 1988). Also, since p45 was most abundant in fractions that induced GVBD, it alone may be sufficient for MPF activity, but required at higher concentrations in oocytes than in the cell-free extracts.

Even though p45 alone may be sufficient to induce metaphase in the cell-free extracts, p32 is still likely to play a role in the pathway of MPF action. Dunphy *et al.* (1988) have shown that MPF-induced nuclear envelope breakdown and chromosome condensation are inhibited in extracts by the $13\times10^3 M_r$ product of the yeast *suc1* gene, p13. In yeast, p13 interacts with $p34^{cdc2}$ (Brizuela *et al.* 1987), and overexpression of *suc1* leads to mitotic delay (Hayles *et al.* 1986*b*). Since p13 also binds *Xenopus* p32, MPF activity may be inhibited by this interaction. The mechanism of this inhibition is still unclear, but does not appear to involve inhibition of protein kinase activity, since both yeast (Brizuela *et al.* 1987) and starfish (Arion *et al.* 1988) p34 retain kinase activity when bound to p13. Although the inhibition of MPF action by p13 can be interpreted to show that p32 is a component of MPF, the possibility that p32 is required for mitotic events that follow the initiation of metaphase, and not actually for initiation, should not be ruled out.

Complexes of $p34^{cdc2/CDC28}$ protein kinase

In highly purified preparations of *Xenopus* MPF both p45 and p32 are co-immunoprecipitated by PSTAIR antibodies (Gautier *et al.* 1988), suggesting that they interact as part of a complex. Similarly, both proteins are retained when crude MPF preparations are applied to an affinity column of p13, which binds p32 (Dunphy *et al.* 1988). The assembly of homologues of $p34^{cdc2}$ and other proteins into multimeric complexes with apparent molecular masses of $150–200\,(\times10^3)\,M_r$ has been demonstrated in *S. cerevisiae* (Wittenberg & Reed, 1988), starfish (Arion *et al.* 1988) and HeLa cells (Draetta & Beach, 1988). The proteins that associate with p34 are different in each case: p40 in *S. cerevisiae*, p45 in *Xenopus*, p62 and p13 in HeLa cells. However, in all cases the p34 in the complex is an active protein kinase that phosphorylates not only exogenous substrates but, perhaps more importantly, other proteins with which it is associated, e.g. p40, p45 or p62. The assembly of multimeric complex, activation of protein kinase activity and phosphorylation of p40 are thought to function in regulating the commitment to cell division ('start') in *S. cerevisiae* (Wittenberg & Reed, 1988). Similarly, it has been proposed that the active protein kinase complex formed between p62, p34 and p13 in HeLa cells is a component of MPF (Draetta & Beach, 1988), although the MPF activity of this complex has yet to be demonstrated. Curiously, p13 is part of this complex, even though it inhibits MPF-induced events *in vitro*. So far, it is unclear from any of these studies precisely what role these complexes play in mitotic entry. Moreover, it is not yet possible to determine whether their formation gives rise to MPF activity, or is a consequence of it.

If the formation of a high molecular weight complex is essential for MPF activity, then the availability of each of the components will influence whether or not cells enter mitosis. Therefore, if proteins that form the complex are more abundant, or interact more readily, in egg extracts than in immature oocytes, it is conceivable that p45 alone may be sufficient to trigger metaphase in the cell-free assay, but not in microinjected oocytes.

Other proteins involved in mitotic control

Mitotic control in *S. pombe* involves the products of several other genes that interact, directly or indirectly, with *cdc2* to make up a regulatory network that controls the timing of cell division (Lee & Nurse, 1988; Russell & Nurse, 1987). Both positive regulators, such as *nim1* and *cdc25*, and negative regulators, such as *wee1*, are involved. *nim1* and *wee1* encode protein kinases, suggesting that protein phosphorylation and dephosphorylation play major roles in the regulatory network. In addition, genetic evidence suggests that *suc1* (Brazuela *et al.* 1987) and *cdc13* (Booher & Beach, 1987) also interact with *cdc2*, and may function in mitosis. Since homologues of *cdc2* and *suc1* have already been identified in higher eukaryotes (Draetta *et al.* 1987; Lee & Nurse, 1987), and since *cdc13* shares a high degree of sequence identity with the cyclins (Solomon *et al.* 1988; Goebl & Byers, 1988), it is probable that homologues of other yeast regulatory genes are also present in all eukaryotes. Analysis of cell cycle mutants in *Aspergillus* has identified other genes, unrelated to known yeast *cdc* genes, that act as positive and negative regulators of mitosis (Osani *et al.* 1988*a*,*b*). Whether homologues of these genes exist in other eukaryotes is not known.

Another family of proteins that undoubtedly plays a key role in the regulation of mitotic entry is the cyclins. Cyclins are characterized by their steady synthesis and accumulation during the cell cycle until metaphase when, after reaching their highest levels, they are rapidly and specifically degraded as cells enter anaphase (Evans *et al.* 1983). Injection of cyclin mRNA into immature oocytes results in GVBD (Swenson *et al.* 1986), and its addition to cell-free extracts induces mitotic entry (Murray *et al.* 1988). The high degree of sequence conservation be-

tween cyclins and *cdc13* argues that cyclins, like the product of *cdc13*, may interact with $p34^{cdc2}$ to regulate cell division. The hypothesis proposed by Solomon *et al.* (1988) that proteins associated with p34, such as p62 in HeLa cells, are related to cyclins is attractive in that it places many of the components thought to be involved in mitotic control in a single multimeric regulatory complex.

In conclusion, gene products that function in the control of mitosis in yeast have counterparts in many eukaryotes, including man. One of these proteins, $p34^{cdc2/CDC28}$, is functionally equivalent to the growth-associated H1 kinase activated during mitosis in proliferating cells. Active $p34^{cdc2/CDC28}$ protein kinase, which is associated with other proteins as part of a high molecular weight complex, may be a component of MPF, although the smallest number of proteins necessary to induce metaphase in each of the MPF assays has not been determined. Whereas these recent results strongly indicate that homologues of yeast *cdc* proteins function in a highly conserved mechanism for mitotic control, their targets, which are more directly involved in nuclear envelope disassembly, chromosome condensation, spindle formation and other mitotic processes, remain unknown.

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