Cell cycle regulation of p34^{cdc2} kinase activity in *Physarum polycephalum*

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Summary

The regulation of the mitotic histone H1 kinase activity has been analyzed during the naturally synchronous cell cycle of *Physarum polycephalum* plasmodia. The universal binding property of the p13^{suc1} *Schizosaccharomyces pombe* gene product was used to precipitate and assay the cdc2 histone H1 kinase activity. The kinase activity peaks at the beginning of metaphase and its decline, which requires protein synthesis, appears to be an early event during the metaphase process. Microtubular poisons, temperature shifts and DNA synthesis inhibitors were used to perturb cell cycle regulatory pathways and characterize their effects on cdc2 kinase activation. Our results suggest that the full activation of the mitotic kinase requires at least two successive triggering signals involving microtubular components and DNA synthesis.

Key words: *Physarum polycephalum*, cell cycle, p34^{cdc2} kinase.

Introduction

The M-phase Promoting Factor (MPF) has been characterized in a large variety of organisms from sea urchin eggs to human mitotic cells (reviewed by Hunt, 1989; Murray and Kirschner, 1989*a*) as an intracellular activity catalyzing the G_2/M-phase transition (Smith and Ecker, 1971; Masui and Market, 1971). Characterization of the components of MPF (Dunphy *et al.* 1988; Gautier *et al.* 1988; Labbé *et al.* 1989; Arion *et al.* 1988) and studies of the genes involved in yeast mitotic control (Beach *et al.* 1982; Booher *et al.* 1989; Moreno *et al.* 1989) have identified in each species a protein homologous to the p34^{cdc2} *Schizosaccharomyces pombe* gene product, which is assumed to be the catalytic subunit of this mitotic protein kinase. To date, histone H1 is the best identified *in vitro* substrate of the cdc2 kinase (Brizuela *et al.* 1989). In the mature form of the enzyme, p34^{cdc2} is stoichiometrically associated with cyclin (Booher and Beach, 1988; Draetta and Beach, 1988; Draetta *et al.* 1989; Pondaven *et al.* 1990; Brizuela *et al.* 1989), a cell cycle regulated protein, originally identified in marine invertebrates (Evans *et al.* 1983). At the G_2/M-phase transition, p34^{cdc2} is dephosphorylated on tyrosine and threonine residues, and the p34^{cdc2}/cyclin complex becomes fully activated (Dunphy and Newport, 1989; Morla *et al.* 1989; Pondaven *et al.* 1990; Jessus *et al.* 1990). Cyclin phosphorylation occurs in metaphase (Meijer *et al.* 1989; Draetta *et al.* 1989) and its destruction is probably responsible for enzyme inactivation. A third component of the complex is p13 the product of the *suc1*$^+$ gene in *S. pombe* (Brizuela *et al.* 1987). The exact role of this protein in the regulation of the enzyme activity is still not clear and subject to investigation (Dunphy and Newport, 1989; Jessus *et al.* 1990). Nevertheless, p13^{suc1} binds to the *cdc2* gene product in *S. pombe in vivo* and *in vitro* (Brizuela *et al.* 1987) and p13 coupled to Sepharose can be used as an affinity matrix for the purification of p34^{cdc2} homologues and associated proteins in every organism studied (Arion *et al.* 1988; Draetta and Beach, 1988; Booher *et al.* 1989; Dunphy *et al.* 1989; Pondaven *et al.* 1990). It is thus a convenient reagent for the assay of p34^{cdc2} kinase activity in a wide range of species.

While the understanding of the activation mechanisms of the p34/cyclin complex is progressing (Dunphy and Newport, 1989; Gould and Nurse, 1989; Pondaven *et al.* 1990; Ducommun *et al.* 1990), the regulation of these events in relation to other cell cycle pathways has not been subjected to intensive investigation. The naturally synchronous plasmodium of the myxomycete *Physarum polycephalum* is a syncytium that can contain 10^8 to 10^9 nuclei dividing every 10 h with perfect synchrony (Howard, 1932; Guttes and Guttes, 1964). This model offers a unique opportunity for following a biochemical event in a single cell during a normal cell cycle or after physical or pharmacological perturbations (Tyson, 1982). Previous studies using this organism have permitted the characterization of the pathways involved in the regulation of tubulin synthesis (Ducommun *et al.*, unpublished data) and degradation (Ducommun and Wright, 1989), and the regulation of thymidine kinase synthesis (Wright and Tollon, 1979, 1988, 1989; Eon-Gerhardt *et al.* 1981*a*,*b*).

Physarum polycephalum was one of the first systems in which the oscillation of histone H1 kinase activities during the cell cycle were described (Bradbury *et al.* 1974; Hardie *et al.* 1976). Furthermore, it has been claimed that addition of partially purified *Physarum* histone H1 kinases or *Physarum* extracts on the external surface of an intact plasmodium advances mitosis (Bradbury *et al.* 1974; Inglis *et al.* 1976; Loidl and Grobner, 1981). More recently, *Physarum* pre-mitotic extracts have been shown to contain a MPF activity able to trigger prophase/metaphase transition after injection in prophase-arrested *Xenopus*

oocytes, whereas post-mitotic extracts (S-phase, since there is no G_1-phase in *Physarum*) contain an inhibitory activity (Adlaka *et al.* 1988). Furthermore, a homolog of $p34^{cdc2}$ has recently been immunologically identified in *Physarum* (Shipley and Sauer, 1989), using antibodies against a consensus conserved sequence (called 'PSTAIR').

In this study, using the universal binding property of $p13^{suc1}$ to $p34^{cdc2}$, we affinity-purified the $p34^{cdc2}$ kinase activity from *Physarum* and we studied its regulation during the cell cycle. Evidence is presented here, first, for the existence of several 'activating signals' involving microtubular components and DNA synthesis in the activation of the mitotic kinase, and second, for the occurrence of a metaphase signal leading to the inactivation of this kinase.

Materials and methods

Physarum *strains, culture and microscopy*

Plasmodia (strain CL) were cultured and prepared as described by Ducommun and Wright (1989). Synchronous giant plasmodia (approximately 10 cm diameter), grown at 22°C except when stated otherwise, were used before or after the third synchronous mitosis. Plasmodial fragments were taken at intervals during the cell cycle and flash-frozen in liquid nitrogen. The timing of the mitotic events was followed on plasmodial smears taken at intervals and observed by phase-contrast microscopy (Zeiss Axiophot with a ×63 objective, ×2 Optovar and a ×4 videolens). Images were recorded with a Lhesa camera (Pasecom) and treated with an image processing system (Sapphire from Quantel) by integrating 200 frames and applying histogram and stretch functions. Screen pictures were taken with a Nikon 35 mm camera (macro-50 mm lens).

p13–Sepharose precipitation

p13 (*S. pombe* $suc1^+$ gene product) was purified from a bacterial expression system as described by Brizuela *et al.* (1987) and coupled to Sepharose (Pharmacia) following the manufacturer's instructions with 5 mg of purified p13 per ml of unpacked beads. Frozen fragments of *Physarum* were thawed and sonicated three times for 10 s (Branson sonicator) in 0.2 ml of buffer I (25 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, 15 mM EGTA (ethyleneglycol-bis-*N,N,N',N'*-tetraacetic acid), 0.1 mM sodium fluoride, 60 mM β-glycerophosphate, 15 mM para-nitrophenylphosphate, 0.1 mM sodium orthovanadate and 0.1 % Triton X-100) kept in ice. Sodium deoxycholate and SDS were added to final concentrations of 0.5 and 0.1 % (w/v), respectively, and after 5 min of incubation on ice, 800 μl of buffer I was added. The soluble protein fraction was recovered by centrifugation for 10 min at 11 000 *g*. After a 30 min preincubation at 4 °C with 30 μl Sepharose CL6B (Pharmacia) and a 10 min centrifugation at 11 000 *g*, to remove any non-specific precipitate, specific precipitations were carried out by incubating the lysate with 30 μl of p13–Sepharose for at least 4 h at 4 °C on a rotator. The complexes were brought down by a 3 min centrifugation at 2000 revs min^{-1} in a Sorvall RT6000B table-top centrifuge. The pellets were then washed three times in buffer II (50 mM Tris–HCl, pH 7.4, 250 mM NaCl, 50 mM sodium fluoride, 5 mM EDTA (ethylenediaminetetraacetic acid), 0.1 mM sodium orthovanadate and 0.1 % Triton X-100) with the same conditions of centrifugation. The final pellets were subsequently treated as described below to assay the kinase activity.

Protein concentration was determined according to the method of Bradford (1976) using the Biorad reagent and bovine serum albumin (BSA) as standard. In most experiments, 500 μg of total protein was used for each determination, except for the experiment shown in Fig. 1 in which 2.5 mg of protein was used for each determination. The following inhibitors of proteases were added to buffers I, II and III: 0.1 mM-PMSF (phenylmethylsulfonyl fluoride), 1 μg ml^{-1} leupeptin, 10 μg ml^{-1} soybean trypsin inhibitor, 1 μg ml^{-1} aprotinin and 10 μg ml^{-1} TPCK (tosyl phenylalanine chloromethyl ketone).

In vitro *phosphorylation and kinase activity*

Proteins precipitated by p13–Sepharose beads were washed (see below) and each sample was equilibrated by an extra wash in kinase assay buffer (50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT (dithiothreitol)). Pellets were resuspended in 30 μl of the same buffer with or without 83 μg ml^{-1} of histone H1 (Boerhinger-Mannheim). When simian virus 40 (SV40) large T antigen was used (kindly provided by Duncan McVey), 0.2 μg T antigen was added to each reaction. After 5 min of equilibration at assay temperature, 10 μl of kinase assay buffer containing 2 μM cold ATP and 5 μCi of [γ-^{32}P]ATP (NEN, 3000 Ci $mmol^{-1}$) was added to each sample. The kinase reaction was carried out for 15 min for histone H1 kinase assays or for 30 min for phosphorylation of the associated proteins in the absence of histone H1. The reactions were stopped by addition of 10 μl of Laemmli sample buffer (Laemmli, 1970) and was quantitated by spotting 15 μl of the reaction mixture on Whatmann 3MM paper followed by TCA (trichloracetic acid) precipitation. The paper was incubated first for 10 min in 10 % (w/v) TCA containing 40 mM sodium pyrophosphate, then washed three times for 10 min in 5 % (w/v) TCA and briefly rinsed in cold (−20 °C) ethanol. Quantitation was done using the AMBIS beta scanner system but can also be done by liquid scintillation. When cdc2-associated protein phosphorylation was examined, the reaction was stopped with 30 μl Laemmli sample buffer, the samples were boiled for 3 min and submitted to electrophoresis according to the method of Laemmli (1970). The gels were then dried and autoradiographed with film X OMAT R (Kodak).

Results

Cell cycle variation of mitotic kinase activity

Synchronous plasmodia, prepared as described in Materials and methods, were collected at intervals during the cell cycle. The stage of the cell cycle was determined by monitoring the nuclear morphology and the occurrence of the different stages of mitosis by phase-contrast microscopy (Fig. 1A). Histone H1 kinase activity was quantitated after precipitation using p13–Sepharose. The level of kinase activity was very low during interphase and only started to rise 30 min before metaphase; it was maximal in metaphase and then dropped very abruptly (Fig. 1B).

It has been demonstrated that phosphorylation of the SV40 large T antigen by $p34^{cdc2}$ kinase activates the initiation of viral DNA replication *in vitro* (McVey *et al.* 1989). Since, in *Physarum*, in the absence of G_1-phase, S-phase initiation occurs at the end of mitosis (3 min after the metaphase/anaphase transition; Beach *et al.* 1980), we investigated the presence of a kinase activity phosphorylating the T antigen in mitotic extracts. Phosphorylation of large T antigen (Fig. 1C) was assayed after p13 precipitation from the same *Physarum* extracts used for determining histone H1 kinase activity (Fig. 1B). A cell cycle variation of kinase activity was observed with a mitotic peak and little activity during interphase (Fig. 1C).

A more accurate determination of the timing of the histone H1 kinase activation during mitosis was obtained by harvesting parts of the same plasmodium at short intervals before and after mitosis and monitoring the stage of mitosis each time. Fig. 2 shows two independent determinations of histone H1 kinase variation in two different plasmodia after p13 precipitation. The peak of histone H1 kinase activity is an early metaphase event, and the level of kinase activity starts to decrease consistently before the occurrence of anaphase (Fig. 2A and B). When the kinase was assayed in the same conditions in the absence of exogenous added substrate, a 60K ($K=10^3 M_r$) band was heavily phosphorylated at the beginning of metaphase, suggesting that it could be a *Physarum*

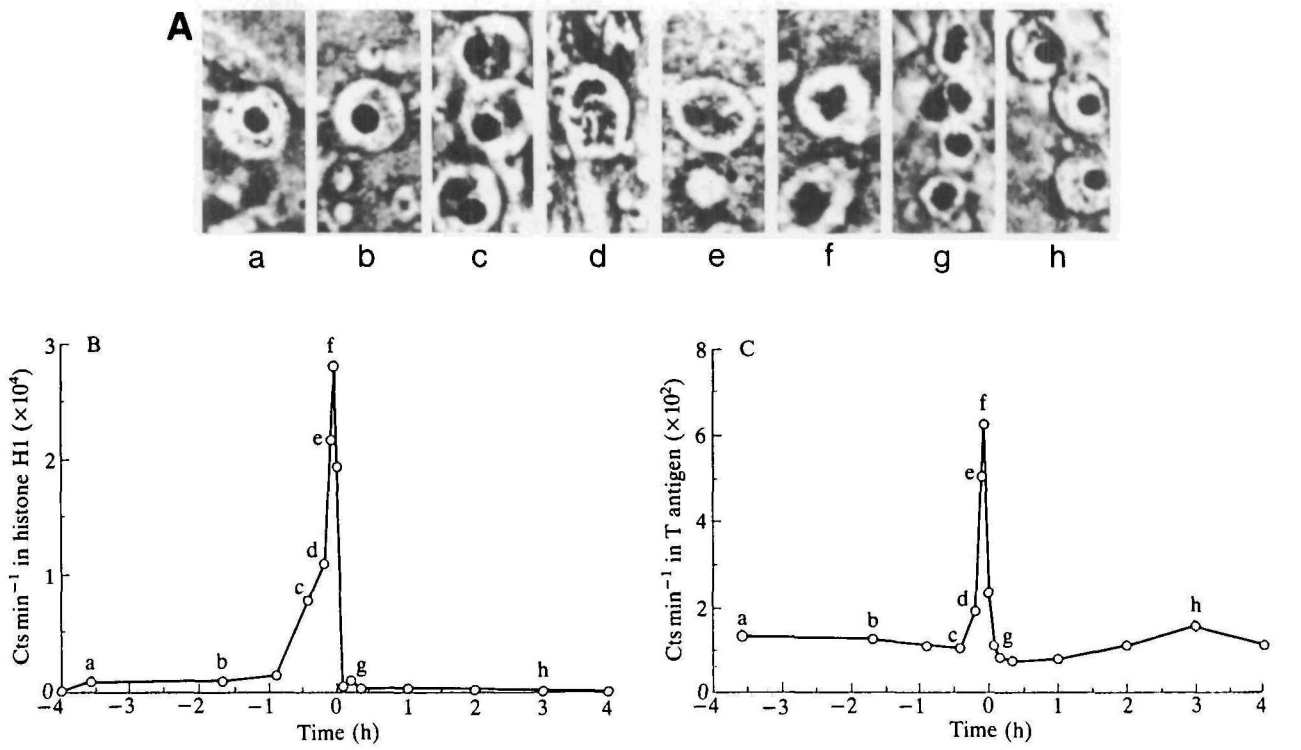

Fig. 1. Cell cycle variations of P34^{cdc2} kinase activity. *Physarum* plasmodia grown at 22 °C were collected at different times in the cell cycle. A. The stages of the cell cycle were determined for each sample by phase-contrast microscopy relative to the third synchronous mitosis. The photographs show the nucleus stage (×2200). During the cell cycle the overall size of the nucleus increases (a–e). At the end of G_2-phase the nucleolus is off center (c,d) and then disappears when the nucleus enters prophase (e). Metaphase stage is typically characterized by metaphasic plate (f). Early S-phase shows small nuclei with condensed chromatin (g) that return to the typical interphase stage after 2 h (h). After p13–Sepharose precipitation of 2.5 mg of proteins of each time point, the kinase activity was assayed using histone H1 (B) or large T antigen (C) as substrates.

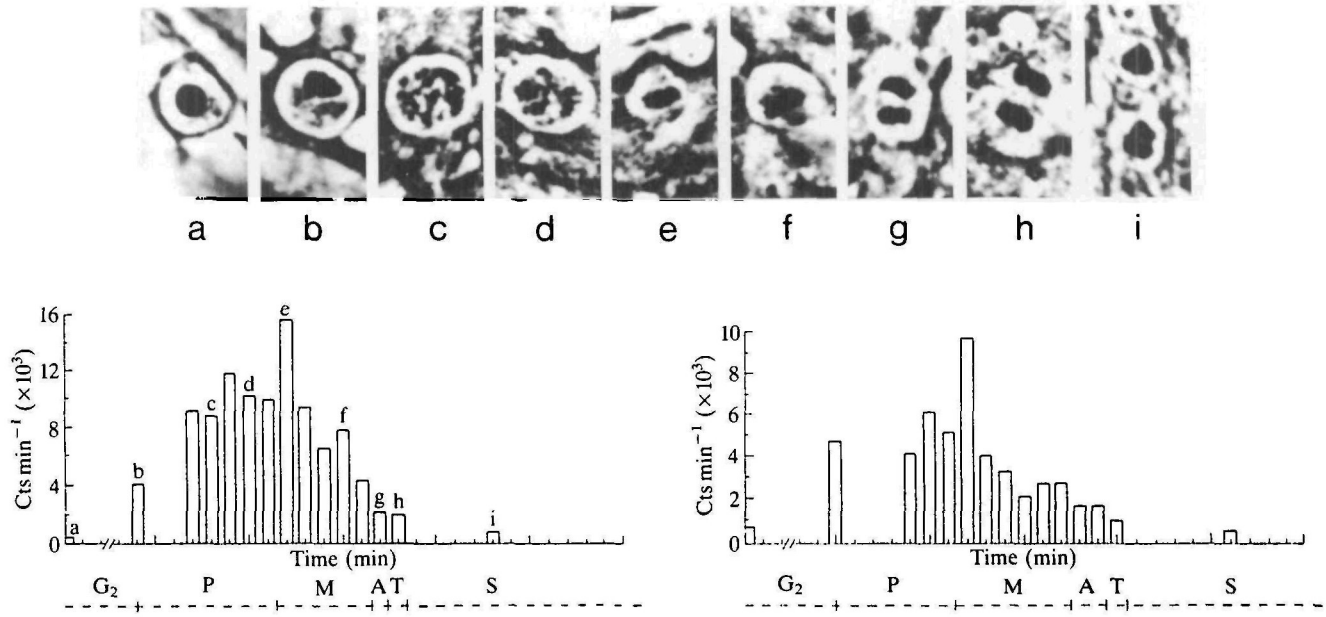

Fig. 2. Variation of histone H1 kinase during mitosis. Fragments of the same plasmodium grown at 22 °C were collected at intervals during the different phases of mitosis indicated on the broken line under each graph (G_2-phase (G_2), prophase (P), metaphase (M), anaphase (A), telophase (T) and S-phase (S)). The histone H1 kinase activity was determined after p13–Sepharose precipitation of 500 μg of protein lysate. Data from two independent plasmodia are shown here. The top line shows the nuclear aspect observed by phase-contrast microscopy for some of the typical stages of mitosis (×2200): G_2-phase 90 min before mitosis (a), early prophase with off-center nucleolus (b), prophase with punctuated aspect of the chromatin (c,d), metaphase plates (e,f), anaphase (g), telophase (h), and early S-phase (i).

cyclin homolog (not shown). Because of the lack of antibodies recognizing cyclin in *Physarum*, we were not able to confirm this hypothesis.

Effects of microtubule poisons

Methyl benzimidazole carbamate (MBC) and griseofulvin, two chemically unrelated microtubular poisons, have been previously shown to perturb the mitotic microtubules of *Physarum* plasmodia (Wright *et al.* 1976; Gull and Trinci, 1974). When a synchronous plasmodium is transferred during G_2-phase to a medium containing 100 μM MBC or 20 μM griseofulvin, protein, DNA and RNA syntheses are virtually unaffected, while the occurrence of the following mitosis is delayed by several hours (Eon-Gerhardt *et al.* 1981*a*). Previous studies have shown that such treatments did not delay the occurrence of periodic tubulin synthesis. In a control plasmodium, tubulin synthesis starts 4 h before mitosis and stops abruptly during this stage. In a treated plasmodium, tubulin synthesis begins as in the control plasmodium (Ducommun *et al.*, unpublished data), even though mitosis is delayed. By contrast, the periodic synthesis of thymidine kinase is delayed (Eon-Gerhardt *et al.* 1981*a*).

The effects of microtubule poisons on $p34^{cdc2}$ kinase activity were investigated. The principle of such experiments consists of transferring a synchronous plasmodium to a medium containing the drug, keeping a small part on untreated medium, and harvesting portions of the treated plasmodium during the subsequent cell cycle. A synchronous plasmodium was transferred 7 h before mitosis to a medium containing 200 μM griseofulvin (Fig. 3A) or 5.5 h before mitosis to a medium containing 100 μM MBC (Fig. 3B). Mitosis was delayed by 2.75 h and 4.5 h, respectively. In both cases, the histone H1 kinase activity was not triggered at the time of the control mitosis, but occurred only at the onset of the treated mitosis (Fig. 3A and B). Therefore, the activation of the histone H1 kinase activity seems to require an event involving the formation of the normal microtubular mitotic cytoskeleton. Upon treatment with MBC an abnormal mitosis occurred (Planques *et al.* 1989). Metaphase persisted for one to two hours with the formation of multipolar spindles, polyasters and condensed chromosomes (see stages d–e, Fig. 3B). The kinase activity decreased before the end of this abnormal metaphase stage, thus suggesting that total completion of metaphase is not necessary to turn off the activation of the $p34^{cdc2}$ kinase.

Effects of aphidicolin

Aphidicolin, an inhibitor of DNA polymerases α and δ, has been shown to be active in *Physarum polycephalum*. When used at a concentration of 200 μM it inhibits DNA synthesis (73 %) whereas RNA and protein syntheses are not affected (Eon-Gerhardt *et al.* 1981*b*). When a plasmodium is treated with aphidicolin up to 3 h before metaphase (from S-phase to mid G_2-phase), mitosis is delayed several hours and the nuclei are blocked in early prophase (Eon-Gerhardt *et al.* 1981*b*).

To investigate the effect of such treatment on the histone H1 kinase activity, a synchronous plasmodium was transferred 4.25 h before the third synchronous mitosis to a medium containing 200 μM aphidicolin (Fig. 4). The occurrence of mitosis in the treated plasmodium was delayed 6.3 h compared to the control (untreated) part of the same plasmodium. The peak of histone H1 kinase activity did not occur at the same time as that of the control mitosis, but was delayed until the occurrence of metaphase in the treated plasmodium.

Effects of protein synthesis inhibition

We investigated the effect of protein synthesis inhibitors

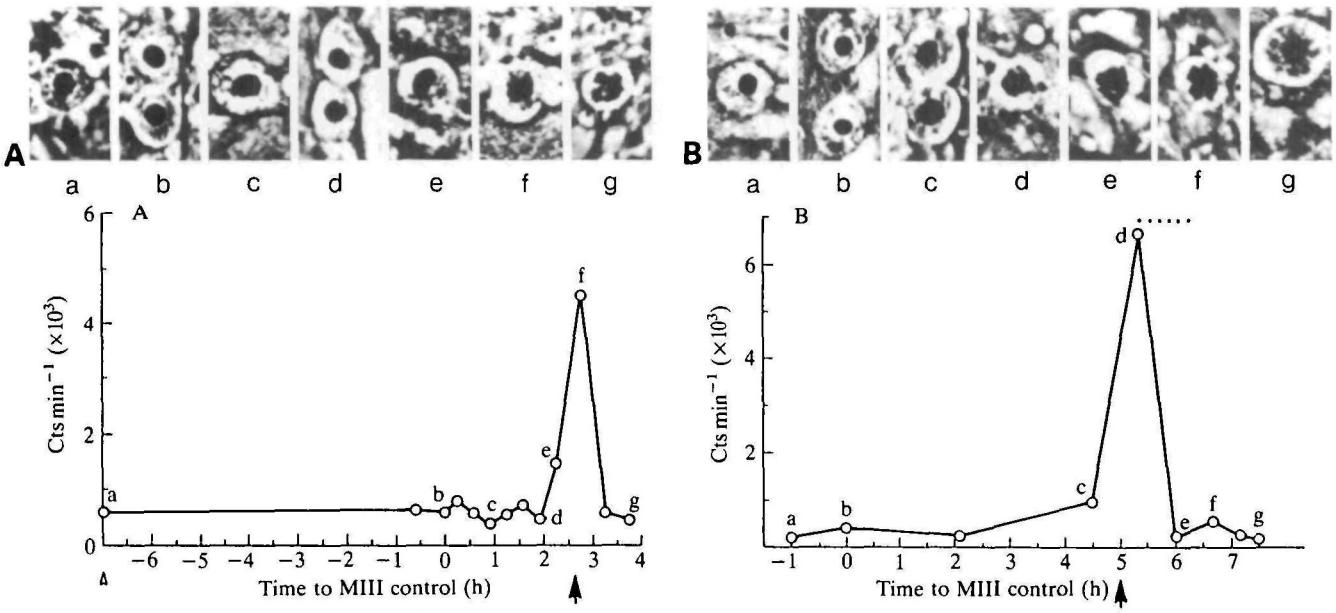

Fig. 3. Effects of treatment with microtubule inhibitors on histone H1 kinase. Variation of histone H1 kinase activity in p13 precipitates from fragments of synchronous plasmodia transferred onto 200 μM griseofulvin 7 h before the control metaphase (A) or 100 μM MBC 5.5 h before the control mitosis (B). The treated mitoses were delayed, respectively, by 2.75 h (A) and 4.5 h (B) (indicated by a black arrow in each panel). The photographs show the morphological aspect of the nuclei observed by phase-contrast microscopy (×2200). A. Plasmodium treated with 200 μM griseofulvin: G_2-phase (a–d), early prophase (e), metaphase (f) and early S-phase (g). B. Plasmodium treated with 100 μM MBC: G_2 phase (a–c), 'condensed chromosomes' (d,e) and S-phase (f,g). The dotted line in B indicates the duration of the condensed chromosomes stage of mitosis treated with MBC. Dimethyl sulfoxide used to dissolve these microtubule poisons does not affect the timing of mitosis, or the timing and the extent of this histone H1 kinase activity (not shown). These results are representative of two independent experiments.

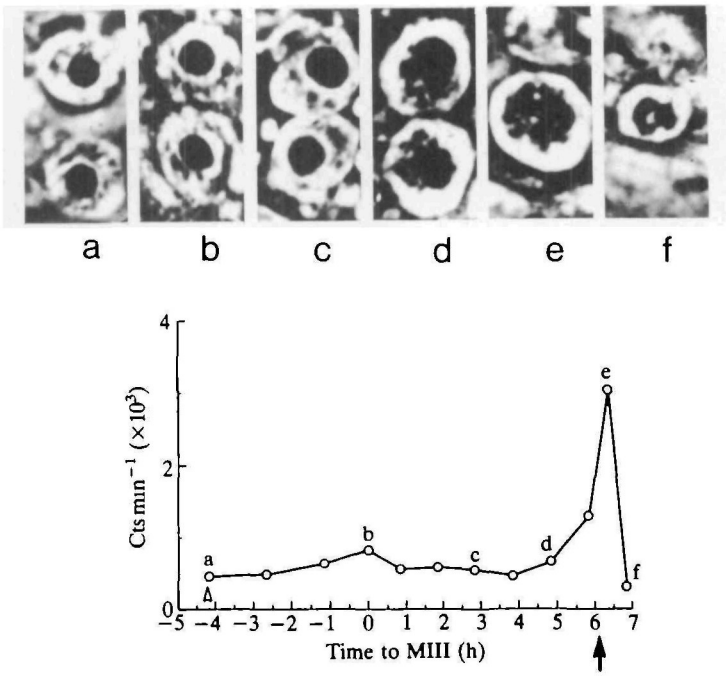

Fig. 4. Effects of treatment with aphidicolin on histone H1 kinase. Variation of histone H1 kinase activity in p13–Sepharose precipitates from fragments of synchronous plasmodium transferred onto 200 μM aphidicolin 4.25 h before metaphase (indicated by an open arrowhead). In this experiment the treated mitosis (indicated by a black arrow) was delayed by 6.3 h. The photographs show the morphological aspect of the nuclei observed by phase-contrast microscopy (×2200): G_2-phase (a–c), early prophase (d), prophase (e) and early S-phase (f).

on the activation as well as on the inactivation of the histone H1 kinase activity. A synchronous plasmodium was transferred 45 min before the third metaphase on a medium containing 150 μM cycloheximide (Fig. 5A). As previously reported, this treatment completely inhibited protein synthesis (Cummins *et al.* 1965). The treated part of the plasmodium never underwent mitosis, and the histone H1 kinase activity stayed low. When a similar treatment was applied to a synchronous plasmodium 0.5 h before the third metaphase, the nuclei entered a metaphase-like stage (Fig. 5B), in which they remained blocked for 2–3 h (Ducommun and Wright, 1989). Under these conditions, the kinase activity was activated and remained at a plateau for 2 h before it declined progressively as nuclei were asynchronously progressing through metaphase and anaphase (Fig. 5B).

Discussion

We have taken advantage of the absolute synchrony of *Physarum* plasmodia to determine accurately the timing of p34^{cdc2} kinase activation and inactivation during the cell cycle and to define their relationship with other cell cycle-regulated pathways.

In *Physarum* synchronous plasmodia, the activation of the kinase occurs before metaphase and requires protein synthesis as shown here (Fig. 5A) and as previously reported in different systems (Hunt, 1989). One hour before metaphase (Fig. 1B), the p34^{cdc2} kinase activity is low and similar to the overall interphase level in late G_2-phase, then the kinase activity increases during prophase. The maximal activity is observed in early metaphase and decreases abruptly thereafter. Among the various cell cycle events (Tyson, 1982) that have been reported to occur in the synchronous plasmodia of *Physarum*, the cyclic increase of two nuclear histone H1 kinase activities has

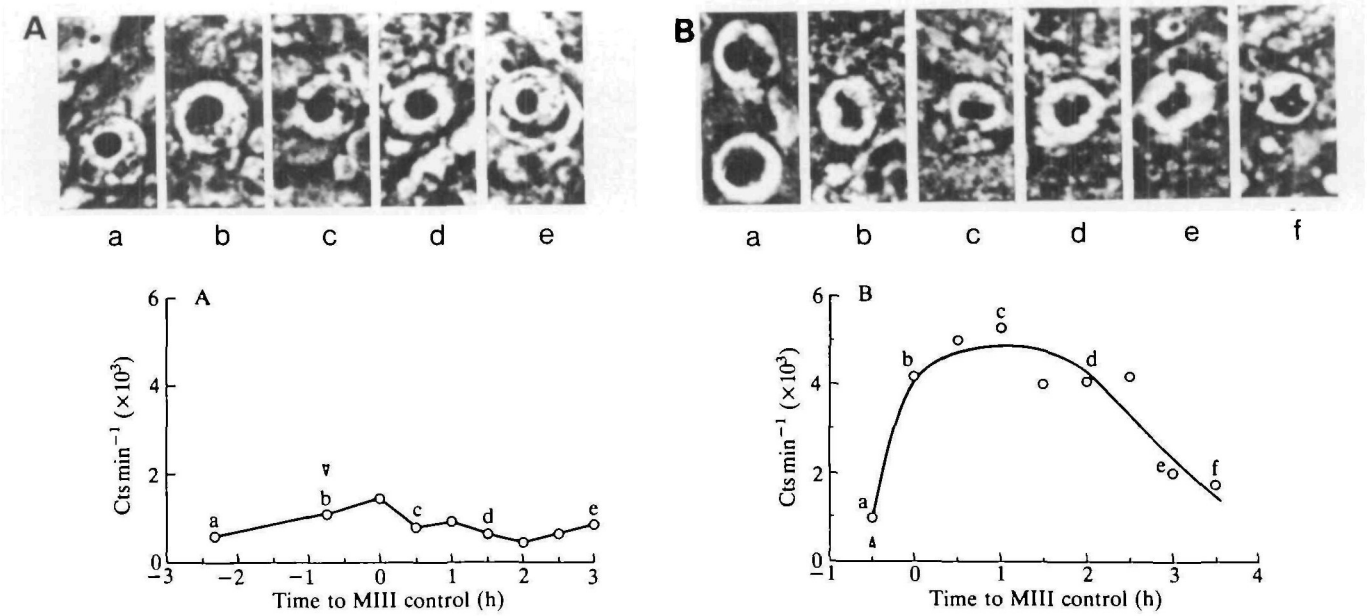

Fig. 5. Effects of treatment with cycloheximide on histone H1 kinase. Variation of histone H1 kinase activity in p13 precipitates from fragments of synchronous plasmodia transferred onto 150 μM cycloheximide 45 min (A) or 30 min (B) before mitosis (indicated by an open arrowhead). A. In the treated portion of the plasmodia the treated mitosis never occurred and the histone H1 kinase determined during 3 h remained at its basal level. B. The nuclei entered mitosis and were blocked in a 'metaphase-like' stage for 2–3 h. The photograph shows the morphological aspect of the nuclei observed by phase-contrast microscopy (×2200) at the indicated times. A. Cycloheximide treatment beginning 45 min before metaphase: G_2-phase (a–e). B. Cycloheximide treatment beginning 30 min before metaphase: early prophase (a), metaphase-like (b–e), anaphase (f).

already received extensive interest (Bradbury *et al.* 1973; Hardie *et al.* 1976). It has been suggested that these nuclear histone H1 kinases could play a role in both histone H1 phosphorylation and chromosome condensation (Bradbury *et al.* 1974; Matthews, 1980). However, these two nuclear histone H1 kinase activities and the $p34^{cdc2}$ kinase activity described in this report do not show a similar timing during the cell cycle. In contrast to the overall variations of $p34^{cdc2}$ kinase activity in plasmodial extract, the two histone H1 kinase activities measured in plasmodial nuclei began to increase at least 3 h before mitosis and reach their maximal values 1 and 2 h before mitosis, respectively, when the $p34^{cdc2}$ kinase was at its low basal level. The two nuclear histone kinase activities decreased thereafter and were very low during mitosis (Hardie *et al.* 1976). These differences raise the possibility that the nuclear histone H1 kinases that have been previously characterized and the $p34^{cdc2}$ histone H1 kinase activities could correspond to distinct kinases.

In order to determine whether the timing of the increase or decrease of $p34^{cdc2}$ kinase activity is correlated with a cell cycle event, we have applied several perturbations to the synchronous plasmodia and determined their effects on the $p34^{cdc2}$ activity.

Treatment with aphidicolin delays mitosis and blocks the nucleus in early prophase (Eon-Gerhardt *et al.* 1981*b*). During this delay the histone H1 kinase is not activated (Fig. 4). This result demonstrates that entry of the nuclei into a very early prophase stage and H1 kinase activation are at least partially independently regulated, but that the activation of the enzyme between prophase and metaphase requires an activating signal indicating completion of DNA synthesis. The existence of such an effect of aphidicolin in G_2-phase has also been reported in fibroblasts (Fukuda and Ohashi, 1983), suggesting a requirement for DNA polymerase α or δ late in G_2-phase.

Among the different microtubular poisons used in various studies, some have been shown to act *in vivo* on tubulin by forming abnormal microtubule-like structures. In mammalian cells, vinblastine forms paracrystallin structures (Bryan, 1971) and, in *Physarum*, MBC and griseofulvin induce the assembly of tubulin into abnormal 'macrotubules' (Wright *et al.* 1976; Gull and Trinci, 1974). In both systems these drugs act by decreasing the level of free tubulin available and consequently increase tubulin synthesis (Ben-Ze'ev *et al.* 1979; Ducommun *et al.*, unpublished data). On the other hand, drugs like nocodazole and colchicine act *in vivo* on mammalian cells by disassembling microtubules and raising the level of free tubulin, which induces a decrease in tubulin synthesis (Ben-Ze'ev *et al.* 1979; Caron and Kirschner, 1986).

In mammalian cells nocodazole induces mitotic arrest with a high level of cdc2 kinase activity (Draetta and Beach, 1988) and this property has been used as a method of increasing the yield of $p34^{cdc2}$ in enzyme purification (Brizuela *et al.* 1989). In *Physarum* (this study), griseofulvin and methyl benzimidazole carbamate delay both mitosis and the triggering of mitotic kinase activity. Thus, different drugs that interfere with microtubules have quite opposite effects, even though each is a potent inhibitor of *in vitro Physarum* tubulin assembly (Quinlan *et al.* 1981).

A resolution of this discrepancy might be that those drugs that inhibit cdc2 activation (e.g. MCB in *Physarum*) sequester tubulin, decreasing the level of functional protein. By contrast, in the case of treatment with nocodazole in mammalian cells, cdc2 is fully activated but the drug acts to disassemble microtubules and raise the level of free tubulin. The present results do suggest that interference with microtubules can inhibit activation of cdc2.

In untreated plasmodia the kinase activity is maximal in early metaphase (Fig. 1) and the histone H1 kinase starts to decline when nuclei are still in metaphase. When a plasmodium is treated with MBC, metaphase is abnormal and lengthened, but the kinase activity declines before completion of this stage. Thus, it is likely that both in untreated plasmodia and plasmodia treated with MBC the signal leading to the inactivation of histone H1 kinase occurs during metaphase and not after completion of metaphase. Cycloheximide treatment of plasmodia in late G_2-phase, blocks the nucleus in a 'metaphase-like' stage (Ducommun and Wright, 1989). During this period, the level of histone H1 kinase activity stays high, suggesting that in this organism the signal permitting kinase inactivation requires protein synthesis. It has been suggested that cyclin degradation triggers the inactivation of the kinase activity (Draetta *et al.* 1989; Murray and Kirschner, 1989*b*) and we presume that protein synthesis is required for cyclin degradation in *Physarum*. This study graphically illustrates the highly asymmetric nature of kinase activation and inactivation. One occurs gradually by post-translational modification of pre-existing components (cyclin/cdc2), whereas the other is due to proteolytic cyclin degradation and is very abrupt.

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