MOLECULAR AND CELLULAR BIOLOGY, Sept. 1989, p. 3860–3868
0270-7306/89/093860-09$02.00/0

Vol. 9, No. 9

Mammalian Growth-Associated H1 Histone Kinase: a Homolog of *cdc2*$^+$/*CDC28* Protein Kinases Controlling Mitotic Entry in Yeast and Frog Cells

THOMAS A. LANGAN,[1]* JEAN GAUTIER,[1] MANFRED LOHKA,[1]† ROBERT HOLLINGSWORTH,[2] SERGIO MORENO,[3] PAUL NURSE,[3] JAMES MALLER,[1] AND ROBERT A. SCLAFANI[2]

Department of Pharmacology[1] and Department of Biochemistry, Biophysics, and Genetics,[2] University of Colorado School of Medicine, Denver, Colorado 80262, and ICRF Cell Cycle Control Laboratory, Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, England[3]

Received 4 April 1989/Accepted 1 June 1989

Mammalian growth-associated H1 histone kinase, an enzyme whose activity is sharply elevated at mitosis, is similar to *cdc2*$^+$ protein kinase from *Schizosaccharomyces pombe* and *CDC28* protein kinase from *Saccharomyces cerevisiae* with respect to immunoreactivity, molecular size, and specificity for phosphorylation sites in H1 histone. Phosphorylation of specific growth-associated sites in H1 histone is catalyzed by yeast *cdc2*$^+$/*CDC28* kinase, as shown by the in vitro thermal lability of this activity in extracts prepared from temperature-sensitive mutants. In addition, highly purified *Xenopus* maturation-promoting factor catalyzes phosphorylation of the same sites in H1 as do the mammalian and yeast kinases. The data indicate that growth-associated H1 kinase is encoded by a mammalian homolog of *cdc2*$^+$/*CDC28* protein kinase, which controls entry into mitosis in yeast and frog cells. Since H1 histone is known to be an in vivo substrate of the mammalian kinase, this suggests that phosphorylation of H1 histone or an H1 histone counterpart is an important component of the mechanism for entry of cells into mitosis.

Among the protein phosphorylation reactions which occur in eucaryotic cells, one of the most prominent is the extensive phosphorylation of H1 histone which takes place during the traverse of the cell cycle. The phosphorylation of H1 histone in growing cells is catalyzed by a Ca^{2+}- and cyclic nucleotide-independent protein kinase, termed growth-associated H1 kinase, whose activity fluctuates throughout the cell cycle in a manner suggesting an involvement in cell cycle control (5, 37, 38, 71, 72). Phosphorylation of H1 histone follows these changes in kinase activity, beginning in late G1 phase, continuing through the S and G2 phases, and rising sharply as cells enter mitosis, after which it falls abruptly (see reference 47 for a review). The sharp elevation of growth-associated H1 phosphorylation and H1 kinase activity as cells enter mitosis led to proposals by Bradbury and others that it triggers or promotes the onset of mitotic chromosome condensation (7, 26, 45). In support of this proposal, it was observed that ingestion of crude preparations of mammalian growth-associated H1 kinase by plasmodia of the slime mold *Physarum polycephalum* advanced the onset of the synchronous waves of mitosis which occur in this organism (6, 32).

Mammalian growth-associated H1 kinase is found only in growing cells, where it is partially bound to chromatin (37, 39, 72). The enzyme catalyzes the phosphorylation of specific serine and threonine residues in H1 histone, with stoichiometry varying from three to six phosphates per molecule, depending on H1 subtype (40). This corresponds to the level of phosphate found in H1 in mitotic cells (24). The sites phosphorylated by the enzyme in vivo and in vitro are localized in the basic carboxyl- and amino-terminal domains of H1 (39, 40, 41), which are the regions of H1 involved in the condensation of chromatin (1). Four of the phosphorylation sites in H1 have been characterized as serine or threonine residues occurring in the consensus sequence Lys-Ser/Thr-Pro-Lys or Lys-Ser/Thr-Pro-X-Lys (39, 42). A protein kinase catalyzing the phosphorylation of these sites in mammalian H1 histone is also present in *P. polycephalum* (14) and *Xenopus* oocytes (46), findings that gave an early indication that this type of enzyme might have a highly conserved function in the mechanism of chromatin condensation.

Extensive studies on the control of cell cycle progression have been carried out in the budding and fission yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively. In each organism, a large number of genes required for cell cycle progression has been identified. In *S. pombe*, the gene *cdc2*$^+$ is required at two points in the cell cycle, first at a point called the start point in late G1, which commits the cell to a round of cell division, and second at a point just prior to entry into mitosis (51, 52). In *S. cerevisiae*, the gene *CDC28* exerts similar control both at the start (56) and at entry into mitosis (54). *CDC28* and *cdc2*$^+$ encode 34-kilodalton (kDa) proteins which are functionally equivalent, since each of these genes can complement mutations in the native gene when introduced into the other species (3, 4). A homolog of *cdc2*$^+$/*CDC28* is also present in the human genome which encodes a protein of 63% identity to the *S. pombe* gene product and which can complement mutations in the *S. pombe cdc2*$^+$ gene (43). Thus, it seems likely that this gene is present in all eucaryotic cells. *CDC28*, *cdc2*$^+$, and the human homolog encode products which have protein kinase activity (16, 59, 61). Recently it has been shown that a protein kinase component of purified *Xenopus* maturation-promoting factor (MPF) and protein kinases present in starfish and clam oocytes, each of which catalyzes in vitro H1 phosphorylation and is associated with the initiation of meiotic or mitotic division, are encoded by homologs of the

* Corresponding author.
† Present address: Department of Cellular and Structural Biology, University of Colorado School of Medicine, Denver, CO 80262.

cdc2$^+$/*CDC28* gene (2, 17, 19, 21, 36). These findings provide evidence that this type of protein kinase participates in a universal mechanism for control of entry of eucaryotic cells into mitosis.

The increase in activity of mammalian growth-associated H1 kinase at mitosis occurs at the time in the cell cycle when the *cdc2*$^+$/*CDC28* gene product is required in yeasts. However, H1 histone has not yet been found in yeasts, despite studies which have clearly identified nucleosome core histones in *S. cerevisiae* (9, 31, 66). If yeasts do contain an H1 histone, it seems possible that they might also contain a growth-associated H1 kinase which, like the *Physarum* enzyme, retains specificity for growth-associated phosphorylation sites in mammalian H1. Here we report that the major H1 kinase activity in yeasts is similar to mammalian growth-associated H1 kinase in its specificity for phosphorylation sites in H1 histone and that this yeast H1 kinase is encoded by the *cdc2*$^+$/*CDC28* gene. Further, the mammalian enzyme is specifically immunoprecipitated by antibody to the *cdc2*$^+$/*CDC28* gene product and contains an immunoreactive 32- to 34-kDa polypeptide. These data show that growth-associated H1 kinase is encoded by a mammalian homolog of the yeast *cdc2*$^+$/*CDC28* gene. These findings provide further evidence for the participation of protein kinases encoded by *cdc2*$^+$/*CDC28* homologs in a highly conserved mechanism for entry of eucaryotic cells into mitosis or meiosis. Since H1 histone is a physiological substrate for mammalian growth-associated kinase (37–41), these findings also suggest that phosphorylation of H1 histone or an H1 histone counterpart is an important component of the mechanism for mitotic or meiotic entry.

MATERIALS AND METHODS

S. cerevisiae* strains, growth conditions, and lysates.** Strains H28C1A1 *MATa** *his7 ura1 cdc28-1*, H17C1A1 *MAT***a** *his7 ura1 cdc17-1*, M16A1 *MAT***a** *bar1 tre1-289 Can1 Sap3 cdc16-1*, and 4503-1-4 *MAT***a** *ura3 his7* were obtained from M. Carson and L. Hartwell. All are congenic with strain A364A (28) and were derived from the original mutant alleles (29, 30). Strains were grown in YEPD medium at 22°C, and cell numbers were determined as described by Sclafani and Fangman (60). Cells were harvested during log phase (2×10^7 cells/ml), spheroplasted with Zymolase 100T (ICN Pharmaceuticals Inc.) at 0.2 mg/ml in SCE (1 M sorbitol, 100 mM sodium citrate, 10 mM EDTA [pH 7.0]) at 4×10^8/ml, washed with SCE, and osmotically disrupted in PK buffer (50 mM Tris hydrochloride [pH 8.0], 50 mM NaCl, 0.1% Tween 20, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride). The protein concentration, determined by the method of Bradford (8) with immunoglobulin G as a standard, was routinely 1 to 2 mg/ml. Extracts were kept at 4°C and were used within 24 h to measure H1 histone phosphorylation.

Protein kinase assays and preparation of phosphorylated H1 histone. Reaction mixtures (100 μl) containing 50 mM Tris hydrochloride (pH 7.4), 5 mM $MgCl_2$, 1 mM dithiothreitol, 1 mg of calf thymus H1 histone per ml (reference 34, method 1), H1 kinase activity to transfer 100 to 500 pmol of phosphate, and 0.5 mM [γ-^{32}P]ATP (specific activity, 30 to 60 cpm/pmol), were incubated for 20 min at 37 or 22°C. Phosphorylated H1 was adsorbed on Whatman P81 phosphocellulose paper (22), washed three times with 75 mM phosphoric acid, and counted by Cerenkov radiation. H1 histone preparations for phosphopeptide analysis were phosphorylated under the same conditions for 60 to 100 min with 0.1 to 0.5 mM [γ-^{32}P]ATP (specific activity, 2,000 cpm/pmol). Phosphorylation by Mono S-purified MPF H1 kinase was carried out in the presence of 100 mM β-glycerophosphate and 10 mM $MgCl_2$. Phenylmethylsulfonyl fluoride (0.25 mM), was added to reaction mixtures containing yeast lysates. Reactions were terminated by the addition of trichloroacetic acid to a final concentration of 5%, and enzyme protein was removed by centrifugation. H1 histone was isolated from the supernatant by precipitation and washing with 25% trichloroacetic acid as previously described (39).

For assay of H1 kinase activity in *S. pombe*, exponentially growing *S. pombe* wild-type *972h-* or temperature-sensitive *cdc2.33h-* cells (10^8) were broken with glass beads in 20 μl of HB buffer (60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM MOPS [morpholinepropanesulfonic acid] [pH 7.2], 15 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 20 μg of leupeptin per ml, 40 μg of aprotinin per ml) containing 15 mM EGTA [ethylene glycerol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid]. The beads were washed with 0.5 ml of the same buffer, and the extract was centrifuged for 15 min at 100,000 rpm in a Beckman TL-100.2 rotor at 4°C. A 4-μl portion of extract supernatant was preincubated with 6 μl of HB buffer containing 5 mM EGTA at 25 or 40°C for 15 min. A 10-μl portion of H1 histone (2 mg/ml) (Boehringer Mannheim Biochemicals)–200 mM [γ-^{32}P]ATP (100 cpm/pmol), was added, and the reaction mixtures were incubated for 20 min at 25 or 40°C. Reactions were terminated by addition of 20 μl of 2× sample buffer.

Enzyme preparations. Mammalian growth-associated H1 histone kinase was purified from washed chromatin of log-phase Novikoff rat hepatoma cells (T. C. Chambers and T. A. Langan, manuscript in preparation). The most purified fractions contained polypeptides of 32 to 34 kDa and of 60 kDa, which are the only proteins present that fractionate with enzyme activity on sucrose density gradients. Enzyme purified through the DEAE-cellulose step of this procedure, as briefly described earlier (40), is stable for extended periods at 4°C and was used in the work reported here. These preparations catalyze transfer of approximately 0.5 μmol of phosphate per min/mg of enzyme to H1 histone. MPF was purified through the TSK column step or the Mono S step as described by Lohka et al. (44).

Phosphopeptide analysis. Chymotrypic and tryptic digestion, gel filtration, and high-voltage paper electrophoresis were carried out as described previously (39, 40), with minor modifications. Briefly, phosphorylated H1 plus 3 mg of carrier calf thymus H1 in 0.5 ml of 25 mM Tris hydrochloride, pH 8.0, was digested with 6 μg of α-chymotrypsin (Worthington Diagnostics) for 1 h at 37°C. The digests were applied to 0.9- by 190-cm columns of Sephadex G-100 and were eluted with 0.02 N HCl at a flow rate of 3 ml/h. Fractions were collected at 30-min intervals, and radioactivity and protein content were determined by Cerenkov counting and UV absorption at 218 nm. Fractions containing chymotryptic peptides were reduced to dryness in a rotary evaporator, dissolved in H_2O, and adjusted to pH 8.0 in a final volume of 40 to 100 μl with 1 M NH_4HCO_3. Digestion with 50 μg of tosyl phenylethyl chloromethyl ketone-treated trypsin (Worthington) was carried out for 1 h at 37°C, and the digests were subjected to high-voltage paper electrophoresis in 0.06 M NH_4CO_3, pH 7.9, as described previously (39, 40). Radioactive peptides were visualized by autoradiography. Phosphoamino acid analyses were carried out, after partial acid hydrolysis of peptides for 3 h at 100°C in 6 N HCl, by

TABLE 1. H1 histone kinase activity in randomly growing and mitotically arrested *S. cerevisiae*

Growth condition of cells[a]	% With following morphology[b]				H1 kinase activity[c]	Fold increase
	A	B	C	D		
Exponential growth	33	23	15	29	18.3	1.00×
Nocodazole	6	3	9	82	54.6	2.98×
cdc16, arrested at 36°C	3	1	0	96	55.2	3.02×

[a] Strain M16A1, containing a temperature-sensitive *cdc16-1* mutation, was grown to 2 × 10^7 cells per ml at 22°C. Cells were arrested in mitosis prior to nuclear migration (65) by addition of nocodazole (10 μg/ml) for 3 h and prior to medial nuclear division (56) by incubation at 36°C for 3 h.

[b] The percentage of different cellular morphologies was determined by phase-contrast microscopy at a total magnification of ×400. A, Unbudded cells; B, cells with small-sized buds; C, cells with medium-sized buds; D, cells with large-sized buds.

[c] Cell lysates were prepared and H1 kinase activity was determined as described in Materials and Methods. Nanomoles of phosphate transferred per 20 min/mg of protein at 37°C.

high-voltage paper electrophoresis in 0.87 M acetic acid–pyridine buffer, pH 3.5.

Antibody preparation and immunochemical assays. The preparation of antisera and affinity-purified antibody to the EGVPSTAIREISLLKE peptide sequence (PSTAIR antibody) (43) and immunoblotting (21) were carried out as previously described. Immunoprecipitates were prepared by using 40 to 50 μl of Novikoff hepatoma growth-associated H1 kinase DEAE-cellulose fraction or of *Xenopus* MPF TSK peak column fractions, and H1 phosphorylation by immunoprecipitates was carried out as described by Gautier et al. (21). Affinity-purified antibody for use in control experiments was preadsorbed with peptide antigen by incubation with 1 ng of peptide per μl of antibody for 1 h at 4°C.

RESULTS

H1 histone kinase activity in yeast. Lysates of randomly growing *S. cerevisiae* contained abundant H1 kinase activity (Table 1). As an initial test of the similarities between this activity and mammalian growth-associated H1 kinase, we compared the levels of H1 kinase in yeasts blocked in mitosis by two different methods with that present in randomly growing cells. *S. cerevisiae* containing a temperature-sensitive *cdc16* mutation was arrested in mitosis either by incubation at a restrictive temperature or by addition of the drug nocodazole (65). As shown in Table 1, the specific activity of the H1 kinase in mitotically arrested yeast cells was elevated threefold, roughly comparable to the four- to sixfold elevation of this enzyme in mammalian cells blocked in mitosis (72). We next examined the phosphorylation of specific sites in H1 histone by crude yeast lysates. Figure 1A shows the pattern of chymotryptic peptides derived from H1 histone phosphorylated by mammalian growth-associated H1 kinase, as resolved by gel filtration on Sephadex G-100. Phosphorylation of H1 by this enzyme is limited to sites present in the carboxyl- and amino-terminal domains of the histone (39, 40). Chymotryptic peptides derived from H1 histone phosphorylated by crude lysates of *S. cerevisiae* are shown in Fig. 1C. The patterns of phosphorylation in the carboxyl-terminal and amino-terminal domains were similar to that seen with the mammalian enzyme; in addition, some phosphorylation of sites in the globular region occurred. Phosphoamino acid analyses of these chymotryptic peptides showed that the yeast lysates catalyze phosphorylation of both serine and threonine residues in the carboxyl and amino termini of H1, as does the mammalian enzyme. Furthermore, the increased phosphorylation of H1 catalyzed by lysates of mitotically arrested cells (Table 1) occurred spe-

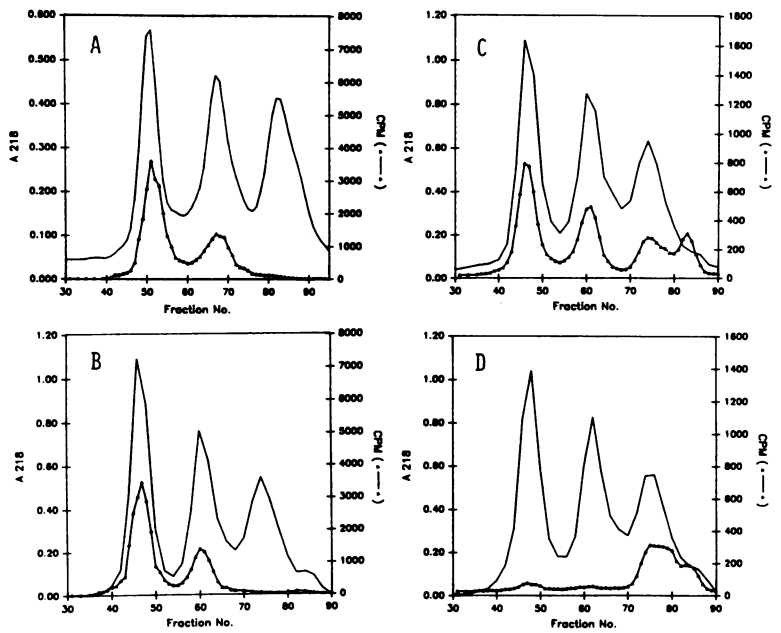

FIG. 1. Gel filtration of chymotryptic peptides derived from phosphorylated H1 histone. Peptides derived from H1 phosphorylated by mammalian growth-associated H1 kinase (A), by enzymatically active immunoprecipitates of the mammalian kinase formed with PSTAIR antibody to the yeast *cdc2*$^+$/*CDC28* gene product (B), by crude lysates of a control *cdc17-1* yeast strain (C), and by crude lysates of a temperature-sensitive *cdc28-2* mutant yeast strain (D) were resolved on Sephadex G-100. H1 histone was phosphorylated at 37°C for 1 h. Reaction mixtures containing yeast lysates were preincubated for 1 h at 42°C in the absence of histone and ATP before phosphorylation of H1, in order to ensure inactivation of temperature-sensitive activity. The peaks represent, in order of elution, chymotryptic fragments derived from the carboxyl-terminal, amino-terminal, and globular domains of H1 histone (12, 40).

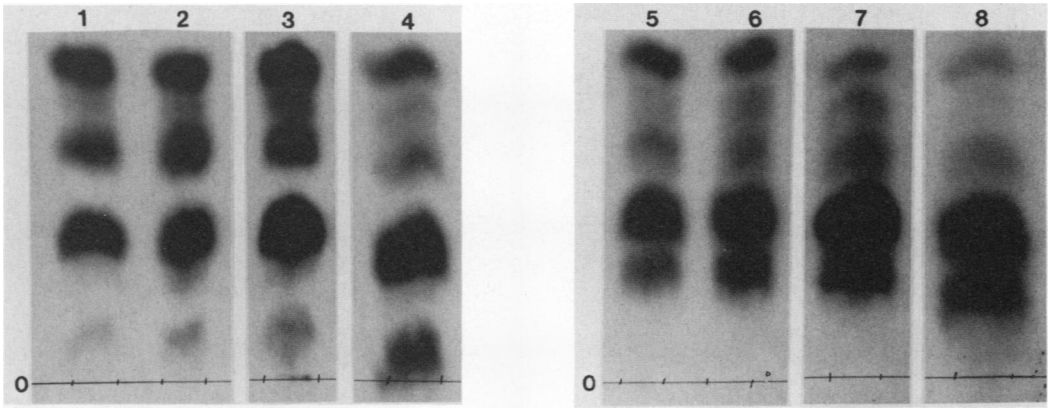

FIG. 2. Tryptic phosphopeptides derived from the carboxyl- and amino-terminal domains of H1 histone phosphorylated by rat, frog, and yeast kinases. Chymotryptic fragments from the carboxyl terminus (lanes 1 through 4) and amino terminus (lanes 5 through 8) of H1 histone phosphorylated by various kinase preparations (see Fig. 1 and 6) were digested with trypsin, and the digests were subjected to high-voltage paper electrophoresis at pH 7.9. Phosphopeptides from H1 phosphorylated by mammalian growth-associated H1 kinase (lanes 1 and 5), by *Xenopus* MPF H1 kinase (lanes 2 and 6), by crude *S. cerevisiae cdc17-1* lysates (lanes 3 and 7), and by enzymatically active immunoprecipitates of the mammalian kinase formed with PSTAIR antibody to the yeast *cdc2*$^+$/*CDC28* gene product (lanes 4 and 8) were visualized by autoradiography. O, Origin. The pattern of phosphopeptides in samples phosphorylated by immunoprecipitates has been shifted down somewhat due to an unusually large amount of electroendosmosis during electrophoresis.

cifically in the carboxyl- and amino-terminal regions of H1 (not shown). In order to further compare the phosphorylation of the carboxyl- and amino-terminal domains of H1 catalyzed by the mammalian and yeast kinases, tryptic digests of the chymotryptic peptides derived from these regions were analyzed by high-voltage paper electrophoresis. The patterns of tryptic phosphopeptides derived from each of these regions of H1 phosphorylated by the mammalian and yeast kinases are qualitatively very similar (Fig. 2, lanes 1, 3, 5, and 7), indicating that the sites phosphorylated by both enzymes are the same. Therefore yeasts contain a protein kinase which is similar to mammalian growth-associated H1 kinase with respect to both elevated activity in mitotic cells and phosphorylation of specific sites in H1 histone.

Temperature-sensitive H1 kinase activity. The *S. cerevisiae* gene *CDC28* encodes a serine-threonine protein kinase (59) that is required at two points in the cell cycle, at the start (56) and at entry into mitosis (54). These points correspond to the points at which growth-associated H1 kinase begins its action and then sharply increases its activity in mammalian cells. We therefore compared the H1 kinase activity in lysates of a temperature-sensitive mutant, *cdc28-1*, to the wild type and to a control yeast strain, *cdc17-1*. When assayed at a restrictive temperature, the specific activity of *cdc28-1* lysates was reduced to 20 to 25% of wild-type and control values (not shown), indicating that the *CDC28* gene encodes a protein kinase with activity towards H1 histone. Figure 1C and D shows the pattern of phosphorylation among chymotryptic peptides derived from H1 phosphorylated by control and temperature-sensitive *cdc28-1* mutant lysates at a restrictive temperature. Phosphorylation of sites in the carboxyl- and amino-terminal regions was eliminated in the mutant lysates (Fig. 1D), indicating that the *CDC28* protein kinase phosphorylates the same sites in H1 as does the mammalian growth-associated H1 kinase. Phosphorylation of sites in the globular domain, however, was unaffected in the *cdc28-1* mutant and is therefore due to a separate protein kinase specific for sites in this region. Phosphorylation of sites in the carboxyl- and amino-terminal domains of H1 is catalyzed by mutant *cdc28-1* lysates at a permissive temperature (22°C). However, the extent of phosphorylation is diminished compared with control lysates (not shown). It is not uncommon for temperature-sensitive enzymes to be less active in vitro at permissive temperatures than the corresponding wild-type enzyme (59, 60).

In a parallel study, we examined the distribution of phosphate among chymotryptic peptides derived from H1 phosphorylated by crude extracts of the fission yeast *S. pombe* and found a pattern identical to that seen with *S. cerevisiae* (not shown). We also examined extracts of *S. pombe* containing a temperature-sensitive mutation in *cdc2*$^+$, the functional homolog of *S. cerevisiae* gene *CDC28* (3, 4). Temperature-sensitive *cdc2*$^+$ mutants contain temper-

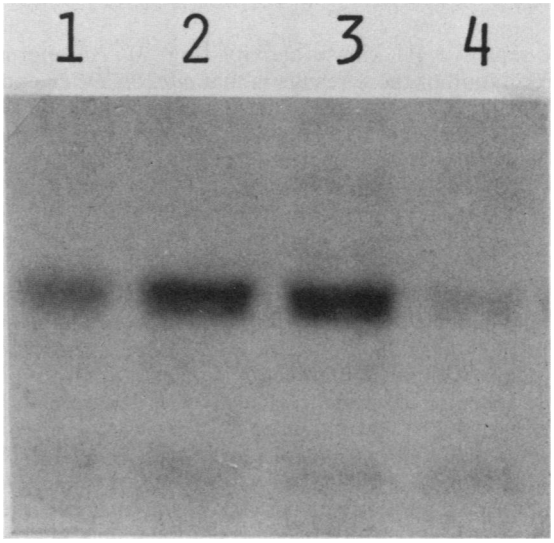

FIG. 3. Temperature-sensitive H1 histone kinase activity in *Schizosaccharomyces pombe cdc2*$^+$ mutants. Exponentially growing *S. pombe* wild-type 972h- or temperature-sensitive *cdc2.33h*- cells (10^8) were broken with glass beads in HB buffer and extracted and assayed for H1 kinase activity at 25 or 40°C as described in Materials and Methods. Terminated reaction mixtures were analyzed on 11% sodium dodecyl sulfate gels. Phosphorylated H1 was visualized by autoradiography. Lane 1, wild type, 25°C; lane 2, wild type, 42°C; lane 3, *cdc2.33h*, 25°C; lane 4, *cdc2.33h*, 40°C.

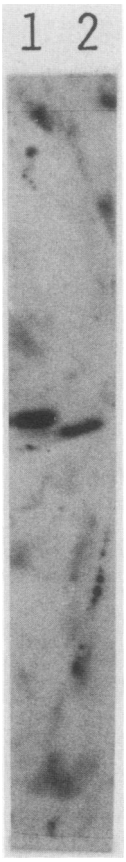

FIG. 4. Immunoblotting of mammalian growth-associated H1 kinase with antibody to the yeast *cdc2*$^+$/*CDC28* gene product. Immunoblotting was carried out with PSTAIR antibody as described in Materials and Methods. Lane 1, crude extract of *Xenopus* oocytes undergoing germinal vesicle breakdown, containing MPF; lane 2, Novikoff rat hepatoma growth-associated H1 histone kinase.

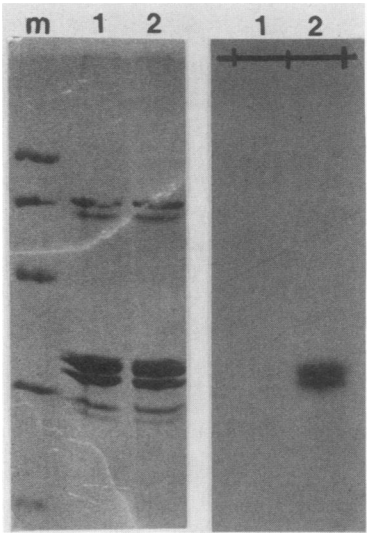

FIG. 5. Phosphorylation of H1 histone by immunoprecipitates of mammalian growth-associated H1 kinase formed with antibodies to the yeast *cdc2*$^+$/*CDC28* gene product. H1 histone was phosphorylated by immune complexes formed with PSTAIR antibody, and the products were analyzed by electrophoresis in 12.5% sodium dodecyl sulfate gels. Coomassie stain, left; autoradiograph, right. Lanes 1, immunoprecipitation with antibody preadsorbed with the PSTAIR peptide antigen. Lanes 2, untreated antibody. m, Molecular weight markers. The radioactive bands in lane 2 are coincident with the H1 doublet visible in the Coomassie stain.

ature-sensitive H1 kinase activity (Fig. 3). An alternative interpretation of these results is that *cdc2*$^+$/*CDC28* encodes an activator of the yeast H1 kinase rather than the H1 kinase itself. The fact that the homolog of *cdc2*$^+$/*CDC28* protein kinase present in highly purified MPF (21) directly phosphorylates H1 on the same sites as the yeast H1 kinase (see below) argues strongly against this possibility. Thus, on the basis of the temperature sensitivity of phosphorylation of the specific growth-associated phosphorylation sites in H1 histone, *cdc2*$^+$/*CDC28* is identified as the gene encoding the protein kinase in yeast cells with properties corresponding to mammalian growth-associated H1 kinase. Subsequent to these observations, Wittenberg and Reed (70) also reported the presence of temperature-sensitive H1 kinase activity in *S. cerevisiae CDC28* mutants.

Immunoreactivity of mammalian growth-associated H1 kinase. *cdc2*$^+$, *CDC28*, and the human homolog of these genes each encode a perfectly conserved 16-amino-acid sequence, EGVPSTAIREISLLKE, which is not found in other protein kinases. Rabbit antibodies raised against this peptide sequence (called PSTAIR antibody) immunoblot a 32- to 34-kDa protein present in crude extracts of *S. pombe* and human cells (43) and of frog and starfish oocytes (21, 36). Figure 4 shows an immunoblot of mammalian growth-associated H1 kinase made with PSTAIR antibody compared with that of a crude *Xenopus* MPF preparation. The MPF preparation contains a major immunoreactive band of 34 kDa, which is also present in highly purified MPF (21), and a minor band of 32 kDa. The immunoreactive component of the mammalian enzyme migrates with the minor 32-kDa band in the MPF preparation. The relationship between these two forms of the protein is not clear at present. Further, immunoprecipitates formed by the PSTAIR antibody with the mammalian enzyme preparation contained H1 kinase activity (Fig. 5, lanes 2). The H1 kinase activity in the immunoprecipitate was closely similar to growth-associated H1 kinase, as shown by the pattern of phosphorylation among chymotryptic peptides (compare Fig. 1A and B) as well as among the tryptic phosphopeptides derived from the chymotrypic fragments (Fig. 2, lanes 1, 4, 5, and 8). Thus, mammalian growth-associated H1 kinase and *cdc2*$^+$/*CDC28* protein kinase are similar with respect to immunoreactivity, molecular size, and specificity for phosphorylation sites in H1 histone.

Growth-associated H1 kinase as a component of MPF. Lohka et al. (44) have purified *Xenopus* MPF to near homogeneity and found that it contains a protein kinase which copurifies with MPF activity and which utilizes H1 histone as a substrate in vitro. Gautier et al. (21) and Dunphy et al. (19), using immunochemical and affinity-column techniques, have shown that the 34-kDa component of MPF is a homolog of the yeast *cdc2*$^+$/*CDC28* gene product. In addition, the H1 kinase activity of purified MPF is specifically immunoprecipitated by antibodies to the conserved PSTAIR peptide sequence encoded by *cdc2*$^+$/*CDC28* genes (21). The finding that mammalian growth-associated H1 kinase is also similar to yeast *cdc2*$^+$/*CDC28* protein kinase indicates that MPF protein kinase and the mammalian kinase are similar enzymes. Analysis of the sites in H1 histone phosphorylated by MPF and by immunoprecipitates of MPF (Fig. 2, lanes 1, 2, 5, and 6; Fig. 6) confirms the similarity of these mamma-

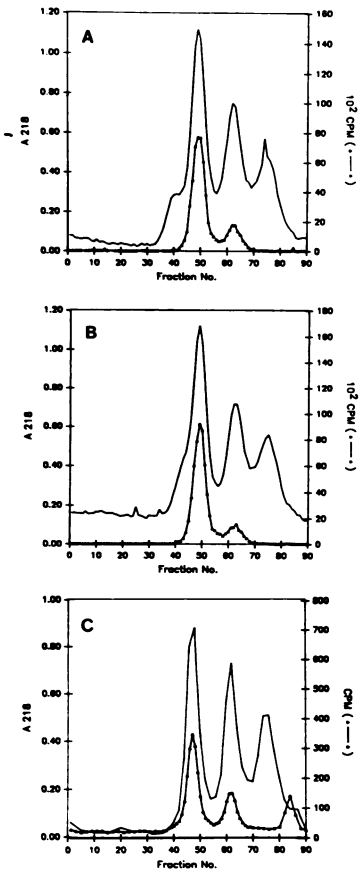

FIG. 6. Gel filtration of chymotryptic peptides derived from H1 histone phosphorylated by *Xenopus* MPF preparations. Peptides derived from H1 phosphorylated by mammalian growth-associated H1 kinase (A), by purified *Xenopus* MPF (B), and by immunoprecipitates of purified MPF formed with PSTAIR antibody to the yeast *cdc2*$^+$/*CDC28* gene product (C) were resolved on Sephadex G-100. The small amount of radioactivity eluting after the last peptide peak in panel C is due to a trace of [γ-^{32}P]ATP not removed during isolation of H1. Other conditions are as described in the legend to Fig. 1.

lian, amphibian, and yeast protein kinases. In addition, the finding that the protein kinase of highly purified MPF and the mammalian H1 kinase have similar properties makes it highly unlikely that the immunoprecipitation of the mammalian enzyme by PSTAIR antibody described above is due to its association with some other immunoreactive protein rather than to a direct recognition of the enzyme by the antibody.

In *Xenopus* oocytes and eggs, a sharp increase in H1 kinase activity during entry into meiotic and mitotic metaphase has been described recently by Dabauvalle et al. (15). By using chymotryptic phosphopeptide analysis, we have found that all of this increase is accounted for by increased phosphorylation of growth-associated sites in H1, indicating that it is due to elevated MPF H1 kinase activity (not shown).

DISCUSSION

The data presented here show that mammalian growth-associated H1 kinase is closely related to protein kinases encoded by *cdc2*$^+$/*CDC28* homologs in yeast and frog cells with respect to specificity for phosphorylation sites in H1 histone, molecular size, and immunoreactivity with antibodies to the *cdc2*$^+$/*CDC28* gene product. This evidence indicates that growth-associated H1 kinase is encoded by a mammalian homolog of the *cdc2*$^+$/*CDC28* gene. Extensive genetic evidence in yeasts and biochemical evidence in *Xenopus* and starfish oocytes indicates that this kinase participates in control of entry into mitosis or meiosis in these organisms. The sharply elevated activity of the enzyme as cells enter mitosis suggests a similar function for growth-associated H1 kinase in mammals. These findings provide further evidence that this type of protein kinase participates in a universal mechanism for control of mitotic entry in eucaryotic cells.

Mechanism of mitotic entry. The physiological substrates for the *cdc2*$^+$/*CDC28* protein kinases in yeasts, frogs, and starfish have not been identified. However, in mammalian cells, H1 histone is known to be a substrate in vivo for growth-associated H1 kinase (37–41). This suggests that H1 histone may also serve as a physiological substrate for this enzyme in other organisms and that the phosphorylation of H1 histone may be an important component of the mechanism for entry of cells into mitosis, perhaps by promoting chromatin condensation as proposed earlier by Bradbury and others (7, 26, 27). Against this view, the apparent absence of H1 histone in budding yeasts (9, 13) suggests that phosphorylation of this substrate is not essential for entry into mitosis. However, the identification of an H1 histone or functional H1 counterpart in organisms distant from mammals is not straightforward, since the properties of the H1 histones present in unicellular eucaryotes vary considerably, and highly active proteases in these organisms have the potential to degrade H1 during isolation (23, 33, 49). It may be noted that the full complement of *S. cerevisiae* nucleosome core histones was identified only after repeated attempts (9, 10).

Chromosome condensation has been clearly demonstrated during mitosis in *S. pombe* (48, 67) and during meiosis in *S. cerevisiae* (18, 35). The existence of condensed chromosomes at mitosis or meiosis may require the presence in both yeasts of a functional counterpart of H1 histone, and the failure to observe mitotic chromosome condensation in *S. cerevisiae* may be due to technical problems. Recently, immunological evidence has been presented that *S. cerevisiae* contains H1 (62). In addition, S. Moreno, J. Hayles, and P. Nurse (manuscript in preparation) have found that overexpression of *cdc2*$^+$ causes a marked increase in the extent of condensation of mitotic chromosomes in *S. pombe*. This finding indicates the presence in *S. pombe* of a chromosomal structural protein with functional properties of an H1 histone counterpart, whose action is modulated by the activity of *cdc2*$^+$ protein kinase. Taken together, the present evidence suggests that induction of chromosome condensation by phosphorylation of H1 histone or an H1 histone counterpart is an important component of the mechanism for entry of eucaryotic cells into mitosis.

Other *cdc2*$^+$/*CDC28* substrates. Although phosphorylation of H1 histone by growth-associated H1 kinase clearly occurs during mitotic entry in mammalian cells, it seems likely that additional substrates which are important for entry into mitosis also exist. It is of interest that DNA topoisomerase II from fission yeasts, which has been implicated in chromosome condensation (68), contains a sequence matching the consensus sequence Lys-Ser/Thr-Pro-Lys (39, 42) for mammalian growth-associated H1 kinase phosphorylation sites (68) and therefore appears to be a potential substrate for *cdc2*$^+$/*CDC28* protein kinases. In vitro, *cdc2*$^+$/*CDC28* pro-

tein kinases catalyze phosphorylation of proteins of 40 kDa, 45 kDa, and 62 kDa with which they are found associated in yeast, *Xenopus*, and mammalian cells, respectively (11, 16, 44, 50), and it seems probable that these proteins are also substrates in vivo. In the case of clam oocyte *cdc2*$^+$/*CDC28*, the kinase is complexed with two proteins of approximately 58 and 56 kDa which have been identified as cyclins A and B (17, 69), proteins which can drive oocytes into mitosis and which are degraded upon exit from mitosis (63, 64). Cyclin B also contains a sequence closely matching the consensus sequences for growth-associated H1 kinase phosphorylation sites and is phosphorylated by clam *cdc2*$^+$/*CDC28* kinase in vitro (69). Recently, evidence has been obtained that the 45-kDa component of *Xenopus* MPF and a 60-kDa component present in rat growth-associated H1 kinase preparations are related to cyclin. (The 45-kDA component of *Xenopus* MPF is immunoprecipitated by antibodies raised against frog cyclin expressed in *Escherichia coli* [J. Gautier, J. Minshull, M. Lohka, T. Hunt, and J. Maller, manuscript in preparation], and a 60-kDa component present in rat growth-associated H1 kinase preparations cross-reacts on Western blots [immunoblot] with antibodies to sea urchin cyclin A [J. Gautier, T. A. Langan, T. Hunt, and J. Maller, unpublished observations].) The function of these associated proteins is not established but it seems likely that they participate in activation or inactivation of *cdc2*$^+$/*CDC28* protein kinase as cells enter and exit metaphase.

Increased phosphorylation of a number of other proteins, such as nuclear lamins and vimentin (20, 53) and H3 histone (27), occurs in association with mitosis, but these are not substrates for *cdc2*$^+$/*CDC28* protein kinases (44; Chambers and Langan, unpublished observations). The phosphorylation and activation of additional protein kinases by *cdc2*$^+$/*CDC28* or the existence of a common mechanism for activation of *cdc2*$^+$/*CDC28* together with other protein kinases could explain the phosphorylation of these multiple substrates at mitosis. The mammalian kinase also phosphorylates other histones of the H1 class, such as H5, H1°, and the testes-specific H1t (Chambers and Langan, unpublished observations). These histones are also phosphorylated in vivo in growing cells, presumably by the same enzyme. A murine microsomal H1 kinase, which may be a cytoplasmic form of the enzyme, also phosphorylates H5 and H1° (57).

***cdc2*$^+$/*CDC28* and cell cycle control.** In *S. cerevisiae*, *CDC28* has been studied primarily in connection with its requirement at the start point in the yeast cell cycle, which commits cells to a round of division (56, 58). Because of the unusual timing of events in the *S. cerevisiae* cell cycle, the start point is in close temporal proximity to mitotic entry, such that the phenotype observed on transfer to a restrictive temperature is almost exclusively an arrest of cells at the start point. Piggot et al., (54) have shown that in *S. cerevisiae* synchronized to increase the proportion of cells between the start point and mitotic entry, transfer of *cdc28* mutant strains to a restrictive temperature results in the arrest of a substantial proportion of cells at entry into mitosis, demonstrating that the *CDC28* gene product is required at the same points in the cell cycle in both *S. cerevisiae* and *S. pombe*. Further, when transferred into *S. pombe*, the *CDC28* gene functions in the same manner as *cdc2*$^+$ in controlling entry into mitosis (4). The fact that *CDC28* protein kinase activity is elevated at mitosis in *S. cerevisiae* (Table 1) is also in keeping with a role for the *CDC28* product at mitosis.

Although recent work has emphasized the role of *cdc2*$^+$/*CDC28* protein kinases in a highly conserved mechanism for mitotic entry, the major points for control of cell cycle progression in mammalian somatic cells are located in G1 (55, 73), where *cdc2*$^+$/*CDC28* genes also regulate progression through the fission and budding yeast cell cycles. Similarly, it is in late G1 that mammalian growth-associated H1 kinase first becomes active in cells released from arrest due to nutrient deprivation (25, 27). Therefore, it will be important to establish whether growth-associated H1 kinase also plays a role in regulating the transition of mammalian cells through G1 after stimulation from quiescence.

ACKNOWLEDGMENTS

We thank Kathy Fassler for secretarial assistance and Alice Alexander for technical help.

This work was supported by Public Health Service grants from the National Institutes of Health to T.A.L., J.L.M., and R.A.S. and by the Imperial Cancer Research Fund fund to P.N. J.G. is on leave from CNRS unit 675, Toulouse, France.

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