Specific members of the GCR protein family have a role in malignant transformation. For example, the ectopic expression of serotonin receptor (5HT1c) in NIH3T3 cells leads to serotonin-dependent transformation of these cells which can give rise to tumours in nude mice[23], and the human *mas* oncogene, which encodes an angiotensin receptor, is also tumorigenic in nude mice[24,25]. Cyclins are required for cellular division, and thus have a key role in cellular proliferation and development[16]. Moreover, one of the cyclins, human D1 (ref. 19), is the *PRAD1* candidate oncogene implicated in the development of certain parathyroid tumours and hepatocarcinomas[26,27], and it is the D-type cyclins to which the HVS *ECLF2* protein seems to be most closely related. Cyclin A can associate with the cellular transcription factor E2F (refs 28, 29) which is likely to be directly involved in the regulation of expression of genes important for cellular proliferation and development, for example c-*myc*, c-*myb* and the EGF receptor gene[30]. It is possible that the HVS cyclin may also associate with cellular transcription factors and thereby influence the expression of cellular and viral genes. Thus, the presence of cyclin and GCR homologues, *ECLF2* protein and *ECRF3* protein, in HVS may be highly relevant to the process of cellular transformation and rapid T-cell proliferation effected by HVS during latent infections of T cells in susceptible hosts. □

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S-phase feedback control in budding yeast independent of tyrosine phosphorylation of p34^{cdc28}

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IN somatic cells, entry into mitosis depends on the completion of DNA synthesis. This dependency is established by S-phase feedback controls that arrest cell division when damaged or unreplicated DNA is present[1]. In the fission yeast *Schizosaccharomyces pombe*, mutations that interfere with the phosphorylation of tyrosine 15 (Y15) of p34^{cdc2}, the protein kinase subunit of maturation promoting factor, accelerate the entry into mitosis and abolish the ability of unreplicated DNA to arrest cells in G2 (ref. 2). Because the tyrosine phosphorylation of p34^{cdc2} is conserved in *S. pombe*[3], *Xenopus*[4], chicken[5] and human[6] cells, the regulation of p34^{cdc2}-Y15 phosphorylation could be a universal mechanism mediating the S-phase feedback control and regulating the initiation of mitosis[7,8]. We have investigated these phenomena in the budding yeast *Saccharomyces cerevisiae*. We report here that the *CDC28* gene product (the *S. cerevisiae* homologue of *cdc2*) is phosphorylated on the equivalent tyrosine (Y19) during S phase but that mutations that prevent tyrosine phosphorylation do not lead to premature mitosis and do not abolish feedback controls. We have therefore demonstrated a mechanism that does not involve tyrosine phosphorylation of p34 by which cells arrest their division in response to the presence of unreplicated or damaged DNA. We speculate that this mechanism may not involve the inactivation of p34 catalytic activity.

We first established that p34^{CDC28} in budding yeast and p34^{cdc2} in fission yeast are phosphorylated on homologous tyrosine residues. We prepared extracts from *S. cerevisiae* cells arrested in S phase with hydroxyurea, precipitated p34, and analysed it by immunoblotting. When precipitates from cells carrying wild-type (wt) *CDC28* were immunoblotted with anti-phosphotyrosine antibodies, a band corresponding to relative molecular mass of 34,000 (M_r 34K) was detected (Fig. 1*b*). In extracts from cells carrying a modified *CDC28* gene that encodes a fusion between p34^{CDC28} and a short epitope tag[9] (Fig. 1*a*), the 34K band was replaced by a 38K band. To show the specificity of the immunoblotting and to localize the phosphorylated site, we constructed a mutant (*cdc28-F*) in which a phenylalanine was substituted for tyrosine 19 (Y19), the residue in p34^{CDC28} that is homologous to the phosphorylated tyrosine 15 of p34^{cdc2} in *S. pombe* (Fig. 1*a*)[3]. When p34 was analysed in extracts from *S. cerevisiae* cells carrying *cdc28-F* in place of wt *CDC28*, no phosphotyrosine was detected (Fig. 1*b*, lane 2) even though p34 was precipitated efficiently in all cases (lanes 5–8). Thus, p34^{CDC28} seems to be phosphorylated on Y19 in cells arrested in S phase.

To determine the cell-cycle dependence of Y19 phosphorylation, we prepared extracts from wt cells arrested at Start with α-factor or arrested in mitosis with the antimicrotubule drug benomyl. No phosphotyrosine was detected on p34 isolated from G1- or M-phase cells (Fig. 1*b* lanes 10 and 12), suggesting that the tyrosine phosphorylation of p34^{CDC28}, like that of p34 in other organisms[7], is restricted to the S and G2 phases of the cell cycle. This biochemical difference also indicates that drugs that inhibit S phase and M phase arrest budding yeast cells at different points in the cell cycle, although the arrest points cannot be distinguished morphologically.

We examined the phenotype of *cdc28-F*, and of another mutant, *cdc28-AF*, which has an additional substitution of threonine to alanine at position 18 (residues homologous to T18 and Y19 coordinately regulate p34^{cdc2} in vertebrate cells[10]). The doubling time, size, and cell cycle distribution (as assayed by fluorescence-activated cell sorting (FACS)) of *cdc28-F* and *cdc28-AF* strains were very similar to wild type, indicating that mitosis was not induced prematurely in the mutants (data not shown).

Do cells carrying *cdc28-F* and *cdc28-AF* mutations arrest normally in response to the presence of damaged DNA? Irradiation produces double-stranded breaks in DNA and, in wild-type cells, results in a cell cycle arrest at a G2 'checkpoint' that is passed only when the DNA damage has been repaired[1]. In cells carrying mutations in genes, such as *RAD9*, that regulate arrest at the checkpoint, division proceeds despite the DNA damage and the cells die[11]. We compared wt *CDC28*, *cdc28-F*, *cdc28-AF* and *rad9* cells for their resistance to X-ray irradiation (Fig. 2*a*). Although *rad9* cells died rapidly, the fraction of survivors was identical in the other strains, indicating that the operation of the *RAD9*-dependent checkpoint does not require phosphorylation of $p34^{CDC28}$ on Y19.

To examine the dependence of cell division on the completion of S phase, we treated cells with sublethal doses of hydroxyurea, which delays the cell cycle by inhibiting DNA synthesis. Cells that are defective in feedback controls triggered by unreplicated DNA should die in the presence of hydroxyurea because they divide before S phase is completed. Wild-type *CDC28*, *cdc28-F* and *cdc28-AF* strains remained highly viable in hydroxyurea (Fig. 2*b*). As a positive control, we examined *mec1* cells (provided by T. Wienert), which do not arrest correctly in the presence of hydroxyurea, and observed that they died rapidly. Thus, the phosphorylation of $p34^{CDC28}$ on Y19 is not essential for the correct operation of S-phase feedback control. To look for more subtle phenotypes, we grew genetically marked *CDC28* and *cdc28-AF* strains together for 100 generations in rich medium, minimal medium and media supplemented with sublethal doses of benomyl and hydroxyurea; we found that the difference in the reproduction rates of wild-type and mutant cells was less than 0.5% per generation in all cases (data not shown).

Reduced tyrosine phosphorylation of $p34^{cdc2}$ makes *S. pombe* cells supersensitive to hydroxyurea[2]. Does the lack of a similar phenotype in *S. cerevisiae* cells carrying *cdc28-F* reflect a difference in the way that the *CDC28* and *cdc2* gene products respond to tyrosine phosphorylation? To investigate this, we expressed *cdc2-F15* in *S. cerevisiae* and *cdc28-F* in *S. pombe*. *S. cerevisiae* cells carrying either a wt *cdc2* gene or the *cdc2-F15* mutant remained viable in the presence of hydroxyurea (Fig. 2*c*). By contrast, *S. pombe* cells carrying *CDC28* grew on hydroxyurea, but those that carried *CDC28-F* did not (Fig. 2*d*) although the rate of killing was quite low (about 10% loss of viability per generation; data not shown). Thus, although budding and fission yeast differ in their sensitivity to the presence of p34 lacking a phosphorylatable tyrosine, this is not caused by differences between the *cdc2* and *CDC28* gene products but must reflect other physiological differences.

In fission yeast, the extent of phosphorylation of Y15 of $p34^{cdc2}$ reflects the balance between the activity of *cdc25* (ref.

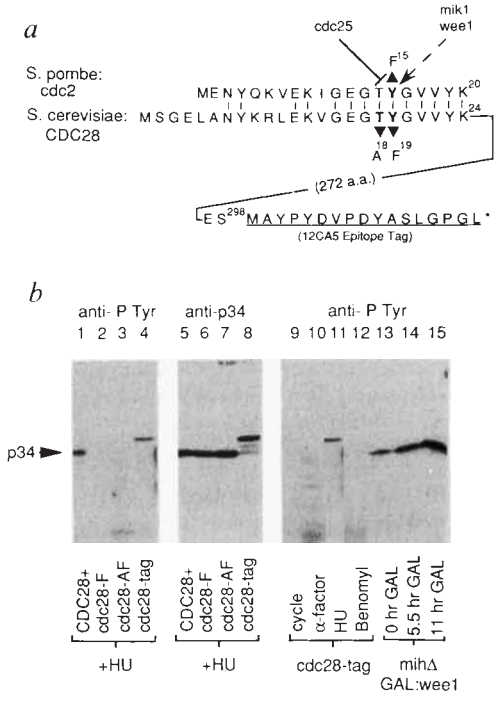

FIG. 1 Regulation of the tyrosine phosphorylation of $p34^{CDC28}$. *a*, A comparison of the amino termini of the products of the *CDC28* gene in *S. cerevisiae* and the *cdc2* gene in *S. pombe* is shown. Bold arrows, positions of mutations that were introduced by site-directed mutagenesis; in *CDC28* these are a tyrosine to phenylalanine substitution at position 19 (*cdc28-F*) and a double mutant combining F19 with a threonine to alanine mutation at position 18 (*cdc28-AF*). In *cdc2*, tyrosine 15 was mutated to phenylalanine (*cdc2-F15*). The opposing action on the phosphorylation of tyrosine 15 of $p34^{cdc2}$ by the phosphatase encoded by the *cdc25* gene[1,2,13] and the kinases encoded by *wee1* (ref. 21) and *mik1* (ref. 15) is indicated schematically. To facilitate the detection of $p34^{CDC28}$ it was fused to a short epitope tag. A gene encoding this epitope-tagged protein seems to function normally because it could be substituted for the endogenous *CDC28* gene without affecting cell growth. The lowest line shows the sequence of the epitope tag; it was fused to the C terminus of $p34^{CDC28}$ using site-directed mutagenesis (non-native sequences are underlined). The epitope is derived from the HA protein of the influenza virus[9] and, when fused to $p34^{CDC28}$, reacts with the 12CA5 monoclonal antibody under both native and denaturing conditions. *b*, Immunoblots of p34 precipitates from extracts of cells carrying wild-type and mutant *CDC28* genes and arrested at various points in the cell cycle. In each panel, noncontiguous lanes from a single exposure of one filter are shown. Precipitates were prepared from samples containing equivalent amounts of total protein using p13-suc1 covalently coupled to agarose beads[4]. p13-suc1 is *S. pombe* protein that binds tightly to p34. Similar results to those shown here have been obtained by immunoprecipitation of tagged p34 with 12CA5. Lanes 1–4, samples from cells carrying wt *CDC28*, the *cdc28-F19* and *cdc28-A18F19* mutations, and epitope-tagged *CDC28* were arrested in S phase with hydroxyurea and probed with anti-phosphotyrosine antibodies; lanes 5–8, the same immunoblot reprobed with an anti-p34 serum to determine the amount of p34 in each sample; lanes 9–15, antiphosphotyrosine immunoblots of samples from cells carrying epitope-tagged *CDC28* and arrested at various points in the cell cycle (9–12) or from cells in which the *MIH1* gene had been deleted (13–15) and *S. pombe wee1* gene overexpressed from the *GAL1* promoter for 5.5 (14) or 11 h (15).

METHODS. Mid-log cultures of *MATa, bar1* cells were grown in rich medium. They were then arrested in G1 by the addition of α-mating factor to 2.5 $\mu g\,ml^{-1}$, in S phase by the addition of hydroxyurea to 15 $mg\,ml^{-1}$ and in mitosis by the addition of benomyl to 15 $\mu g\,ml^{-1}$. α-factor and hydroxyurea treatment were for 3 h at 30 °C, and benomyl for 3 h at 25 °C; the synchrony of the arrests was established by microscopic examination of the cells. The samples in lanes 13–15 were prepared from *MATa* cells carrying a disruption of the *MIH1* gene and several integrated copies of a construct that fuses the *GAL1/10* promoter to the *S. pombe wee1* coding sequence. *MIH1* was disrupted using a *LEU2* marker (*mihΔLEU2*) and the *GAL1:wee1* construct was integrated at the *URA3* locus as described previously[16]. These cells were grown in raffinose and the expression of the *wee1* gene induced in mid-log cultures by the addition of galactose. Extracts were prepared by mechanical disruption using glass beads as described previously[22] in breakage buffer containing 50 mM MOPS, pH 7.2, 15 mM $MgCl_2$, 10 mM EGTA, 25 mM NaF, 50 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, 0.5 mM *N*-tosyl-L-phenylalanine chloromethyl ketone and 1 $\mu g\,ml^{-1}$ each of leupeptin and chemostatin. Total protein (0.5 mg) was diluted into 0.25 ml in a buffer similar to breakage buffer but containing 50 mM Tris, pH 7.4 instead of MOPS and supplemented with 250 mM NaCl and 0.1% Triton. p13 beads (20 μl) prepared as previously described[4] were added to each sample, incubated at 4 °C for 2 h, and washed with 50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.05% deoxycholate, 0.1% SDS and then eluted with SDS sample buffer, run on a 15% SDS-polyacrylamide gel and transferred to nitrocellulose. Filters were blocked, incubated with undiluted culture supernatant (supplemented with Tween 20 to 0.2%) from cells expressing the 4G10 antiphosphotyrosine monoclonal antibody[23] and antibody conjugates detected by incubation with ^{125}I-labelled goat anti-mouse serum; filters were then stripped, reprobed with a polyclonal anti-$p34^{CDC28}$ antibody (a gift from Ray Deshaies) and detected with alkaline phosphatase-conjugated secondary antibodies.

12), which encodes a tyrosine phosphatase[13,14], and a pair of functionally redundant kinases encoded by the *wee1* and *mik1* genes[15]. Enoch and Nurse have proposed that *cdc25* mediates the S-phase feedback control in *S. pombe*[8]. Thus, one explanation for our failure to find a phenotype associated with the *cdc28-AF* mutation in budding yeast is that p34^{CDC28} has sites of phosphorylation in addition to Y19 (and T18) that are targets of *cdc25/wee1* action. We tested this possibility by taking advantage of the observation that the simultaneous deletion of *MIH1* (a homologue of the *S. pombe cdc25* gene[16]) and overexpression of *wee1* arrests *S. cerevisiae* cells in a G2-like state with hyperphosphorylated p34 (Fig. 1*b*, lanes 13–15)[16]. If this arrest is due primarily to the phosphorylation of p34^{CDC28} on T18 and Y19, the *cdc28-AF* mutation should suppress the growth arrest. In agreement with this, we find almost complete suppression of the arrest by *cdc28-AF*, and somewhat weaker suppression by *cdc28-F* (Fig. 3). Moreover, suppression by *cdc28-AF* is dominant to *CDC28*, in a manner analogous to the dominance of *cdc2-F15* to *cdc2* in *S. pombe*[3]. Thus, the arrest imposed by the overexpression of *wee1* and deletion of *MIH1* seems to involve the hyperphosphorylation of T18 and Y19 of p34^{CDC28}.

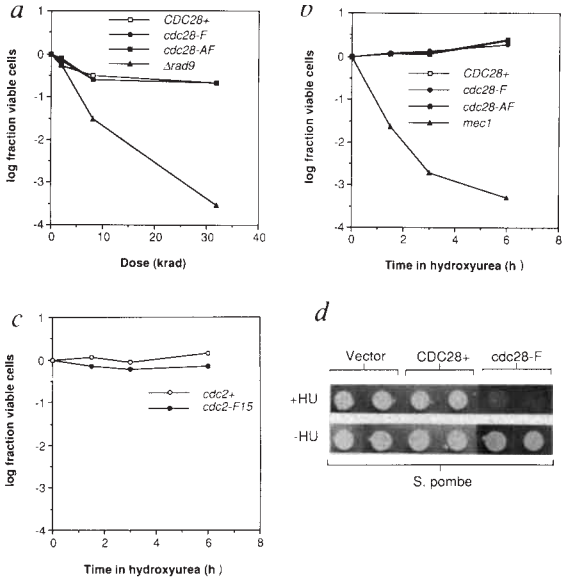

FIG. 2 Effect of p34 mutations on the viability of budding and fission yeast following DNA damage or the inhibition of DNA synthesis. *a*, Cell survival was determined for asynchronous liquid cultures of *S. cerevisiae* cells in which double-stranded breaks in DNA were introduced by exposure to X-ray irradiation. Curves are shown for strains carrying either a wt *CDC28* allele, or *cdc28-F19 or cdc28-A18F19* alleles in place of wt *CDC28* and also for *CDC28* strains carrying a complete disruption of the *RAD9* gene[11] as indicated. *b*, Survival in 10 mg ml^{-1} hydroxyurea of *S. cerevisiae* strains containing various *CDC28* alleles or the hydroxyurea-supersensitive *mec1* mutation (T. Weinert, personal communication). In the absence of hydroxyurea, cell number increased six to eightfold, demonstrating that hydroxyurea treatment substantially lengthens the cell cycle. *c*, Survival in 10 mg ml^{-1} hydroxyurea of *S. cerevisiae* cells containing a complete disruption of the endogenous *CDC28* locus and a high-copy number (2 micron) plasmid that carries a fusion gene linking the *CDC28* promoter to the coding sequence of a wild-type *S. pombe cdc2* cDNA or of a *cdc2-F15* mutation. *d*, Growth on solid media lacking leucine of wild-type *S. pombe* cells containing a high copy number *LEU2* plasmid with no insert (vector), a wild-type *CDC28* gene (CDC28+), or a gene with the *cdc28-F19* mutation (cdc28-F) in the presence or absence of 1 mg ml^{-1} hydroxyurea. A single exposure of non-contiguous patches of cells from a single plate is shown.
METHODS. Following different doses of X-ray irradiation, or different lengths of treatment with hydroxyurea, cells were plated on solid media and the fraction of viable cells calculated by counting colonies after 3 days of growth at 25 °C; the viability of untreated cells at time 0 was set at 100%. The data were collected from cultures treated in parallel and each data point represents the average of two or more determinations which typically varied by less than 10%.

This suggests that the failure of the *cdc28-AF* mutation to inactivate S-phase feedback control in *S. cerevisiae* is not a consequence of the presence of additional sites of *MIH1* action on p34^{CDC28}.

It is thought that high levels of tyrosine phosphorylation of p34 in G2 inhibit the kinase[7] and, in cells containing unreplicated DNA, arrest division by eliminating p34 activity[8]. To investigate this in budding yeast, we measured p34^{CDC28} activity, using histone H1 as a substrate, in extracts from cells arrested at various points in the cell cycle. We found that p34 activity is extremely low in cells arrested at Start with α-factor, increases at least 20-fold in cells arrested in S phase with hydroxyurea and rises a further three-fold in cells arrested in mitosis with benomyl (Fig. 4*a*). Similar regulation is observed in *cdc* mutants[17] arrested at the non-permissive temperature (Fig. 4*b*). Thus, the activity of p34^{CDC28} seems to be high during S phase even though Y19 phosphorylation is also maximal. Arrest caused by unreplicated DNA does not seem to involve the elimination of p34^{CDC28} activity, at least as measured *in vitro*. Mutations that prevent the tyrosine phosphorylation of p34^{CDC28} do not increase kinase activity in S-phase arrested cells (Fig. 4*c*); conversely, conditions that induce the hyperphosphorylation of p34 and arrest cells in G2 (ref. 16) (the deletion of *MIH1* and overexpression of *wee1*, Fig. 3) do not seem to reduce kinase activity below that found in S phase (Fig. 4*c*). These data suggest that in *S. cerevisiae*, in contrast to what has been proposed previously[7], Y19 phosphorylation may regulate cell-cycle progression by a mechanism that does not simply involve the elimination of the catalytic activity of p34.

We have shown that p34^{CDC28} is phosphorylated in S phase on Y19, a site that is homologous to the tyrosine modified in other p34 proteins and that Y19, and possibly an adjacent threonine, are the principal sites of action of *MIH1*. But mutations that prevent the phosphorylation of p34^{CDC28} on T18 and Y19 do not accelerate mitosis and do not disrupt the feedback controls that link cell division with the completion of DNA synthesis. Despite the ubiquity in eucaryotes of the tyrosine phosphorylation of p34 (ref. 7), and the conservation, even in budding yeast, of the *wee1/cdc25* pathway[16], tyrosine phosphorylation of p34 does not seem to constitute the universal rate-limiting regulator of the entry into M phase.

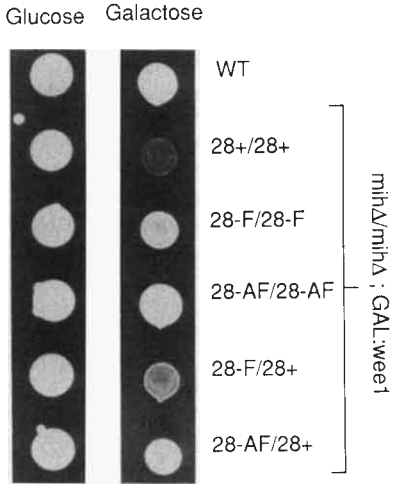

FIG. 3 Effect of *CDC28* mutations on the G2-like arrest of budding yeast cells lacking *MIH1* and overexpressing *wee1*. Growth on solid media of diploid strains that are wild-type (WT) or that are homozygous for a disruption of the *mih1* gene, that overexpress the *S. pombe wee*1 protein from an integrated *GAL:wee1* fusion construct (present in several linked copies; see Fig. 1) and that carry various combinations of *CDC28* alleles. 28+, Wild type *CDC28* locus; 28-F, *cdc28-F19* mutation; 28-AF, *cdc28-A18F19* mutation. *Wee1* expression is strongly induced on plates containing galactose and is repressed on plates containing glucose; both types of plates also contained 1mM sodium orthovanadate.

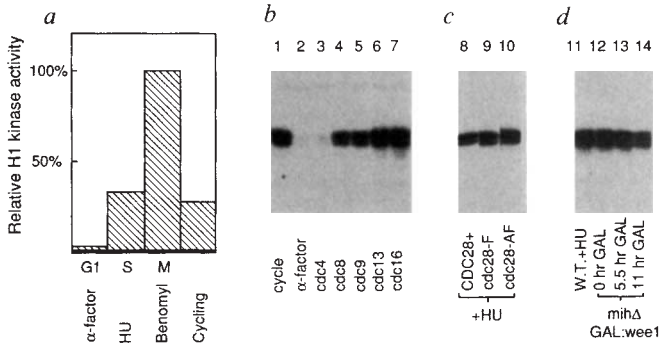

FIG. 4 Kinase activity of p34^{CDC28} in cells arrested at various stages in the cell cycle. *a*, The kinase activity of p34^{CDC28} in cells arrested in G1 and S phase is shown as a percentage of the activity in mitosis (defined as 100%). Cultures of cells carrying tagged *CDC28* were arrested with α-factor, hydroxyurea and benomyl, and extracts prepared as described in Fig. 1; p34 was immunoprecipitated with 12CA5 antibodies and kinase activity measured using histone H1 as a substrate essentially as described[22]. The solid bar (1.5%) represents the background level of activity in precipitates from cell extracts that contain untagged p34^{CDC28}. Each data point represents an average of three or more determinations done at different extract concentrations. *b*, The kinase activity of p34^{CDC28} precipitated with p13 beads from extracts of cells carrying temperature-sensitive mutations in genes required for progression through the cell cycle (*cdc* genes[17]) after arrest at 37 °C for 3 h *cdc4* prevents the initiation of DNA synthesis, *cdc8* blocks DNA synthesis, *cdc9* is defective in DNA ligase, *cdc13* arrests cells in G2, and *cdc16* arrests cells in mitosis. Analysis of these precipitates, and those of *c* below, by immunoblotting with anti-p34^{CDC28} antiserum shows that they contain equivalent amounts of p34. Each panel shows samples assayed in parallel; the activities of samples in different panels cannot be compared directly. *c*, p34^{CDC28} kinase activity in p13 precipitates from extracts of cells carrying various *CDC28* alleles and arrested with hydroxyurea (lanes 8–11), or *d*, of cells carrying the *mih1ΔLEU2* mutation and the *GAL1: wee1* fusion construct (Fig. 1) in conditions in which *wee1* expression is repressed (lane 12) or has been induced for 5.5 h (lane 13) or 11 h (lane 14). The extracts used in lanes 12–14 are identical to those in lanes 12–15 of Fig. 1.

Why does interfering with the phosphorylation of p34 on tyrosine have different effects on feedback control in fission and budding yeasts? Perhaps there exist several parallel controls (one of which may be tyrosine phosphorylation of p34) whose relative importance varies between the two organisms. Alternatively, it is possible that S-phase feedback control in *S. pombe* operates primarily through a mechanism other than the regulation of Y15 phosphorylation, but that *cdc2-F15* acts to overwhelm this restraining mechanism by strongly accelerating the entry into mitosis. The observation that the overproduction of *string* (a homologue of *S. pombe cdc25*) in *Drosophila* does not induce mitosis in cells containing unreplicated DNA[18], suggests that other organisms may not require the tyrosine phosphorylation of p34 for feedback control.

We have demonstrated the existence of a mechanism independent of the tyrosine phosphorylation of p34^{CDC28} by which *S. cerevisiae* cells that contain unreplicated or damaged DNA arrest their division and regulate the entry into mitosis. One possibility is that this mechanism requires an uncharacterized modification of the maturation promoting factor (MPF) complex. But two pieces of evidence suggest that it does not simply involve a second inhibitory modification of p34^{CDC28}. First, in a screen of 8,000 heavily mutagenized plasmids that carry *cdc28-F*, we were unable to identify any hydroxyurea-sensitive *CDC28* alleles (L. Godley and P.K.S., unpublished observations). Second, T18 and Y19 are the only residues in p34^{CDC28} whose phosphorylation rises during S phase[19]. A second possibility is that there exists a regulator of mitosis other than MPF and that this regulator is rate limiting in *S. cerevisiae* and is the primary target of feedback controls. The nimA kinase of *Aspergillus nidulans*, which appears to be unrelated to the MPF kinase but is a key activator of mitosis, may be such a regulator[20]. □

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Regulation of p34^{CDC28} tyrosine phosphorylation is not required for entry into mitosis in *S. cerevisiae*

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PROGRESSION from G2 to M phase in eukaryotes requires activation of a protein kinase composed of p34$^{cdc2/CDC28}$ associated with G1-specific cyclins (reviewed in ref. 1). In some organisms the activation of the kinase at the G2/M boundary is due to dephosphorylation of a highly conserved tyrosine residue at position 15 (Y15) of the cdc2 protein[2–6]. Here we report that in the budding yeast *Saccharomyces cervisiae*, p34^{CDC28} also undergoes cell-cycle regulated dephosphorylation on an equivalent tyrosine residue (Y19). However, in contrast to previous observations in *S. pombe*[6], *Xenopus*[2,3] and mammalian cells[4,5], dephosphorylation of Y19 is not required for the activation of the CDC28/cyclin kinase. Furthermore, mutation of this tyrosine residue does not affect dependence of mitosis on DNA synthesis nor does it abolish G2 arrest induced by DNA damage. Our data imply that regulated phosphorylation of this tyrosine residue is not the 'universal' means by which the onset of mitosis is determined. We propose that there are other unidentified controls that regulate entry into mitosis.

To investigate whether entry into mitosis in *S. cerevisiae* is associated with dephosphorylation of p34^{CDC28} we compared the tryptic phosphopeptide maps of p34^{CDC28} in various *cdc* mutants arrested at different stages of the cell cycle. Among the phosphopeptides derived from p34^{CDC28}, phosphorylation of only one major peptide (peptide 1) is cell-cycle regulated (Fig. 1). Although peptide 1 is absent in cells arrested in G1 by

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