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Human p53 Inhibits Growth in *Schizosaccharomyces pombe*

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Overexpression of wild-type p53 in mammalian cells blocks growth. We show here that the overexpression of wild-type human p53 in the fission yeast *Schizosaccharomyces pombe* also blocks growth, whereas the overexpression of mutant forms of p53 does not. The p53 polypeptide is located in the nucleus and is phosphorylated at both the cdc2 site and the casein kinase II site in *S. pombe*. A new dominant mutation of p53, resulting in the change of a cysteine to an arginine at amino acid residue 141, was identified. The results presented here demonstrate that *S. pombe* could provide a simple system for studying the mechanism of action of human p53.

The p53 gene encodes a nuclear phosphoprotein that can function as a tumor suppressor (for a review, see references 21, 23, 24, and 33). p53 was first identified as a cellular protein that forms complexes with the large tumor antigen of simian virus 40 in simian virus 40-infected cells (22, 25). Subsequently, it was demonstrated that p53 formed complexes with the 55-kDa E1B protein of adenovirus (41) and the E6 protein of human papillomavirus (43). The targeting of p53 by DNA tumor virus proteins implied a role for p53 in the process of cellular transformation.

Initially, the p53 gene was characterized as an oncogene since it was able to immortalize some cells (9, 10, 17, 18, 39) and cooperate with activated *ras* oncogene to transform rat cells (37). The p53 genes used in these studies were later found to harbor point mutations. It is now clear that the expression of authentic wild-type p53 actually suppresses transformation by activated *ras* (12, 17). The apparent paradox that p53 is able to act as both a dominant transforming oncogene and a tumor suppressor gene can be explained by the existence of dominant mutations in the p53 gene. This idea is supported by the observation that certain mutant p53 proteins can cooperate with activated *ras* to transform rat cells even in the presence of the endogenous wild-type p53 (12, 17, 23).

The p53 polypeptide is phosphorylated in at least four sites in vivo (29, 40). Two of these sites have been mapped. One is serine 315 of human p53, and the other is serine 389 of murine p53. Human p53 is phosphorylated at serine 315 by the cell cycle-regulated cyclin A- and cyclin B-cdc2 kinases (4). Murine p53 is phosphorylated on serine 389 by the mitogenically stimulated casein kinase II (28). The consequences of these phosphorylation events are not known, but they suggest that p53 function may be regulated by phosphorylation during the cell cycle. This is consistent with the observation that overexpression of wild-type p53 in rat embryo fibroblasts (26, 31) and human glioblastoma cells (27) results in a growth arrest in the G_1 phase of the cell cycle. The mechanism of this p53-induced cell cycle arrest is not known.

As outlined above, there is an increasing body of evidence suggesting that p53 plays an important role in the cell as a tumor suppressor and as a possible regulator of the cell cycle. To investigate the mechanism of p53 action, we have set up a simple system in the fission yeast *Schizosaccharomyces pombe*. We reasoned that if wild-type p53 could function in *S. pombe* as it does in mammalian cells, we could use this yeast to identify components of the p53 pathway. In this paper, we provide evidence that human p53 behaves in a specific manner in *S. pombe*.

MATERIALS AND METHODS

Strains and media. *S. pombe* SP130 (*h*$^{-s}$ *leu1.32 ade6.210*) or SP224 (*h*$^{+N}$ *leu1.32 ura4 ade6.216*) were used for all the experiments reported here. *S. pombe* strains were grown in standard PMA medium (3). Amino acids, uracil, and adenine were added to minimal medium at 75 μg/ml. Thiamine hydrochloride (Sigma) was prepared in H_2O as a 20 mM stock solution and stored at −20°C for up to 1 week. Thiamine was added to autoclaved PMA liquid or solid medium at a final concentration of 10 μM.

Plasmid constructs. A cDNA in Bluescript SK+ of wild-type p53 was generously provided by B. Vogelstein, Johns Hopkins University. *Nde*I and *Bam*HI restriction sites were placed at the initiating methionine and just after the termination codon, respectively, by the polymerase chain reaction. The polymerase chain reaction was performed as follows. Target DNA (1 μg) was added to a reaction mixture containing 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 100 pmol of each oligonucleotide (GGGCATATGGAGGAGCCGCAGTCA and GGGGGATC CAAAATGGCAGGGAGGGA), and 1 U of *Taq* polymerase (Cetus). Five cycles of 94°C for 1.5 min, 55°C for 3 min, and 72°C for 5 min were carried out by using an Eppendorf Thermocycler. The resulting product (ca. 1,200 bp) was gel purified, digested with *Nde*I and *Bam*HI, and subcloned into *Nde*I- and *Bam*HI-digested pREP1 (27). The plasmid was designated p53wtREP1. A cDNA of the human p53 gene that contains a point mutation at amino acid 273 of an arginine to a histidine (16) was generously provided by E. Harlow, Massachusetts General Hospital Cancer Center. This cDNA, His-273, was subcloned into pREP1 exactly as described above. This plasmid was designated p53273REP1. Point mutations at amino acid residues 175, 315, and 392 were introduced by oligonucleotide-directed in vitro mutagenesis (Amersham). The changes were all verified by dideoxynucleotide sequencing with the Sequenase system

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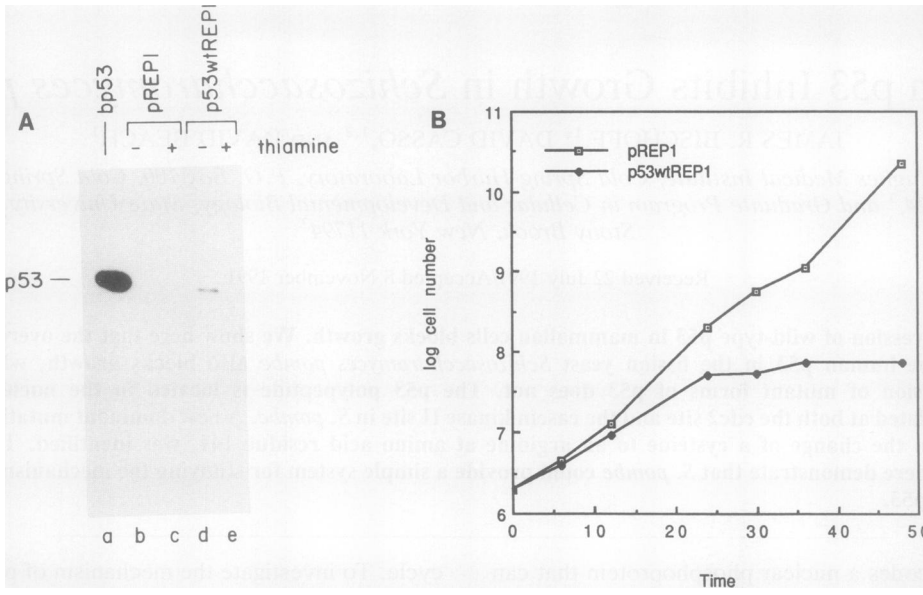

FIG. 1. Expression of wild-type human p53 inhibits growth in *S. pombe*. (A) Immunoblot of lysates prepared from wild-type *S. pombe* (SP130) transformed with either the expression vector pREP1 (lanes b and c) or p53wtREP1 (lanes d and e), grown in the presence (+) or absence (−) of 20 μM thiamine. Lane a (bp53) contains 0.2 μg of human wild-type p53 immunopurified from a bacterial overexpression system (3a). The blot was probed with PAb421, a monoclonal antibody to p53 (15). (B) Growth curve of SP130 containing either pREP1 or p53wtREP1 grown in the absence of thiamine.

(U.S. Biochemical Corp.). The resulting plasmids were named as follows: p53175REP1, a change of an arginine to a histidine at residue 175; p53ala315, a change of a serine to an alanine at residue 315; p53asp315, a change of a serine to an aspartate at residue 315; p53ala392, a change of a serine to an alanine at residue 392; and p53asp392, a change of a serine to an aspartate at residue 392.

***S. pombe* transformations.** Transformation of *S. pombe* was carried out by the method of Okazaki et al. (36), with the following modification: after heat shock, the cells were allowed to recover in nonselective medium for 2 h prior to plating on selective medium.

Western blots (immunoblots) and tryptic peptide maps. Preparation of protein lysates from *S. pombe* and metabolic labeling of *S. pombe* with $^{32}P_i$ (ICN) were done as previously described (5). Western immunoblots and two-dimensional tryptic maps of ^{32}P-labeled p53 were done as previously described (4).

Immunofluorescence. Immunofluorescence was carried out by the method of Booher et al. (5), except for the following modifications: the primary antibody (PAb421) was used at a 1:6 dilution, and either fluorescein-conjugated (Amersham) or rhodamine-conjugated (Boehringer) secondary antibody was used at a dilution of 1:100.

Gene replacement. The wild-type human p53 cDNA with the *nmt1* promoter and polyadenylation signal was digested from p53wtREP1 with *Sac*I and *Pst*I, blunted with T4 DNA polymerase, and ligated into the *Stu*I site of the *S. pombe ura4* gene in pUC18. The p53-disrupted *ura4* was excised from pUC18 by a partial *Sph*I digest and transformed into SP130. Cells from the transformation were plated onto minimal medium supplemented with uracil, leucine, adenine, and 2 mg of 5-fluoroorotic acid per ml (Pharmacia). Only cells that have lost the *ura4* gene will grow on plates containing 5-fluoroorotic acid. Southern and Western blots were performed on colonies that were uracil auxotrophs. A strain, SP982 (*h⁻ leu1.32 ura4::p53 ade6.210*), was obtained in which the *ura4* gene was disrupted with the cDNA of wild-type p53 under control of the *nmt1* promoter and in which the expression of the p53 polypeptide inhibited growth.

RESULTS

Overexpression of wild-type human p53 inhibits growth in *S. pombe*. A cDNA of wild-type human p53 was subcloned downstream of the *nmt1* promoter. The *nmt1* promoter is repressed when *S. pombe* is grown in minimal medium supplemented with thiamine and is derepressed when *S. pombe* is grown in thiamine-free medium (27). When this construct was introduced into a wild-type *S. pombe* strain, the p53 polypeptide was expressed, resulting in an inhibition of growth (Fig. 1). Similar results have been observed with mammalian cell systems (8, 26, 30, 31).

To determine whether the inhibition of growth depicted in Fig. 1 was due to the p53 polypeptide or to a nonspecific effect of overexpression of a foreign protein, two mutant p53 genes were introduced separately into *S. pombe*. One mutant was a change of an arginine to a histidine at amino acid residue 273 (His-273), and the other mutant was also a change of an arginine to a histidine, but at amino acid residue 175 (His-175). These mutations in p53 are frequently found in tumor biopsies (1, 34). Wild-type p53 and the two mutant polypeptides were expressed to similar levels in *S. pombe* (Fig. 2A). The polypeptides had different effects on the growth of *S. pombe* (Fig. 2C and D). Although after 24 h of expression wild-type p53 caused *S. pombe* to stop dividing, two mutant p53 polypeptides caused *S. pombe* to grow slowly relative to the same strain grown under conditions which repressed the *nmt1* promoter. The His-175 mutant polypeptide had the smallest effect on the growth of *S. pombe*. The His-273 polypeptide was intermediate between wild-type p53 and His-175 p53 with respect to its growth-inhibitory effect. Similar results have been obtained with

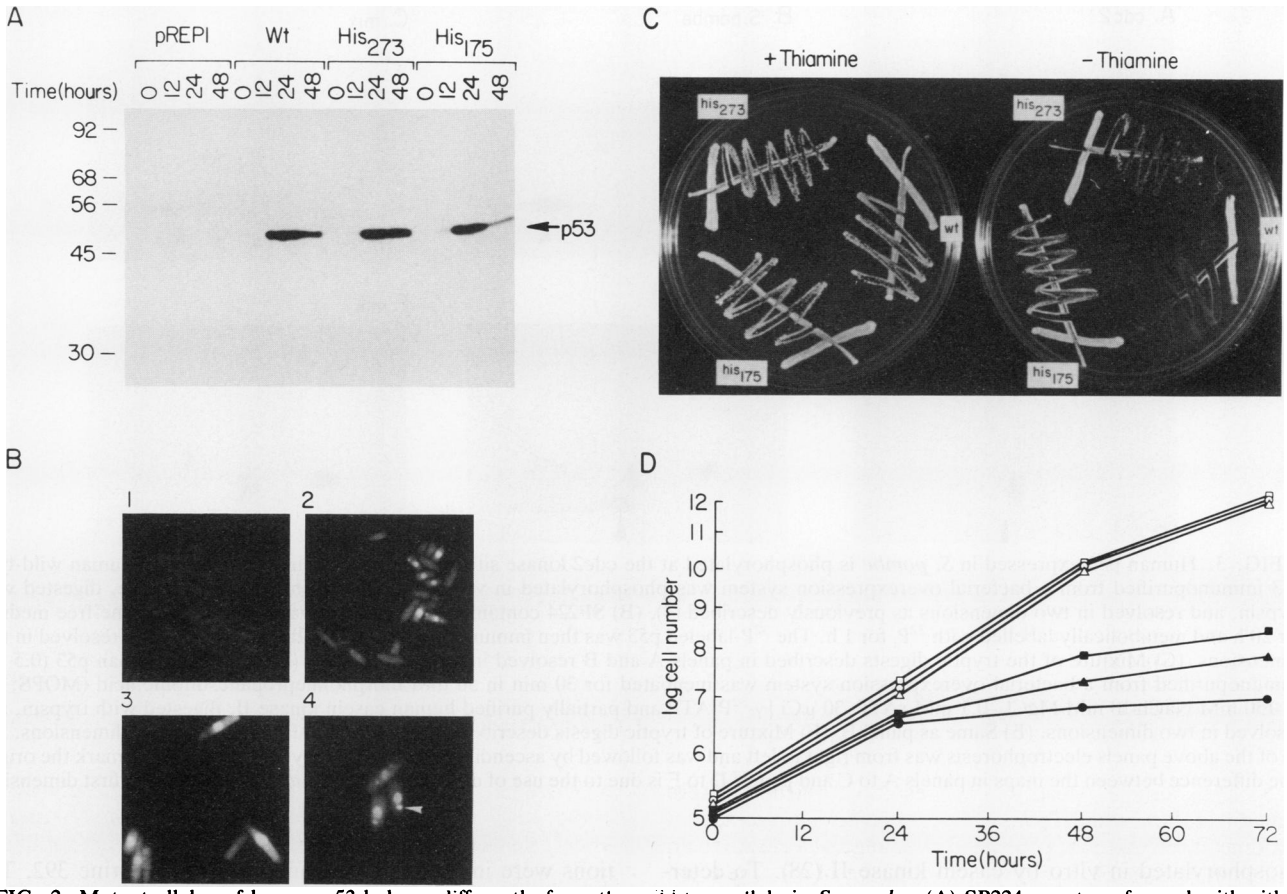

FIG. 2. Mutant alleles of human p53 behave differently from the wild-type allele in *S. pombe*. (A) SP224 was transformed with either pREP1, p53wtREP1, p53273REP1, or p53175REP1. The transformants were grown in thiamine-free medium for 0, 12, 24, and 48 h, harvested, and lysed. Total protein (100 μg) was immunoblotted and probed with PAb421. pREP1, Wt, His_{273}, and His_{175} refer to cells transformed by pREP1, p53wtREP1, p53273REP1, and p53175REP1, respectively. The numbers at left indicate the positions of molecular mass markers of 92,000, 68,000, 56,000, 45,000, and 30,000 Da. (B) Immunofluorescence of *S. pombe* with PAb421. Panel 1, cells containing p53wtREP1 grown in medium containing thiamine. Panel 2, cells expressing wild-type p53. Panel 3, cells expressing the His-273 mutant of p53. Panel 4, cells expressing the His-175 mutant of p53; the arrow indicates extranuclear staining. (C) A single colony of SP224 which contained either pREP1, p53wtREP1, p53273REP1, or p53175REP1 was struck out on thiamine-containing or thiamine-free plates. The plates were incubated for 3 days at 32°C. (D) Growth curves of SP224 containing either p53wtREP1 (●, ○), p53273REP1 (▲, △), or p53175REP1 (■, □) in the presence (open symbols) or absence (closed symbols) of 20 μM thiamine.

Saccharomyces cerevisiae (11, 34a). For unknown reasons the growth-inhibitory effect of p53 is more severe when the cells are grown in liquid medium (Fig. 2C and D).

The inhibition of growth of *S. pombe* by wild-type p53 was accompanied by a morphological phenotype (data not shown). After 24 h of wild-type p53 expression, the cells formed multisepta. Some cells also became branched. The cells were phase bright, which is an indication of being in stationary phase. His-273 and His-175 also induced the formation of multisepta, but to a lesser degree. Because of this phenotype, we were unable to determine by flow cytometry the phase of the cell cycle in which these cells were blocked.

In mammalian cells, p53 is predominantly a nuclear protein (14, 26). To determine whether this is so in *S. pombe*, immunofluorescence of the cells described above was carried out. p53 protein was not detected in *S. pombe* when the *nmt1* promoter was repressed (Fig. 2B, panel 1). The wild-type protein was localized to the nucleus, and the mutant polypeptides exhibited more extranuclear staining than the wild-type p53 did (Fig. 2C, panels 2, 3, and 4). The His-175 protein was localized to an unknown cytoplasmic compartment (Fig. 2B, panel 4, arrowhead) 10 times more frequently than the wild-type and His-273 polypeptides were. This might be due to the association of the His-175 polypeptide with the *S. pombe* homolog of heat shock protein 70 (hsc70) (data not shown). In mammalian cells the His-175 mutant associates with hsc70 (23) and is thought to be a hallmark of p53 proteins that are activated for transformation (12, 13). Two recent reports have indicated that p53 must be in the nucleus to exert its growth-inhibitory effect (14, 26). The partial cytoplasmic localization of the His-273 and His-175 polypeptides could explain their weaker growth-inhibitory effect in *S. pombe*. The two mutant proteins had a shorter half-life than the wild-type protein (data not shown); this may also be due to their partial cytoplasmic localization.

Phosphorylation of p53 in *S. pombe*. The p53 protein is phosphorylated on at least four residues in vivo (29, 40). One phosphorylation site on human p53 is serine 315, which is phosphorylated in vitro by the cell cycle-regulated cdc2 kinases (4). Another phosphorylation site at residue 389 of murine p53 (the equivalent of serine 392 of human p53) is

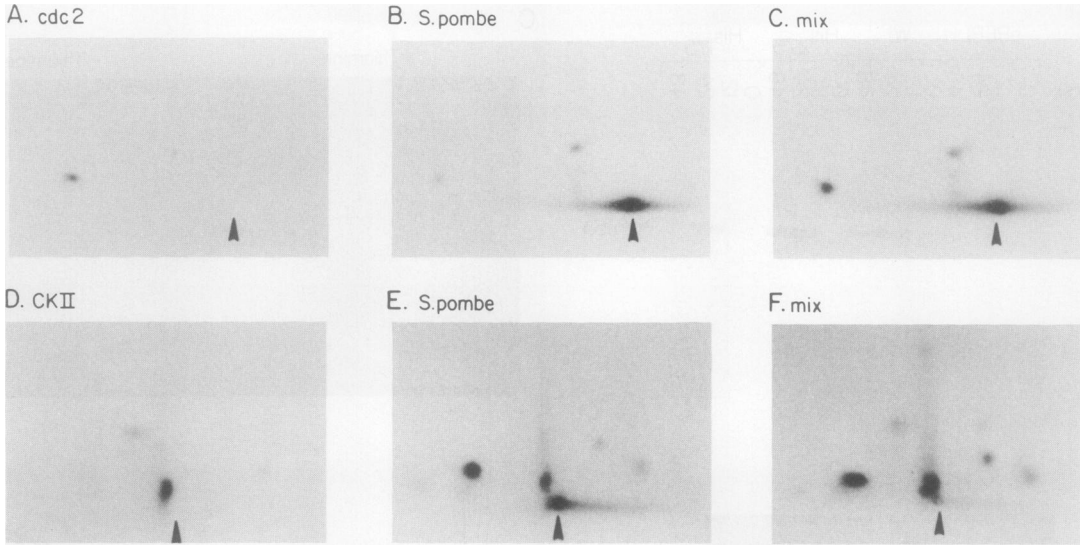

FIG. 3. Human p53 expressed in *S. pombe* is phosphorylated at the cdc2 kinase site and the casein kinase II site. (A) Human wild-type p53 immunopurified from a bacterial overexpression system was phosphorylated in vitro with purified human cdc2 kinase, digested with trypsin, and resolved in two dimensions as previously described (4). (B) SP224 containing p53wtREP1 was grown in thiamine-free medium for 20 h and metabolically labelled with $^{32}P_i$ for 1 h. The ^{32}P-labeled p53 was then immunopurified, digested with trypsin, and resolved in two dimensions. (C) Mixture of the tryptic digests described in panels A and B resolved in two dimensions. (D) Wild-type human p53 (0.5 μg) immunopurified from a bacterial overexpression system was incubated for 30 min in 50 mM morpholinepropanesulfonic acid (MOPS; pH 7)–150 mM NaCl–10 mM $MgCl_2$–0.1 mM rATP–30 μCi [γ-^{32}P]ATP and partially purified human casein kinase II, digested with trypsin, and resolved in two dimensions. (E) Same as panel B. (F) Mixture of tryptic digests described in panels D and E resolved in two dimensions. For all of the above panels electrophoresis was from right to left and was followed by ascending chromatography. The arrowheads mark the origin. The difference between the maps in panels A to C and panels D to F is due to the use of different electrophoresis buffers in the first dimension.

phosphorylated in vitro by casein kinase II (28). To determine whether these sites were phosphorylated when wild-type human p53 protein was expressed in *S. pombe*, the following experiment was done. Wild-type human p53 purified from a bacterial overexpression system was ^{32}P labeled in vitro by either purified human cdc2 kinase (4) or purified human casein kinase II. At the same time, human p53 was metabolically labeled by $^{32}P_i$ in *S. pombe*. The ^{32}P-labeled p53 polypeptides were immunopurified, digested with trypsin, and resolved in two dimensions. The p53 protein was phosphorylated at one major site in vitro by human cdc2 kinase (Fig. 3A). A tryptic map of p53 metabolically phosphorylated in vivo by *S. pombe* reveals three major phosphopeptides (Fig. 3B) and, upon longer exposures, an additional three minor phosphopeptides (Fig. 3E). When a tryptic digest of metabolically labeled p53 protein was mixed with the in vitro-labeled p53 protein, no unique phosphopeptides were observed and the in vitro-labeled phosphopeptide comigrated in two dimensions with an in vivo-labeled phosphopeptide (Fig. 3B and C). Casein kinase II also phosphorylated one major peptide of human p53 in vitro (Fig. 3D). When this in vitro-labeled material was mixed with the in vivo-labeled material, the in vitro-labeled phosphopeptide comigrated with a peptide phosphorylated in vivo (Fig. 3D and E). The two phosphopeptides just above and to the left of the origin in Fig. 3F are probably the result of a partial tryptic digest of the serine 392-containing peptide, since both these spots disappear in the Ala-392 mutant; for an explanation, see below. Thus, cdc2 kinase and casein kinase II phosphorylated p53 in vitro on sites that were also phosphorylated in vivo in *S. pombe*.

To determine whether these phosphorylation sites play a role in the growth-inhibitory effect of p53, these sites were altered by oligonucleotide-directed mutagenesis. Two mutations were introduced at serine 315 and at serine 392. The phosphorylation sites were changed by making alanine (Ala-315 and Ala-392) and aspartate (Asp-315 and Asp-392) substitutions. The aspartate mutations were made to provide a negative charge at the phosphorylation sites to mimic constitutive phosphorylation at serine 315 and serine 392. The mutations were confirmed by DNA sequencing (data not shown). The four mutant cDNAs under control of the *nmt1* promoter were introduced individually into *S. pombe*, and two-dimensional phosphotryptic mappings of immunopurified ^{32}P-labeled p53 were performed. The alanine substitution at 315 removed the cdc2 kinase site (Fig. 4B, upper arrowhead), and the alanine substitution at 392 removed the casein kinase II site (Fig. 4C, upper arrowhead). The aspartate mutations yielded maps identical to those of the alanine mutations (data not shown). Thus, the mutations removed the phosphorylation sites identified above.

The effect of these mutations on the ability of p53 to inhibit growth was examined. The Ala-315, Asp-315, Ala-392, and Asp-392 mutations inhibited growth to the same extent that wild-type p53 did in *S. pombe* (data not shown). Thus, phosphorylation of p53 at Ser-315 and Ser-392 did not affect the ability of p53 to block growth in *S. pombe*.

Identification of a new dominant mutant allele of human p53. It has been shown indirectly that certain mutant forms of p53 are dominant to and can inactivate wild-type p53. To provide a rapid assay to directly identify mutant alleles of p53 that are dominant to wild-type p53, we constructed a *S. pombe* strain which contained a single copy of wild-type human p53 under control of the *nmt1* promoter integrated into the genome. This strain has been designated SP982. The expression of wild-type p53 in SP982 inhibited growth (data not shown). Several different mutant alleles of human p53 were introduced into SP982 to determine whether they could

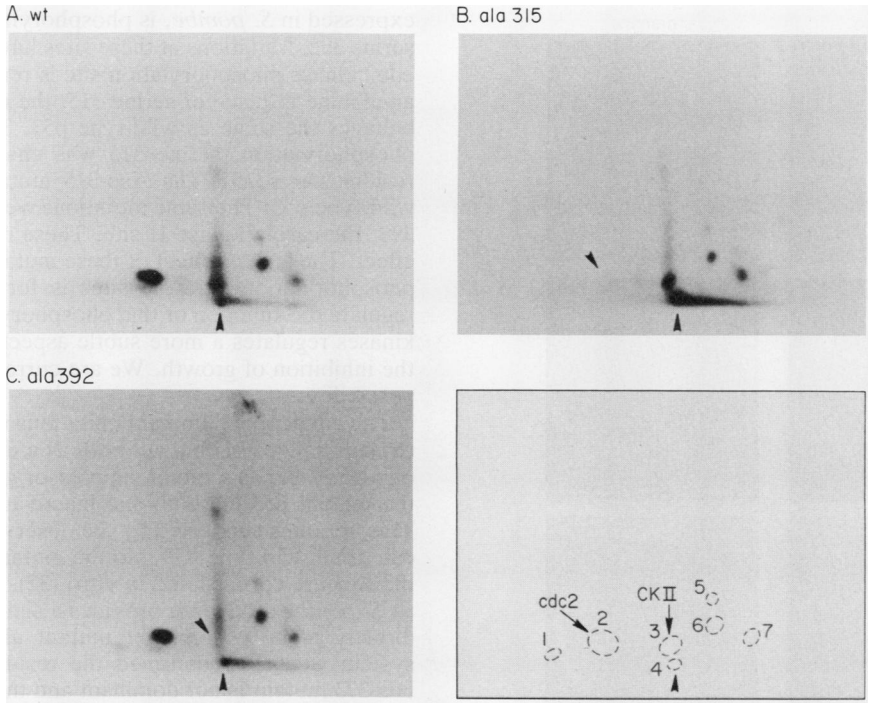

FIG. 4. Mutations at serine 315 and serine 392 remove phosphorylation sites on human p53. SP224 containing either p53wtREP1, p53ala315, or p53ala392 was grown in thiamine-free medium for 20 h and then metabolically labeled with $^{32}P_i$ for 1 h. The ^{32}P-labeled p53 was then immunopurified, digested with trypsin, and resolved in two dimensions. (A) Two-dimensional tryptic map of wild-type (wt) human p53 labeled in *S. pombe*; the arrowhead indicates the origin. (B) Two-dimensional tryptic map of ^{32}P-labeled p53ala315. The lower arrowhead indicates the origin, and the upper arrowhead designates the lost phosphopeptide. (C) Two-dimensional tryptic map of ^{32}P-labeled p53ala392. The lower arrowhead indicates the origin, and the upper arrowhead indicates the lost phosphopeptide. (D) Cartoon of the tryptic phosphopeptides of human p53 phosphorylated in *S. pombe*. The cdc2 kinase and casein kinase II (CKII) phosphopeptides are indicated.

release *S. pombe* from the inhibitory effects of wild-type p53. The expression vector alone and the expression vector containing wild-type p53 did not rescue *S. pombe* from the effects of wild-type p53 (Fig. 5A and B). A mutation at residue 273 (His-273) which does not inhibit growth as severely as wild-type p53 does in *S. pombe* (Fig. 2C) was not dominant to wild-type p53 (Fig. 5C), consistent with observations made with mammalian cells (7). Two mutations, His-175 and Arg-141, were dominant in this assay (Fig. 5D and E). The His-175 mutant has been shown to be dominant to wild-type p53 in mammalian cells (23). The Arg-141 mutation was obtained fortuitously during an in vitro mutagenesis reaction while creating the Asp-392 mutant. The mutation is the change of a cysteine to an arginine at amino acid residue 141. This mutation had the least effect on the growth of *S. pombe* (data not shown). These two mutants were not dominant when assayed in liquid medium; therefore it was not possible to accurately quantitate their relative dominance to wild-type p53.

DISCUSSION

Three points have been made in this communication. Wild-type human p53 blocks growth in the fission yeast *S. pombe*, whereas transforming mutants of p53 do not. Phosphorylation at Ser-315 and Ser-392 does not regulate p53 function, and a mutation at amino acid residue 141 is dominant to the wild-type p53.

The antiproliferative effect of wild-type p53 in mammalian cells is well established (8, 26, 28, 31). Here, we extend those observations to include lower eukaryotes. The following observations suggest that human p53 is functioning in a specific manner in *S. pombe*. As in mammalian cells, wild-type human p53 in *S. pombe* is localized to the nucleus, its overexpression inhibits growth, and it can associate with simian virus 40 T antigen to form a stable complex (3a). Mutant forms of p53 exhibit a weaker antigrowth effect in *S. pombe*. These mutant proteins do not inhibit growth in mammalian cells, but can cooperate with activated *ras* to transform cells. The His-175 mutant cooperates with activated *ras* to transform cells 3 to 10 times better than the His-273 mutant does (23). Thus, the ability of these mutants to assist *ras* in the transformation of mammalian cells correlates inversely with their ability to inhibit growth in *S. pombe*. p53 in *S. pombe* is phosphorylated at two sites that have been identified in mammalian cells. Finally, the His-273 mutant, which has been shown to be recessive to wild-type p53 in mammalian cells, is recessive in *S. pombe* whereas the His-175 mutant, which has been shown to be dominant in mammalian cells, is also dominant in *S. pombe*.

The mechanism of growth inhibition by wild-type p53 in *S. pombe* is not known. The amino terminus of the p53 polypeptide has been shown to possess a powerful transcriptional transactivation domain (11, 38). In addition, p53 can bind to specific DNA sequences (2, 20). The consequences of p53 binding at these sequences are not known. Perhaps wild-type p53 inhibits growth in *S. pombe* by titrating an essential transcription factor and/or by imposing irregular transcriptional control on an important gene(s).

Two kinases which are involved in cell growth have been shown to phosphorylate p53 at sites that are phosphorylated in vivo. The cdc2 kinase, which has been shown to be

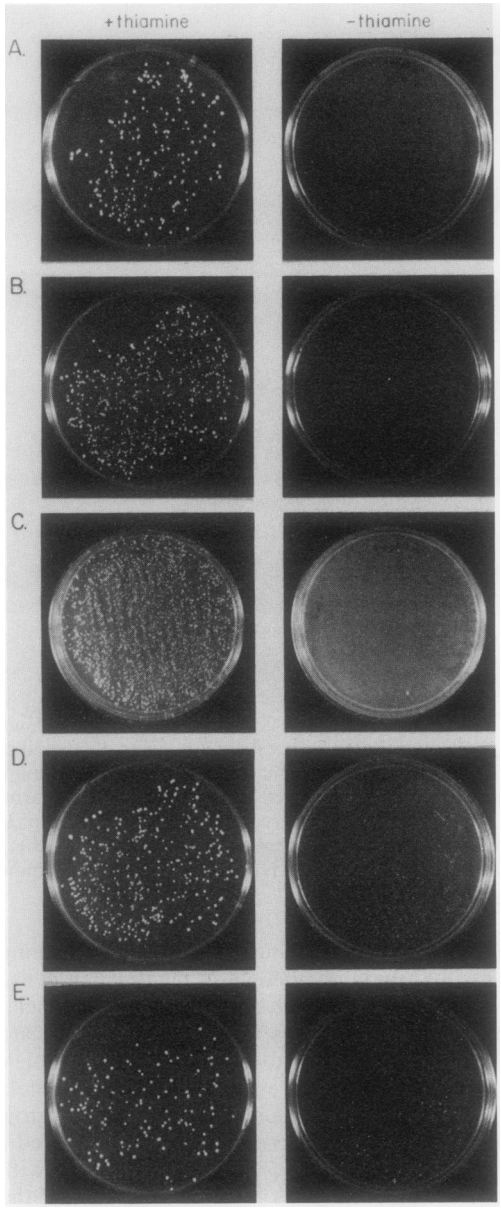

FIG. 5. A change of a cysteine to an arginine at residue 141 creates a dominant allele of p53. SP982 was precultured for 24 h in MBA medium (36) containing thiamine. The cells were washed twice with sterile H_2O and inoculated into thiamine-free MBA medium at a density of 10^5 cells per ml. The culture was incubated at 32°C for 20 h, and 10^9 cells were prepared for transformation (36). (A) SP982 transformed with pREP1. (B) SP982 transformed with p53wtREP1. (C) SP982 transformed with p53273REP1. (D) SP982 transformed with p53175REP1. (E) SP982 transformed with p53arg141. The transformation mixture was divided into two aliquots, plated on thiamine-containing (left) or thiamine-free (right) plates, and incubated for 5 days at 32°C.

required at the start of DNA synthesis and at mitosis in yeasts (35), phosphorylates human p53 on serine 315 (4). Casein kinase II, whose activity is stimulated by various mitogens (6, 19, 42), phosphorylates murine p53 on serine 389 (28). Here, it is shown that casein kinase II phosphorylates human p53 on serine 392, the equivalent of murine serine 389. We have shown that human wild-type p53, when expressed in *S. pombe*, is phosphorylated on serine 315 and serine 392. Mutations at these sites have no effect. When the cdc2 kinase phosphorylation site is removed by substituting an alanine in place of serine 315, the mutated p53 (Ala-315) behaves the same as wild-type p53. To mimic constitutive phosphorylation, serine 315 was changed to an aspartate residue (Asp-315). The Asp-315 mutant also behaves like wild-type p53. The same mutations were also made at serine 392, the casein kinase II site. These mutations also had no effect. The lack of effect of these mutations could mean that phosphorylation at these sites is fortuitous and does not regulate p53 function or that phosphorylation of p53 by these kinases regulates a more subtle aspect of p53 function than the inhibition of growth. We are currently investigating this possibility.

The concept of dominant mutations in p53 was put forth to explain how p53 could act both as a dominant transforming oncogene and as a tumor suppressor gene. It is thought that the mutant p53 binds to and inactivates the wild-type p53. This notion is supported by the observation that mutant p53 can drive wild-type p53 into the mutant conformation when the two are cotranslated in vitro (32). We have constructed an *S. pombe* strain that provides a simple and rapid assay to directly identify dominant mutant alleles of p53. In this system we have confirmed the results of others that the His-273 mutant is not dominant and that the His-175 mutant is dominant to wild-type p53 (7, 23). We show that a change of a cysteine to an arginine at residue 141 is dominant to wild-type p53. Interestingly, a p53 mutation at residue 141 (a change to atyrosine) has been isolated from a colon carcinoma (34). This is the first direct demonstration of dominant mutant alleles of human p53. As a cautionary note, it should be stated here that the mutant alleles were probably present in more than one copy per cell; it was not determined whether one copy of His-175 or Arg-141 was dominant to wild-type p53.

The fact that one can identify dominant mutant alleles of p53 by using *S. pombe* suggests that this system can be used to isolate genes which directly interact with p53 and perhaps even to identify genes which function along the p53 pathway. We are currently pursuing this avenue of investigation.

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