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Negative regulation of mitosis in fission yeast by catalytically inactive *pyp1* and *pyp2* mutants

(cell cycle regulation/protein tyrosine phosphatases/*Schizosaccharomyces pombe*)

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ABSTRACT **The *Schizosaccharomyces pombe* genes *pyp1*$^+$ and *pyp2*$^+$ encode protein tyrosine phosphatases (PTPases) that act as negative regulators of mitosis upstream of the *wee1*$^+$/*mik1*$^+$ pathway. Here we provide evidence that *pyp1*$^+$ and *pyp2*$^+$ function independently of *cdr1*$^+$(*nim1*$^+$) in the inhibition of mitosis and that the wee1 kinase is not a direct substrate of either PTPase. In a *pyp1*::*ura4 cdc25-22* genetic background, overexpression of either the N-terminal domain of *pyp1*$^+$ or a catalytically inactive mutant, *pyp1*C470S, causes cell cycle arrest. This phenotype reverses the suppression of a *cdc25* temperature-sensitive mutation at 35°C caused by a *pyp1* disruption. Furthermore, *pyp1*C470S and a catalytically inactive mutant of *pyp2*, *pyp2*C630S, induce mitotic delay as do their wild-type counterparts. Analysis of *pyp1*$^+$ and *pyp2*$^+$ further reveals that the *in vitro* PTPase activity of pyp1 and pyp2, as well as their biological activity, is dependent on the presence of N-terminal sequences that are not normally considered part of PTPase catalytic domains.**

The initiation of mitosis is a highly conserved process among eukaryotes, including the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (for review, see ref. 1). It is regulated by the activity of an evolutionarily conserved complex referred to as maturation or M-phase promoting factor consisting of a serine/threonine-specific protein kinase known as p34^{cdc2} (for review, see ref. 2) and associated cell-cycle-regulated proteins. In late G_2 phase, p34^{cdc2} is maximally phosphorylated on Tyr-15 (3). To initiate mitosis in fission yeast, which is accompanied by the rapid activation of the p34^{cdc2}–cyclin complex, this Tyr residue has to be dephosphorylated by a phosphatase known as cdc25 (4–8). The activity of p34^{cdc2} is negatively regulated by the *wee1*$^+$/*mik1*$^+$ pathway (9, 10). We have shown that the two fission yeast protein tyrosine phosphatases (PTPases) pyp1 and pyp2 possess intrinsic tyrosine phosphatase activity (11) and that the overexpression of either gene leads to mitotic delay (12, 13). This phenotype is similar to that displayed by cells overexpressing the negative regulators of mitosis, *wee1*$^+$ and *mik1*$^+$ (9, 10). Since *pyp1*$^+$ and *pyp2*$^+$ function is dependent on a functional *wee1*$^+$ gene product (13), both PTPases act as negative regulators upstream of the *wee1*$^+$/*mik1*$^+$ pathway.

In view of the apparent conservation of the major cell cycle control elements among eukaryotes and the well-defined mitotic network in *S. pombe*, we were interested in characterizing further the mitotic function of *pyp1*$^+$ and *pyp2*$^+$ by analyzing the contribution of various structural domains of both enzymes to their biological activity. Our data demonstrate that enzymes that possess a crucial role in the negative regulation of mitosis can function even in the absence of catalytic activity, demonstrating unique features of pyp1 and pyp2 among the known PTPases.

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MATERIALS AND METHODS

***S. pombe* Strains and Media.** The following *S. pombe* strains were used in this study: h$^+$ *leu1-32 ura4-D18 ade6-M216* (FWP 165; F. Winston, Harvard Medical School, Boston), h$^+$ *leu1-32 ura4-D16 cdc25-22* (P. Russell, Scripps Institute, La Jolla, CA), h$^-$ *leu1-32 ura4-D18 wee1-50* (P. Russell), h$^-$ *pyp1*::*ura4 leu1-32 ura4-D18 cdc25-22* (SOP18) (13), h$^+$ *pyp1*::*ura4 leu1-32 ura4-D18 ade6-M216* (SOP 14) (13), *adh-nim1*$^+$ *leu2 ura4-D18* (P. Russell), and *nim1*::*LEU2 ura4-D18* (P. Russell). *S. pombe* strains were grown in complex medium, YEA (14), or minimal medium (EMM) with or without 20 μM thiamine. *S. pombe* cells were transformed as described (15).

Cloning and Expression. Before subcloning into the *S. pombe* pREP3 expression vector, it was modified to eliminate the initiator methionine provided by the pREP3 polylinker sequence: the vector was linearized with *Bal* I, *Bam*HI linkers were attached, the DNA was digested with *Bam*HI, and the vector was circularized with T4 DNA ligase.

A *S. pombe pyp1*$^+$ cDNA fragment (12) was subcloned into a *Bgl* II restriction site of a modified version of the *S. pombe* expression vector pREP1 lacking the ATG provided by the polylinker as described (13, 16), to generate pREP1-*pyp1*$^+$.

A mutant of wild-type *pyp1*$^+$ containing Ser instead of Cys at amino acid 470 (*pyp1*C470S) was generated by site-directed mutagenesis using a kit and following the manufacturer's protocol (Amersham). The mutagenic oligonucleotide 5′-TACTATTGTGCACAGATCTGCCGGTGTT-3′, which contains an artifically introduced *Bgl* II restriction site (underlined), was used to introduce this mutation. The mutation was confirmed by restriction digestion with *Bgl* II and DNA sequencing and the fragment was subcloned into pREP3.

A mutant of wild-type *pyp1*$^+$ containing Lys instead of Arg at amino acid 476 (*pyp1*R476K) was generated with the mutagenic oligonucleotide 5′-GCCGGTGTTGGTAAGA-CAGGAACCTTT-3′, as described for *pyp1*C470S. The mutation was confirmed by DNA sequencing, and the fragment was subcloned into pREP3 to generate pREP3-pyp1R476K.

To generate *pyp1* deletion mutants, we employed PCR using the following oligonucleotide primers. Each oligonucleotide primer contained appropriate restriction sites (underlined) to clone these fragments into the *S. pombe* multicopy plasmids pREP1 or pREP3 and into pGEX-2T (17) or pGEX-KG (18) for expression in bacteria. Primers: *pyp1*ΔN, 5′ primer (5′-GCGGGATCCCATATGTTTGGCAGTGC-TACAGTC-3′; amino acids 235–240) and 3′ primer (5′-GCGGGATCCGAATTCTCATGTTAAAACCGG-3′);

Abbreviation: PTPase, protein tyrosine phosphatase.

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*pyp1*CD, 5′ primer (5′-GCGGGATCCCATATGAAAAACAGATATACCGAC-3′; amino acids 297–303) and same 3′ primer as for *pyp1*ΔN; *pyp1*ND, 5′ primer (5′-GCGGGATCCCATATGAATTTTTCAACCGGT-3′) and 3′ primer (5′-GCGGGATCCTCACTTATACGATGTATTACT-3′; amino acids 291–296); *pyp1*235/296, same 5′ primer as for *pyp1*ΔN and the same 3′ primer as for *pyp1*ND.

A mutant of wild-type *pyp2*$^+$ containing Ser instead of Cys at position 630 (*pyp2*C630S) was constructed as described for *pyp1*C470S. The mutagenic oligonucleotide 5′-CAATGTTCGTTCACAGCTCAGCAGGCGTA-3′ contains an artificially introduced *Alu* I restriction site (underlined), and the mutation was confirmed by restriction digestion with *Alu* I and DNA sequencing.

A mutant wild-type *pyp2*$^+$ containing Lys instead of Arg at position 636 (*pyp2*R636K) was constructed with the mutagenic oligonucleotide 5′-GCAGGCGTAGGAAAAACTGGTACTTTTA-3′ as described for *pyp1*C470S. The mutation was confirmed by DNA sequencing.

A *pyp2*$^+$ cDNA fragment, in the pBluescript vector (pBS-pyp2$^+$), was digested with *Spe* I and *Bam*HI to generate a *pyp2*$^+$ fragment with a truncation of the first 345 N-terminal amino acids. The ends were filled-in with the Klenow fragment of DNA polymerase I, *Bam*HI linkers were attached, the construct was digested with *Bam*HI, and the fragment was subcloned into the modified pREP3 vector to generate pREP3-pyp2ΔN(1) and into pGEX-KG to generate pGEX-KG-pyp2ΔN(1). Further *pyp2* deletion mutants were generated by PCR using the following pairs of oligonucleotide primers: *pyp2*ΔN(2), 5′ primer (5′-GCGGGATCCTATAAGTTTAAAAGACTTGAGG-3′; amino acids 425–430) and 3′ primer (5′-GCGGGATCCTTAAGTCATCAAGGGCTTGGA-3′); *pyp2*CD, 5′ primer (5′-GCGGGATCCAAAAATCGTTACACAGATATCG-3′; amino acids 461–467) and 3′ primer was identical to that used for *pyp2*ΔN(2); *pyp2*ND, 5′ primer (5′-GCGGGATCCATGCTCCATCTTCTGTCTAAA-3′) and 3′ primer (5′-GCGGGATCCTTATCTTGAATTGGAAGTGGA-3′; amino acids 455–460).

To ensure that the amplified PCR products contained no *Taq* polymerase-induced mutations, their nucleotide sequences were determined by DNA sequencing to confirm wild-type products.

Purification of PTPases Expressed in Bacteria. Bacterial pGEX-2T and pGEX-KG expression plasmids containing either *pyp1* or *pyp2* mutants were transformed into the *Escherichia coli* JM109, and the glutathione-*S*-transferase fusion proteins were purified in a single glutathione affinity chromatography step as described (19), except that the purified proteins were not subjected to cleavage by thrombin.

Hydrolysis of *p*-Nitrophenyl Phosphate. The reactions were carried out in 200 μl containing 25 mM Hepes (pH 7.3), 5 mM EDTA, 10 mM dithiothreitol, and 20 mM *p*-nitrophenyl phosphate (Sigma) at 30°C either in the absence or presence of 1 mM sodium orthovanadate, a specific PTPase inhibitor. PTPase activity was determined both as a function of enzyme concentration (1 μg, 2.5 μg, or 5 μg) and as a function of time (15 min, 30 min, or 60 min). Addition of 200 μl of 1 M sodium carbonate terminated the reactions. The hydrolysis of *p*-nitrophenyl phosphate was monitored by determining the absorbance at 410 nm.

^{32}P-Labeled Raytide. ^{32}P-labeled Raytide (1 × 10^5 cpm; Oncogene Science), exclusively labeled on Tyr by p43$^{v\text{-}abl}$ (Oncogene Science), was incubated with bacterially expressed PTPases (1 μg, 2.5 μg, or 5 μg) for various amounts of time (30 min, 90 min, or 180 min) in phosphatase buffer containing 25 mM Hepes (pH 7.3), 5 mM EDTA, and 10 mM dithiothreitol, in the presence or absence of 1 mM sodium orthovanadate, at 30°C in 50 μl. The reaction was stopped and quantified as described (20).

RESULTS

Mutations of Invariant Residues in the Catalytic Sites of pyp1 and pyp2 Do Not Inhibit Their Ability to Induce Mitotic Delay. To study the effects of mutations in the catalytic domains of pyp1 and pyp2, we introduced a series of mutations into highly conserved amino acids in this region. These mutants were assayed for *in vitro* PTPase activity using both *p*-nitrophenyl phosphate, a phosphotyrosine-related chromogenic molecule, and [^{32}P]Tyr-labeled Raytide as substrates. Since the majority of full-length pyp1 and pyp2 expressed in bacteria is insoluble (11), we routinely used N-terminal truncations of both proteins. Furthermore, these mutants were overexpressed under the control of the inducible *nmt1* promoter (16) in a variety of *S. pombe* strains listed in Table 1.

The highly conserved Cys residue located within the signature motif found in the catalytic domains of all known PTPases is directly involved in the formation of a thiophosphate intermediate during catalysis and is essential for catalytic activity *in vitro* (21–23). To determine whether this Cys residue is also crucial for the capacity of pyp1 and pyp2 to induce mitotic delay, we altered Cys-470 of pyp1 and Cys-630 of pyp2 to Ser by site-directed mutagenesis. Overexpression of either *pyp1*C470S or *pyp2*C630S resulted in cell elongation in both wild-type and *cdc25-22* cells. The *cdc25-22* cells formed slower growing colonies compared to control cells transformed with pREP1 (Fig. 1 and Table 1). In a *pyp1*::*ura4*

Table 1. Phenotypes of *S. pombe* wild-type (FWP165) cells, *pyp1*::*ura4* cells, *cdc25-22* cells, and *pyp1*::*ura4 cdc25-22* cells after the overexpression of *pyp1* mutants or *pyp2* mutants under the control of the inducible *nmt1* promoter

	S. pombe strain phenotype					
	Wild-type	*pyp1*::*ura4*	*cdc25-22*		*pyp1*::*ura4 cdc25-22*	
	32°C	32°C	25°C	35°C	25°C	35°C
pyp1 construct						
*pyp1*WT	+	+	−	−	±	−
*pyp1*C470S	++	+	++	−	±	−
*pyp1*R476K	+	ND	+	−	±	−
*pyp1*ΔN^{234}	+	ND	−	−	+	−
*pyp1*CD^{296}	+++	ND	+++	−	+++	+++
*pyp1*ND^{297}	+++	++	++	−	++	−
*pyp1*235/296	+++	ND	+++	−	+++	+++
pREP1	+++	+++	+++	−	+++	+++
pyp2 construct						
*pyp2*WT	+		−	−	±	−
*pyp2*C630S	++		+	−	+	−
*pyp2*R636K	++		+	−	ND	ND
*pyp2*ΔN(1)^{345}	+		+	−	ND	ND
*pyp2*ΔN(2)^{424}	+++		+++	−	+++	+++
*pyp2*CD^{460}	+++		+++	−	+++	+++
*pyp2*ND^{461*}	+++		+++	−	+++	+++
pREP1	+++		+++	−	+++	+++

Transformed cells were grown to late logarithmic phase in EMM containing 20 μM thiamine at 32°C (wild-type and *pyp1*::*ura4* cells) or 25°C (*cdc25-22* and *pyp1*::*ura4 cdc25-22* cells), washed twice in sterile H_2O, diluted into EMM lacking thiamine, incubated further at 32°C (wild-type and *pyp1*::*ura4* cells) or 25°C (*cdc25-22* and *pyp1*::*ura4 cdc25-22* cells), and incubated further for 40 h. *pyp1*::*ura4 cdc25-22* cells were also shifted to 35°C and grown for another 40 h. +, Highly elongated cells (50–100%) with very slow colony formation; ++, cells display some degree of cell elongation (25–50%) and slower colony formation compared to control cells; +++, the phenotype of the transformed cells, as judged by cell size and their ability to form colonies on plate, is indistinguishable from that of nontransformed cells; ±, cells are very highly elongated (100–200%), some *cdc*$^-$; −, cell division cycle (*cdc*)-arrested cells; ND, not determined.

cdc25-22 genetic background, however, the overexpression of either mutant resulted in very highly elongated cells, and for *pyp1*C470S, this phenotype was hardly distinguishable from a cdc$^-$ phenotype (Table 1). These results indicate that mutation of this Cys residue does not abolish the biological activity of either enzyme but decreases its efficiency. Consistent with data reported for other PTPases (24–27), however, alteration of the Cys residue in the catalytic site of either enzyme completely abolished its *in vitro* PTPase activity (data not shown).

Since the Arg residue located within the PTPase signature motif has also been defined as an invariant residue that is crucial for *in vitro* PTPase activity (5, 28), we altered Arg-476 of pyp1 and Arg-636 of pyp2 to Lys by site-directed mutagenesis. The overexpression of these mutants also resulted in cell elongation. The mutant *pyp1*R476K influenced growth to a greater extent than did *pyp1*C470S, as judged by the ability of transformed cells to form colonies, whereas *pyp2*C630S and *pyp2*R636K showed no such difference (Fig. 1 and Table 1). In contrast to other reports (5, 28), however, alteration of the Arg residue located in the catalytic site of pyp1 did not completely inactivate the enzyme but significantly reduced its *in vitro* PTPase activity (data not shown).

Biological Activity of *pyp1*$^+$ and *pyp2*$^+$ Is Dependent on a Minimal N-Terminal Region. The overexpression of *pyp1*$^+$ and *pyp2*$^+$ causes mitotic delay in a *S. pombe* wild-type strain and leads to cell cycle arrest in a *cdc25-22* strain at the permissive temperature (13, 29). The same phenotypes were observed after the overexpression of *pyp1*ΔN in wild-type and *cdc25-22* cells (Fig. 2 and Table 1). These results demonstrate that the first 234 N-terminal amino acids (79% of the N terminus) of pyp1 are not crucial for its ability to induce mitotic delay. However, under the same conditions, the overexpression of *pyp1*CD, which lacks the remaining 60 N-terminal amino acids (residues 235–296), caused no morphological changes in either a wild-type or a *cdc25-22* strain (Fig. 1 and Table 1). This indicates that the putative catalytic domain of pyp1 is not sufficient to induce mitotic delay. To determine whether *pyp1*CD has lost its ability to induce mitotic delay but may be able to rescue a *cdc25-22* mutation, we shifted *cdc25-22* cells expressing *pyp1*CD to the restrictive temperature of 35°C. However, the overexpression of *pyp1*CD did not rescue the lethality of the *cdc25-22* mutation, which demonstrates that *pyp1*CD cannot substitute for the mutated *cdc25* gene (Table 1).

The overexpression of *pyp2*ΔN(1), which lacks the first 345 N-terminal amino acids (75% of the N terminus) of pyp2, led to highly elongated cells and slow growing colonies, a phenotype similar to that observed after the overexpression of *pyp2*$^+$. The phenotype was less severe when *pyp2*ΔN(1) was overexpressed in *cdc25-22* cells at the permissive temperature, compared to the cell cycle arrest caused by *pyp2*$^+$ overexpression in this strain. However, the overexpression of *pyp2*ΔN(2), which lacks the first 424 N-terminal amino acids (92% of the N terminus), displayed no detectable phenotypical changes in either wild-type or *cdc25-22* cells, which indicates, as for *pyp1*$^+$, that a minimal N-terminal region of pyp2 is required for its biological activity (Table 1). Furthermore, also consistent with *pyp1*$^+$, overexpression of the catalytic domain of *pyp2*$^+$ (*pyp2*CD) displayed no detectable phenotypical changes in either genetic background, and overexpression of *pyp2*CD failed to rescue the lethality of *cdc25-22* mutation at the restrictive temperature. These results correlate with the *in vitro* PTPase activity displayed by these mutants: *pyp1*ΔN and *pyp2*ΔN(1) had enzymatic activity similar to that determined for wild-type pyp1 and pyp2 proteins (11), whereas *pyp1*CD, *pyp2*CD, and *pyp2*ΔN(2) displayed no activity (data not shown).

***pyp2*$^+$ Activates the *wee1*$^+$/*mik1*$^+$ Pathway Independent of *cdr1*$^+$(*nim1*$^+$) Activity.** *Cdr1*$^+$(*nim1*$^+$) functions as a dosage-dependent inducer of mitosis upstream of *wee1*$^+$ (30–33). A *pyp1*::*ura4 nim1*::*LEU2* double disruption reverses the mitotic delay observed in a *nim1*-deleted strain, indicating that *pyp1*$^+$ and *cdr1*$^+$(*nim1*$^+$) regulate mitosis by independent mechanisms (29). We were further interested in investigating whether *pyp2*$^+$ interacts with *cdr1*$^+$(*nim1*$^+$) in the regulation of *wee1*$^+$/*mik1*$^+$ pathway. Therefore, we overexpressed *pyp2*$^+$ under the control of the inducible *nmt1* promoter in a strain carrying a *nim1* deletion. If *pyp2*$^+$ were to activate the *wee1*$^+$/*mik1*$^+$ pathway by inhibiting the cdr1(nim1) kinase, then overexpression of *pyp2*$^+$ would not be expected to be additive to the mitotic delay caused by a *nim1* deletion (32). We found, however, that the overexpression of *pyp2*$^+$ resulted in very highly elongated cells (100–200%), which were unable to form colonies, equivalent to a cdc$^-$ phenotype, whereas the loss of nim1 function caused only a moderate delay of mitosis, with a cell length at division of ≈18 μm (this study and ref. 32). Second, we overexpressed *pyp2*$^+$ in a strain that carries an additional integrated *cdr1*$^+$(*nim1*$^+$) copy under the control of the *adh* promoter. Fission yeast cells that

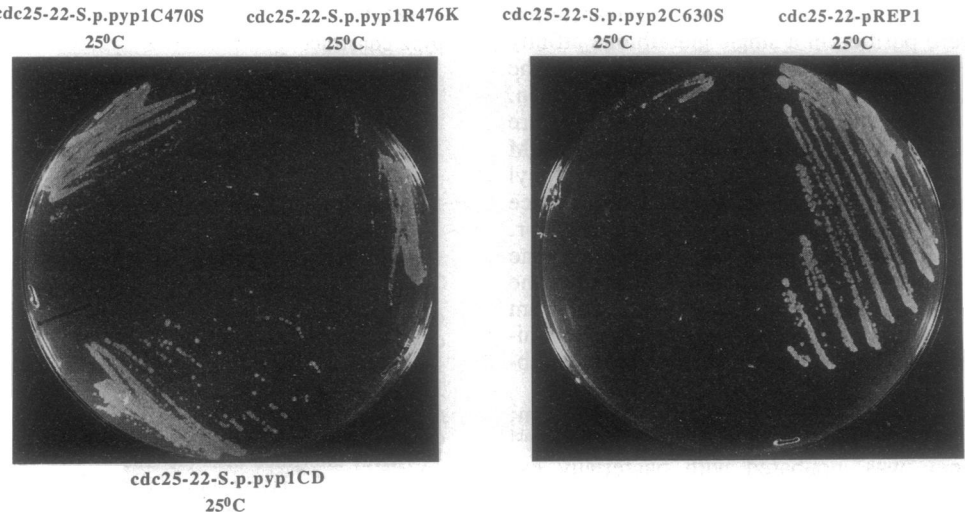

FIG. 1. Overexpression of *pyp1*R476K, *pyp1*C470S, and *pyp2*C630S in *cdc25-22* cells severely impairs the formation of single colonies. The *cdc25-22* cells overexpressing these mutants under the control of the inducible *nmt1* promoter were streaked on EMM plates lacking thiamine and incubated for 3–4 days at 25°C. Under the same experimental conditions, the colony formation of *cdc25-22* cells overexpressing *pyp1*CD or of *cdc25-22* cells transformed with the pREP1 expression vector was not affected. S.p., *S. pombe*.

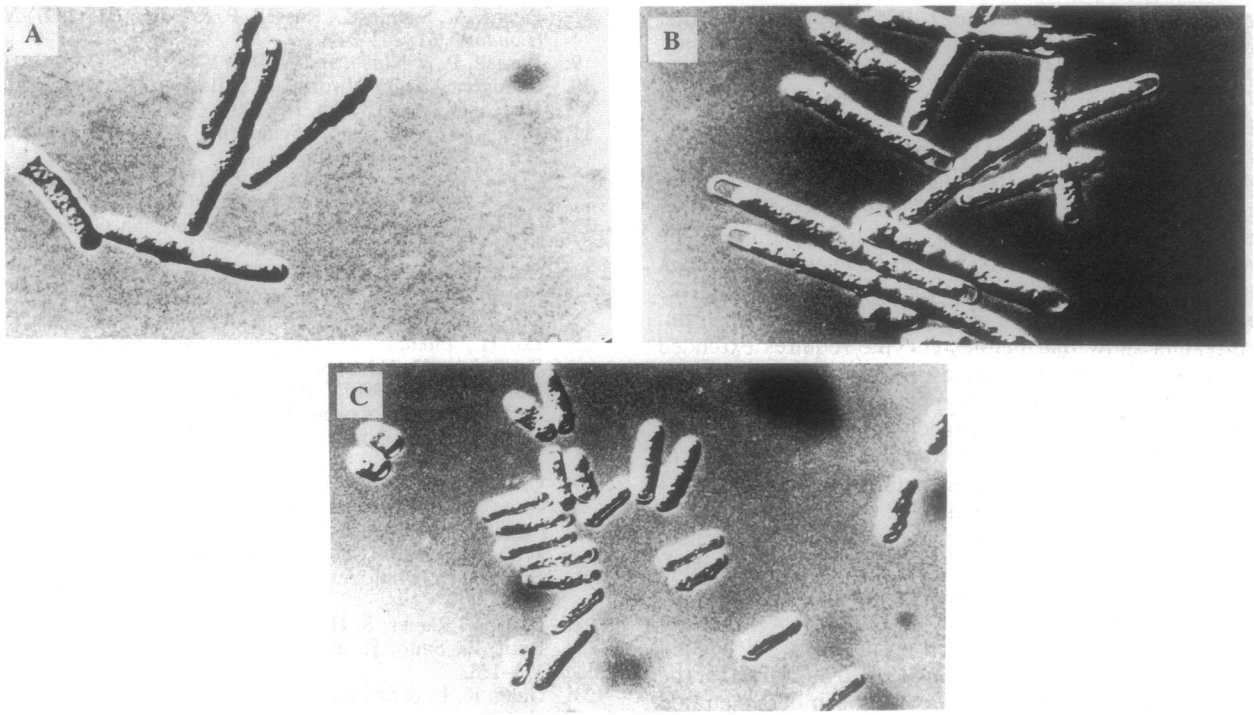

FIG. 2. Overexpression of *pyp1*ΔN in wild-type (FWP165) cells (*A*) results in highly elongated cells. This phenotype is identical to that observed after the overexpression of *pyp1*$^+$ (*B*) (this study and refs. 12 and 13), whereas the transformation of wild-type cells with pREP1 (*C*) caused no phenotypical changes. Wild-type cells were transformed with pREP1 overexpressing either *pyp1*ΔN or *pyp1*$^+$ under the control of the inducible *nmt1* promoter or pREP1 containing no insert. The transformed cells were grown to late logarithmic phase at 32°C in EMM containing 20 μM thiamine, washed twice in sterile H_2O, diluted into EMM lacking thiamine, and incubated further at 32°C for 40 h.

overexpress *cdr1*$^+$(*nim1*$^+$) initiate mitosis at a significantly reduced cell size, which causes a wee phenotype (this study and ref. 32). In this genetic background, we observed a complete reversion of the G_2-phase delay caused by overexpression of *pyp2*$^+$. The vast majority of the cells displayed a wee phenotype, with a small minority of cells displaying either a semi-wee or wild-type phenotype.

DISCUSSION

Our previous studies regarding *pyp1*$^+$ and *pyp2*$^+$ function as negative regulators of mitosis were unable to distinguish whether these enzymes operate by directly activating the wee1 kinase, by inhibiting the cdr1(nim1) kinase or by functioning independently of both mechanisms. Based on data presented in this study, we suggest the following sequence of events of how *pyp1*$^+$ and *pyp2*$^+$ might be involved in the negative regulation of mitosis: pyp1/pyp2 ⊣ X ⊣ wee1/mik1 ⊣ cdc2. This suggests (*i*) that the wee1 kinase is not the physiological substrate of either PTPase and (*ii*) that *pyp1*$^+$ and *pyp2*$^+$ do not act through *cdr1*$^+$ (*nim1*$^+$), which has been shown to directly inhibit the wee1 kinase (30, 31, 33).

Several lines of evidence originally suggested that the wee1 kinase might be a physiological substrate of pyp1 and/or pyp2 (11, 13), although in unsynchronized fission yeast cells, p107^{wee1} is apparently phosphorylated exclusively on Ser residues (34). If the wee1 kinase were an *in vivo* substrate of either PTPase, then the overexpressed catalytically inactive *pyp1* and *pyp2* mutants such as *pyp1*C470S and *pyp2*C630S would bind to and titrate out the wee1 kinase, causing a wee phenotype. Our results, however, indicate a cell cycle delay. The fact that these mutants still retain significant biological activity as judged by their ability to induce mitotic delay, despite their obvious loss of catalytic activity as demonstrated by *in vitro* PTPase assays, suggests that these mutant proteins titrate out Tyr-phosphorylated proteins that are involved in the negative regulation of the *wee1*$^+$/*mik1*$^+$ pathway. These results, however, do not indicate that catalytic activity is not important for *pyp1*$^+$ and *pyp2*$^+$ biological activity. All strains used in this study contained at least one genomic *pyp* copy, and this genomic copy obviously still performs its function. Indeed, catalytic activity appears to be essential for *pyp* function, since we were unable to suppress the lethality of a *pyp1 pyp2* double mutant by overexpressing *pyp1*C470S and *pyp2*C630S from the pREP1 multicopy plasmid (S.O., unpublished data).

Our interpretation that catalytically inactive mutants of either PTPase titrate crucial physiological substrate(s) is further supported by Sun *et al.* (35), who reported similar results for a catalytically inactive mutant of MPK-1, MPK-1C258S, and Bliska *et al.* (36), who showed that YopHC430A, a catalytically inactive mutant of the *Yersinia pseudotuberculosis* YopH PTPase, has a higher affinity for binding Tyr-phosphorylated substrates than does its wild-type counterpart. There are also reports, however, that demonstrate that PTPases carrying a mutation of this highly conserved Cys residue completely lose their *in vivo* activity (5, 24).

We further predict that neither *pyp1*$^+$ nor *pyp2*$^+$ act through *cdr1*$^+$(*nim1*$^+$), which is supported by the following evidence. (*i*) The overexpression of *pyp2*$^+$ in a *nim1* deletion strain led to very highly elongated cells, with the majority of these cells unable to form colonies. (*ii*) The overexpression of *nim1*$^+$ completely reversed the mitotic delay caused by the overexpression of *pyp2*$^+$. (*iii*) A *pyp1* disruption reverses the mitotic delay observed in a *nim1*-deleted strain (29).

Therefore, we postulate a gene "X" that is functionally redundant with *cdr1*$^+$(*nim1*$^+$) (32, 37) to inhibit the *wee1*$^+$/*mik1*$^+$ pathway. In this pathway, *pyp1*$^+$ and/or *pyp2*$^+$ would inhibit the function of gene X. In fission yeast, the presence of functional redundant gene pairs such as *wee1*$^+$/*mik1*$^+$ (9, 10), *cdc25*$^+$/*pyp3*$^+$ (38, 39), and *pyp1*$^+$/*pyp2*$^+$ (12, 13, 29), is a common phenomenon. Negative regulation of p107^{wee1} by cdr1(nim1)-mediated C-terminal phosphorylation has been shown (30, 31, 33). Furthermore, mitosis-specific hyperphos-

phorylation of the N terminus of p107^{wee1} by a kinase other than cdr1/nim1 was detected in *Xenopus* egg extracts (40), which resulted in a dramatic decrease in the ability of p107^{wee1} to mediate the inhibitory Tyr-phosphorylation of cdc2.

pyp1 and pyp2 contain extended N-terminal regions of 296 and 460 amino acids, respectively, but these regions reveal no sequence motifs indicative of their function, as is the case for several other PTPases (for review, see refs. 41 and 42). The overexpression of *pyp1* and *pyp2* mutants with significant N-terminal deletions in both a wild-type and a *cdc25-22* genetic background resulted in mitotic delay in either strain. These results show that neither enzyme requires extended regions of its N-terminal domain (≈80%) to induce mitotic delay. Therefore, these regions apparently do not affect the substrate specificity or the intracellular localization; however, a minimal N-terminal region is absolutely required for biological function, since the mere catalytic domains of pyp1 and pyp2 are biologically inactive. In the course of this investigation, we have narrowed these minimal N-terminal regions to 62 and 115 amino acids for pyp1 and pyp2, respectively, and our data concerning *pyp2*ΔN(2) indicate further that an N-terminal region of 36 amino acids is not sufficient to support biological activity of pyp2.

The minimal N-terminal region appears to be crucial for the correct folding of either PTPase, as has been demonstrated for HPTPβ (43). Since the overexpression of *pyp1*CD and *pyp2*CD yielded no detectable morphological changes in either a wild-type or a *cdc25-22* genetic background, we also determined whether they might still have the ability to rescue a lethal *cdc25-22* mutation. We found that they were unable to rescue this mutation, and cells, therefore, arrested at 35°C with a cdc$^-$ phenotype. These results are consistent with our data observed for the *in vitro* PTPase activity of *pyp1*CD and *pyp2*CD and support the hypothesis that a minimal N-terminal region is required to display catalytic activity.

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