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The Fission Yeast Genes *pyp1*$^+$ and *pyp2*$^+$ Encode Protein Tyrosine Phosphatases That Negatively Regulate Mitosis

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We have used degenerate oligonucleotide probes based on sequences conserved among known protein tyrosine phosphatases (PTPases) to identify two *Schizosaccharomyces pombe* genes encoding PTPases. We previously described the cloning of *pyp1*$^+$ (S. Ottilie, J. Chernoff, G. Hannig, C. S. Hoffman, and R. L. Erikson, Proc. Natl. Acad. Sci. USA 88:3455-3459, 1991), and here we describe a second gene, called *pyp2*$^+$. The C terminus of each protein contains sequences conserved in the apparent catalytic domains of all known PTPases. Disruption of *pyp2*$^+$ results in viable cells, as was the case for *pyp1*$^+$, whereas disruption of *pyp2*$^+$ and *pyp1*$^+$ results in synthetic lethality. Overexpression of either *pyp1*$^+$ or *pyp2*$^+$ in wild-type strains leads to a delay in mitosis but is suppressed by a *wee1-50* mutation at 35°C or a *cdc2-1w* mutation. A *pyp1* disruption suppresses the temperature-sensitive lethality of a *cdc25-22* mutation. Our data suggest that *pyp1*$^+$ and *pyp2*$^+$ act as negative regulators of mitosis upstream of the *wee1*$^+$/*mik1*$^+$ pathway.

The structural and functional diversity of protein tyrosine kinases (PTKs) has been a major center of attention for over a decade because of the role of PTKs in oncogenesis, cell proliferation, and cell differentiation (26). Studies on reversible tyrosine phosphorylation of proteins have suffered, however, from a notable absence of characterized phosphatases specific to tyrosine dephosphorylation (PTPases). In contrast to PTKs, the genes for which were initially captured by avian retroviruses or became available because of the relative abundance of some growth factor receptors, the PTPases required extensive purification because of their low abundance (27). The sequencing of a purified human placental PTPase termed PTP1B led to a major breakthrough in our understanding of this class of enzymes (4). They are structurally unrelated to serine/threonine-specific phosphatases and have an extraordinarily high level of activity in vitro. Molecular cloning results to date indicate that the PTPase family may be as diverse as the PTK family (2, 5, 7, 21). Some members are entirely cytoplasmic in location, whereas others have transmembrane sequences and N-terminal extracellular domains (7, 48, 49). These proteins may be analogous to receptor tyrosine kinases, with their PTPase activity regulated by extracellular ligands. Therefore, studies of this gene family are likely to be as rewarding as studies of PTKs.

A key regulator of the eukaryotic cell cycle is p34^{cdc2} (for a review, see reference 35), a serine/threonine-specific protein kinase. The *Schizosaccharomyces pombe* homolog p34^{cdc2}, encoded by the *cdc2*$^+$ gene (24), responsible for the G_1/S and G_2/M phase transition, is regulated in its activity throughout the cell cycle by phosphorylation (33, 47), in particular of a tyrosine residue within the ATP-binding site (18). At the G_2/M transition in the cell cycle, dephosphorylation of this tyrosine (Tyr-15) is associated with rapid activation of the capacity of p34^{cdc2} to phosphorylate exogenous substrates such as histone H1. Although the PTK that phosphorylates p34^{cdc2} has not been unambiguously identified, the *wee1*$^+$ gene product, p107, which negatively regulates p34^{cdc2} activity, is a strong candidate in *S. pombe* (12, 39, 43). More recent data indicate that the *wee1*$^+$ gene product, along with that of a newly identified gene, *mik1*$^+$, cooperates in the tyrosine phosphorylation of p34^{cdc2} (30). Genetic data indicate that p107^{wee1} is negatively regulated by the protein kinase encoded by *cdr1*$^+$ (*nim1*$^+$) (13, 42). The product of *cdc25*$^+$, p80^{cdc25}, is required for activation of p34^{cdc2} (41) and is believed to oppose the *wee1*$^+$/*mik1*$^+$ pathway. Although its sequence shows only weak similarity to the known PTPases (34), it has been recently reported to have PTPase activity (8, 16, 29, 32).

We have previously identified an *S. pombe* gene, *pyp1*$^+$, that encodes a protein with structural and functional features of a PTPase (38). The product of *pyp1*$^+$ is not required for viability of haploid cells, suggesting there may be genes with complementary functions resulting in cell viability in the absence of *pyp1*$^+$. In *Saccharomyces cerevisiae*, two genes encoding PTPases have also recently been identified (20, 37). In this report, we describe a second gene, *pyp2*$^+$, with the potential to express a PTPase-like protein and show that the disruption of both *pyp1*$^+$ and *pyp2*$^+$ genes results in synthetic lethality. Our unpublished data also show that the pyp1 and pyp2 proteins produced in bacteria have the capacity to dephosphorylate tyrosine-containing peptides. In addition, genetic data and overexpression studies indicate that these two genes act as negative regulators of mitosis.

MATERIALS AND METHODS

Isolation of *pyp2*$^+$ cDNA clone. Oligonucleotide synthesis and polymerase chain reactions (PCR) were performed as previously described (38). A total of 20,000 colonies of an *S. pombe* cDNA library (50) were screened by using a 300-bp *pyp2*$^+$ probe labeled with [α-^{32}P]dCTP to 5 × 10^8 cpm/μg by the random-priming technique (14). The filters were hybridized overnight at 65°C in 1% NaCl to 1% sodium dodecyl sulfate (SDS) and washed twice at 65°C in 0.2× SSC (1×

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SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS for 30 min. Autoradiography was performed by overnight exposure of the filters to Kodak XAR film. The *Eco*RI insert from pUC18-*pyp2*$^+$ was subcloned into pBluescript SK$^+$ (Stratagene), and nested deletions were constructed by standard protocols (23). Sequencing reactions were performed by the chain termination technique, using double-stranded DNA templates (45).

Southern and Northern (RNA) blot analysis. Genomic DNA from *S. pombe* cells was isolated by using the protocol of Hoffman and Winston (25). DNA (3 μg) was digested with restriction enzymes, electrophoresed in a 1% agarose gel (GIBCO/BRL), and transferred to a Zetabind membrane (Cuno, Inc.) by capillary blotting. The filters were hybridized overnight under high-stringency conditions at 65°C in 0.5 M sodium phosphate (pH 7.2)–7% SDS–1% bovine serum albumin and probed with either a 1.8-kb *Bal*I fragment from *pyp2*$^+$ or a 1.8-kb *Hin*dIII fragment from the *ura4*$^+$ gene labeled with [α-^{32}P]dCTP.

Total RNA from *S. pombe* cells was obtained by using the protocol of Carlson and Botstein (3). Poly(A)$^+$ RNA was purified by using the Pharmacia mRNA purification kit according to the manufacturer's protocol. Three micrograms of poly(A)$^+$ RNA was electrophoresed as described previously (38), and the filter was hybridized under high-stringency conditions at 65°C in 0.5 M sodium phosphate (pH 7.2)–7% SDS–1% bovine serum albumin and probed with either a 1.8-kb *Bal*I fragment from *pyp2*$^+$ or a 1.8-kb *Hin*dIII fragment from the *ura4*$^+$ gene labeled with [α-^{32}P]dCTP.

Pulsed-field gel electrophoresis analysis. Agarose plugs containing *S. pombe* chromosomal DNA (Bio-Rad) were digested with the restriction enzyme *Not*I (New England BioLabs) overnight and electrophoresed in a 1.0% agarose gel (GIBCO/BRL) containing 0.5× Tris-borate-EDTA buffer, using a Bio-Rad CHEF DRII pulsed-field gel electrophoresis system. The running conditions were 200 V with a 60-s switch time for 18 h and 90-s switch time for 12 h. To determine the size of the separated *Not*I fragments, we ran *S. cerevisiae* chromosomal DNA (Bio-Rad) and λ marker from New England BioLabs. The gel was blotted to a Zetabind membrane (Cuno, Inc.) by capillary blotting. The filter was hybridized under high-stringency conditions and probed with either a *pyp1*$^+$- or *pyp2*$^+$-specific probe.

In vivo gene disruption. All strains used in this study are listed in Table 1. *S. pombe* cells were grown in complex medium, YEA (22), or essential minimal medium (EMM), supplemented as required. Plasmid pCG1 (19), containing the *S. pombe ura4*$^+$ gene (1), was digested with *Hin*dIII; the 1.8-kb fragment was filled in with Klenow polymerase and deoxynucleoside triphosphates and blunt-end ligated into the *Eco*RV site of the *pyp2*$^+$ cDNA clone, thereby replacing a 0.9-kb *Eco*RV fragment that presumably codes for the catalytic domain of *pyp2*$^+$. This construct (ppyp2::ura4$^+$) was digested with *Bal*I, and the resulting *pyp2::ura4*$^+$ insert was used to transform the haploid *S. pombe* strain FWP172 to uracil prototrophy. Stable Ura$^+$ transformants were isolated, and it was shown by Southern hybridization, using a 1.8-kb *Bal*I fragment of *pyp2*$^+$ as a probe, that strain SOP11 contains the disrupted *pyp2* allele.

Analysis of *pyp1 pyp2* double mutants. To obtain an h$^+$ *pyp1::ura4* strain, strain SOP13 was crossed to strain FWP165, and the progeny were analyzed to isolate strain SOP14. Strains SOP14 and SOP11 were crossed on YPD plates (46). After incubation at 25°C for 3 days, tetrads were dissected, and the viable colonies were replica plated onto either YEA plates or SC-minus-uracil plates. DNA from these colonies was isolated, digested with *Bal*I and *Aat*II, electrophoresed, blotted, and hybridized under high-stringency conditions with either a *pyp1*$^+$- or *pyp2*$^+$-specific fragment. The phenotype of the double mutants was determined by microscopic analysis.

TABLE 1. *S. pombe* strains

Strain	Genotype	Source
972	h^{-s}	
FWP165	h$^+$ *leu1-32 ura4-D18 ade6-M216*	F. Winston
FWP172	h− *leu1-32 ura4-D18 ade6-M210*	F. Winston
SOP11	h− *pyp2::ura4 leu1-32 ura4-D18 ade6-M210*	This study
SOP13	h− *pyp1::ura4 leu1-32 ura4-D18 ade6-M216*	This study
SOP14	h$^+$ *pyp1::ura4 leu1-32 ura4-D18 ade6-M216*	This study
SOP18	h− *pyp1::ura4 leu1-32 ura4-D18 cdc25-22*	This study
SOP24	h$^+$ *pyp2::ura4 leu1-32 ura4-D18 cdc25-22*	This study
SOP31	h$^+$ *pyp1::ura4 leu1-32 ura4-D18 cdc2-1w*	This study
SOP32	h$^+$ *pyp1::ura4 leu1-32 ura4-D18 cdc2-3w*	This study
SOP35	h$^+$ *pyp1::ura4 leu-1-32 ura4-D18 wee1-50*	This study
SOP39	h$^+$ *pyp1::ura4 leu1-32 ura4-D18 cdc2-33*	This study
SOP51	h− *pyp2::ura4 leu1-32 ura4-D18 wee1-50*	This study
SOP54	h$^+$ *pyp2::ura4 leu1-32 ura4-D18 cdc 2-1w*	This study
SOP59	h$^+$ *pyp2::ura4 leu1-32 ura4-D18 cdc2-33*	This study
SOP60	h$^+$ *pyp2::ura4 leu1-32 ura4-D18 cdc2-3w*	This study
	h$^+$ *leu1-32 ura4-D18 cdc25-22*	P. Russell
	h− *leu1-32 ura4-D18 wee1-50*	P. Russell
	h− *leu1-32 ura4-D18 cdc2-1w*	P. Russell
	h− *leu1-32 ura4-D18 cdc2-3w*	P. Russell
	h− *ura4-D18 cdc2-33*	P. Russell

Overexpression of *pyp1*$^+$ and *pyp2*$^+$. The full-length coding regions of *pyp1*$^+$ and *pyp2*$^+$ were subcloned into a *Bgl*II restriction site of a modified version of the *S. pombe* expression vector pREP-1 (31). To eliminate the initiator methionine provided by the *Nde*I restriction site of the pREP-1 polylinker, the vector was linearized with *Nde*I, the ends were filled in with Klenow DNA polymerase, *Bgl*II linkers were attached and digested, and the vector was circularized with T4 DNA ligase.

S. pombe strains were transformed with these plasmids and selected for leucine prototrophy. Transformed cells were inoculated at 25 or 35°C in EMM containing thiamine (20 μM), grown to late log phase, washed twice with sterile H_2O, and diluted into thiamine-free medium.

Genetic analysis of *pyp1*$^+$ and *pyp2*$^+$. Strains containing either a *pyp1* or *pyp2* disrupted gene were crossed to the strains listed in Table 3, using standard genetic procedures (22). For each cross, at least 10 tetrads were dissected and the phenotype of the progeny was determined. Cells were inoculated into liquid YEA medium at 25 and 35°C and grown to mid-log phase, and the length of cells displaying a complete septum was measured by using a phase-contrast microscope (Olympus) with an eyepiece micrometer; 24 to 36 cells from each strain were measured at both temperatures.

Nucleotide sequence accession number. The sequence reported in this paper has been deposited in the GenBank data base (accession number X59599).

RESULTS

Cloning and sequence of the *S. pombe pyp2*$^+$ gene. The DNA and protein sequences of all known PTPases were analyzed to identify consensus regions to which degenerate oligonucleotides could be synthesized. Using appropriately designed primers (38), a DNA fragment of approximately 300

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      GTTTCACCAACGAGTTCTTCCCCATGGACGTTACTCCCAATGACGGTTCCGCTCAATTCGTTGTCTACAACATGTCTGGTAACCCCAAGA   90
      AGCAAACCACCATCATCCAAGAATACAAAACTTTTAATTTTGTTTATTTTCTGTTTATTAAAAATTTATTTATTTGCTTGTTTGATTTCG  180
      TCTCTTTCAATCAACAAGTTTCCTCATTCATCCTTGTTCACGTCGCCCAGGAGTTTCTTTGTCTTATATTTTTGACTCCTTGATTGAACT  270
      CGCAAAAAGTCAATAATTGTTATTTTTATACAGTTTCTTCTCTCTTACGTTTCCATTTTGTTTATATTCTTTTTTTCCGAAAGCACACTG  360
      TAAATAACTCAAAAAATTTCTCTTAACCCGCACACCTCTTCACACTCGCCTGCTATCTTTTTTTTTCGTTTTAATTTGTGTGCCATTTTA  450
      AAAGTTTCCTGTATATATATTTTAGCTTGGCCAATCTTGTCTCTGTCCCTTTTACTTAGGATATAACATCTTCCAAGGTGTTTTCAAAAA  540
      TTTTGTGCTACTATGCTCCATCTTCTGTCTAAAGACGAATTTAACTCTACTCTTAAGTCTTTTGAAGAACAAACCGAGAGCGTTTCTTGG  630
  1            M  L  H  L  L  S  K  D  E  F  N  S  T  L  K  S  F  E  E  Q  T  E  S  V  S  W
      ATTATTGATTTGCGCCTGCACTCCAAATATGCTGTTAGCCATATAAAAAATGCCATCAACGTTTCTCTTCCAACTGCGTTGTTACGTCGT  720
 27   I  I  D  L  R  L  H  S  K  Y  A  V  S  H  I  K  N  A  I  N  V  S  L  P  T  A  L  L  R  R
      CCGTCTTTTGACATTGGAAAGGTTTTCGCCTGTATAAAATGCAACGTAAAAGTCTCGTTGGATGAAATTAATGCCATATTCCTATACGAC  810
 57   P  S  F  D  I  G  K  V  F  A  C  I  K  C  N  V  K  V  S  L  D  E  I  N  A  I  F  L  Y  D
      TCTAGCATGGCTGGCATGAACCGTATTTATGATTTGGTACAGAAGTTTCGACGTGGTGGATATTCCAAAAAAATTTATTTATTAAGCAAT  900
 87   S  S  M  A  G  M  N  R  I  Y  D  L  V  Q  K  F  R  R  G  G  Y  S  K  K  I  Y  L  L  S  N
      GGATTTGAAGCCTTTGCTTCCTCTCATCCGGACGCCATTGTCTCTACCGAAATGGTCAAGGAGTCGGTCCCATACAAAATTGACATCAAT  990
117   G  F  E  A  F  A  S  S  H  P  D  A  I  V  S  T  E  M  V  K  E  S  V  P  Y  K  I  D  I  N
      GAGAATTGCAAGCTGGATATCCTTCATTTATCCGATCCATCTGCGGTTTCTACCCCTATTTCACCAGATTATAGCTTTCCATTGAGAGTT 1080
147   E  N  C  K  L  D  I  L  H  L  S  D  P  S  A  V  S  T  P  I  S  P  D  Y  S  F  P  L  R  V
      CCTATTAACATCCCACCACCTTTATGCACACCTTCGGTAGTCTCCGATACCTTTAGTGAGTTCGCGAGTCATGCGGAATACCCTGGATTT 1170
177   P  I  N  I  P  P  P  L  C  T  P  S  V  V  S  D  T  F  S  E  F  A  S  H  A  E  Y  P  G  F
      TCAGGTTTAACACCGTTTTCGATTCACTCTCCTACTGCTTCTTCTGTTCGTTCGTGCCAATCTATATATGGTTCACCCCTCTCTCCCCCA 1260
207   S  G  L  T  P  F  S  I  H  S  P  T  A  S  S  V  R  S  C  Q  S  I  Y  G  S  P  L  S  P  P
      AATTCAGCTTTTCAAGCTGAAATGCCATATTTTCCAATCTCTCCCGCCATTTCTTGCGCATCTTCTTGTCCTAGTACGCCTGATGAACAG 1350
237   N  S  A  F  Q  A  E  M  P  Y  F  P  I  S  P  A  I  S  C  A  S  S  C  P  S  T  P  D  E  Q
      AAAAACTTTTTTATCGTAGGCAATGCCCCTCAGCAAACTCCTGCCAGGCCATCTCTACGATCGGTGCCTTCTTATCCCTCCTCGAATAAT 1440
267   K  N  F  F  I  V  G  N  A  P  Q  Q  T  P  A  R  P  S  L  R  S  V  P  S  Y  P  S  S  N  N
      CAGAGACGGCCTTCTGCTTCTCGCGTTCGTAGCTTTAGCAACTATGTTAAATCCAGCAACGTCGTCAATCCAAGTTTGTCTCAAGCTTCC 1530
297   Q  R  R  P  S  A  S  R  V  R  S  F  S  N  Y  V  K  S  S  N  V  V  N  P  S  L  S  Q  A  S
      TTGGAAATTATTCCACGGAAGTCAATGAAACGTGATAGCAATGCACAGAATGATGGTACTAGTACGATGACAAGCAAACTTAAACCATCT 1620
327   L  E  I  I  P  R  K  S  M  K  R  D  S  N  A  Q  N  D  G  T  S  T  M  T  S  K  L  K  P  S
      GTTGGTTTATCAAACACACGAGATGCTCCAAAACCAGGCGGTCTAAGAAGAGCTAACAAACCGTGCTTTAATAAAGAGACCAAGGGAAGC 1710
357   V  G  L  S  N  T  R  D  A  P  K  P  G  G  L  R  R  A  N  K  P  C  F  N  K  E  T  K  G  S
      ATTTTCTCCAAGGAAAACAAAGGACCCTTTACTTGTAATCCCTGGGGTGCCAAAAAGGTTTCTCCTCCTCCTTGTGAGGTGCTTGCGGAT 1800
387   I  F  S  K  E  N  K  G  P  F  T  C  N  P  W  G  A  K  K  V  S  P  P  P  C  E  V  L  A  D
      TTAAATACTGCTTCTATTTTTTATAAGTTTAAAAGACTTGAGGAAATGGAAATGACTAGATCCCTAGCGTTTAATGACAGTAAATCTGAT 1890
417   L  N  T  A  S  I  F  Y  K  F  K  R  L  E  E  M  E  M  T  R  S  L  A  F  N  D  S  K  S  D
      TGGTGCTGTTTAGCTTCCAGCCGCTCCACTTCCATTTCAAGAAAAAATCGTTACACAGATATCGTGCCCTACGATAAAACAAGAGTCCGT 1980
447   W  C  C  L  A  S  S  R  S  T  S  I  S  R  K  N  R  Y  T  D  I  V  P  Y  D  K  T  R  V  R
      CTAGCCGTTCCAAAAGGATGTTCTGATTATATTAATGCTTCACATATAGACGTTGGAAATAAAAAATATATTGCCTGCCAGGCCCCTAAG 2070
477   L  A  V  P  K  G  C  S  D  Y  I  N  A  S  H  I  D  V  G  N  K  K  Y  I  A  C  Q  A  P  K
      CCGGGAACTCTTTTAGACTTTTGGGAAATGGTTTGGCATAACTCAGGAACAAATGGTGTTATCGTAATGCTCACAAATCTGTATGAGGCG 2160
507   P  G  T  L  L  D  F  W  E  M  V  W  H  N  S  G  T  N  G  V  I  V  M  L  T  N  L  Y  E  A
      GGAAGTGAGAAATGTTCTCAATATTGGCCAGATAACAAAGATCACGCATTATGCTTGGAAGGCGGATTACGCATATCTGTTCAAAAATAT 2250
537   G  S  E [K  C  S  Q  Y  W  P] D  N  K  D  H  A  L  C  L  E  G  G  L  R  I  S  V  Q  K  Y
      GAAACCTTTGAAGATTTGAAGGTCAACACTCATTTGTTTCGATTGGATAAACCTAATGGTCCTCCAAAGTATATACATCACTTTTGGGTG 2340
567   E  T  F  E  D  L  K  V  N  T  H  L  F  R  L  D  K  P  N  G  P  P  K  Y  I  H  H  F  W  V
      CACACGTGGTTTGACAAAACCCATCCAGATATTGAAAGCATCACGGGAATCATACGTTGTATTGATAAGGTTCCCAATGATGGACCAATG 2430
597   H  T  W  F  D  K  T  H  P  D  I  E  S  I  T  G  I  I  R  C  I  D  K  V  P  N  D  G  P  M
      TTCGTTCACTGTTCAGCAGGCGTAGGACGCACTGGTACTTTTATTGCTGTAGACCAAATACTTCAGGTACCAAAAAACATTTTACCCAAG 2520
627   F [V  H  C  S  A  G  V  G] R  T  G  T  F  I  A  V  D  Q  I  L  Q  V  P  K  N  I  L  P  K
      ACGACCAATTTGGAAGATTCAAAAGATTTCATATTCAATTGTGTTAACTCGTTGAGATCACAACGGATGAAAATGGTTCAAAACTTTGAG 2610
657   T  T  N  L  E  D  S  K  D  F  I  F  N  C  V  N  S  L  R  S  Q  R  M  K  M  V  Q  N  F  E
      CAATTCAAATTTCTCTACGACGTCGTGGATTATTTAAATAGCGGCGTTAACCAGGCTTCCAAGCCCTTGATGACTTAACGAAACGACTGT 2700
687   Q  F  K  F  L  Y  D  V  V  D  Y  L  N  S  G  V  N  Q  A  S  K  P  L  M  T  *
      TCTTTAATTTTCTGTGTTTGTACACACTATGTTCTATTTATGTGAGATTGTGTAATTCCTCATTTTTTACATTATCTGACGCGAAGGTTA 2790
      TGTTAAAAATACTTAACCACACTCTCCGGTAATCATGTGTAATTCCTTCCTTATTGTTGTTAAAAATTGATTTTATCTCATGCGTGTAAT 2880
      GAATGTTTATATCTAGTTATTAATGAATCTTGTTTATGTTGGAAAAAAAAAA                                       2932
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FIG. 1. Nucleotide and predicted amino acid sequences of *pyp2*$^+$. An upstream termination codon is underlined. The amino acids corresponding to the degenerate oligonucleotides used for PCR are boxed. The termination codon is marked with an asterisk. Nucleotides are numbered at the right; amino acids are numbered at the left.

bp was amplified from an *S. pombe* cDNA library (50). This fragment was then subcloned, and six independent isolates were sequenced. In each case, the nucleic acid sequences were identical, except for regions corresponding to the degenerate primers used for PCR amplification. The predicted amino acid sequence for this fragment conforms to all known PTPase consensus sequences. Therefore, it is likely that this sequence represents a portion of an authentic yeast PTPase.

The DNA fragment produced by the PCR amplification was used to screen the *S. pombe* cDNA library in order to isolate a full-length clone. Two distinct clones, designated *pyp1*$^+$ (38) and *pyp2*$^+$, were isolated in this way. The *pyp2*$^+$ cDNA (Fig. 1) encompasses all of the sequences found in the 300-bp PCR product, differing at only a few nucleotides corresponding to the primers used in the amplification. The 2,932-bp *pyp2*$^+$ cDNA contains a long 5′ leader sequence (552 bp) upstream of the putative initiation codon, followed by a long open reading frame, a short 3′ untranslated region, and a polyadenylate tract. Because only a few yeast mRNAs contain 5′ untranslated regions of this length (6), we considered the possibility that the cDNA insert was in fact a chimeric molecule, formed from an inadvertent concatenation of unrelated cDNAs during library construction. We believe that this is unlikely, however, as PCR analysis of genomic DNA with use of primers derived from various

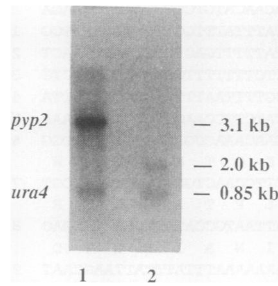

FIG. 2. Northern blot analysis of *pyp2* transcripts. Poly(A)$^+$ RNA was isolated from strains 972 (lane 1) and SOP11 (lane 2), separated on a 1.2% agarose–6% formaldehyde gel, and transferred to a Nytran membrane. The filter was hybridized with a α-^{32}P-labeled 1.8-kb *Bal*I fragment (*pyp2$^+$*) and the 1.8-kb *Hin*dIII *ura4$^+$* fragment. Each lane was loaded with 5 μg of poly(A)$^+$ RNA.

regions of the cDNA insert yields the same lengths as from the *pyp2$^+$* cDNA (data not shown). In addition, Northern blot analysis of *S. pombe* RNA with use of a full-length *pyp2$^+$* probe reveals a single transcript of about 3.1 kb (Fig. 2), consistent with the observed size of the cDNA insert. Therefore, we conclude that the cDNA clone accurately reflects an authentic mRNA.

Homology between pyp2 and PTPases. The *pyp2$^+$* sequence predicts a protein of 711 amino acids. The carboxy terminus of the protein bears striking homology to the conserved (and presumed catalytic) domain of all known PTPases, whereas the amino terminus is unrelated to any protein sequences found in current data banks. There are no extended hydrophobic regions compatible with transmembrane structures. The predicted pyp1 and pyp2 amino acid sequences are particularly similar to one another at the carboxyl terminus (Fig. 3). Over the lengths of the entire proteins, the two yeast PTPases are 34% identical and 56% similar. A sequence alignment of pyp1, pyp2, and human PTP1B (Fig. 3) indicates a strong sequence similarity between the putative catalytic domains of pyp1 and pyp2 and the human PTP1B sequence. pyp1 and pyp2 protein sequences terminate immediately after the putative catalytic domain. In human leukocyte common antigen-related protein D1 (48), human PTP1B (5), and human T-cell phosphatase (7), this domain is followed by at least another 150

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     pyp1  MNFSNGSKSS TFTIAPSGSC IALPPQRGVA TSKYAVHASC LQEYLDKEAW KDDTLIIDLR PVSEFSKSRI KGSVNLSLPA TLIKRPAFSV ARII...... ..........
     pyp2  .......... .......... ....MLHLLS KDEFNSTLKS FEEQTESVSW .....IIDLR LHSKYAVSHI KNAINVSLPT ALLRRPSFDI GKVFACIKCN VKVSLDEINA
    PTP1B  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

     pyp1  .....SNLHD VDDKRDFQN. ..WQEFS... .......... .......... .......... .......... .......... .........S ILVCVPAWIA NYVTNAEVIG
     pyp2  IFLYDSSMAG MNRIYDLVQK FRRGGYSKKI YLLSNGFEAF ASSHPDAIVS TEMVKESVPY KIDINENCKL DILHLSDPSA VSTPISPDYS FPLRVPINIP PPLCTPSVVS
    PTP1B  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

     pyp1  EKFRKESYSG DFGILD..LD YSKVSGKYPS V.....IDNS PVKSKLGALP SARPRLSYSA AQTAPISLSS ...EGSDYFS R...PPPTPN VAGL...... ..........
     pyp2  DTFSEFASHA EYPGFSGLTP FSIHSPTASS VRSCQSIYGS PLSPPNSAFQ AEMPYFPISP AISCASSCPS TPDEQKNFFI VGNAPQQTPA RPSLRSVPSY PSSNNQRRPS
    PTP1B  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

     pyp1  .....SLNNF F......... .......... .......... .......... .......... ...CPLPE.. ........NK DNKSS..... ........PF GSATVQTPCL
     pyp2  ASRVRSFSNY VKSSNVVNPS LSQASLEIIP RKSMKRDSNA QNDGTSTMTS KLKPSVGLSN TRDAPKPGGL RRANKPCFNK ETKGSIFSKE NKGPFTCNPW GAKKVSPP..
    PTP1B  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

     pyp1  HSVPDAFTNP DVATLYQKFL RLQSLEHQRL VSCSDRNSQW STV....... DSLSNTSY.K ......KNRY TDIVPYNCTR VHLKRTSPSE LDYINASFIK TET..SNYIA
     pyp2  ..PCEVLADL NTASIFYKFK RLEEMEMTRS LAFNDSKSDW CCL....... ASSRSTSISR ......KNRY TDIVPYDKTR VRLAVPKGCS .DYINASHID VGN..KKYIA
    PTP1B  .......... .......... ....MEMEKE FEQIDKSGSW AAIYQDIRHE ASDFPCRVAK LPKNKNRNRY RDVSPFDHSR IKLHQEDN.. .DYINASLIK MEEAQRSYIL
Consensus  ---------- ---------- -----E---- ----D----W ---------- -S-------- -------NRY -D--P----R --L------- -DYINAS-I- -------YI-

     pyp1  CQGSISRSIS DFWHMVWDNV ENIGTIVMLG SLFEAGREMC TAYWPSNGIG DKQVYG.DYC VKQISEENVD NSRFILRKFE IQNANFPSVK KVHHYQYPNW SDCNSPENVK
     pyp2  CQAPKPGTLL DFWEMVWHNS GTNGVIVMLT NLYEAGSEKC SQYWPDNKDH ALCLEG.GLR ISVQKYETFE DLKVNTHLFR LDKPNGPP.K YIHHFWVHTW FDKTHPD.IE
    PTP1B  TQGPLPNTCG HFWEMVWEQK SR..GVVMLN RVMEKGSLKC AQYWPQKEEK EMIFEDTNLK LTLISEDI.. KSYYTVRQLE LENLTTQETR EILHFHYTTW PDFGVPESPA
Consensus  -Q-------- -FW-MVW--- ------VML- ---E-G---C --YWP----- ---------- ---------- ---------- ---------- ---H-----W -D---P----

     pyp1  SMVEFLKYVN N....SHGSG NTIVHCSAGV GRTGTFIVLD TILRFPESKL SGFNPSVADS SDVVFQLVDH IRKQRMKMVQ TFTQFKYVY. DLID....SL QKS..QVHFP
     pyp2  SITGIIRCID K....VPNDG PMFVHCSAGV GRTGTFIAVD QILQVPKNIL PK.TTNLEDS KDFIFNCVNS LRSQRMKMVQ NFEQFKFLY. DVVD....YL NSGVNQASKP
    PTP1B  SFLNFLFKVR ESGSLSPEHG PVVVHCSAGI GRSGTFCLAD TCLLLMDKRK ...DPSSVDI KKVLLEM... .RKFRMGLIQ TADQLRFSYL AVIEGAKFIM GDSSVQDQWK
Consensus  S--------- ---------G ---VHCSAG- GR-GTF---D --L------- --------D- ---------- -R--RM---Q ---Q----Y- ---------- -----Q----

     pyp1  VLT....... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     pyp2  LMT....... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    PTP1B  ELSHEDLEPP PEHIPPPPRP PKRILEPHNG KCREFFPNHQ WVKEETQEDK DCPIKEEKGS PLNAAPYGIE SMSQDTEVRS RVVGGSLRGA QAASPAKGEP SLPEKDEDHA
Consensus  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

     pyp1  .......... .......... .......... ....
     pyp2  .......... .......... .......... ....
    PTP1B  LSYWKPFLVN MCVATVLTAG AYLCYRFLFN SNT.
Consensus  ---------- ---------- ---------- ----
```

FIG. 3. Sequence alignment of pyp1, pyp2, and human PTP1B. The consensus sequence represents amino acids present in all three sequences.

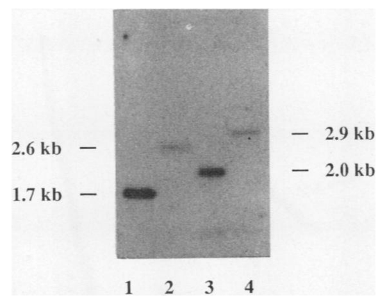

FIG. 4. Southern blot analysis of the *pyp2* gene disruption. Genomic DNA from strains FWP172 (lanes 1 and 3) and SOP11 carrying the disrupted *pyp2* allele (lanes 2 and 4) was isolated, digested with the restriction enzymes *Bal*I (lanes 1 and 2) and *Afl*II-*Aat*II (lanes 3 and 4), separated by agarose gel electrophoresis, transferred to a Zetabind membrane, and probed with a α-^{32}P-labeled 1.8-kb *Bal*I fragment of *pyp2*$^+$. Each lane was loaded with 5 μg of DNA.

TABLE 2. Meiotic segregation pattern of SOP14 × SOP11[a]

No. of viable spores	No. of tetrads		
	Parental ditype (4:0 Ura$^+$/Ura$^-$ segregation)	Tetratype (3:1 Ura$^+$/Ura$^-$ segregation)	Nonparental ditype (2:2 Ura$^+$/Ura$^-$ segregation)
4	58	0	0
3	16[b]	36[c]	0

[a] A total of 122 asci were dissected, and the phenotype of the spores was determined. The relatively high number of tetrads with four viable spores with a 4:0 Ura$^+$/Ura$^-$ segregation indicates linkage (see text) and allowed assignment as parental ditype tetrads. Not included in the data are 12 tetrads with two viable spores, 8 with a 2:0 Ura$^+$/Ura$^-$ segregation and 4 with a 1:1 Ura$^+$/Ura$^-$ segregation.

[b] Random spore inviability resulted in some tetrads with three viable spores and a 3:0 Ura$^+$/Ura$^-$ segregation. On the basis of Southern blot analysis of two asci of this type, they were assigned as parental ditype tetrads.

[c] Asci with three viable spores and a 2:1 Ura$^+$/Ura$^-$ segregation were assigned as tetratype tetrads on the basis of Southern blot analysis of progeny derived from 10 asci of this type.

amino acids, whereas many members of the *cdc25* family, such as the products of human *cdc25* (44), the *Drosophila* homolog, string (9), and *S. pombe cdc25*$^+$ (41), share a short C-terminal region following the catalytic domain. It was recently shown (15) that the C-terminal region of the human PTPase family contains a stretch of amino acids similar to a cyclin B sequence that may be important for full PTPase activity. This sequence is absent in pyp1 and pyp2.

In vivo gene disruption of *pyp2*$^+$. To determine whether or not *pyp2*$^+$ is essential for cell viability, we constructed a null allele by the one-step gene disruption method (40). A 0.9-kb *Eco*RV fragment containing most of the putative catalytic domain of *pyp2*$^+$ was excised and replaced by the *S. pombe ura4*$^+$ gene (1, 19), creating plasmid ppyp2::ura4$^+$.

We transformed the haploid *S. pombe* strain FWP172 with a *Bal*I fragment of plasmid ppyp2::ura4$^+$. Transformants were screened for a stable Ura$^+$ phenotype; DNA from strain SOP11 was subjected to Southern hybridization analysis using a *pyp2*$^+$-specific probe (Fig. 4) and a *ura4*$^+$-specific probe (data not shown) and shown to contain a *pyp2* disrupted gene. Cells with the disrupted allele of *pyp2*$^+$ showed no significant alteration in morphology or cell length compared with wild-type cells. Therefore, the *pyp2*$^+$ gene is not essential for *S. pombe* growth, and the disruption does not cause advancement of mitosis, as observed for the *pyp1* disruption (see Table 3).

Poly(A)$^+$ RNA was isolated from wild-type strain 972 and strain SOP11 to determine whether a partial *pyp2*$^+$ gene product is synthesized in cells carrying the *pyp2* disruption. Northern blots were hybridized with a *pyp2*$^+$ fragment and with a fragment of the *ura4*$^+$ gene. With use of the *pyp2*$^+$ probe, a 3.1-kb band was detected in RNA from wild-type cells, and a 0.85-kb mRNA was identified with the *ura4*$^+$ gene. Northern analysis of RNA from SOP11 cells containing the disrupted *pyp2* gene detected no 3.1-kb mRNA, but bands of 2.0 and about 0.85 kb were identified with the *pyp2*$^+$ and *ura4*$^+$ probes, respectively (Fig. 2). These data indicate that no functional *pyp2*$^+$ product could be produced in SOP11.

***pyp1 pyp2* double mutants are lethal.** Since *pyp1*$^+$ and *pyp2*$^+$ are nonessential genes for *S. pombe* growth, we investigated the phenotype of a double mutant strain. Strains SOP14 and SOP11, carrying disrupted alleles of *pyp1*$^+$ and *pyp2*$^+$, respectively, were crossed, and 122 tetrads were dissected (Table 2). Fifty-eight tetrads resulted in four viable spores, all displaying the Ura$^+$ phenotype, and they can therefore be classified as spores from a parental ditype tetrad. We did not observe tetrads with four viable spores and a 3:1 Ura$^+$/Ura$^-$ segregation. Thirty-six tetrads resulted in three viable spores with a 2:1 segregation of Ura$^+$ to Ura$^-$ progeny. DNA from 30 colonies derived from 10 tetrads of this type was subjected to Southern hybridization analysis using the *pyp1*$^+$ and *pyp2*$^+$ probes. In each case, we confirmed that cells derived from the Ura$^+$ progeny carried a single *pyp1* or *pyp2* disruption, whereas cells from the Ura$^-$ progeny carried both wild-type alleles. It is therefore likely that the spores that result in nonviable cells contain both disrupted *pyp* genes, suggesting that a double mutant is lethal.

To determine the phenotype of the double mutants, we analyzed microscopically the spores that derived from asci that yielded three viable spores with a 2:1 Ura$^+$/Ura$^-$ segregation. Spores with the presumed double disruption that germinated underwent a few cell divisions and resulted in very small cells, suggesting premature mitosis. Because of this behavior, the double-mutant phenotype cannot be further analyzed at this time. It should be noted, however, that this phenotype is similar to a deletion of the mitotic inhibitor *wee1*$^+$/*mik1*$^+$, which yields very small cells and is lethal (30).

Genomic mapping of *pyp1*$^+$ and *pyp2*$^+$. The fact that 74 tetrads were parental ditypes suggests that the two PTPase genes are linked. Therefore, to analyze their genomic localization and possible linkage, we performed pulsed-field gel electrophoresis of *Not*I-digested *S. pombe* chromosomal DNA. According to Fan et al. (10), a complete *Not*I digest of *S. pombe* chromosomal DNA results in 17 fragments. *pyp1*$^+$- and *pyp2*$^+$-specific probes hybridized to the same DNA fragment of about 530 kb, presumably localized at the tip of the right arm of chromosome I (data not shown). The fact that the two *pyp*-specific probes hybridize to the same *Not*I fragment supports our assumption that these two genes are linked.

Overexpression of *pyp1*$^+$ and *pyp2*$^+$ delays mitosis. To determine the role of *pyp1*$^+$ and *pyp2*$^+$ in regulation of the cell cycle, *pyp1*$^+$ and *pyp2*$^+$ were expressed under the control of the nmt promoter (31) either in a wild-type strain or in strains with mutations involved in cell cycle regulation (*cdc25-22*, *wee1-50*, *cdc2-1w*, *cdc2-3w*, and *cdc2-33*). The overexpression of either *pyp1*$^+$ or *pyp2*$^+$ led to significant cell elongation in a wild-type background and to cell division

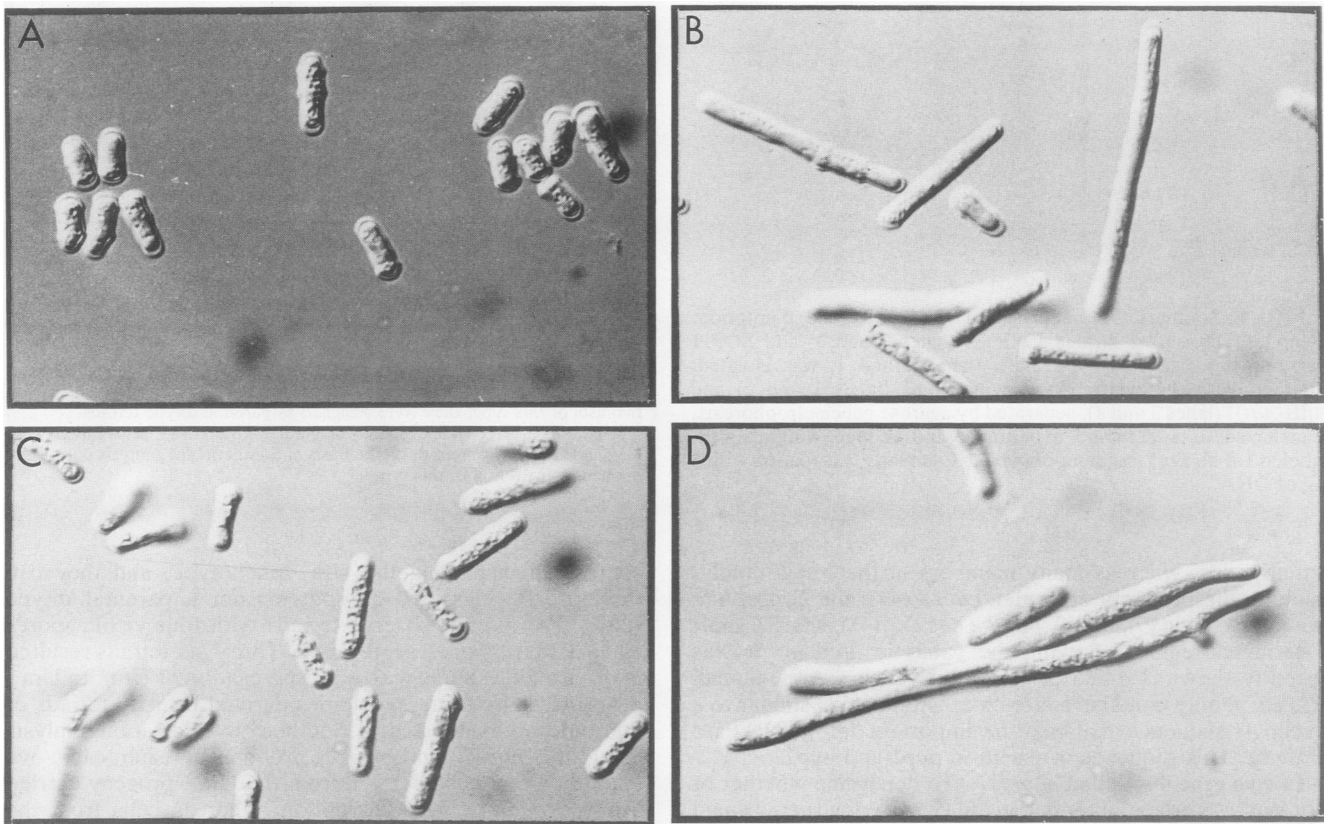

FIG. 5. Overexpression of *pyp1*$^+$ in wild-type (FWP172) and *cdc25-22* cells. Wild-type (A and B) and *cdc25-22* (C and D) cells were transformed with the plasmid expressing *pyp1*$^+$ under the control of the *S. pombe* nmt promoter. Transformed cells were grown in EMM containing thiamine (20 μM) to late log phase, washed twice, diluted into EMM containing either 20 μM thiamine (A and C) or no thiamine (B and D), and incubated at 25°C.

arrest in a *cdc25-22* strain at both the permissive and nonpermissive temperatures (Fig. 5). This result suggests that neither the *pyp1* nor *pyp2* gene product can substitute for the *S. pombe cdc25*$^+$ gene product as described for the human T-cell phosphatase (17). Therefore, neither pyp1 or pyp2 protein appears to have the capacity to dephosphorylate p34^{cdc2} as is the case for human T-cell phosphatase and the *cdc25* gene product.

The overexpression of either *pyp1*$^+$ or *pyp2*$^+$ led to cell elongation in all strains tested except a *cdc2-1w* strain, in which we found only modest increases in cell length. This strain carries a gain-of-function mutation in the *cdc2*$^+$ gene that is relatively insensitive to overexpression of *wee1*$^+$ (43). In contrast, the overexpression of *pyp1*$^+$ and *pyp2*$^+$ in a *cdc2-3w* strain carrying another gain-of-function mutation that is sensitive to *wee1*$^+$ overexpression (43) led to elongated cells. Thus, *cdc2* mutations that respond to *wee1*$^+$ respond to *pyp1*$^+$ and *pyp2*$^+$.

In wild-type cells, the overexpression of *wee1*$^+$ leads to the inhibition of cell division (43). To study the potential interaction of the *wee1*$^+$ gene product with *pyp1*$^+$ or *pyp2*$^+$, either *pyp1*$^+$ or *pyp2*$^+$ was overexpressed in a *wee1-50* strain. Expression of either *pyp1*$^+$ or *pyp2*$^+$ in *wee1-50* cells in thiamine-free medium at 25°C led to cell elongation. This delay in mitosis was suppressed when cells were incubated at 35°C before induction (Fig. 6), which demonstrated that *pyp1*$^+$ and *pyp2*$^+$ require a functional *wee1*$^+$ gene product in order to inhibit mitosis. It is therefore likely that *pyp1*$^+$ and *pyp2*$^+$ act as negative regulators of mitosis upstream of *wee1*$^+$.

A *pyp1* disruption, but not a *pyp2* disruption, suppresses a *cdc25* temperature-sensitive mutation. These data suggested that *pyp1*$^+$ and *pyp2*$^+$ act as negative regulators of mitosis upstream of *wee1*$^+$. Moreover, we have noted that a *pyp1* disruption leads to a reduction in the length of wild-type cells (Table 3), a semi-wee phenotype. We therefore determined the potential of *pyp1* and *pyp2* deletion mutants to influence strains with other cell cycle mutations. *pyp1*- and *pyp2*-disrupted strains were crossed to strains with mutations involved in cell cycle regulation (Table 3). If *pyp1*$^+$ and *pyp2*$^+$ act upstream of *wee1*$^+$ as suggested by the overexpression data, mutations in *cdc2* should be unresponsive to their disruptions. The data in Table 3 show that *pyp1* or *pyp2* disruption does not alter the phenotype of a *cdc2-33* strain at 35°C and that neither a *cdc2w-3w* nor a *cdc2-1w* strain showed a detectable change in phenotype upon *pyp* disruption. A *cdc2-3w* mutation is, however, lethal in a *wee1-50* strain, in which the double mutant enters mitosis at 35°C prematurely and undergoes "mitotic catastrophe" (43). In contrast, the disruption of either *pyp1*$^+$ or *pyp2*$^+$ in a *wee1-50* background resulted in a wee or semi-wee phenotype at 25 or 35°C.

The *pyp1* disruption rescued *cdc25-22* at 35°C (Table 3 and Fig. 7) and thus is similar in phenotype to the loss of *wee1*$^+$ function (11). A *pyp2* disruption did not rescue a *cdc25-22* mutation at 35°C, however, and the cell length reduction at

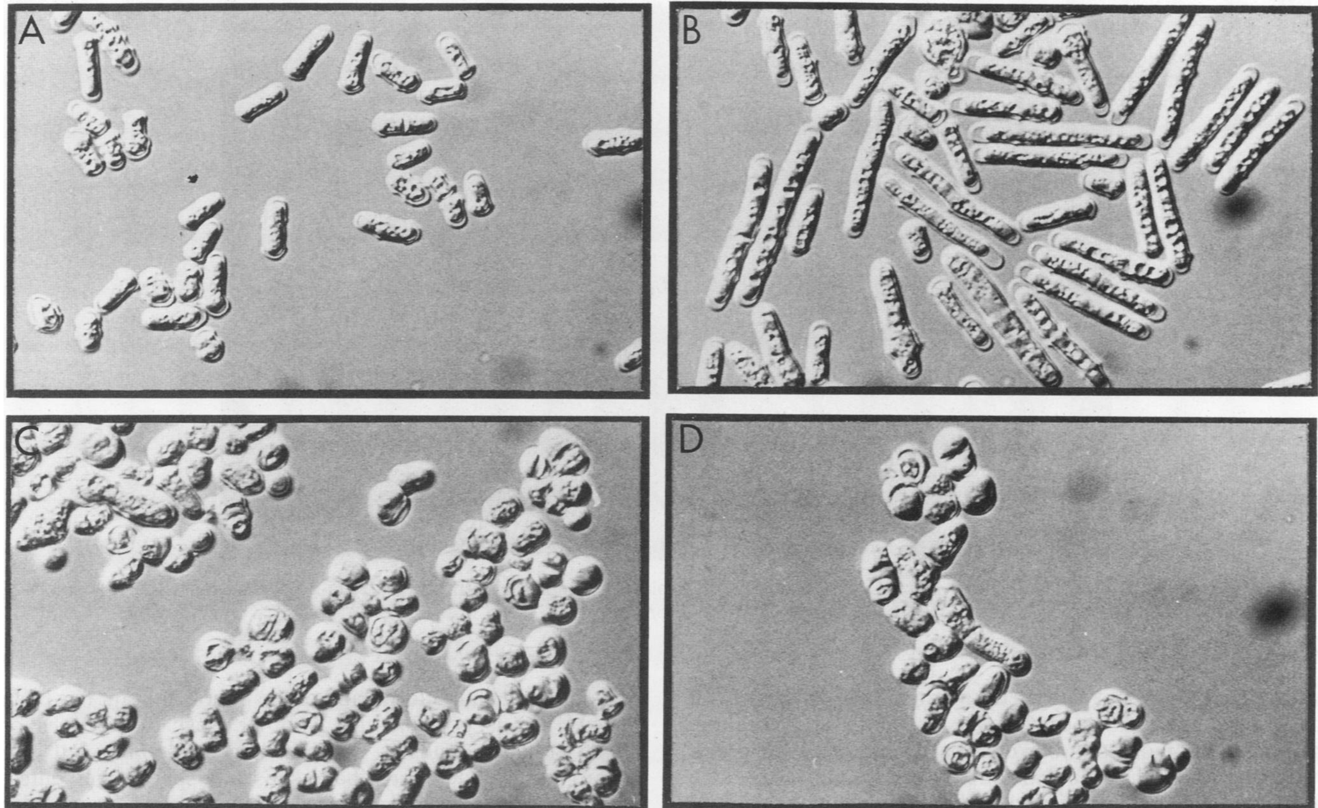

FIG. 6. Overexpression of *pyp1*$^+$ in *wee1-50* cells. *wee1-50* cells were transformed with *pyp1*$^+$ under the control of the nmt promoter. Transformed cells were grown in EMM containing thiamine (20 μM) at 25 and 35°C to late log phase. Cells were washed twice and diluted into EMM containing either 20 μM thiamine (A and C) or no thiamine (B and D) and further incubated at either 25°C (A and B) or 35°C (C and D).

25°C for *pyp2::ura4*$^+$ *cdc25-22* (20.5 ± 1.5 μm) was not as dramatic as for *pyp1::ura4*$^+$ *cdc25-22* (15.6 ± 1.6 μm), which is about wild-type length. These data indicate that a *pyp1* disruption causes advancement of mitosis and can rescue a *cdc25-22* mutation as previously described for a *wee1* deletion (11). *pyp2*$^+$ has a less dominant role, since a *pyp2* disruption neither advances mitosis nor rescues a *cdc25-22* mutation at 35°C.

DISCUSSION

In this report, we describe the cloning of a second gene from *S. pombe*, called *pyp2*$^+$, encoding a protein with sequence similarity to animal cell protein PTPases. In a previous report (38), we described *pyp1*$^+$, which is distinct from *pyp2*$^+$ but also encodes a PTPase-like protein. Disruption of *pyp1*$^+$ leads to an advancement of mitosis, whereas the disruption of *pyp2*$^+$ has no obvious impact on cell growth and proliferation. The disruption of both genes results in synthetic lethality. We also show that these gene products negatively regulate mitosis, possibly by stimulating a pathway counteracted by *cdc25*$^+$ function and upstream of the *wee1*$^+$ pathway.

The *pyp1*$^+$ and *pyp2*$^+$ gene products show a major difference in M_r, with pyp1 consisting of 550 amino acids and pyp2 consisting of 711 amino acids. Within the catalytic domains, they have 59% similarity and 41% identity, which is approximately the identity between pyp1 and human PTP1B (37%). The two genes exhibit sequence similarity in the N-terminal

TABLE 3. Genetic analysis[a]

Strain	Mean cell length (μm) at:	
	25°C	35°C
pyp1$^+$ *pyp2*$^+$ (FWP172)	14.7 ± 1.1	15.7 ± 1.7
pyp1::ura4 (SOP14)	11.3 ± 0.9	12.4 ± 1.1
pyp2::ura4 (SOP11)	14.6 ± 1.5	14.6 ± 0.8
cdc25-22	23.5 ± 2.2	cdc$^-$
cdc25-22 pyp1::ura4 (SOP18)	15.6 ± 1.6	23.0 ± 3.7
cdc25-22 pyp2::ura4 (SOP24)	20.5 ± 1.5	cdc$^-$
wee1-50	11.7 ± 1.4	8.2 ± 1.2
wee1-50 pyp1::ura4 (SOP35)	8.9 ± 1.1	8.1 ± 1.4
wee1-50 pyp2::ura4 (SOP51)	9.5 ± 1.2	8.7 ± 1.2
cdc2-3w	10.8 ± 1.6	9.0 ± 1.2
cdc2-3w pyp1::ura4 (SOP32)	8.9 ± 1.0	8.5 ± 1.0
cdc2-3w pyp2::ura4 (SOP60)	10.50 ± 1.3	9.5 ± 0.9
cdc2-1w	10.2 ± 1.6	8.1 ± 1.2
cdc2-1w pyp1::ura4 (SOP31)	8.1 ± 0.9	7.9 ± 1.0
cdc2-1w pyp2::ura4 (SOP54)	9.5 ± 1.2	8.6 ± 1.3
cdc2-33	13.7 ± 1.0	cdc$^-$
cdc2-33 pyp1::ura4 (SOP39)	12.8 ± 1.0	cdc$^-$
cdc2-33 pyp2::ura4 (SOP59)	14.8 ± 1.5	cdc$^-$

[a] Strains were grown in YEA medium (mid-log phase) at 25 and 35°C, and the length of cells displaying a septum was determined; 24 to 36 cells from each strain were measured at both temperatures.

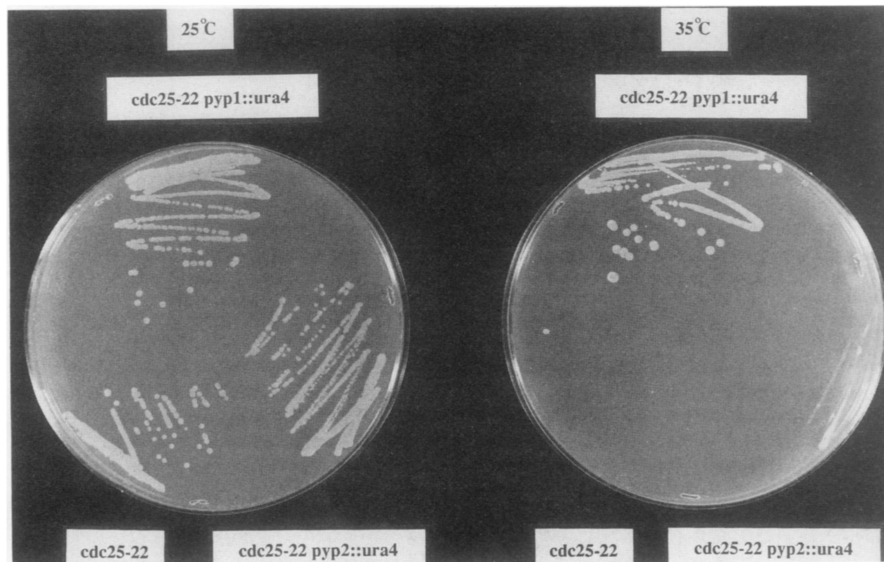

FIG. 7. Rescue of *cdc25-22* through a *pyp1* disruption. A YEA plate was spread with strain *cdc25-22 pyp1::ura4* (SOP18), *cdc25-22 pyp2::ura4* (SOP24), or *cdc25-22* and incubated at either 25 or 35°C for 4 days.

half, but we have not detected any significant similarity of the N-terminal regions of the yeast proteins and potential mammalian homologs in our data base searches. Our previous experiments using *p*-nitrophenyl phosphate (a tyrosine homolog) as a substrate failed to show phosphatase activity associated with the pyp1 or pyp2 protein; however, recently we have obtained evidence that the glutathione *S*-transferase fusion proteins of pyp1 and pyp2 purified from bacteria dephosphorylate phosphotyrosine-labeled peptide substrates and thus may serve as PTPases in vivo (22a).

The relatively weak conservation (41% identity) of these PTPase-like proteins is in sharp contrast to the strong homologies between the type 1 (PP1) and type 2A (PP2A) serine/threonine-specific protein phosphatases, whose sequences are highly conserved. In *S. pombe*, each class of serine/threonine enzymes is encoded by two genes. The two classes of serine/threonine-specific phosphatases (PP1 and PP2A) do not have complementary functions in *S. pombe*. A type 1 phosphatase is encoded by *dis2* and participates in chromosome disjoining, and mutants of the gene fail to exit mitosis. The type 2A phosphatases are also required at some other, as yet unidentified point in the cell cycle (28, 36). The two PP1 gene products are approximately 80% identical, as are the two PP2A gene products, and the yeast PP1 and PP2A proteins are also highly similar to their mammalian homologs. Thus, the pyp1 and pyp2 proteins are more highly divergent than are the serine/threonine phosphatases.

Although a *pyp1* disruption has no impact on cell viability, it leads to an advancement in mitosis, a semi-wee phenotype. After sporulation of diploid cells carrying single disruptions of *pyp1*$^+$ and *pyp2*$^+$, we have failed to detect spores that resulted in viable haploid cells with disruptions in both genes. These results suggest that only one functional gene is required for viability and that the products of *pyp1*$^+$ and *pyp2*$^+$ evidently participate in similar biochemical pathways in *S. pombe*, despite their distinct structures. We tried to determine the phenotype of a *pyp1 pyp2* double mutant microscopically. Spores with the presumed double mutation that germinated underwent a few cell divisions and yielded very small cells. Because of this unconditional phenotype, no additional characterization of the double mutant has been possible. It is possible that another class of PTPases or *cdc25*$^+$-related genes is expressed in *S. pombe*, but any other gene products must have substrate specificities different from those of pyp1 and pyp2. A search for genes similar in sequence to *pyp1*$^+$ and *pyp2*$^+$ with techniques such as low-stringency screening of Southern blots and genomic libraries remains to be completed. In this regard, it may be relevant that the *S. cerevisiae* PTPases recently cloned (20, 37) are only distantly related to the *pyp1*$^+$ and *pyp2*$^+$ products.

The overexpression of *pyp1*$^+$ or *pyp2*$^+$ leads to a delay of mitosis in all strains tested with the exception of *cdc2-1w*, a phenotype similar to that reported for overexpression of the negative regulators of mitosis, *wee1*$^+$ (43) and *mik1*$^+$ (30). These data suggest that *pyp1*$^+$ and *pyp2*$^+$ may also act as negative regulators of mitosis in a dose-dependent manner, as do *wee1*$^+$ and *mik1*$^+$. We therefore investigated *pyp1*$^+$ and *pyp2*$^+$ overexpression in a *wee1-50* background. At the permissive temperature, *wee1-50* cells overexpressing either *pyp1*$^+$ or *pyp2*$^+$ elongate, but this phenotype is suppressed at 35°C. These data indicate that both *pyp1*$^+$ and *pyp2*$^+$ require *wee1*$^+$ function to arrest the cell cycle and that they act upstream of the *wee1*$^+$/*mik1*$^+$ step. The fact that the semilethal effect of overexpressed *pyp1*$^+$ and *pyp2*$^+$ is largely suppressed in a strain carrying *cdc2-1w*, a mutation that is insensitive to *wee1*$^+$ regulation, further supports the idea that *wee1*$^+$ mediates *pyp1* and *pyp2* function.

These experiments further show that a *pyp1* disruption suppresses the lethality of a *cdc25-22* mutation at 35°C, as was described for a *wee1* disruption (11); however, a *cdc25-22* mutation at 35°C is not suppressed by a *pyp2* deletion. Thus, in this genetic background, *pyp1* and *pyp2* disruptions have a suppressor capacity analogous to that described for *wee1*$^+$ and *mik1*$^+$. A *cdc25-22* mutation is rescued by a *wee1* deletion at 35°C but not by a *mik1* disruption (30). Taken together, these data suggest a model outlined in Fig. 8, whereby the pyp1 and pyp2 proteins may activate negative regulators of mitosis, such as the *wee1*$^+$ product, or inhibit the inducer of mitosis, *cdr1*$^+$ (*nim1*$^+$).

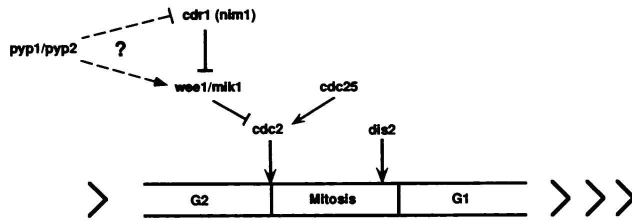

FIG. 8. Proposed model of *pyp1*$^+$ and *pyp2*$^+$ interaction.

Our genetic data cannot distinguish between these two possibilities and require further investigation of the pathways regulating *wee1*$^+$/*mik1*$^+$ activity.

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