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A fission-yeast gene encoding a protein with features of protein-tyrosine-phosphatases

(*Schizosaccharomyces pombe*/tyrosine phosphorylation/conserved sequences/cell cycle control)

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ABSTRACT **Degenerate oligonucleotide probes encoding sequences conserved among mammalian protein-tyrosine-phosphatases (PTPases) were used to amplify DNA fragments from a *Schizosaccharomyces pombe* cDNA library by polymerase chain reaction (PCR) methods. A cloned PCR product predicted peptide sequences similar to those found in PTPases but not identical to any published sequences. A *S. pombe* gene, designated *pyp1*$^+$, was identified in a cDNA library with this PCR probe, cloned, and sequenced. The sequence of the gene predicted a 550-amino acid protein with M_r 61,586, which includes amino acid sequences that are highly conserved in mammalian PTPases. Disruption of the *pyp1*$^+$ gene resulted in viable cells. Overexpression of the *pyp1*$^+$ gene in *S. pombe* permitted detection of a protein of apparent M_r 63,000.**

Reversible protein phosphorylation has long been regarded as an important regulatory mechanism, beginning with the seminal studies on glycogen metabolism and the discovery of cAMP and cAMP-dependent protein kinase (see ref. 1 for a review). Thus, the amplification of an extracellular signal in metabolic pathways as the result of both the positive and the negative influence of protein phosphorylation became apparent. Subsequently, it became clear that the phosphoprotein phosphatases are also important regulatory components of these pathways (for a review, see ref. 2). The majority of the phosphorylation in such metabolic cycles appears to involve serine, and to a lesser extent threonine, residues.

The discovery that some oncogene products and peptide growth factor receptors are also protein kinases emphasized the importance of protein phosphorylation in cell proliferation events as well. In contrast to the activity of previously characterized enzymes, most, but not all, of these protein kinases phosphorylate tyrosine residues (3). The considerable attention to this class of protein kinases has demonstrated their extensive diversity in expression and function during cell proliferation and differentiation (4). Early studies of protein-tyrosine-phosphatases (PTPases; ref. 5) led to a general understanding of their properties, but the sequencing of the major human placental PTPase (PTPase 1B; ref. 6) opened new research avenues for study of these enzymes. The availability of this protein sequence information has led quickly to the molecular cloning of numerous cDNAs that apparently encode enzymes with the capacity to dephosphorylate phosphotyrosine (7–12). These data also revealed that previously characterized proteins such as leukocyte common antigen (LCA, also known as CD45) and LCA-related protein (LAR) contain C-terminal domains with PTPase activity (13, 14). This family of enzymes, which may eventually prove to be as structurally diverse as protein-tyrosine kinases, falls into two classes: (*i*) transmembrane proteins with extracellular domains that are likely to function as receptors for extracellular signals and (*ii*) proteins that may be membrane-associated but are entirely cytoplasmic.

The interplay of serine/threonine and tyrosine reversible phosphorylation has recently been featured in the regulation of the highly conserved protein kinase p34^{cdc2}. This protein kinase is a critical component of the molecular network that controls the cell cycle (15). Tyrosine phosphorylation within the ATP-binding site of the M_r 34,000 *cdc2* gene product, p34^{cdc2}, of the fission yeast *Schizosaccharomyces pombe* negatively regulates its capacity to phosphorylate other substrates *in vitro* on serine or threonine (16). To date, the protein kinase(s) responsible for phosphorylation of this tyrosine residue is uncertain, although the *wee1* gene product is one candidate (17, 18). The product of the *S. pombe* gene *cdc25*, p80, is required for dephosphorylation and activation of p34^{cdc2} in cells (19, 20) and therefore may inhibit the relevant protein kinase or stimulate the dephosphorylation of the tyrosine residues; however, the sequence of p80^{cdc25} does not resemble that of mammalian PTPases, and thus p80 is unlikely to directly dephosphorylate p34^{cdc2}.

In this communication, we report the molecular cloning of a gene from the yeast *S. pombe*, denoted *pyp1*$^+$, that encodes a protein with a sequence similar to that of mammalian PTPases, and present a preliminary characterization of its expression.‡

MATERIALS AND METHODS

***S. pombe* Strains.** *S. pombe* cells were grown in complex medium, YEA (21), or in synthetic complete (SC) medium lacking uracil (22). The haploid strains FWP165 (h$^+$ *ura4-D18 leu1-32 ade6-M216*) and FWP172 (h$^-$ *ura4-D18 leu1-32 ade6-M210*) were used to generate the diploid strain SOP1 used for gene disruption. The wild-type strain 972 (h$^-$) was used.

Oligonucleotides. Oligonucleotides for the polymerase chain reactions (PCRs) and sequencing reactions were synthesized on a MilliGen/Biosearch (Burlington, MA) apparatus and were purified with phenyl solid-phase extraction (J. T. Baker) columns (23).

PCR. The following degenerate oligonucleotides were used for PCRs: primer F, 5′-CGG-AAT-TC-AA(A/G)-TG(T/C)-(G/C)(A/C)N-(G/C)A(A/G)-TA(T/C)-TGG-CC-3′ (sense), and primer R, 5′-CGC-GGA-TCC-CC-NA(C/T)-NCC-NGC-NGA-(G/A)CA-(A/G)TG-NAC (antisense), which contain restriction sites for *Eco*RI and *Bam*HI, respectively, followed by the codons for the amino acids KC(A/D/H)(Q/E)YWP and VHCSAG(V/I)G of the conserved domain of PTPases. DNA amplification was carried out on a Perkin–Elmer thermal cycler in 50 μl containing 50 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.01% gelatin, 0.1% Triton X-100, 6 μM each primer, 2.5 units of

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Abbreviation: PTPase, protein-tyrosine-phosphatase.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession number M63257).

Thermus aquaticus (*Taq*) DNA polymerase, and 10 ng of DNA of a *S. pombe* cDNA library as a template (24). Thirty cycles of amplification were performed (94°C for 45 sec, 45°C for 1 min, and 72°C for 1 min), and 10% of the product was analyzed by electrophoresis in a 1.5% agarose gel (GIBCO/BRL). The product [≈300 base pairs (bp)] was isolated on glass beads and reamplified. The reamplified band was isolated in a 1.5% agarose gel, purified, digested with *Bam*HI and *Eco*RI, and ligated into pBluescript SK+ (Stratagene). The sequence of the 300-bp PCR product conformed to that expected to encode PTPases but was not identical to any known isolates.

Isolation of cDNA Clones. The 300-bp PCR product was labeled with [α-^{32}P]dCTP (New England Nuclear) to a specific activity of 1–5 × 10^8 cpm/μg by the random-priming technique (25). Twenty thousand colonies of a *S. pombe* cDNA library (24) were screened under low-stringency conditions [hybridization conditions, 35% formamide in buffer A (1 M NaCl/50 mM Tris·HCl, pH 7.5/5× Denhardt's solution/1% SDS) at 42°C; washing conditions, 2× standard saline citrate (SSC)/1% SDS at 50°C]. The inserts of 20 recombinant clones were excised by restriction digestion with *Eco*RI, separated by agarose gel electrophoresis, and transferred to Zetabind membrane (Cuno) by capillary blotting with 0.4 M NaOH/0.6 M NaCl. The filter was hybridized overnight at 42°C under moderate-stringency conditions (40% formamide in buffer A at 42°C) with the [α-^{32}P]dCTP-labeled PCR product and washed with 0.2× SSC/1% SDS at 60°C.

DNA Sequencing and Peptide Comparisons. The sequence of clone 13 (*pyp1*$^+$) was determined by the dideoxy chain-termination technique (26) using oligonucleotide primers. The University of Wisconsin Genetics Computer Group programs were used to compile and analyze the sequence data. Alignments were performed using the GAP comparison program, version 6.0, with a gap weight of 1.2 and a length weight of 0.08 (27).

Southern Blot Analysis. Genomic DNA from *S. pombe* cells was obtained using the protocol of Hoffman and Winston (28). DNA (3 μg) was digested with *Eco*RI, electrophoresed in a 1% agarose gel (GIBCO/BRL), and transferred to Zetabind membrane by capillary blotting. The filters were hybridized overnight under high-stringency conditions (50% formamide in buffer A at 42°C) with an [α-^{32}P]dCTP-labeled 1.9-kilobase (kb) *Hin*dIII–*Eco*RI fragment of clone 13 (*pyp1*$^+$) and washed with 0.2× SSC/1% SDS. Autoradiographs were obtained by exposing the blots as above.

Northern Blot Analysis. Total RNA from *S. pombe* cells was obtained using the protocol of Carlson and Botstein (29). RNA (10 μg) was electrophoresed in a 1.2% agarose/6% formaldehyde gel, transferred to a Nytran membrane (Schleicher & Schuell) by capillary blotting with 10× SSC, and baked at 80°C for 2 hr. The filter was hybridized overnight at 65°C in 0.5 M sodium phosphate, pH 7.2/7% SDS/1% bovine serum albumin with an [α-^{32}P]dCTP-labeled 1.9-kb *Hin*dIII–*Eco*RI cDNA fragment and washed at 65°C with 0.2× SSC/1% SDS at 65°C. Blots were exposed as above for 18 hr.

Gene Disruption. The plasmid pCG1 (30), containing the *S. pombe ura4* gene, was digested with *Hin*dIII, and the 1.8-kb *ura4* fragment was filled in with Klenow polymerase and dNTPs and blunt-end ligated into the *Bal* I site of the *pyp1*$^+$ cDNA clone. This construct was digested with *Hin*dIII/*Eco*RI, and the resulting *pyp1*$^+$::*ura4* insert was used to transform the *S. pombe* diploid strain SOP1 by the protocol of Ito *et al.* (31). Stable Ura$^+$ transformants were detected by repeatedly plating for single colonies on SC plates lacking uracil. DNA from these colonies was isolated, digested with *Eco*RI, electrophoresed, blotted, and hybridized under high stringency with a 1.9-kb *Hin*dIII–*Eco*RI fragment, as described above. Transformants with one disrupted phosphatase allele were sporulated on YPD plates (22). Ascus spores were dissected into tetrads on YEA plates and the viability of the spores was analyzed.

pyp1 Protein Expression, Antiserum, and Immunoprecipitation. The *pyp1*$^+$ gene product was expressed in *Escherichia coli* using pET (32) or pGEX (33) vectors. Protein was purified from inclusion bodies and used for immunization of rabbits as described (34). For overexpression, the *pyp1*$^+$ gene was cleaved with *Ssp* I and *Eco*RV and cloned in the sense and antisense orientations relative to the *adh* promoter by using *Bam*HI linkers. The pART2 vector with the *S. pombe adh* promoter was a gift from Paul Russell (17). *S. pombe* cells carrying the vector with the *pyp1*$^+$ gene in sense or antisense orientation were labeled with [^{35}S]methionine (50 μCi/ml; 1 μCi = 37 kBq) for 4 hr in minimal medium lacking methionine and cysteine, centrifuged, washed once in H_2O, and rapidly frozen in a liquid nitrogen bath. Cells were broken by vortex mixing for 2–4 min with glass beads in lysis buffer [25 mM Tris·HCl, pH 7.5/15 mM EGTA/15 mM $MgCl_2$/1 mM dithiothreitol containing soybean trypsin inhibitor (10 μg/ml), leupeptin (1 mM), and aprotinin (1 μg/ml)]. The lysate was boiled for 2 min after the addition of SDS to 0.5% and then diluted 5- to 10-fold with RIPA buffer without SDS (150 mM NaCl/50 mM Tris·HCl, pH 7.2/1% Triton X-100/1% sodium deoxycholate). Immunoprecipitation of radiolabeled protein was carried out as described (34).

RESULTS

Isolation and Characterization of a Putative *S. pombe* PTPase Gene, *pyp1*$^+$. Twenty cDNA clones were selected after low-stringency screening of the cDNA library. The clones with the longest inserts that displayed the strongest hybridization signals to the PCR probe were selected for sequencing. Sequence analysis of the region of the clone 13 (*pyp1*$^+$) insert corresponding to the 300-bp PCR probe revealed that this clone did not include an exact sequence match to the PCR product, but it did predict protein sequences that were highly conserved in mammalian PTPases. The sequencing and characterization of a *S. pombe* gene corresponding to the exact sequence of the PCR product will be described in a separate communication. The DNA sequence of an 1887-bp *Ssp* I–*Eco*RV subclone of clone 13 (*pyp1*$^+$) and its predicted protein sequence is presented in Fig. 1. It contains a 1650-bp open reading frame that potentially encodes a protein consisting of 550 amino acids corresponding to M_r 61,586. The first 5′ ATG triplet within this sequence is preceded by an adenine residue in position −3, which is generally consistent with the consensus sequence for the initiation of translation in higher eukaryotes (35, 36) and is also highly conserved in the budding yeast *Saccharomyces cerevisiae* (37). The open reading frame is terminated by a stop codon at nucleotide 1689 within this sequence.

The *pyp1*$^+$ DNA sequence predicts some peptide sequences that are identical to those shared by many members of the PTPase family (Fig. 2; amino acids conserved in PTPases are marked by stars). The identical peptides are found within a domain with a high degree of sequence similarity to mammalian PTPases. Within this putative catalytic domain, the pyp1 protein (residues 282–550) is 61% similar and 37% identical to PTPase 1B (residues 27–295).

Disruption of the *pyp1*$^+$ Gene *in Vivo*. In order to test for function of the *S. pombe pyp1*$^+$ gene, the *S. pombe ura4* gene was inserted into the single *Bal* I site of *pyp1*$^+$, thereby disrupting the coding region within the putative catalytic domain at amino acid 383. A diploid *S. pombe* strain was constructed and transformed with the *pyp1*$^+$::*ura4* fragment. Stable uracil-prototrophic colonies were isolated, and transformants containing one disrupted allele of *pyp1*$^+$ and one

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  1  AATATTCTTAATACAAAACATTCATCTAAAAAACCACGATGAATTTTTCAAACGGTTCAAAATCGTCTACTTTTACAATTGCTCCTTCGGGTTCATGTATTGCTTTACCTCCTCAGCGG  119
                                                M  N  F  S  N  G  S  K  S  S  T  F  T  I  A  P  S  G  S  C  I  A  L  P  P  Q  R

120  GGTGTAGCAACGAGTAAGTATGCTGTTCATGCTTCTTGCCTCCAAGAATATCTCGACAAGGAAGCTTGGAAGGATGACACATTGATCATCGACCTTCGTCCCGTATCCGAGTTTTCCAAA  239
       G  V  A  T  S  K  Y  A  V  H  A  S  C  L  Q  E  Y  L  D  K  E  A  W  K  D  D  T  L  I  I  D  L  R  P  V  S  E  F  S  K

240  TCTAGAATTAAGGGCTCCGTCAATCTTTCTTTACCTGCAACCTTAATTAAGCGTCCTGCCTTTTCGGTCGCGCGTATCATCAGCAATCTTCACGACGTCGACGACAAGAGAGATTTTCAA  359
       S  R  I  K  G  S  V  N  L  S  L  P  A  T  L  I  K  R  P  A  F  S  V  A  R  I  I  S  N  L  H  D  V  D  D  K  R  D  F  Q

360  AACTGGCAAGAGTTTTCATCCATTCTTGTTTGTGTTCCTGCTTGGATTGCTAACTACGTAACGAACGCTGAGGTAATTGGTGAAAAATTTAGAAAAGAATCCTATTCTGGTGACTTCGGT  479
       N  W  Q  E  F  S  S  I  L  V  C  V  P  A  W  I  A  N  Y  V  T  N  A  E  V  I  G  E  K  F  R  K  E  S  Y  S  G  D  F  G

480  ATTTTAGATTTAGACTATTCGAAAGTCTCGGGCAAGTATCCATCTGTGATTGACAACTCTCCTGTAAAAAGTAAATTAGGTGCTTTGCCTAGCGCTCGCCCCAGATTGTCTTACTCTGCT  599
       I  L  D  L  D  Y  S  K  V  S  G  K  Y  P  S  V  I  D  N  S  P  V  K  S  K  L  G  A  L  P  S  A  R  P  R  L  S  Y  S  A

600  GCTCAAACTGCTCCTATCTCTTTGTCCAGCGAAGGCTCCGATTACTTCTCTCGTCCTCCTCCCACTCCTAATGTTGCCGGTTTGTCTTTAAATAACTTTTCTGTCCTCTACCTGAGAAC  719
       A  Q  T  A  P  I  S  L  S  S  E  G  S  D  Y  F  S  R  P  P  P  T  P  N  V  A  G  L  S  L  N  N  F  F  C  P  L  P  E  N

720  AAGGACAACAAATCTTCTCCATTTGGCAGTGCTACAGTCCAGACACCCTGCTTACACAGTGTGCCCGATGCTTTTACCAATCCAGATGTTGCGACCCTCTACCAGAAGTTCTTGCGCCTG  839
       K  D  N  K  S  S  P  F  G  S  A  T  V  Q  T  P  C  L  H  S  V  P  D  A  F  T  N  P  D  V  A  T  L  Y  Q  K  F  L  R  L

840  CAATCATTGGAGCATCAACGTCTAGTTTCTTGTTCGGATCGTAATTCTCAGTGGAGTACCGTTGATTCATTGAGTAATACATCGTATAAGAAAAACAGATATACCGACATTGTTCCTTAC  959
       Q  S  L  E  H  Q  R  L  V  S  C  S  D  R  N  S  Q  W  S  T  V  D  S  L  S  N  T  S  Y  K  K  N  R  Y  T  D  I  V  P  Y

960  AATTGTACTCGTGTGCATTTGAAAAGAACTAGCCCTTCAGAGCTCGATTATATCAATGCTTCCTTTATCAAAACTGAAACTTCAAATTACATCGCTTGCCAAGGTTCTATTTCTCGTTCT  1079
       N  C  T  R  V  H  L  K  R  T  S  P  S  E  L  D  Y  I  N  A  S  F  I  K  T  E  T  S  N  Y  I  A  C  Q  G  S  I  S  R  S

1080 ATTAGCGATTTCTGGCACATGGTATGGGACAATGTGGAGAATATAGGCACTATTGTTATGCTTGGTTCATTGTTCGAGGCTGGCCGCGAAATGTGTACTGCATATTGGCCAAGTAATGGT  1199
       I  S  D  F  W  H  M  V  W  D  N  V  E  N  I  G  T  I  V  M  L  G  S  L  F  E  A  G  R  E  M  C  T  A  Y  W  P  S  N  G

1200 ATTGGAGATAAACAAGTGTATGGAGATTACTGTGTCAAACAAATTTCGGAGGAAAATGTCGATAATTCTCGATTCATTTTGCGAAAGTTTGAAATTCAGAATGCCAATTTTCCTTCAGTT  1319
       I  G  D  K  Q  V  Y  G  D  Y  C  V  K  Q  I  S  E  E  N  V  D  N  S  R  F  I  L  R  K  F  E  I  Q  N  A  N  F  P  S  V

1320 AAAAAAGTACATCACTATCAATATCCTAATTGGTCTGATTGTAATTCTCCTGAAAACGTGAAATCTATGGTTGAGTTCTTAAAATATGTGAACAACTCTCACGGATCAGGAAATACTATT  1439
       K  K  V  H  H  Y  Q  Y  P  N  W  S  D  C  N  S  P  E  N  V  K  S  M  V  E  F  L  K  Y  V  N  N  S  H  G  S  G  N  T  I

1440 GTGCACTGCTCTGCCGGTGTTGGTCGGACAGGAACCTTTATTGTACTAGATACGATCCTACGCTTCCCAGAAAGCAAACTTTCTGGTTTCAATCCTTCTGTCGCCGATTCTTCAGACGTC  1559
       V  H  C  S  A  G  V  G  R  T  G  T  F  I  V  L  D  T  I  L  R  F  P  E  S  K  L  S  G  F  N  P  S  V  A  D  S  S  D  V

1560 GTTTTCCAGTTGGTTGATCATATCCGAAAGCAGCGGATGAAGATGGTTCAAACCTTCACACAATTTAAATATGTGTATGACTTGATCGATTCTTTGCAAAAATCTCAAGTTCATTTCCCG  1679
       V  F  Q  L  V  D  H  I  R  K  Q  R  M  K  M  V  Q  T  F  T  Q  F  K  Y  V  Y  D  L  I  D  S  L  Q  K  S  Q  V  H  F  P

1680 GTTTTAACATGAAATTTTTGACTGGATTTTTCTTGGCAATATATATTCGTGTTTTAATCGATTCCTTTATTTCTTGTACTTGTAAAGTGTCTTTTTTTTTACATTTGCATTTGAATTTAC  1799
       V  L  T  *

1800 TGTCAATGTTTGTGAAATTCAGTTGCTTTAATCACGTTGTCTTTTATTTCAAAAAGTATATTTGAGAACTAGGCTTTTTAATGATATC                                1887
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FIG. 1. The DNA sequence of an *Ssp* I–*Eco*RV subclone of the *S. pombe pyp1*$^+$ gene and its predicted amino acid sequence.

wild-type copy were identified by Southern hybridization analysis (Fig. 3).

Sporulation of these cells was induced and several tetrads were dissected. DNA was isolated from cultures derived from uracil-independent cells, digested with *Eco*RI, and analyzed by Southern hybridization to confirm the presence of the disrupted *pyp1*$^+$ allele. In a Southern blot (Fig. 3*A*), *Eco*RI-digested DNA from the diploid strain SOP2, a derivative of SOP1, carrying a disrupted and a wild-type allele of *pyp1*$^+$, showed the expected pattern (lane 2), a 2.2-kb band,

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Pyp1     1 MNFSNGSKSSTFTIAPSGSCIALPPQRGVATSKYAVHASCLQEYLDKEAWKDDTLIIDLRPVSEFSKSRIKGSVNLSLPATLIKRPAFSVARIISNLHDVDDKRDFQNWQ  110
PTP 1B   1 ..............................................................................................................  1

Pyp1   111 EFSSILVCVPAWIANYVTNAEVIGEKFRKESYSGDFGILDLDYSKVSGKYPSVIDNSPVKSKLGALPSARPRLSYSAAQTAPISLSSEGSDYFSRPPPTPNVAGLSLNNF  220
                                                                                                                  :.::.
PTP 1B   1..............................................................................................................MEMEKE    6

                                                                                                  **                ****
Pyp1   221 FCPLPENKDNKSSPFGSATVQTPCLHSVPDAFTNPDVATLYQKFLRLQSLEHQRLVSCSDRNSQW.STVDSLSNTSYKKNRYTDIVPYNCTRVHLKRTSPSELDYINASF  329
           |    |. | ||:.:                      |.:||. :|     |:        .|:: : |..|.... .:||| |: |:: .|:.|..   .: ||||||:
PTP 1B     7 F....EQID.KSGSW.....................AAIYQD.IR.....HE........ASDFPCRVAKLPKNK.NRNRYRDVSPFDHSRIKLHQ...EDNDYINASL   71

                          ** *                       *  **
Pyp1   330 IKTETSN..YIACQGSISRSISDFWHMVWDNVENIGTIVMLGSLFEAGREMCTAYWPSNGIGDKQ.VYGDYCVK..QISEENVDNSRFILRKFEIQNANFPSVKKVHHYQ  434
           || |...  || .||.:....:.||.|||:  :.  .:|||..::| |. .|..||| .  ::|: :::|  :|   |||: : .| :.:|.:|::| . ...:.: |::
PTP 1B    72 IKMEEAQRSYILTQGPLPNTCGHFWEMVWE..QKSRGVVMLNRVMEKGSLKCAQYWPQK..EEKEMIFEDTNLKLTLISED.I.KSYYTVRQLELENLTTQETREILHFH  175

           *    *                          ** **                                                  *
Pyp1   435 YPNWSDCNSPENVKSMVEFL.KYVNNSHGS.....GNTIVHCSAGVGRTGTFIVLDTILRFPESKLSGFNPSVADSSDVVFQLVDHIRKQRMKMVQTFTQFKYVY.DLID  537
           |..|.| . ||.. |:::|| | |.:| ||     | .:|||||:||.|||.: ||.| : :.:    :|| .| ..|::::    || || ::|| .|::: | .:|:
PTP 1B    176 YTTWPDFGVPESPASFLNFLFK.VRES.GSLSPEHGPVVVHCSAGIGRSGTFCLADTCLLLMDKRK...DPSSVDIKKVLLEM....RKFRMGLIQTADQLRFSYLAVIE  276

Pyp1   538 S....LQKSQV..HFPVLT*..........................................................................................  550
           :    :..| |  ::. |.
PTP 1B     277 GAKFIMGDSSVQDQWKELSHEDLEPPPEHIPPPPRPPKRILEPHNGKCREFFPNHQWVKEETQEDKDCPIKEEKGSPLNAAPYGIESMSQDTEVRSRVVGGSLRGAQAAS  386

Pyp1   550 ..........................................  550

PTP 1B     387 PAKGEPSLPEKDEDHALSYWKPFLVNMCVATVLTAGAYLCYRFLFNSNT 435
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FIG. 2. Amino acid sequence alignment of the *pyp1*$^+$ gene product (upper row) and the human placental PTPase 1B (lower row). Gaps were introduced to achieve maximal sequence similarity. Vertical lines indicate identity, and colons (:) and periods (.) indicate conserved changes according to the described programs (27). Amino acids highly conserved in all PTPases are marked with stars. Residues 282–550 of pyp1 protein are 61% similar and 37% identical to PTPase 1B residues 27–295. The underlined sequences were used to design the degenerate PCR primers.

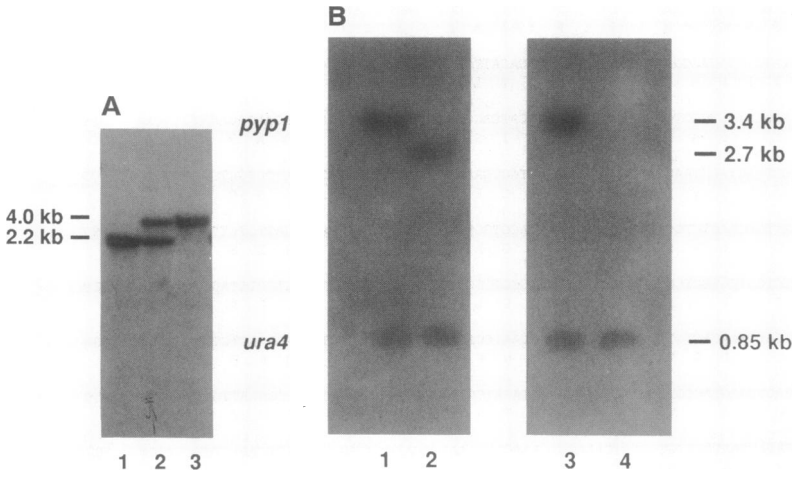

FIG. 3. (*A*) Southern blot analysis of the *pyp1*$^+$ gene disruption. DNA was isolated from strains indicated below, digested with *Eco*RI, electrophoresed, transferred to Zetabind membrane, and probed with ^{32}P-labeled 1.9-kb *Hin*dIII–*Eco*RI fragment of *pyp1*$^+$. Lane 1, wild-type strain 972; lane 2, diploid strain SOP2, carrying one disrupted and one wild-type allele of *pyp1*$^+$; lane 3, cells from the uracil-prototrophic haploid strain SOP13, carrying a disrupted allele of *pyp1*$^+$. Each lane was loaded with 5 μg of DNA. (*B*) Northern blot analysis of *pyp1*$^+$ transcripts. Total RNA (10 μg per lane) was resolved in a 1.2% agarose/6% formaldehyde gel and transferred to a Nytran membrane. Lanes 1 and 3, RNA from wild-type strain 972; lanes 2 and 4, RNA from strain SOP13, carrying a disrupted *pyp1*$^+$ gene. Lanes 1 and 2 were probed with a ^{32}P-labeled *Hin*dIII–*Eco*RI fragment. Lanes 3 and 4 were probed with a ^{32}P-labeled *Bal* I–*Eco*RV fragment. In addition, all lanes were hybridized with the *ura4* fragment.

which was also present in DNA from strain 972 (lane 1), and an additional band of 4.0 kb, representing the *pyp1*$^+$ allele shifted by 1.8 kb, the length of the *ura4* fragment. *Eco*RI-digested DNA from strain SOP13, derived from the *pyp1*::*ura4* spore, displayed only a 4.0-kb band, representing the disrupted *pyp1*$^+$ allele (lane 3). The 4.0-kb DNA fragment also hybridized to a radiolabeled *ura4* probe (data not shown). The viability of these cells containing the disrupted *pyp1*$^+$ allele indicates that the *pyp1*$^+$ gene is not essential for growth of *S. pombe* cells.

Expression of *pyp1*$^+$ mRNA. Total RNA samples from strains 972 and SOP13 were electrophoresed, blotted, and probed with a 1.9-kb *Hin*dIII–*Eco*RI fragment of the *pyp1*$^+$ clone. A single RNA band of about 3.4 kb was detected from wild-type cells (Fig. 3*B*, lane 1). The RNA from *pyp1*$^+$::*ura4* cells revealed an RNA hybridizing to the same probe, with a reduced size of 2.7 kb (lane 2). RNA from these cells was also probed with a *pyp1*$^+$ DNA fragment 3′ to the *ura4* insert (*Bal* I–*Eco*RV fragment). This probe detected the 3.4-kb mRNA in wild-type cells (lane 3); however, it did not hybridize to the 2.7-kb RNA transcript (lane 4), indicating that the insertion of the *ura4* gene causes transcriptional termination within the *pyp1*$^+$ gene. Therefore, the *pyp1*$^+$::*ura4* cells are unable to encode a functional protein. A radiolabeled *ura4* gene fragment detected a 0.85-kb mRNA in all four tracks.

Overexpression of *pyp1*$^+$ in *S. pombe*. Initial experiments with the antiserum raised in rabbits against antigen produced in *E. coli* failed to convincingly detect a specific protein in *S. pombe* labeled with [^{35}S]methionine, although it did specifically react with the bacterially produced protein (data not shown). Expression of *pyp1*$^+$ under control of the *adh* promoter, however, did result in protein expression readily detected in *S. pombe* cells (Fig. 4). Extracts from *adh–pyp1*$^+$ cells immunoprecipitated with preimmune and immune antiserum revealed a specific protein of $M_r \approx 63{,}000$ (lanes 1 and 2). In contrast, when the *pyp1*$^+$ gene was placed in the antisense orientation, no protein was detected (lanes 3 and 4). Immunoprecipitation of the M_r 63,000 protein can be blocked by preincubation of the antiserum with unlabeled *pyp1*$^+$ gene product produced in *E. coli* (data not shown). A 7-fold longer exposure of lanes 3 and 4 did not reveal a M_r 63,000 protein. Experiments carried out concurrently with wild-type cells, using equal exposures of immunoprecipitated protein, did not reveal a specific M_r 63,000 protein. Therefore, although *pyp1*$^+$ mRNA is readily detected, the protein expressed in unsynchronized cells does not appear to be abundant.

DISCUSSION

In this communication, we describe the molecular cloning of a *S. pombe* gene encoding a protein related to mammalian PTPases. Degenerate oligonucleotides with sequences based on highly conserved amino acid sequences in PTPases were used for PCR-based synthesis of probes for screening a *S. pombe* cDNA library. Two distinct clones were obtained, one of which, *pyp1*$^+$, is described here. The sequence over ≈200 amino acids at the C terminus of the pyp1 protein contains conserved amino acid sequences found in many mammalian PTPases that are believed to correspond to the catalytic domain. Unlike human PTPase 1B and several other phosphatases, the C terminus of the pyp1 protein evidently coincides with the C terminus of the catalytic domain, whereas PTPase 1B has an additional 140 amino acids C-terminal to the putative catalytic domain. The predicted sequence of the pyp1 protein includes only a single apparent catalytic domain, unlike CD45. Nor is there evidence of sequences that indicate it is a transmembrane protein.

Although viable cells were obtained from spores with a disrupted *pyp1* gene, we have not completely evaluated their growth and proliferative behavior. We have isolated a second, *pyp1*$^+$-like gene from *S. pombe* (unpublished data), and it is possible its protein product has a function complementary to that of the pyp1 protein, resulting in viable cells when

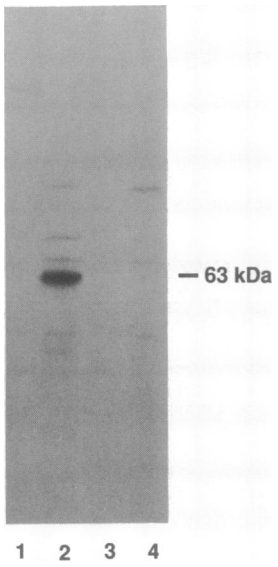

FIG. 4. Detection of pyp1 protein in *S. pombe* cell lysates. The *pyp1*$^+$ gene in sense (lanes 1 and 2) or antisense (lanes 3 and 4) orientation relative to the *adh* promoter was used for expression in *S. pombe*. Approximately 2×10^7 cpm of radiolabeled protein was immunoprecipitated with preimmune serum (lanes 1 and 3) or immune serum (lanes 2 and 4). Exposure was for 15 hr at −70°C after sodium salicylate treatment of the gel.

pypl$^+$ is disrupted. The presence of redundant serine/threonine phosphatases has already been clearly demonstrated in *S. pombe* (38, 39). In these cases, disruption of one of the two genes encoding protein phosphatases type 1 and type 2A (PP1 and PP2A, respectively) has no major influence on cell growth, whereas disruption of both results in nonviable spores. These studies also revealed that a PP1 gene product cannot complement a PP2A gene product, although *in vitro* these enzymes have a broad specificity with overlapping substrates. The two *S. pombe* PP2A sequences are nearly 80% identical and in turn are nearly 80% identical to mammalian enzymes. This is in distinct contrast to *pypl*$^+$, which is related to PTPase 1B only within the putative catalytic domain. In this regard, the yeast gene product is akin to most mammalian PTPases, which differ greatly beyond certain highly conserved sequences within the catalytic domain (11). It should be noted that we have not searched for other related genes in a systematic manner, and therefore other proteins of this class may exist in *S. pombe*.

We have failed thus far to demonstrate significant PTPase activity associated with the *pypl*$^+$ gene product expressed in bacteria. Human PTPase 1B expressed in parallel in these experiments yielded highly active enzyme preparations. This result raises the possibility that, although the pypl protein has a sequence expected for a PTPase, it has an unrelated function. Preliminary results have indicated, however, that extracts from the *adh–pypl*$^+$ overexpressor cells described in Fig. 4 show greatly increased capacity to dephosphorylate *p*-nitrophenyl phosphate, an analog of phosphotyrosine, at pH 7.5, which may be an indication of pypl PTPase activity. These results suggest that modification of pypl protein by another *S. pombe* gene product is required for activation of its phosphatase activity. For example, the activity of the p80^{cdc25} protein is required in *S. pombe* to initiate dephosphorylation of p34^{cdc2} (16). Therefore, addition of the *cdc25* gene product may be necessary in order to measure pypl protein function *in vitro*.

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