Molecular Biology of the Cell
Vol. 4, 303–313, March 1993

Isolation and Characterization of the Fission Yeast Protein Phosphatase Gene *ppe1*$^+$ Involved in Cell Shape Control and Mitosis

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Submitted November 16, 1992; Accepted January 22, 1993

We isolated a fission yeast putative protein serine/threonine phosphatase gene designated *ppe1*$^+$ by hybridization. The predicted amino acid sequence is similar to those of the fission yeast ppa2 (53% identity) and dis2 (39%) phosphatases, and highly similar to those of the budding yeast SIT4 (72%), Drosophila PPV (68%) and rabbit PPX (61%) phosphatases. Antibodies against ppe1 protein identified a 37-kd polypeptide in fission yeast. A gene disruption (designated Δ*ppe1*) caused cold-sensitive lethality and short, pear-shaped cells. These phenotypes were fully suppressed by a plasmid carrying *ppe1*$^+$. Three classes of multicopy suppressor genes for Δ*ppe1* were identified as follows: 1) *ppa1*$^+$ and *ppa2*$^+$ encoding type 2A-like phosphatases, 2) mitotically essential *dis3*$^+$ similar to the budding yeast *SSD1/SRK1*, a suppressor for *sit4*, and 3) *pck1*$^+$ coding for a protein kinase C-like kinase. Consistently, the budding yeast *SIT4* gene was also a multicopy suppressor for Δ*ppe1*. Phosphatase ppe1 may play a role in cell morphogenesis and mitosis by either regulating or being regulated by these multicopy suppressor gene products. Consistent with this hypothesis, double mutants *ppe1-ppa2* and *ppe1-pck1* are lethal at the permissive temperature.

INTRODUCTION

The protein ser/thr phosphatases are a class of enzymes that catalyze dephosphorylation of protein phosphoserine or phosphothreonine and are present ubiquitously in eukaryotes. It was only recently established that these phosphatases are essential in the cell division cycle. Mutations in the phosphatase genes were identified among mutants defective in mitosis for the filamentous fungus *Aspergillus nidulans* (Doonan and Morris, 1989), the fission yeast *Schizosaccharomyces pombe* (Ohkura *et al.*, 1989), and the fruit fly *Drosophila* (Axton *et al.*, 1990). The phosphatase genes identified are highly similar to the mammalian type 1 protein phosphatase catalytic subunit (designated PP1; Cohen, 1989). The amino acid sequence identity is ~80% between fission yeast dis2 and mammalian PP1. Definitive evidence for essentiality of the PP1-like genes in cell division was provided in fission yeast by the lethality of the double gene disruption of *dis2*$^+$ and *sds21*$^+$ both of which encode polypeptides highly similar to PP1 (Ohkura *et al.*, 1989).

Phosphatase genes encoding polypeptides highly similar to mammalian type 2A catalytic subunits (designated PP2A) exist in fission and budding yeasts (Kinoshita *et al.*, 1990; Sneddon *et al.*, 1990; Ronne *et al.*, 1991; Sutton *et al.*, 1991). The amino acid sequence identity is ~80%. The fission yeast *ppa1*$^+$ and *ppa2*$^+$ genes encoding PP2A-like polypeptides are by themselves non-essential for viability but share an essential function for cell viability (Kinoshita *et al.*, 1990); the single gene disruptions were viable, but the double disruption causes lethality. PP1- and PP2A-like *dis2*$^+$ and *ppa2*$^+$ genes, however, do not share an essential function; the double mutant of *ppa2* and *dis2* can grow (Kinoshita *et al.*, 1990). Consistent with this conclusion, *dis2* and *ppa2* mutants exhibit strikingly different phenotypes (Ohkura *et al.*, 1989; Kinoshita *et al.*, 1990), suggesting that their functions in cell division cycle are

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distinct. Other genes encoding type 2B (calcineurin)-like phosphatases were reported in budding yeast (Cyert *et al.*, 1991; Liu *et al.*, 1991); deletion mutants are viable, suggesting that they are not essential for cell growth.

One question that these studies raise is how many protein ser/thr phosphatases exist in one organism to counteract the many protein ser/thr kinases (reviewed by Cohen *et al.*, 1990). A large number of protein kinases have been found (Hunter, 1987), but only a relatively small number of phosphatases have been reported (Cohen, 1989). Protein phosphatases may have a variety of regulators that generate the specificity for diverse substrates, or there may be many more phosphatase genes awaiting discovery. An approach to find more phosphatase genes is to isolate the candidate DNA sequences by hybridization or polymerase chain reaction with the use of a consensus sequence. As the amino acid sequence of the catalytic domains of different phosphatases are conserved (approximate identity is 40% between dis2 and ppa2), genomic DNAs or cDNAs having the consensus sequences can be obtained. Such attempts have been made (e.g., Cohen *et al.*, 1990; da Cruz e Silva *et al.*, 1991; Posas *et al.*, 1992).

In the present paper, we report isolation and characterization of a novel phosphatase gene in fission yeast, designated *ppe1*$^+$. It encodes a 37-kd polypeptide, and the predicted amino acid sequence is similar to that of the budding yeast SIT4 (Arndt *et al.*, 1989), the fruit fly PPV (Cohen *et al.*, 1990) and the rabbit PPX phosphatases (da Cruz e Silva *et al.*, 1991). It is not absolutely essential for viability, but its disruption mutant is cold sensitive. The loss of *ppe1*$^+$ causes a defect in cell shape and length. The phosphatase encoded by *ppe1*$^+$ may be implicated in multiple aspects of cell regulation as the cs phenotype of *ppe1* mutant is suppressed by the high dosage of 2A-like phosphatase genes *ppa1*$^+$ and *ppa2*$^+$, a PKC-related kinase *pck1*$^+$, and a 110-kd protein-encoding *dis3*$^+$ required for sister chromatid separation. In addition, Matsumoto and Beach (1993) have demonstrated that *esp1* mutations suppress the temperature sensitive phenotype of *pim1*, and the *esp1*$^+$ gene is identical to *ppe1*$^+$. The *pim1*$^+$ gene is similar to the human RCC1 gene (Ohtsubo *et al.*, 1987) involved in chromosome condensation and signal transduction (Matsumoto and Beach, 1991).

MATERIALS AND METHODS

Strains and Media

S. pombe haploid strains HM123 (*h*$^-$ *leu1*), *wee1-50*, *cdc25-22*, (Nurse *et al.*, 1976), and *dis3-54* (Ohkura *et al.*, 1988) were employed. Gene disruptant strains Δ*ppa1* (*h*$^-$ *leu1 ura4 ppa1::ura4*$^+$), Δ*ppa2* (*h*$^-$ *leu1 ura4 ppa2::ura4*$^+$), Δ*dis2*, and Δ*sds21* were previously constructed (Ohkura *et al.*, 1989; Kinoshita *et al.*, 1990). The gene disruption of *pck1*$^+$ is *h*$^+$ *leu1 ura4 his2 pck1::ura4*$^+$ (Toda *et al.*, unpublished observation). The rich yeast extract, peptone, dextrose (YPD) medium contains 1% yeast extract, 2% glucose, and 2% polypeptone. The synthetic EMM2 medium was as described (Mitchison, 1970); 2% agar was added for plating.

Plasmids and Gene Disruption

Plasmid pPH101 containing the *ppe1*$^+$ gene (formerly called *ppx1*$^+$, Kinoshita *et al.*, 1990) was isolated by hybridization with the use of a 20-mer mixed oligonucleotide (Kinoshita *et al.*, 1990). Plasmids used for suppression of Δ*ppe1* were described by Ohkura *et al.* (1989), Kinoshita *et al.* (1990, 1991b), and Ohkura and Yanagida (1991). Plasmids CB182 and pSRK1-2 containing the budding yeast *SIT4* (Arndt *et al.*, 1989) and *SRK1/SSD1* (Sutton *et al.*, 1991; Wilson *et al.*, 1991), respectively, were the gifts of Drs. K. Arndt and K. Tatchell.

The *ppe1*$^+$ gene was disrupted by one-step gene replacement (Rothstein, 1983). A 1.9 kb genomic DNA fragment containing the *ppe1*$^+$ gene was ligated with the *Kpn* I fragment of the *S. pombe ura4*$^+$ gene inserted at the *Kpn* I site in the coding region of *ppe1*$^+$ gene. A linearized *Eco*RI fragment (one end in the vector sequence) was used for transformation of the diploid host strain *h*$^-$/*h*$^+$ *leu1/leu1 ura4/ura4* +/ *his2 ade6-210/ade6-216*.

Fluorescence Microscopy

S. pombe cells in liquid culture were collected by a low-speed centrifugation and stained with the DNA-specific fluorescent probe 4,6-diamidino-2-phenylindole (DAPI) by the procedure described previously (Toda *et al.*, 1981). Indirect-immunofluorescence microscopy was performed (Hagan and Hyams, 1988) by using anti-tubulin antibodies TAT1 (Woods *et al.*, 1989).

Nucleotide Sequencing and Southern Hybridization

Nucleotide sequencing was done by the dideoxy method (Sanger *et al.*, 1977). pPH211 and pPH221 contained the insert of 4.1 kb genomic DNA fragment in Bluescript. Standard procedures for sequencing and hybridization (Maniatis *et al.*, 1982) were followed.

Overexpression of ppe1$^+$

Two oligonucleotides, a 21-mer (5′-CCGCATATGTTTGTCTTGGAT-3′) and a 24-mer (5′-CGCGGATCCTTAGATGAAATACTC-3′) were made. The 21-mer contained the *Nde* I restriction site in front of the initiation codon of the *ppe1*$^+$ gene. The 24-mer contained the *Bam* HI site just after the termination codon of the *ppe1*$^+$ gene in the noncoding strand. These were used as the primers for polymerase chain reaction (PCR). The resulting 1-kb DNA fragment was ligated into pREP1 (Maundrell, 1990), which had been restricted with *Nde* I and *Bam* HI and treated with alkaline phosphatase. pREP1 contains the inducible promoter *nmt1;* the resulting plasmid pXP102 expresses the entire coding region of the *ppe1*$^+$ gene in the absence of thiamine. pXP102 was used for transformation of the wild-type HM123, and transformants were grown at 33°C in synthetic EMM2 (+thiamine, final concentration 2 μM) medium and then transferred to the same medium lacking thiamine at 33°C. The cell concentration was adjusted to 1.0 × 10^5/ml. Aliquots of the culture were taken at 0, 8, 10, 12, 14, 16, and 18 h.

FACScan Analysis

The procedures described by Costello *et al.* (1986) were followed. Haploid wild-type cells were used as a control. *S. pombe* cells (1–5 × 10^7) were collected, washed twice in distilled water, resuspended in 3 ml distilled water, slowly mixed with 7 ml ethanol with vortexing, and incubated at 4°C for 12 h. Cells were washed and resuspended in 1 ml 50 mM sodium citrate (pH 7.0). RNase A (final concentration, 0.5 mg/ml) (Sigma, St. Louis, MO) was added and incubated at 37°C for 2 h. Cells were stained by propidium iodide (final concentration, 12.5 μg/ml) and analyzed by FACScan (Beckton Dickinson, San Jose, CA).

Preparation of Fusion Proteins and Antisera

Two fusion proteins were made by using the T7 promoter (Studier and Moffat, 1986). pXP101 and pUM32 contain the entire coding

region and the carboxyl group (COOH) terminal half of *ppe1*$^+$, respectively. The 21-mer and 24-mer oligo nucleotides described above were used for PCR of plasmid pPH101, and the resulting 1 kb DNA fragment was ligated into pAR3038 (Studier and Moffat, 1986; containing the T7 promoter), which had been treated with *Nde* I/*Bam* HI restriction enzymes and bacterial alkaline phosphatase. pXP101 thus made was introduced into *Escherichia coli* BL21 and produced a 37 kd full length protein. The *Nde* I linker (5'-CCATATGG-3') was introduced at the *Ssp* I site in the *ppe1*$^+$ coding region and used for ligation behind the T7 promoter. The resulting COOH-half polypeptide (168 residues, 20 kd) was produced in *E. coli* BL21. These fusion proteins were purified and injected into rabbits by the procedures described previously (Hirano *et al.*, 1988). Antibodies were affinity-purified. Immunoblots were performed according to Towbin *et al.* (1979).

RESULTS

ppe1$^+$ *Gene Codes for a Protein Phosphatase*

We isolated the *ppe1*$^+$ gene (formerly called *ppx1*$^+$; Kinoshita *et al.*, 1990) from a fission yeast genomic DNA library by hybridization by using a 20-mer mixed oligonucleotide probe encoding the consensus amino acid sequence for 2A-like phosphatases. Two type 2A-related phosphatase genes *ppa1*$^+$ and *ppa2*$^+$ obtained in this manner were previously reported (Kinoshita *et al.*, 1990). The nucleotide sequence of the DNA fragment from a third gene, *ppe1*$^+$, was determined (Figure 1a), predicting a coding region consisting of 305 amino acids. The sequence is identical to that of *esp1*$^+$ identified by Matsumoto and Beach (1993). Genomic Southern hybridization using *ppe1*$^+$ as the probe showed only one set of hybridizing bands with the expected sizes.

The amino acid sequence comparison among different protein ser/thr phosphatases in fission yeast indicated that ppe1 is similar to type 2A-like ppa2 (Kinoshita *et al.*, 1990) and type 1-like dis2 (Ohkura *et al.*, 1989) phosphatases (Figure 1b). It is more similar to ppa2 (53% identical) than dis2 (39% identical). Comparison with phosphatases of different organisms indicated that ppe1 is highly similar to the *Saccharomyces cerevisiae* SIT4 phosphatase (Arndt *et al.*, 1989; 72% identical), the *Drosophila* PPV (Cohen *et al.*, 1990; 68% identical) and the rabbit PPX (da Cruz e Silva *et al.*, 1988; 61% identical). The amino acid sequence similarity between ppe1 and these three phosphatase was found over the entire coding regions except the short terminal or near-terminal sequences. The COOH-terminal residues YFL/I, however, are conserved among ppa2, ppe1, PPV and PPX phosphatases.

ppe1$^+$ *Gene is Required at Low Temperature*

To investigate whether the *ppe1*$^+$ gene is essential for viability, one-step gene disruption was done, by using a 1.9-kb fragment containing the *S. pombe ura4*$^+$ gene as the marker (Figure 2a). The resulting Ura$^+$ heterozygous diploid transformants produced four viable spores at 33°C. Genomic Southern hybridization of the heterozygous diploid (+/Δ) and haploid segregants (+ or Δ) produced new restriction bands with the expected sizes (8.5-kb *Bgl* II and 2.0 and 2.1-kb *Hin*dIII) for gene disruption of *ppe1*$^+$ (Figure 2b). These results indicate that the *ppe1*$^+$ gene is non-essential for viability.

Cell growth of the haploid gene disruptant (designated Δ*ppe1*), however, is not normal. The cell division of Δ*ppe1* is slow at 33°C. The doubling time of Δ*ppe1* is 3.5 h in rich YPD medium at 33°C, whereas the wild-type doubling time is 2.2 h under the same culture conditions. Furthermore, as also described by Matsumoto and Beach (1993), the growth of Δ*ppe1* is cold-sensitive. Small colonies exist at 26°C and no colony is seen at 20°C, as shown in Figure 3a. Introduction of a multicopy plasmid carrying the *ppe1*$^+$ gene complements the cs phenotype so that the absence of the *ppe1*$^+$ gene must cause the cs lethal phenotype.

Multicopy Suppressor Genes for the cs Phenotype of Δppe1

Various phosphatases, kinases and related genes were examined for ability to suppress the cs phenotype of Δ*ppe1*. As shown in Figure 3b and Table 1, we found that high gene dosage of the following four *S. pombe* genes suppresses the cs phenotype, in that colonies were produced in the transformants at 26 and 20°C. Multicopy plasmids carrying *ppa1*$^+$ or *ppa2*$^+$ phosphatase genes (Kinoshita *et al.*, 1990) suppress the growth phenotype of Δ*ppe1*. Their amino acid sequences are ~80% identical. Multicopy plasmids carrying other phosphatase genes, *dis2*$^+$ or *sds21*$^+$ (Ohkura *et al.*, 1989) or the regulator *sds22*$^+$ for *dis2*$^+$ and *sds21*$^+$ (Ohkura and Yanagida, 1991; Stone *et al.*, 1993) do not suppress the cs phenotype of Δ*ppe1*.

Cells carrying a multicopy plasmid with the *dis3*$^+$ gene (Kinoshita *et al.*, 1991a) also produce colonies at 20°C (Figure 3b and Table 1). Hence the high gene dosage of *dis3*$^+$ suppresses the cs lethality of Δ*ppe1*. This may be analogous to the case of the *S. cerevisiae SSD1* (Sutton *et al.*, 1991), the overexpression of which suppresses the phenotype of *sit4* phosphatase mutants. The amino acid sequence of the *dis3*$^+$ gene product was previously shown to be similar to SSD1/SRK1 (Kinoshita *et al.*, 1991a; Sutton *et al.*, 1991; Wilson *et al.*, 1991). *dis3*$^+$ was previously shown to be related to the protein phosphatase *dis2*$^+$ as the double cs mutant *dis2 dis3* was lethal at the permissive temperature (Kinoshita *et al.*, 1991a) and as the mitotic phenotype of *dis2-11* and *dis3-54* is highly similar (Ohkura *et al.*, 1988).

Surprisingly, a multicopy plasmid carrying the protein kinase C-related *pck1*$^+$ gene (Toda *et al.*, 1993) was found to suppress the cs phenotype of Δ*ppe1*. The pck1 and pck2 kinases have the consensus sequences for mammalian protein kinase C, and a cell morphology phenotype similar to Δ*ppe1* (see below) was found for the deletion mutant of *pck2*$^+$.

a

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                                  TCTAGAAGAGATCCTTGAATAATTTGTGCTACAACG   -1
ATGTTTGACTTGGATGAATGGATCGCTACAGTAAGGAAGTGTAAATATCTTCCAGAACAC  +60
M  F  D  L  D  E  W  I  A  T  V  R  K  C  K  Y  L  P  E  H    20

CAGTTGAAACGATTGTGTGAGATGGTAAAAGTTATTTTAATGGAGGAGTCCAATATCCAG +120
Q  L  K  R  L  C  E  M  V  K  V  I  L  M  E  E  S  N  I  Q    40

CCTGTGCGGACTCCCGTTACTGTTTGTGGTGATATACATGGTCAGTTTTACGACCTTTTG +180
P  V  R  T  P  V  T  V  C  G  D  I  H  G  Q  F  Y  D  L  L    60

GAACTGTTTCGAGTAGGTGGAGAATTGCCAAGCACAAATTATATATTTATGGGTGATTTT +240
E  L  F  R  V  G  G  E  L  P  S  T  N  Y  I  F  M  G  D  F    80

GTGGATCGAGGTTATTTTAGCTTAGAAACTTTTACGTTATTTATGCTTTTGAAAGCAAGG +300
V  D  R  G  Y  F  S  L  E  T  F  T  L  F  M  L  L  K  A  R   100
KpnI
TACCCAGATAAAATTACATTGCTTCGTGGTAATCACGAAAGTCGCCAGATCACTCAAGTC +360
Y  P  D  K  I  T  L  L  R  G  N  H  E  S  R  Q  I  T  Q  V   120
                                                       SspI
TACGGGTTTTATGATGAGTGTCAAACCAAATATGGAAATGCAAATGTTTGGAAATATTGT +420
Y  G  F  Y  D  E  C  Q  T  K  Y  G  N  A  N  V  W  K  Y  C   140

TGCCAAGTTTTTGATTTTTTGACTTTGGCAGCGGTTATCGATAACAAAATTTTATGCGTG +480
C  Q  V  F  D  F  L  T  L  A  A  V  I  D  N  K  I  L  C  V   160

CATGGTGGATTATCTCCGGAAGTTCGCACATTGGATCAGATTCGTATTCTTGCGCGTGCT +540
H  G  G  L  S  P  E  V  R  T  L  D  Q  I  R  I  L  A  R  A   180

CAAGAAATTCCCCATGAAGGCTCATTTTGCGATTTGATGTGGTCTGATCCAGAGGATATT +600
Q  E  I  P  H  E  G  S  F  C  D  L  M  W  S  D  P  E  D  I   200

GAATCTTGGACTGTTTCGCCTCGGGGAGCTGGATGGTTGTTTGGTTCTAAAGTTACTACC +660
E  S  W  T  V  S  P  R  G  A  G  W  L  F  G  S  K  V  T  T   220

GAATTTAGTCAAATCAACGACTTGACATTGATTGCTCGTGCTCATCAACTTGTTCAAGAG +720
E  F  S  Q  I  N  D  L  T  L  I  A  R  A  H  Q  L  V  Q  E   240

GGCTACAAATATCATTTTGCCGATAAAAATCTTGTTACCGTTTGGAGTGCTCCCAATTAC +780
G  Y  K  Y  H  F  A  D  K  N  L  V  T  V  W  S  A  P  N  Y   260

TGCTATAGATGTGGAAATGTGGCAAGTGTTATGAAAGTAGATGAATCTTTGGAACCGGAA +840
C  Y  R  C  G  N  V  A  S  V  M  K  V  D  E  S  L  E  P  E   280

TTTCGTATTTTCTCGGCTGTTGCCGACGAAGATCGTACCGTTCCCCCTAGCAGAAAAAGA +900
F  R  I  F  S  A  V  A  D  E  D  R  T  V  P  P  S  R  K  R   300

AGCGAGTATTTCATCTAACTTTGGTATGTGCAAGTCAAATATCTTGTTTTTTATGGCACT +960
S  E  Y  F  I  *                                              305

CATATACCGAATTC                                               +974
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b

Figure 1. Nucleotide and predicted amino acid sequences of *ppe1*$^+$. (a) The nucleotide sequence of the cloned *ppe1*$^+$ gene is shown with the predicted amino acid sequence. (b) Comparison of the amino acid sequences of the budding yeast SIT4, ppe1, ppa2, and dis2 phosphatases. Identical amino acid residues are hatched. The nucleotide sequence of *ppe1*$^+$ will appear in the DDBJ, EMBL, and GenBank Nucleotide Sequence Databases with accession number D13712.

These high gene dosage suppressors may be classified into three types, namely, phosphatase, kinase, and a mitotically essential phosphatase-related protein. Suppression by *ppa1*$^+$, *ppa2*$^+$, *dis3*$^+$, and *pck1*$^+$ genes is partially compared with that by *ppe1*$^+$ itself; the colony size produced at 20 and 26°C is smaller than that of wild-type or Δ*ppe1* cells transformed with a plasmid carrying the *ppe1*$^+$ gene.

Budding Yeast SIT4 Phosphatase Gene is also a Multicopy Suppressor

A multicopy plasmid carrying the *S. cerevisiae SIT4* gene (a gift of Dr. K. Arndt) suppresses the cs phenotype of Δ*ppe1* (Figure 3b, Table 1). Suppression was clearly seen both at 20 and 26°C, but the degree of suppression was partial, judging from the size of colonies formed. The budding yeast *SSD1/SRK1* gene (a gift of Dr. K. Tatchell)

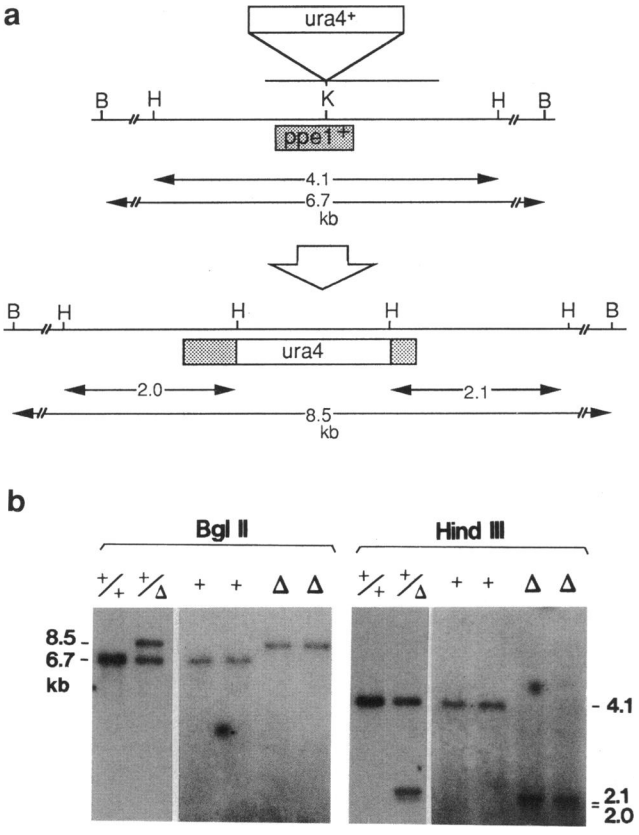

Figure 2. Gene disruption of *ppe1*$^+$. (a) A genomic DNA fragment containing the *ppe1*$^+$ gene was restricted with *Kpn* I (indicated by K) into which was ligated the *S. pombe ura4*$^+$ gene (the fragment ends contained the sites of *Kpn* I and *Hin*dIII (indicated by H). This fragment was used for transformation of a diploid strain. Expected restriction sizes for the chromosomal integrant are shown; 8.5 kb by *Bgl* II (indicated by B) and 2.0- and 2.1-kb bands by *Hin*dIII. (b) Genomic Southern hybridization of *S. pombe* wild-type diploid (indicated by +/+), heterozygous diploid (+/Δ), and haploid segregants (+ or Δ) restricted with *Bgl* II (left) or *Hin*dIII (right). The probe used was a 1.9-kb fragment containing the *ppe1*$^+$ gene. Haploid disruption segregants were viable at 33°C and their genomic DNA produced the hybridization bands at the expected sizes.

failed to suppress the cs phenotype of Δ*ppe1*. However, it was not determined whether *SSD1/SRK1* was expressed in *S. pombe* under the experimental condition employed.

Cell Shape and Length are Altered in Δppe1

The cell length and shape of Δ*ppe1* was strikingly different from the wild type (Figure 4, a and b); Δ*ppe1* cells were short and round.

The average cell length of Δ*ppe1* is 7.6 and 9.6 μm at 26 and 33°C, respectively. The average cell length (7.6 μm) of slowly growing Δ*ppe1* at 26°C is comparable to that (7.0 μm; Fantes, 1981; Russell and Nurse, 1987) of *wee1-50* at 36°C and much shorter than the wild type average cell length (11.0 μm). Growth-arrested Δ*ppe1* cells at 20°C were also short with roughly the same average length as that at 26°C. However, Δ*ppe1* cells are distinct from *wee1*, as their cell width is larger (~1.5 times) than that of *wee1*. Furthermore the cell shape of Δ*ppe1* is round, often pear-shaped, or somewhat irregular. The cell shape of *wee1* mutants is rod-like, as are wild-type cells (Fantes, 1981).

DAPI-stained Δ*ppe1* cells are shown in Figure 4b. Neither highly condensed nor abnormally segregated chromosomes have been observed. The frequency of mitotic cells in a culture of Δ*ppe1* at 26°C is similar to that of wild-type cells. Hence obvious mitotic defect was not found in Δ*ppe1* cells.

The DNA contents of Δ*ppe1* cells at 33, 26, and 20°C were analyzed by the FACScan method (Costello *et al.*, 1986). As shown in Figure 4c, exponentially growing Δ*ppe1* cells at 33°C revealed a single peak. The position of the peak was shifted from that of exponentially growing wild-type cells containing the G2 DNA content to the direction opposite that for the G1 DNA content. The peak position for G1 DNA content was determined by using nitrogen-starved wild-type cells or *cdc10* mutant incubated at 36°C (Kinoshita *et al.*, 1991a). The shift was probably due to changes in cell shape and length rather than in DNA content. Hence a culture of Δ*ppe1* cells at the permissive temperature is likely to consist mostly of G2 cells. Similar FACScan patterns were obtained for slowly growing or arrested cells at 26 and 20°C, respectively.

Suppression of the Cell Shape Phenotype by Multicopy Suppressor Genes

We investigated whether multicopy suppressor genes for the cs phenotype of Δ*ppe1* could also suppress the cell length and shape phenotype of Δ*ppe1*. Transformants of Δ*ppe1* cells carrying the multicopy vector plasmid (indicated by vector), or multicopy plasmids with *ppe1*$^+$, the budding yeast *SIT4*, *ppa2*$^+$, *pck1*$^+$, or *dis3*$^+$ gene are shown in Figure 4a. Δ*ppe1* cells carrying the vector plasmid were short and pear-shaped, whereas Δ*ppe1* cells carrying plasmids with the *ppe1*$^+$ gene were normal in shape and length; cells are rod-like rather than pear-shaped. The cell size distribution was also like the wild type. Transformants of Δ*ppe1* by plasmids with the *SIT4*, *ppa2*$^+$, *pck1*$^+$, or *dis3*$^+$ gene still contained small fractions of short and/or pear-shaped cells, but the majority of cells were normal or nearly normal. High gene dosage of *SIT4* has a strong effect on the cell length and shape; the cell shape of Δ*ppe1* cells became mostly normal. However, a fraction of cells were still small and round. Three genes, *ppa2*$^+$, *pck1*$^+$, and *dis3*$^+$ showed similar suppression patterns; that is, abnormality of Δ*ppe1* cells in both cell shape and length partly remained in the transformants.

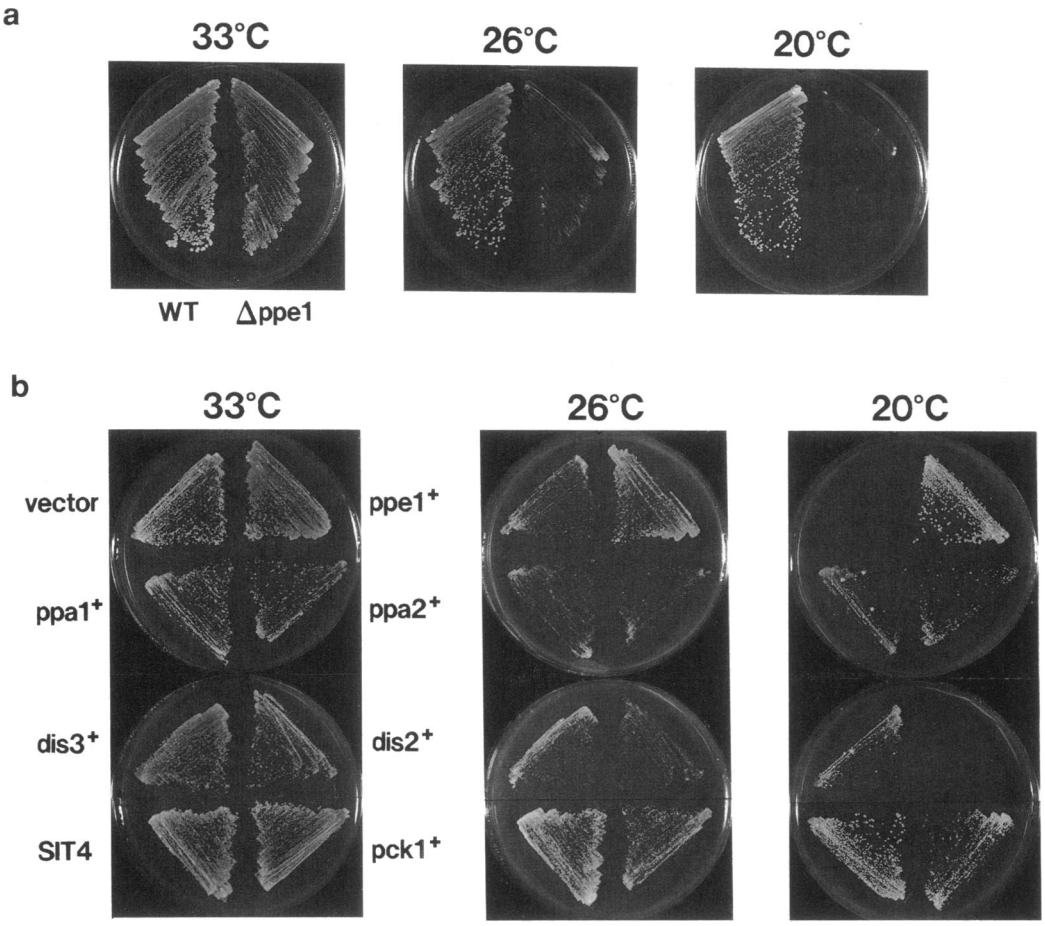

Figure 3. Cold sensitive phenotype of Δ*ppe1* and its extragenic suppressors. (a) Wild-type (wt) and Δ*ppe1* disruption mutant cells (Δppe1) were streaked on plates at 33, 26, and 20°C. Δ*ppe1* fails to produce colonies at 20°C and produces small colonies at 26°C so that Δ*ppe1* is cold-sensitive. (b) Δ*ppe1* disruption mutant cells were transformed with different multicopy plasmids, and transformant cells were streaked on plates at 33, 26, and 20°C. Results of 8 transformant cells carrying different plasmids are shown. Cells carrying multicopy vector plasmid (vector) failed to produce colonies at 20°C, while cells carrying plasmid with the *ppe1*$^+$ gene (ppe1$^+$) grew normally at 20°C. The Δ*ppe1* cs phenotype was suppressed by several multicopy plasmids carrying *ppa1*$^+$, *ppa2*$^+$, *dis3*$^+$, and *pck1*$^+$ genes but not by *dis2*$^+$, *sds21*$^+$, and *sds22*$^+$. The high gene dosage of the *S. cerevisiae SIT4* gene also suppressed the cs phenotype of Δ*ppe1*.

Other Phosphatase Genes Functionally Overlap with ppe1$^+$

The Δ*ppe1* deletion mutant was crossed with phosphatase deletion mutants, Δ*ppa1*, Δ*ppa2*, Δ*dis2*, or Δ*sds21*, and the growth properties of resulting double mutants were examined. As Δ*ppe1* cells were poor in conjugation and hardly formed spores when crossed with wild type, transformants with plasmid carrying the *ppe1*$^+$ gene were used for crossing and, after tetrad dissection, haploid segregants, which lost plasmid were examined. As shown in Table 2, the double mutant Δ*ppe1* Δ*ppa2* failed to produce colonies, suggesting that *ppe1*$^+$ and *ppa2*$^+$ genes might share an essential function. Other double gene disruptants, Δ*ppe1*-Δ*ppa1*, Δ*ppe1*-Δ*dis2*, and Δ*ppe1*-Δ*sds21* were viable and produced colonies. However, two of the double mutants did not grow like the Δ*ppe1* single mutant; the doubling times of Δ*ppe1*-Δ*ppa1* and Δ*ppe1*-Δ*dis2* were both 5.2 h, whereas that of single Δ*ppe1* and double Δ*ppe1* Δ*sds21* was 3.5 h. The double gene disruptant Δ*ppe1*-Δ*ppa2* germinated but was arrested after a few rounds of cell division. The cell shape and morphology of these arrested cells were similar to those of the single Δ*ppe1* disruptant.

A cross was made between Δ*ppe1* and the Δ*pck1* kinase deletion mutant; the double mutant was lethal. Hence *ppe1*$^+$ phosphatase and *pck1*$^+$ kinase may share an essential function. The functional relationship between *ppe1*$^+$ and *pck1*$^+$ is further strengthened by the staurosporine sensitivity of Δ*ppe1* as shown in Table 3. Wild-type fission yeast is sensitive to staurosporine, a kinase inhibitor, and is not able to grow in the presence of 0.5 μg/ml drug (Toda *et al.*, 1991). Δ*ppe1* becomes more sensitive to the drug (unable to grow in 0.3 μg/ml drug concentration) than wild type, whereas high-

Table 1. Multicopy suppressors of the cs phenotype of Δ*ppe1*

Genes carried by multicopy plasmid	Colony formation at 20°C
ppe1$^+$	++
ppa1$^+$	+
ppa2$^+$	+
dis2$^+$	−
sds21$^+$	−
sds22$^+$	−
dis3$^+$	+
pck1$^+$	+
Budding yeast *SIT4*	+
Budding yeast *SSD1*	−
None, vector	−

Δ*ppe1* cells were transformed with multicopy plasmids carrying the indicated genes, and resulting transformants obtained at 33°C were streaked on EMM2 plates and incubated at 33 and 20°C for 4 and 10 d, respectively. The colony formation of transformants at 20°C is shown in the Table. The colony formation at 33°C was normal.

copy expression of either the *ppe1*$^+$ or *pck1*$^+$ gene suppresses the drug hypersensitivity. The loss of the *ppe1*$^+$ gene may reduce the activity or the level of a kinase involved in determining the staurosporine sensitivity.

Characterization of the ppe1$^+$ *Gene Product*

To characterize the gene product of *ppe1*$^+$, we raised antiserum against a full length polypeptide made in *E. coli* using the T7 promoter. As shown in Figure 5, wild-type extracts produce a polypeptide band at the position of 37 kd, which is completely absent in Δ*ppe1* deletion mutant extracts. When wild-type extracts of cells containing multicopy plasmid with the *ppe1*$^+$ gene were used, the band intensity did not increase significantly, but a small increase (clearly seen in original blotting patterns) was reproducibly observed. We concluded that the antibodies specifically detected the gene product of *ppe1*$^+$.

Effect of Overexpression of ppe1$^+$ *on Cell Division*

The fission yeast *nmt1* inducible promoter (Maundrell, 1990) was employed for overexpression of *ppe1*$^+$. To this end, the *nmt1* promoter was ligated in front of the initiation codon of *ppe1*$^+$, and the resulting plasmid was introduced into *S. pombe* wild-type cells. In the presence of thiamine, the *nmt1* promoter is shut off, whereas in the absence of thiamine, expression is induced (Maundrell, 1990). On plates, wild-type cells carrying a plasmid with the *nmt-ppe1*$^+$ gene produce colonies in the presence or the absence of thiamine. The size of colonies is smaller in the absence than in the presence of thiamine.

In liquid culture, because intracellular thiamine depletion requires several cell divisions, induced expres-

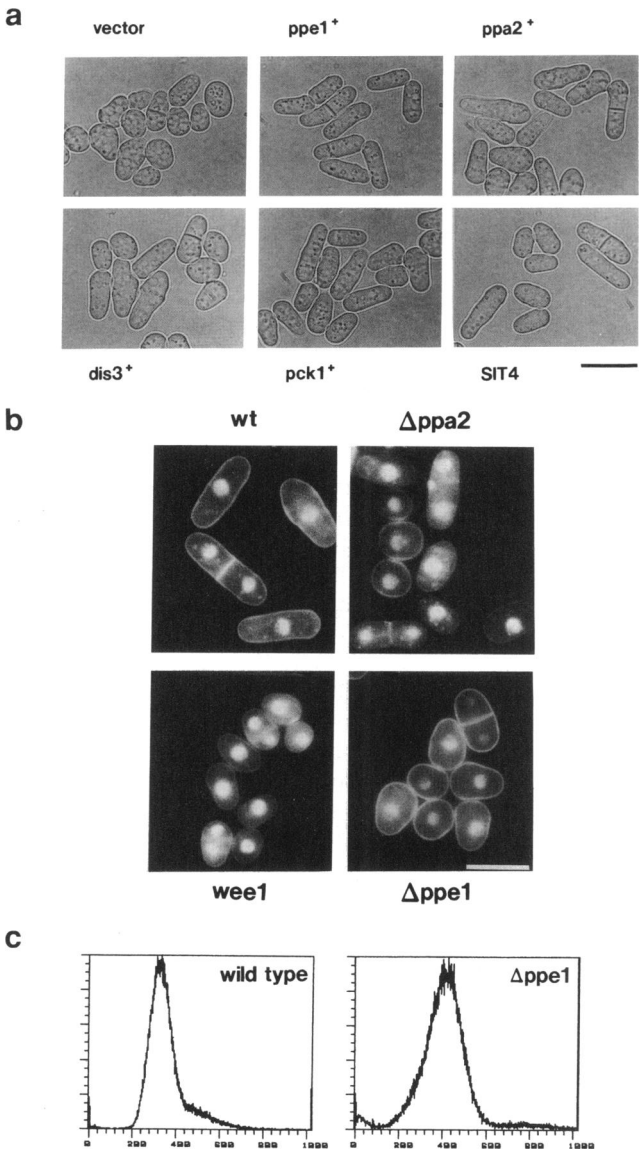

Figure 4. Cell morphology and the DNA content of Δ*ppe1*. (a) Δ*ppe1* disruption mutant cells were transformed with 6 different multicopy plasmids, and the phase contrast micrographs of transformant cells grown at 33°C are shown. Vector; Δ*ppe1* cells carrying multicopy vector plasmid. They were short and round. ppe1$^+$; Δ*ppe1* cells carrying multicopy plasmid with the *ppe1*$^+$ gene. Their cell shape and length are indistinguishable from wild type. ppa2$^+$, dis3$^+$, and pck1$^+$; Δ*ppe1* cells carrying multicopy plasmids with the *ppa2*$^+$, *dis3*$^+$, or *pck1*$^+$ gene. SIT4; Δ*ppe1* cells carrying multicopy plasmid with the *S. cerevisiae SIT4* gene. These 4 extragenic suppressors, which complemented the cs phenotype of Δ*ppe1*, partially suppressed the cell shape and length phenotype of Δ*ppe1*. The wild-type *S. pombe* cells are not shown but are indistinguishable from cells shown in the panel indicated by ppe1$^+$. The bar indicates 10 μm. (b) DAPI-stained fluorescence micrographs of wild type (wt), Δ*ppa2*, *wee1-50* and Δ*ppe1* cells grown at 33°C. Cells of Δ*ppa2* and *wee1-50* are shown to be short in cell size (Russell and Nurse, 1987; Kinoshita *et al.*, 1990). Normal looking nuclear chromatin structure was found in DAPI-stained Δ*ppe1* cells. The bar indicates 10 μm. (c) FACScan analysis of exponentially growing wild-type and Δ*ppe1* cells. Cells containing 2C DNA are abundant.

Table 2. Growth property of the double mutants between Δ*ppe1* and other phosphatase disruptions

Strains	Colony formation at 33°C	Doubling time (h)
Wild type	+++	2.2
Δ*ppe1*	++	3.5
Δ*ppe1* Δ*ppa1*	+	5.2
Δ*ppe1* Δ*ppa2*	−	Lethal
Δ*ppe1* Δ*dis2*	+	5.2
Δ*ppe1* Δ*sds21*	++	3.5
Δ*ppe1* Δ*pck1*	−	Lethal

The doubling time was estimated in YPD liquid cultures at 33°C.

Table 3. Sensitivity of Δ*ppe1* to staurosporine

Strains	Staurosporine (μg/ml)	Colony formation
Wild type	0.5	−
	0.3	+
	0.2	+
Δ*ppe1*	0.5	−
	0.3	−
	0.2	+
Δ*ppe1* with OP*ppe1*$^+$	0.5	−
	0.3	+
Δ*ppe1* with OP*pck1*$^+$	0.5	−
	0.3	+

OP*ppe1*$^+$ and OP*pck1*$^+$ represent multicopy plasmids carrying the *ppe1*$^+$ and *pck1*$^+$ gene, respectively.

sion was observed only after 10 h. The amount of ppe1 protein estimated by immunoblotting (Figure 6a) increased after 12 h. Significant protein degradation of ppe1 was also detected. In parallel with the overproduction of ppe1 protein, the rate of cell number increase was sharply reduced to 30% after 12 h. DAPI-stain fluorescence microscopy showed that many cells in the absence of thiamine after 16 h are elongated, containing the single nucleus (Figure 6b). Nuclear chromatin is somewhat altered (possibly condensed) from the wild type hemispherical chromatin region (see Figure 4b). Immunofluorescence microscopy using anti-tubulin antibody TAT-1 (Woods *et al.*, 1989), however, displayed cytoplasmic microtubules in the absence of thiamine (Figure 6c). Such cytoplasmic array is characteristic of the interphase cells (Hagan and Hyams, 1988). The spindle structure was hardly seen, suggesting that ppe1-overexpressing cells may not enter mitosis. In the presence of thiamine, however, both interphase and mitotic cells, respectively, revealing cytoplasmic and spindle (indicated by the arrow in Figure 6C, left) microtubules are seen.

DISCUSSION

We report in the present paper isolation and characterization of the *ppe1*$^+$ phosphatase gene of fission yeast. It is not absolutely required for viability, but the disruption mutant displays a cs lethal phenotype. Even at the permissive temperature, cells grow significantly more slowly than wild type. ppe1 appears to have a cellular function distinct among protein ser/thr phosphatase genes so far identified in fission yeast; gene disruption mutant cells of *ppe1*$^+$ produce an aberrant cell shape. Because this cell morphology phenotype has not been found in other protein ser/thr phosphatase mutants identified in fission yeast (Booher and Beach, 1989; Ohkura *et al.*, 1989; Kinoshita *et al.*, 1990, 1991b; Yanagida *et al.*, 1992; Yoshida *et al.*, unpublished observation), but seen even in growing Δ*ppe1* cells at the permissive temperature, the *ppe1*$^+$ gene seems to play a role in controlling cell shape in fission yeast. However, little is known about how ppe1 phosphatase is actually involved in forming the rod-shaped cell morphology of fission yeast. Because plasmids carrying the *ppe1*$^+$ gene fully complemented the cell shape phenotype, the loss of *ppe1*$^+$ is solely responsible for the phenotypes observed. The PKC-related *pck1*$^+$ gene might have an overlapping function in cell shape control (see below).

The cell length of Δ*ppe1* is strikingly reduced compared with that of the wild type. The average cell length of Δ*ppe1* at the restrictive temperature is comparable to that of *wee1-50* mutant (Fantes, 1981; Russell and Nurse, 1986, 1987). However, Δ*ppe1* cells were distinct from *wee1-50* because the cell width of Δ*ppe1* was much larger than that of wild type. Therefore, the total cell volume of Δ*ppe1* at the restrictive temperature is roughly the same as that of wild type. The cell volume of *wee1-50* is approximately one-half that of wild type. From this difference, the short cell length phenotype of Δ*ppe1* is not thought to represent the same phenomenon as that of the *wee* phenotype. The double mutant *wee1-50*

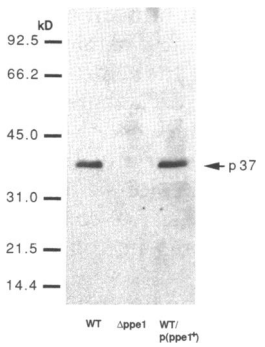

Figure 5. Identification of ppe1 polypeptide using anti-ppe1 antibodies. Extracts of *S. pombe* wild-type cells (wt), disruption mutant (Δ*ppe1*), and wild-type cells carrying multicopy plasmid with the *ppe1*$^+$ (WT/p[ppe1$^+$]) gene were run in SDS-PAGE and immunoblotted with the use of anti-ppe1 antibodies. The 37-kd polypeptide was detected in the lanes of wild type and wild type with multicopy plasmid but not in deletion mutant. Molecular weight markers used are phosphorylase B (92.5 kd), bovine serum albumin (66.2 kb), ovalbumin (45.0 kd), carbonic anhydrase (31.0 kd), soybean trypsin inhibitor (21.5 kd), and lysozyme (14.4 kd).

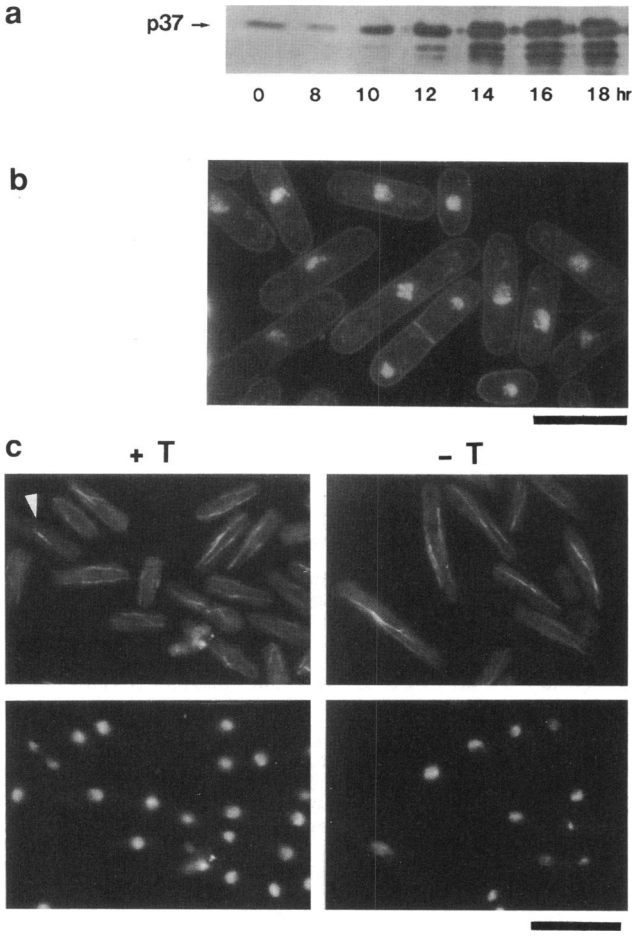

Figure 6. Overexpression of *ppe1*$^+$. (a) The *ppe1*$^+$ gene was ligated with the inducible promoter *nmt1*$^+$ and overexpressed in the absence of thiamine. The level of ppe1 protein (▼) with p37 shown in the immunoblotting patterns was increased after 12 h. (b) DAPI-stained cells carrying nmt-ppe1 plasmid, which overexpress the *ppe1*$^+$ gene in the absence of thiamine. Elongated cells containing the nucleus with apparently altered chromatin structure are frequently seen. The bar indicates 10 μm. (c) Immunofluorescence micrographs of wild-type cells carrying nmt-ppe1 plasmid in the presence (indicated by +T) or the absence (−T) of thiamine. Micrographs were taken for cells after 16 h. Top, anti-tubulin TAT-1 staining; bottom, DAPI staining. Elongated cells produced in the absence of thiamine contained cytoplasmic microtubules. Cells with the mitotic spindle were hardly seen in the absence of thiamine. A cell with the mitotic spindle in the presence of thiamine is indicated by the arrow. The bar indicates 10 μm.

Δ*ppe1* is viable so that they do not share an essential function. The cell length of Δ*ppe1* is shorter at 26 than at 33°C. There might exist a correlative relationship between cs lethal phenotype and cell length shortening.

An abnormal cell shape was not observed in mutants of the budding yeast *SIT4* gene (Arndt *et al.*, 1989; Sutton *et al.*, 1991), which might be a functional homologue of *ppe1*$^+$, judging from predicted amino acid sequence identity and mutant phenotype suppression. The amino acid identity between SIT4 and ppe1 phosphatases is high (72%), and the Δ*ppe1* mutant phenotype is suppressed by the high gene dosage of *SIT4*. Note, however, that the cell shape of wild-type budding yeast is round so that the mechanism to produce the rod-shaped cells might be specific to fission yeast and not present in budding yeast. Alternatively, the lack of ppe1/SIT4 phosphatase possibly causes aberration in cytoskeletal structures, and that causes a gross change in cell shape for fission yeast. Another significant difference between *sit4* and *ppe1* mutants was found in the measured DNA contents under the restrictive conditions. Cells with a G2 DNA content were accumulated in Δ*ppe1*, but cells with a G1 DNA content were found in *sit4* mutants (Sutton *et al.*, 1991). The reason for this difference is not understood. *Drosophila* PPV and rabbit PPX phosphatases (Cohen *et al.*, 1990) are also highly similar to ppe1, but their cellular roles are unknown. Some abnormality in cell-shape control was reported in type 2A catalytic (Ronne *et al.*, 1991) and regulatory subunits (Healy *et al.*, 1991).

The multiple phenotypes for Δ*ppe1* are consistent with the action of multicopy suppressor genes, which are classified into three groups, namely, 2A-related phosphatases ppa1 and ppa2, a mitotically required protein dis3 and a PKC-related kinase, pck1. In addition to these, Matsumoto and Beach (1993) show that mutations in the fission yeast *pim1*$^+$ gene, which is similar to human *RCC1* (Matsumoto and Beach, 1991), were suppressed by the loss of *ppe1*$^+$ function. Only future investigations will reveal the actual relationship between these diverse gene products (Figure 7). The following discussion is intended to establish the presence of relationships rather than to explain molecular interactions among them.

Because the double mutant *ppa2-ppe1* is lethal, these different phosphatases may share the substrates that are essential for viability. The amino acid sequence identity between ppe1 and ppa2 is ~50%, significantly higher than that between ppe1 and dis2. The loss of the *ppa2*$^+$ gene produces a semi-wee phenotype (Kinoshita *et al.*, 1990). Substrate proteins related to cell length control might be regulated by both ppe1 and

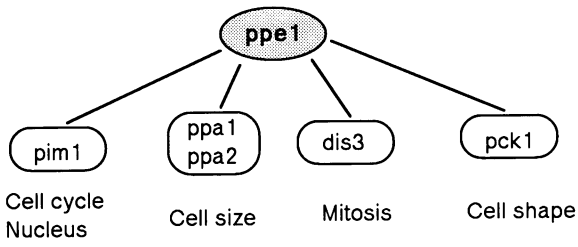

Figure 7. Relationship between *ppe1* phosphatase mutation and high-gene dosage suppressors, *ppa1*$^+$, *ppa2*$^+$, *dis3*$^+$, and *pck1*$^+$ genes identified in the present study. ts *pim1* mutation was suppressed by mutation or disruption of the *ppe1*$^+$ gene (Matsumoto and Beach, 1993).

ppa2 phosphatases. The inactivation or hyperactivation of such substrate protein functions by the lack of ppa2 and/or ppe1 phosphatases might cause the inability to reach normal cell length.

High gene dosage of *dis3*$^+$ suppresses the disruption phenotype of *ppe1*$^+$. The *dis3* mutation is defective in mitotic chromosome disjunction, showing the failure to exit from mitosis, the phenotype commonly found in *dis1* and *dis2* (Ohkura *et al.*, 1988). The *dis3*$^+$ gene encoding an essential 110-kd protein (Kinoshita *et al.*, 1991a) is possibly related to the *dis2*$^+$ gene, because the double mutant *dis2-dis3* is lethal at the permissive temperature. The *dis2*$^+$ gene encodes the catalytic unit of type 1-like phosphatase (Ohkura *et al.*, 1989; Kinoshita *et al.*, 1990). The relationship between *dis3*$^+$ and *ppe1*$^+$ was not unexpected because the budding yeast *sit4* mutations were suppressed by *SSD1/SRK1*, which has a significant sequence similarity to *dis3*$^+$ (Kinoshita *et al.*, 1991a; Sutton *et al.*, 1991; Wilson *et al.*, 1991). Hence ppe1 phosphatase is related to dis3 protein, but the actual relationship between ppe1 and dis3 is unknown. The double mutant *dis3-ppe1* has not been constructed due to the difficulty in crossing between them. Characteristic abnormal mitosis that causes *dis* mutants to fail in sister chromatid separation (Ohkura *et al.*, 1988) is not observed in Δ*ppe1* cells at 20°C so that the reason why Δ*ppe1* is suppressed by multicopy plasmid carrying the *dis3*$^+$ gene is unclear. The molecular function of dis3 protein is little understood; dis3 protein is enriched in the nucleus and essential for viability (Kinoshita *et al.*, 1991a). It remains to be determined whether the dis3 directly interacts with ppe1 and related phosphatases. Alternatively, dis3 protein might interact with pim1 or related proteins such as spi1 (Matsumoto and Beach, 1991). Only future investigation can establish the involvement of ppe1 phosphatase in mitosis.

High gene dosage of PKC-related *pck1*$^+$ also suppresses the disruption phenotype of *ppe1*$^+$. The link between ppe1 phosphatase and pck1 kinase is strengthened by the phenotypic similarity between Δ*ppe1* and Δ*pck2* in producing pear-shaped cells (Toda *et al.*, 1993). Furthermore, the synthetic lethality of the double mutant Δ*ppe1*-Δ*pck1* gene disruptants suggest that these genes share an essential function. The phosphatase ppe1 may directly or indirectly up-regulate the activity of pck1 kinase. This hypothesis is consistent with the fact that Δ*ppe1* is hypersensitive to staurosporine. Alternatively, pck1 kinase possibly up-regulates ppe1 phosphatase. These two genes appear to be implicated in cell-shape control.

Matsumoto and Beach (1993) show that ts *pim1* mutants are suppressed by mutations in the *esp1*$^+$ gene that is identical to *ppe1*$^+$. pim1 is likely to be a GTP-exchange factor that interacts with the GTP-binding protein spi1 (Matsumoto and Beach, 1991 and 1993). The loss of *pim1*$^+$ gene function causes hyperchromosome condensation like in a mutant of the mammalian RCC1 gene (Ohtsubo *et al.*, 1987). pim1 or spi1 might be phosphorylated, and its dephosphorylation possibly regulated by ppe1. In short, we report a novel fission yeast phosphatase ppe1 that is involved in cell-shape control and possibly also in cell length, mitosis, and signal transduction.

ACKNOWLEDGMENTS

We thank Drs. Tomohiro Matsumoto and David Beach for communicating their results prior to publication; Drs. Kim Arndt and Kelly Tatchell for plasmids; and Dr. Keith Gull for antitubulin antibody. This work was supported by grants from the Human Frontier Science Program and the Ministry of Education, Science and Culture of Japan.

REFERENCES

Arndt, K.T., Styles, C.A., and Fink, G.R. (1989). A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell *56*, 527–537.

Axton, J.M., Dombradi, V., Cohen, P.T.W., and Glover, D.M. (1990). One of the protein phosphatase 1 isozymes in Drosophila is essential for mitosis. Cell *63*, 33–46.

Booher, R., and Beach, D. (1989). Involvement of a type 1 protein phosphatase encoded by *bws1*$^+$ in fission yeast mitotic control. Cell *57*, 1009–1016.

Cohen, P. (1989). The structure and regulation of protein phosphatases. Annu. Rev. Biochem. *58*, 453–508.

Cohen, P.T.W., Brewis, N.D., Hughes, V., and Mann, D.J. (1990). Protein serine/threonine phosphatases; an expanding family. FEBS Lett. *268*, 353–359.

Costello, G., Rodgers, L., and Beach, D. (1986). Fission yeast enters the stationary phase G0 state from either mitotic G1 or G2. Curr. Genet. *11*, 119–125.

Cyert, M.S., Kunisawa, R., Kaim, D., and Thorner, J. (1991). Yeast has homologs (*CNA1* and *CNA2* gene products) of mammalian calcineurin, a calmodulin-regulated phosphoprotein phosphatase. Proc. Natl. Acad. Sci. USA *88*, 7376–7380.

da Cruz e Silva, E.F., Hughes, V., McDonald, P., Stark, M.J.R., and Cohen, P.T.W. (1991). Protein phosphatase $2B_W$ and protein phosphatase Z are *Saccharomyces cerevisiae* enzymes. Biochim. Biophys. Acta *1089*, 269–272.

Doonan, J.H., and Morris, N.R. (1989). The *bimG* gene of *Aspergillus nidulans*, required for completion of anaphase, encodes a homolog of mammalian phosphoprotein phosphatase 1. Cell *57*, 987–996.

Fantes, P. (1981). Isolation of cell size mutants of a fission yeast by a new selective method. J. Bacteriol. *146*, 746–754.

Hagan, I.M., and Hyams, J.S. (1988). The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. *89*, 343–357.

Healy, A.M., Zolnierwicz, S., Stapleton, A.E., Goebl, M., DePaoli-Roach, A.A., and Pringle, J.R. (1991). *CDC55*, a *Saccharomyces cerevisiae* gene involved in cellular morphogenesis: identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase. Mol. Cell. Biol. *11*, 5767–5780.

Hirano, T., Hiraoka, Y., and Yanagida, M. (1988). A temperature-sensitive mutation of the *Schizosaccharomyces pombe* gene *nuc2*$^+$ that encodes a nuclear scaffold-like protein blocks spindle elongation in mitotic anaphase. J. Cell Biol. *106*, 1171–1183.

Hunter, T. (1987). A thousand and one protein kinases. Cell *50*, 823–829.

Kinoshita, N., Goebl, M., and Yanagida, M. (1991a). The fission yeast *dis3*$^+$ gene encodes a 110-kDa essential protein implicated in mitotic control. Mol. Cell. Biol. *11*, 5839–5847.

Kinoshita, N., Ohkura, H., and Yanagida, M. (1990). Distinct, essential roles of type 1 and 2A protein phosphatases in the control of the fission yeast cell division cycle. Cell *63*, 405–415.

Kinoshita, N., Yamano, H., Le Bouffant-Sladechek, F., Kurooka, H., Ohkura, H., Stone, E.M., Takeuchi, M., Toda, T., Yoshida, T., and Yanagida, M. (1991b). Sister-chromatid separation and protein dephosphorylation in mitosis. Cold Spring Harbor Symposium on Quantitative Biology "The Cell Cycle" *57*, 621–628.

Liu, Y., Ishii, S., Tokai, M., Tsutsumi, H., Ohki, O., Akada, R., Tanaka, K., Tsuchiya, E., Fukui, S., and Miyakawa, T. (1991). The *Saccharomyces cerevisiae* genes (*CMP1* and *CMP2*) encoding calmodulin-binding proteins homologous to the catalytic subunit of mammalian protein phosphatase 2B. Mol. Gen. Genet. *227*, 52–59.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular cloning: A laboratory manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Matsumoto, T., and Beach, D. (1991). Premature initiation of mitosis in yeast lacking *RCC1* or an interacting GTPase. Cell *66*, 347–360.

Matsumoto, T., and Beach, D. (1993). Interaction of RCC1/GTPase mitotic checkpoint with a protein phosphatase. Mol. Biol. Cell *4*, 337–345.

Maundrell, K. (1990). *nmt1* of fission yeast: a highly transcribed gene completely repressed by thiamine. J. Biol. Chem. *265*, 10857–10864.

Mitchsion, J.M. (1970). Physiological and cytological methods for *Schizosaccharomyces pombe*. Methods Cell Physiol. *4*, 131–165.

Nurse, P., Thuriaux, P., and Nasmyth, K. (1976). Genetic control of the cell cycle in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. *146*, 167–178.

Ohkura, H., Adachi, Y., Kinoshita, N., Niwa, O., Toda, T., and Yanagida, M. (1988). Cold-sensitive and caffeine-supersensitive mutants of the *Schizosaccharomyces pombe dis* genes implicated in sister chromatid separation during mitosis. EMBO J. *7*, 1465–1473.

Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T., and Yanagida, M. (1989). The fission yeast *dis2*$^+$ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. Cell *57*, 997–1007.

Ohkura, H., and Yanagida, M. (1991). Fission yeast *sds22*$^+$ gene is essential for a mid-mitotic transition and encodes a leucine-rich repeat protein which positively modulates type 1 protein phosphatase. Cell *64*, 149–157.

Ohtsubo, M., Kai, R., Furuno, N., Sekiguchi, T., Sekiguchi, M., Hayashida, H., Kuma, K., Miyata, T., Fukushige, S., Murotsu, T., Matsubara, K., and Nishimoto, T. (1987). Isolation and characterization of the active cDNA of the human cell cycle gene (*RCC1*) involved in the regulation of onset of chromosome condensation. Genes Dev. *1*, 585–593.

Posas, F., Casamayor, A., Morral, N., and Arinõ, J. (1992). Molecular cloning and analysis of a yeast protein phosphatase with an unusual amino-terminal region. J. Biol. Chem. *267*, 11734–11740.

Ronne, H., Carlber, M., Hu, G., and Nehlin, J.O. (1991). Protein phosphatase 2A in *Saccharomyces cerevisiae:* effects on cell growth and bud morphogenesis. Mol. Cell. Biol. *11*, 4876–4884.

Rothstein, R.J. (1983). One-step gene disruption in yeast. Methods Enzymol. *101*, 202–211.

Russell, P., and Nurse, P. (1986). *cdc25*$^+$ functions as an inducer in the mitotic control of fission yeast. Cell *45*, 145–153.

Russell, P., and Nurse, P. (1987). Negative regulation of mitosis by *wee1*$^+$, a gene encoding a protein kinase homolog. Cell *49*, 559–567.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Sneddon, A., Cohen, P.T.W., and Stark, M.J.R. (1990). *Saccharomyces cerevisiae* protein phosphatase 2A performs an essential cellular function and is encoded by two genes. EMBO J. *9*, 4339–4346.

Stone, E.M., Yamano, H., Kinoshita, N., and Yanagida, M. (1993). Mitotic regulation of protein phosphatases by the fission yeast sds22 protein. Current Biol. *3*, 13–26.

Studier, F.W., and Moffatt, B.A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. *189*, 113–130.

Sutton, A., Immanuel, D., and Arndt, K.T. (1991). The SIT4 protein phosphatase functions in late G1 for progression into S phase. Mol. Cell. Biol. *11*, 2133–2148.

Toda, T., Shimanuki, M., and Yanagida, M. (1991). Fission yeast genes that confer resistance to staurosporine encode an AP-1-like transcription factor and a protein kinase related to the mammalian ERK1/MAP2 and budding yeast FUS3 and KSS1 kinases. Genes Dev. *5*, 60–73.

Toda, T., Shimanuki, M., and Yanagida, M. (1993). Two novel protein kinase C-related genes of fission yeast are essential for cell viability and implicated in cell shape control. EMBO J. *12*, (in press).

Toda, T., Yamamoto, M., and Yanagida, M. (1981). Sequential alterations in the nuclear chromatin region during mitosis of the fission yeast *Schizosaccharomyces pombe:* video fluorescence microscopy of synchronously growing wild-type and cold sensitive *cdc* mutants by using a DNA-binding fluorescent probe. J. Cell Sci. *52*, 271–287.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets; procedure and some applications. Proc. Natl. Acad. Sci. USA *76*, 4350–4354.

Wilson, R.B., Brenner, A.A., White, T.B., Engler, M.J., Gaughran, J.P., and Tachell, K. (1991). The *Saccharomyces cerevisiae SRK1* gene, a suppressor of *bcy1* and *ins1*, may be involved in protein phosphatase function. Mol. Cell. Biol. *8*, 505–510.

Woods, A., Sherwin, T., Sasse, R., McRae, T.H., Baines, A.J., and Gull, K. (1989). Definition of individual components within the cytoskeleton of *Trypanosoma brucei* by a library of monoclonal antibodies. J. Cell Sci. *93*, 491–500.

Yanagida, M., Kinoshita, N., Stone, E.M., and Yamano, H. (1992). Protein phosphatases and cell division cycle control. In: Regulation of the Eukaryotic Cell Cycle, Chichester, UK: John Wiley & Sons, 130–146.