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TPD1 of *Saccharomyces cerevisiae* Encodes a Protein Phosphatase 2C-Like Activity Implicated in tRNA Splicing and Cell Separation

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The *Saccharomyces cerevisiae TPD1* gene has been implicated in tRNA splicing because a *tpd1-1* mutant strain accumulates unspliced precursor tRNAs at high temperatures (W. H. van Zyl, N. Wills, and J. R. Broach, Genetics 123:55–68, 1989). The wild-type *TPD1* gene was cloned by complementation of the *tpd1-1* mutation and shown to encode a protein with substantial homology to protein phosphatase 2C (PP2C) of higher eukaryotes. Expression of Tpd1p in *Escherichia coli* results in PP2C-like activity. Strains deleted for the *TPD1* gene exhibit multiple phenotypes: temperature-sensitive growth, accumulation of unspliced precursor tRNAs, sporulation defects, and failure of cell separation during mitotic growth. On the basis of the presence of these observable phenotypes and the fact that Tpd1p accounts for a small percentage of the observed PP2C activity, we argue that Tpd1p is a unique member of the PP2C family.

Eukaryotic organisms use phosphorylation to regulate a wide variety of essential processes, including translation, metabolism, and cell division. The phosphorylation state of proteins is controlled by a competition between two highly conserved groups of enzymes: protein kinases and protein phosphatases. These two groups include both tyrosine-specific enzymes and serine/threonine-specific enzymes (5, 13, 27, 28).

Serine/threonine protein phosphatases can be divided into four major families (protein phosphatase 1 [PP1], PP2A, PP2B, and PP2C) based on their substrate specificity, metal ion requirements, and the effects of certain inhibitors on their activity (5, 30, 67). Organisms ranging from yeasts to higher eukaryotes express all four families of protein phosphatases (8). Protein phosphatases appear to be structurally and functionally conserved. For example, the catalytic subunit of PP1 is 81% identical in *Saccharomyces cerevisiae* and rabbits (15). In both *S. cerevisiae* and mammals, PP1 regulates glycogen metabolism by dephosphorylating glycogen synthase and phosphorylase (6, 15), and in each case this activity is regulated by a highly conserved G subunit (6, 16). Similarly, PP2A in both *S. cerevisiae* and vertebrates is composed of a highly conserved catalytic subunit and two regulatory subunits (5, 8, 54). PP2A acts to regulate the onset of mitosis in humans by dephosphorylating and inactivating maturation-promoting factor (34). In *S. cerevisiae*, PP2A plays a similar but not identical role. Overproduction of the catalytic subunit (59) or deletion of regulatory subunits causes defects in cytokinesis, resulting in elongated cells (20, 74).

Although the majority of serine/threonine protein phosphatase activity can be accounted for by the four major families of protein phosphatases, at least 14 different species of protein phosphatases have been discovered in mammals and *S. cerevisiae* (4). In *S. cerevisiae*, some of the families such as PP2A (65) and PP2B (11, 35) are represented by two functionally redundant catalytic subunits, either of which can be deleted without an observable phenotype. Others such as *SIT4* (1) are unique members of the major families, since deletion of the gene causes distinct phenotypes.

Of the four families of protein phosphatases, PP2C is the least well understood. PP2C has been defined biochemically as a Mg^{2+}-dependent protein phosphatase that is resistant to the inhibitor okadaic acid (5, 7). PP2C exists as two enzymatically indistinguishable isozymes in higher eukaryotes, and the cDNAs encoding these proteins have been cloned from organisms such as rabbits, rats, and humans (38, 44, 45, 69, 76). PP2C species from these higher eukaryotes are between 95 and 99% identical. PP2C has been implicated in the regulation of lipid metabolism in higher eukaryotes by catalyzing the dephosphorylation and inactivation of AMP-activated protein kinase (47, 48). This prevents the AMP-activated protein kinase from phosphorylating and inactivating three key enzymes involved in lipid metabolism: 3-hydroxy-3-methyl glutaryl coenzyme A reductase, acetyl coenzyme A carboxylase, and hormone-sensitive lipase (for review, see reference 19).

We show here that the *S. cerevisiae TPD1* gene encodes a protein phosphatase similar to PP2C. *tpd1-1* was isolated in a screen for mutants defective in de novo tRNA production. *tpd1-1* mutants are temperature sensitive for growth and accumulate unspliced precursor tRNAs at the nonpermissive temperature (74). We report here the isolation of the *TPD1* gene and the characterization of its product. The predicted amino acid sequence of Tpd1p is substantially homologous to PP2C of higher eukaryotes, and expression of *TPD1* in *Escherichia coli* results in protein phosphatase activity similar to that of PP2C. Similar observations have been reported recently by Maeda et al. (36), who isolated *TPD1* on the basis of synthetic lethality with mutations in yeast protein tyrosine phosphatase genes and showed that it encoded a protein with PP2C activity. In this report, we further show that deletion of *TPD1*, like the original mutation, results in temperature-sensitive growth and the accumulation of unspliced precursor tRNAs. It also causes a defect in sporulation and a failure of cell separation during mitotic growth. *S. cerevisiae* has at least two different PP2C species, since cells deleted for *TPD1* exhibit these distinct phenotypes yet still have substantial PP2C activity.

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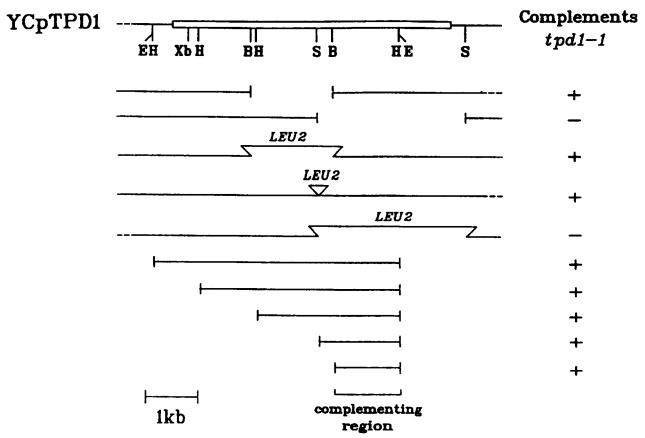

FIG. 1. Localization of the *TPD1* coding region. A restriction map of the cloned yeast genomic DNA (open bar) carried on plasmid YCpTPD1 is shown at the top. The solid line represents flanking YCp50 sequences. Below are diagrams of deletion and insertion derivatives of YCpTPD1 and subclones of the genomic DNA into YCp50, constructed as described in Materials and Methods and transformed into strain Y1782. Solid lines indicate sequences present on the plasmids. Plasmids that yielded Ura$^+$ transformants capable of growth at 37°C were scored positive for complementation. Restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; Xb, *Xba*I.

MATERIALS AND METHODS

Strains and plasmids. The *E. coli* strains used for cloning procedures were JM101 [Δ(*lac-pro*) *supE thi*/F′ *traD36 proAB lacI*^{q} *lacZ*Δ*M15*], MC1066 [Δ(*lacIPOZYA*)*X74 galU galK* StrA^r *hsdR trpC9830 leuB6 pyrF74*::Tn*5*] and RZ510 {F′ *lacI*^{SQ} *lacZU118 pro* [Δ(*lac pro*) *ara str recA56 srl*)]}.

The library of yeast genomic DNA used to clone *TPD1* was obtained from Mark Rose (60). This library was a partial *Sau*3A fragment DNA pool prepared from strain S288C in the vector YCp50. Plasmid YCpTPD1 consists of a 6.7-kb partial *Sau*3A fragment spanning *TPD1* cloned into the *Bam*HI site of YCp50 (Fig. 1). Deletion derivatives of the plasmid were constructed by digestion with *Bam*HI or *Sal*I and religation at low dilution. Insertion derivatives were obtained by ligating the 2.2-kb *Sal*I-*Xho*I or the 2.8-kb *Bgl*II fragment from plasmid YEp13 spanning *LEU2* (3) into *Sal*I- or *Bam*HI-digested YCpTPD1 DNA, respectively. Specific fragments from plasmid YCpTPD1 were tested for *TPD1*-complementing activity by appropriate subcloning into YCp50.

Plasmid pUC-TPD1 consists of the 1.25-kb *Bam*HI-*Eco*RI fragment spanning *TPD1* from YCpTPD1 cloned into the equivalent sites in plasmid pUC19. The *tpd1*::*LEU2-1* allele was created by using a synthetic linker to convert the *Stu*I site in the *TPD1* gene in pUC-TPD1 to an *Xho*I site, to generate plasmid pUC-TPD1-Xho, and then inserting the 2.2-kb *Xho*I-*Sal*I *LEU2* fragment into the newly created *Xho*I site. The *tpd1*::*LEU2-2* allele was created by digesting pUC-TPD1 with *Stu*I and *Sna*BI and inserting an *Xho*I linker. The *LEU2* gene was inserted as a 2.2-kb *Sal*I-*Xho*I fragment in the created *Xho*I site. The *tpd1*::*LEU2-3* allele was obtained by digesting pUC-TPD1 with *Bcl*I and inserting a 2.8-kb *Bgl*II fragment *LEU2* gene from YEp13. Finally, the *tpd1*::*LEU2-4* allele was created by digesting plasmid pUC-TPD1-Xho (see above) with *Xho*I and *Bcl*I and then inserting the *LEU2* gene as a 2.5-kb *Bgl*II-*Sal*I fragment from YEp13.

Plasmid pAR20 consists of a 3.6-kb *Hin*dIII-to-*Cla*I fragment spanning the yeast *SIR4* gene cloned into plasmid pSP64.

All *S. cerevisiae* strains used in this study are described in Table 1. Strain Y1782 was obtained by excision of the circularly integrated *LEU2-GAL10-lacZ-URA3* plasmid from strain Y1412 (listed in reference 74 as strain TPD1) by selecting a 5-fluoroorotic-acid-resistant Leu$^-$ Ura$^-$ revertant of the parent strain (2). Strains Y1778 to Y1781 were obtained by transforming strain Y1029 to Leu$^+$ with *Hin*dIII-plus-*Bam*HI-

TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference or source
Y1407	*MATα leu2*::*LEU2-GAL10-lacZ*$_o$*-URA3 his3Δ1 hml*::*SUP4*$_o$ *hmr*::*SUC2 sir4-1 ade2-1 ura3-52*	72
Y1412	*MATα leu2*::*LEU2-GAL10-lacZ*$_o$*-URA3 his3Δ1 hml*::*SUP4*$_o$ *hmr*::*SUC2 sir4-1 ade2-1 ura3-52 tpd1-1*	72
Y1782	*MATα leu2-3,112 his3Δ1 hml*::*SUP4*$_o$ *hmr*::*SUC2 sir4-1 ade2-1 ura3-52 tpd1-1*	This study
Y1029	*MATa*/*MATα ura3-52*/*ura3-52 his3Δ1*/*his3Δ1 leu2-3,112*/*leu2-3,112 trp1-289*/*TRP1 lys2-1*/*lys2-1 ADE2*/*ade2-1*	14
Y1778	As Y1029, *TPD1*/*tpd1*::*LEU2-1*	This study
Y1779	As Y1029, *TPD1*/*tpd1*::*LEU2-2*	This study
Y1780	As Y1029, *TPD1*/*tpd1*::*LEU2-3*	This study
Y1781	As Y1029, *TPD1*/*tpd1*::*LEU2-4*	This study
Y1783	*MATa ura3-52 his3Δ1 leu2-3,112 trp1-289 lys2-1 tpd1*::*LEU2-4*	This study
Y1784	*MATα ura3-52 his3Δ1 leu2-3,112 lys2-1 ade2-1 tpd1*::*LEU2-4*	This study
S150-2B	*MATa leu2-3,112 ura3-52 gal2 trp1-289 his3Δ1*	Laboratory stocks
YPH81	*MATa ura3-52 trp1Δ1 lys2 ade2-101*	Phil Hieter
SC465	*MATa ura3-52 leu2-3,112 ade2-101 lys2-801*	Laboratory stock
SC277	*MATα ura3-52 leu2-3,112 trp1-1 sen1-1*	Laboratory stock
OR14-17b	*MATa SUP27 can1-100 ilv1-2 leu2-1 trp1-1 lys1-1 met*	Genetic Stock Center
8201-7A	*MATa lys14*	Genetic Stock Center
SC350	*MATa ura3-52 leu2-3,112 ade2-101 lys2-801 tpd1*::*LEU2-3*	This study
MR19	*MATa ura3-52 leu2-3,112 ade2-101 lys2-801* [YEpTPD1]	This study
MR20	*MATa ura3-52 leu2-3,112 ade2-101 lys2-801 tpd1*::*LEU2-3* [YEpTPD1]	This study
SC466	*MATα ura3-52 leu2-3,112 lys2-801 his3Δ200*	This study
SC441	*MATα ura3-52 leu2-3,112 lys2-801 his3Δ200 tpd1*::*LEU2-3*	This study
SC551	*MATα lys14 trp1-1 leu2-3,112*	This study
SC527	*MATa ura3-52 ilv1-2 leu2-3,112 tpd1*::*LEU2-3*	This study
SC472	*MATa*/*MATα ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112 lys2-801*/*lys2-801 ade2-101*/*ADE2 HIS3*/*his3Δ200*	This study
SC475	*MATa*/*MATα ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112 lys2-801*/*lys2-801 ade2-101*/*ADE2 HIS3*/*his3Δ200 tpd1*::*LEU2-3*/*tpd1*::*LEU2-3*	This study

digested pUC19/tpd3 plasmid DNA carrying the indicated *tpd1*::*LEU2* allele. Similarly, strains SC350, MR20, and SC441 were obtained by transforming strains SC465, MR19, and SC466, respectively, with a *Bam*HI-*Hin*dIII fragment spanning the *tpd1*::*LEU2-3* allele. All integrations were confirmed by Southern analysis (23, 37). Strain SC527 was obtained as a segregant from a cross between strains OR14-17b and SC441, and strain SC551 was obtained as a segregant from a cross between strains 8201-7A and SC277. Yeast strains were grown on either YEP (1% yeast extract, 2% Bacto Peptone) or SC (0.67% Difco yeast nitrogen base, supplemented with amino acids, adenine, and uracil as described previously [61]) to which an indicated carbon source has been added to 2%. Strains Y1783 and Y1784 were haploid segregants from strain Y1781.

Other nucleic acid manipulations. Transformations of *S. cerevisiae* and *E. coli* were performed as described by Ito et al. (31) and Silhavy et al. (64), respectively. Recovery of plasmids from yeast cells was performed as described by Nasmyth and Reed (49).

Sequence analysis. DNA sequence of the 1.5-kb *Eco*RI-to-*Bam*HI fragment spanning *TPD1* was obtained by the chain termination method of Sanger et al. (62), using single-stranded templates from subclones of the fragment onto M13 phage derivative mp11 or mp18 (46). The entire sequences of both strands of this fragment were determined. Sequence comparisons were performed with FASTA and BESTFIT programs of the Genetics Computer Group package from the University of Wisconsin.

Northern (RNA) analysis for tRNA intermediates. Cultures were grown in YEPD medium to 10^7 cells per ml and then shifted to 37°C in a shaking water bath for appropriate time intervals. Samples (3.0 ml) were removed at hourly intervals over a period of 6 h and immediately chilled on ice. Cells were collected by centrifugation, and low-molecular-weight RNAs were extracted with phenol at 65°C as described previously (32). RNA species were resolved on 10% polyacrylamide–7 M urea gels, and Northern blotting analysis, as well as oligonucleotide preparation with subsequent ^{32}P end labeling, was performed as described by van Zyl et al. (74).

Yeast chromosome fractionation. Intact yeast chromosomes were prepared as described by Rose et al. (61) and separated in 0.5× Tris-borate-EDTA on a contour-clamped homogeneous electric field gel apparatus by electrophoresis for 14 h at 200 V with a 120-s pulse frequency and then for 10 hr at 200 V with a 90-s pulse frequency.

Protein phosphatase assays. Protein phosphatase activity was measured as described by McGowan and Cohen (45) with ^{32}P-casein (bovine; Sigma) prepared by using cyclic AMP (cAMP)-dependent protein kinase (10 U/μl; Sigma) and 50 μCi of [γ-^{32}P]ATP (3,000 Ci/mmol; NEN). Reaction mixtures of 30 μl contained 50 mM Tris-Cl (pH 7.5), 0.5 mM EDTA, 2 mM EGTA, 100 μg of bovine serum albumin per 3 μg of ^{32}P-casein (1.2×10^3 cpm/μg of protein), a source of Tpd1p, and where appropriate divalent cations (10 mM) and okadaic acid (5 μM). Reaction mixtures were incubated at 30°C for 10 min, protein was precipitated, and supernatants were counted as described previously (45).

Expression and partial purification of Tpd1p from *E. coli*. *TPD1* was cloned into the *E. coli* expression vector pTTQ8 under control of the hybrid *trp-lac* promoter (66). An *Eco*RI restriction site was placed just 5′ to the beginning of the *TPD1* open reading frame by PCR, allowing *TPD1* to be inserted in frame with the ATG and in proper alignment with the Shine-Dalgarno sequence of the vector. In so doing, an additional asparagine residue was added as the second amino acid of the protein. The 3′ end of the resulting clone (pTTQ8-TPD1), from the unique *Nde*I site at position 41 to the end of the coding region, was replaced with the corresponding fragment from the original *TPD1* clone. The remaining 5′ end of the clone, derived from PCR, was sequenced by using double-stranded DNA as a template.

pTTQ8-TPD1 was transformed into strain EMP52 (relevant genotype, *lacI*^{SQ}), and Tpd1p was expressed in mid-log-phase cells by induction with 1 mM isopropylthiogalactopyranoside (IPTG) for 1.5 h. Cells (500 ml, 1.0×10^9 cells per ml) were harvested, resuspended in 3 ml of lysis buffer (50 mM Tris-Cl [pH 7.5], 1 mM EDTA, 5 mM dithiothreitol, 200 mM NaCl, 10% sucrose), sonicated for 2 min in 20-s bursts, supplemented with phenylmethylsulfonyl fluoride to 1 mM, and centrifuged to remove cellular debris. Supernatants were aliquoted and either frozen on dry ice or used immediately; freezing had no effect on activity.

Tpd1p was partially purified from *E. coli* extracts by chromatography on DE-52. Extract (1 ml) was diluted to 50 mM NaCl with lysis buffer and loaded onto a DE-52 (Whatman) column (2 by 10 cm), and protein was eluted with a 90-ml gradient from 50 mM to 1 M NaCl. Fractions (3 ml) were collected and assayed for protein phosphatase activity. Active fractions were dialyzed into buffer containing 20 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1 mM dithiothreitol, 200 mM NaCl, and 50% glycerol and stored at −20°C.

Preparation of yeast extracts. Isogenic strains with different gene dosages of *TPD1* were grown in selective media and shifted to YEPD for two generations, and then 150 ml (6×10^7 cells per ml) was harvested to make extracts. Cells were resuspended in 1 ml of extraction buffer [50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 5 mM dithiothreitol, 200 mM NaCl, 0.8 M $(NH_4)_2SO_4$, 10% glycerol], disrupted with glass beads (Biospec Products, Bartlesville, Okla.) at 4°C for 3.0 min in 20-s bursts, supplemented with phenylmethylsulfonyl fluoride to 1 mM, and centrifuged to remove cellular debris. Supernatants were aliquoted and either frozen on dry ice or used immediately; freezing had no effect on activity. To assay tRNA splicing activity, isogenic strains differing only at the *TPD1* locus were grown to 4×10^7 cells per ml and harvested. Extracts were made from spheroplasts to measure tRNA endonuclease activity (22) or from glass beads, as described above, to measure tRNA ligase (56) or 2′-phosphotransferase (40) activity.

Microscopy. Cells to be examined were harvested by centrifugation for 2 min at 2,000 rpm. Cells were washed once with water, fixed for 2 h in 4% formaldehyde, washed with water, and resuspended in 1 μg of 4,6′-diamidino-2-phenylindole (DAPI) per ml. Cells were washed with water, spread on polylysine-coated slides, and mounted under a coverslip in 80% glycerol. Microscopy was performed with an Olympus model BH-2 microscope and a 40× APL (numerical aperture, 0.65) objective. Photographs were obtained with an Olympus C-35AD2 camera and autofocus unit.

For treatment with zymolyase, cells were harvested as described above, washed in phosphate buffer (50 mM $KHPO_4$ [pH 6.6]), fixed for 2 h in phosphate buffer plus 4% formaldehyde, washed, and resuspended in sorbitol buffer (50 mM $KHPO_4$ [pH 6.6], 1.2 M sorbitol). Zymolyase (1/30 volume, 10 mg/ml) was added, and the cells were incubated at room temperature for 1 h. Cells were washed in phosphate buffer, mounted, and photographed with the optics specified above.

For time-lapse photography, cells were grown in YEPD to a density of 2×10^6 cells per ml, sonicated briefly, and then spread on YEPD–2% Bacto Agar previously poured over a metal grid placed on a microscope slide. The slide was incubated at the indicated temperature in a humidified cham-

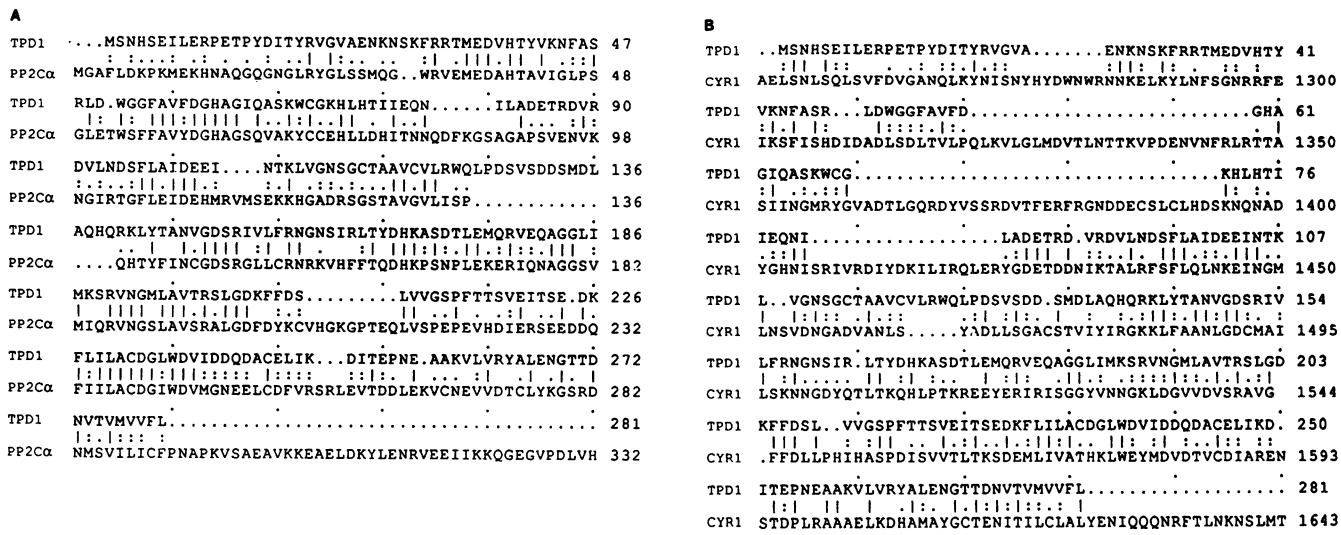

FIG. 2. Comparison of the Tpd1p amino acid sequence with those of mammalian PP2C and yeast adenylate cyclase. A homology search using the BLAST service of National Center for Biotechnology Information showed homology between the predicted amino acid sequence of *TPD1* and mammalian PP2C and yeast adenylate cyclase sequences. The GAP program of the Genetics Computer Group computer package was then used to align the Tpd1p sequence with the amino acid sequences of rat PP2Cα (A) and yeast adenylate cyclase (B).

ber, and several fields were photographed repeatedly at the times indicated. The grid provided a frame of reference to allow identification of the same field at subsequent times. Photomicroscopy was performed on a Zeiss Photomicroscope III by phase contrast, using a Neofluar 25× (numerical aperture, 0.60) objective.

RESULTS

The *TPD1* gene encodes a protein with homology to PP2C. We previously isolated and characterized several yeast mutants deficient in tRNA biosynthesis (72, 74). Since we anticipated that mutational inactivation of tRNA biosynthesis would be lethal, we recovered only strains with conditional lethal defects. One such strain carried a recessive, temperature-sensitive allele of a gene that we designated *TPD1* and accumulates unspliced tRNA intermediates when transferred to the nonpermissive temperature.

We cloned *TPD1* by a standard complementation protocol. By selecting for temperature-resistant colonies following transformation of the *ura3 tpd1-1* strain Y1782 with a random yeast genomic library carried on a YCp50 vector, we recovered two overlapping DNA fragments that complemented *tpd1-1*. This overlapping region could be recovered as a 4.7-kb *Eco*RI fragment (Fig. 1). To confirm that this region encompasses *TPD1*, we used the 4.7-kb *Eco*RI fragment to direct homologous integration of a *URA3*-containing plasmid into the genome of the *tpd1-1 ura3* strain Y1782. Several such transformants were crossed with the *TPD1 ura3* strain S150-2B. For more than 20 tetrads analyzed from these crosses, the *URA3* marker always segregated 2:2 and no temperature-sensitive segregants were observed. Thus, we concluded that the complementing fragments carried *TPD1*.

To localize the *TPD1* coding region within the cloned 4.7-kb *Eco*RI yeast fragment, we constructed various deletions of and insertions into plasmid YCpTPD1 and subcloned different fragments from YCpTPD1 into YCp50. These constructs were tested for the ability to complement the *tpd1-1* mutation in strain Y1782. As noted from the results of this analysis (Fig. 1), *TPD1*-complementing activity resided within a 1.25-kb *Eco*RI-*Bam*HI fragment. This fragment was sequenced and found to contain a single extended open reading frame, extending 843 bp from an ATG codon 412 bp downstream of the *Eco*RI site to a TAA codon 11 bp upstream of the *Bam*HI site. The sequence is identical to that of *PTC1* recently reported by Maeda et al. (36).

Computer searches using the BLAST network service provided by the National Center for Biotechnology Information revealed that the predicted amino acid sequence of *TPD1* is highly homologous to PP2C sequences of larger eukaryotes (Fig. 2A). Tpd1p is 34% identical and 53% homologous to PP2Cα of rat liver over its entire length and is correspondingly similar to PP2C of other vertebrates. Tpd1p also exhibited striking homology to a central domain of yeast adenylate cyclase, exhibiting 32% identity and 60% homology over a 200-amino-acid stretch (Fig. 2B). The region of adenylate cyclase to which Tpd1p exhibits homology lies between the central, Ras-responsive domain and the carboxy-terminal catalytic domain and is not associated with any known function of the protein. Mutant adenylyl cyclase proteins containing amino acid insertions in this domain exhibit both normal catalytic activity and normal Ras responsiveness.

Tpd1p has PP2C-like activity. Given the similarity of Tpd1p to mammalian PP2C, we examined whether Tpd1p possessed protein phosphatase activity. We synthesized Tpd1p in *E. coli*, in which there is little endogenous protein phosphatase activity (70). Strains expressing Tpd1p showed a low but reproducible Mg^{2+}-dependent protein phosphatase activity, and this activity was not observed in extracts derived from either uninduced strains or strains lacking *TPD1* (data not shown). Tpd1p phosphatase activity in crude extracts is 3- to 10-fold more active when Mn^{2+} is substituted for Mg^{2+}, and partially purified Tpd1p retains the same Mn^{2+}-to-Mg^{2+} activity ratio as seen in crude extracts (Fig. 3). Mg^{2+} and Mn^{2+} are the only commonly occurring metal ions capable of eliciting Tpd1p protein phosphatase activity (Table 2). Finally, Tpd1p protein phosphatase activity was resistant to EGTA and okadaic acid in the presence of either Mg^{2+} or Mn^{2+} (data not shown). These properties are diagnostic of PP2C from mammalian cells

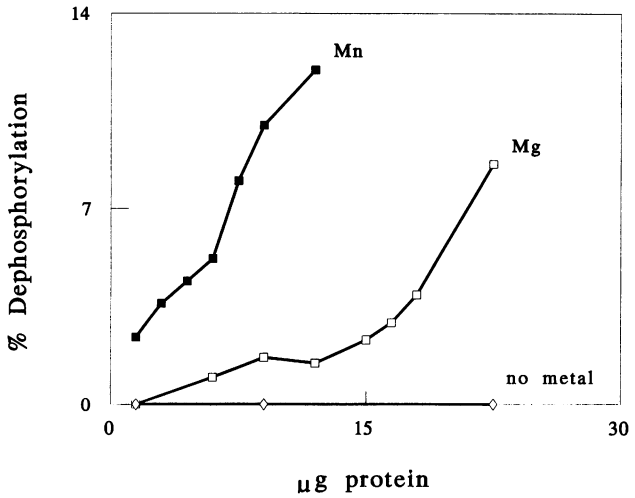

FIG. 3. Protein phosphatase activity of partially purified Tpd1p. DE-52-purified Tpd1p was assayed for protein phosphatase activity, using ^{32}P-casein (0.6 fmol of ^{32}P, 3,000 Ci/mmol) and either Mg^{2+} or Mn^{2+} as indicated. Percent dephosphorylation is corrected for the amount of $^{32}P_i$ released in the presence of buffer alone.

(7, 8) and demonstrate that Tpd1p possesses a PP2C-like activity.

Tpd1p is not the only PP2C species in *S. cerevisiae*. Extracts prepared from *tpd1*::*LEU2* yeast strains have nearly as much PP2C activity as extracts prepared from wild-type strains (data not shown). Furthermore, extracts prepared from strains which overproduce Tpd1p protein phosphatase do not have significantly more PP2C activity than extracts prepared from wild-type strains (data not shown). This is true whether Mn^{2+} or Mg^{2+} is used as the metal cation. Thus, at least one other PP2C species is present in yeast cells, and this species comprises most of the detectable PP2C activity extractable from the cell.

***TPD1* is conditionally required for growth.** As a preliminary step in our genetic analysis of *TPD1*, we determined its genetic map position. *TPD1* was physically mapped to chromosome IV by performing a Southern analysis on separated intact yeast chromosomes of yeast strain YPH81 (61). The same nylon membrane was also probed with the *SIR4* sequence obtained from plasmid pAR20. *SIR4* maps to chromosome IV (39), and the *SIR4* probe highlighted the same chromosomal band as *TPD1*, confirming its assignment to chromosome IV (data not shown). Meiotic recombination data from a diploid strain heterozygous for *lys14*, *trp1*, and *tpd1*::*LEU2* showed that *tpd1*::*LEU2* mapped to the left arm of chromosome IV, closely linked to the centromere (Table 3). Analysis of 110 tetrads yielded map distances of 10 centimorgans (cM) between *lys14* and *tpd1*::*LEU2*, 9 cM between *lys14* and *trp1*, and 1 cM between *trp1* and *tpd1*::*LEU2* and suggested a map order of *tpd1*::*LEU2*, *CEN4*, *trp1*, *lys14*. These data position *tpd1*::*LEU2* near the gene *crl18*. The *CRL18* gene was identified by alleles that conferred resistance to low-level cycloheximide (42). While *tpd1*::*LEU2* strains are sensitive to 0.5 μg of cycloheximide per ml, we have been able to recover alleles of *TPD1* that confer dominant resistance to 2 μg of cycloheximide per ml (data not shown). Thus, since *TPD1* and *CRL18* map to the same location and since alleles of the two genes yield the same phenotype, the two genes are likely to be the same.

Strains carrying *tpd1-1* are temperature sensitive for growth. To test whether *TPD1* is essential for mitotic growth, we created four different *LEU2* disruptions in the *TPD1* open reading frame (Fig. 4) and used them to disrupt one of the two homologs of *TPD1* in the diploid Y1029. Diploid transformants with the different integrated constructs were sporulated, and 10 asci of each were dissected. The Leu^+ haploid progeny that contained the *tpd1*::*LEU2* disruption grew almost as well as the Leu^- *TPD1* haploid strains at 23°C. The *tpd1*::*LEU2* haploid progeny grew much slower than the *TPD1* haploid strains at 30°C and not at all at 35°C (Fig. 4 and 5). When the Leu^+ haploid strains with the *tpd1*::*LEU2* disruptions were streaked on fresh YEPD plates at 37°C, they failed to grow.

TABLE 2. Effects of various metals on Tpd1p activity[a]

Metal	% Dephosphorylation[b]
Mg^{2+}	5
Mn^{2+}	1
Ca^{2+}	13
Cu^{2+}	<1
Zn^{2+}	<1
Fe^{3+}	<1
Ni^{2+}	<1
None	1

[a] Tpd1p was expressed in and partially purified from *E. coli* as described in Materials and Methods. Protein phosphatase assays were performed as described in Materials and Methods with 0.66 fmol of ^{32}P-casein (3,000 Ci/mmol) in the presence of various divalent cations as indicated.

[b] Corrected for release of $^{32}P_i$ in the presence of buffer alone.

TABLE 3. Meiotic mapping data for *TPD1*[a]

Loci	No. of tetrads		
	PD	NPD	TT
trp1 × *tpd1*::*LEU2-3*	108	0	2
lys14 × *tpd1*::*LEU2-3*	93	1	16
lys14 × *trp1*	95	1	14

[a] Tetrad analysis was performed on 110 asci from a cross between strains SC551 and SC527 to determine recombination frequencies between markers *lys14*, *trp1-1*, and *tpd1*::*LEU2*. PD, parental ditype; NPD, nonparental ditype; TT, tetratype.

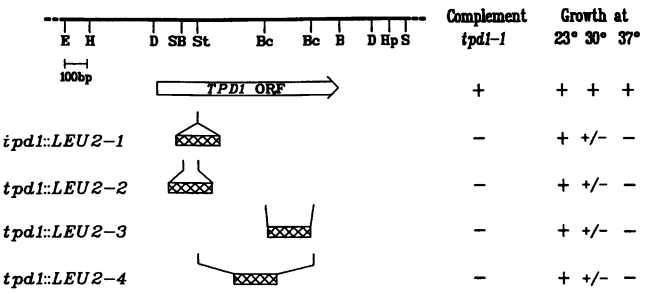

FIG. 4. *LEU2* disruptions of the *TPD1* open reading frame. Four sets of disruptions of the *TPD1* open reading frame (ORF) were constructed by insertion of the *LEU2* gene in the *Sna*BI, *Stu*I, and *Bcl*I sites as described in Materials and Methods. The *tpd1*::*LEU2* disruptions were cloned as *Hin*dIII-*Sal*I fragments into YCp50, and their abilities to complement the *tpd1-1* mutation were determined. The *tpd1*::*LEU2* disruptions were also integrated into the chromosome of strain Y1029. Transformants of Y1029 were sporulated and dissected, and the haploid spore clones with the *tpd1*::*LEU2* disruptions were scored for viability at 23, 30, and 35°C. Restriction sites: B, *Bam*HI; Bc, *Bcl*I; D, *Dra*I; E, *Eco*RI; Hp, *Hpa*I; S, *Sal*I; SB, *Sna*BI; St, *Stu*I.

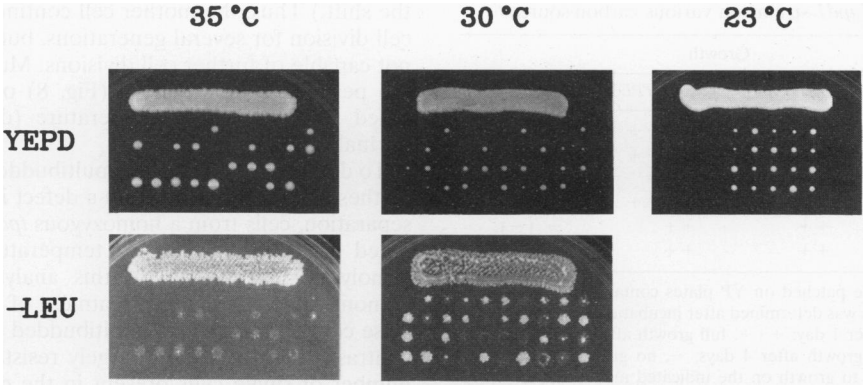

FIG. 5. Tetrad analysis of Y1029 transformants containing *tpd1*::*LEU2* disruptions. Strain Y1029 transformed with *tpd1*::*LEU2-1* was sporulated, and asci were dissected on YEPD. The plates were incubated at 35, 30, or 23°C until viable spores developed, after which they were replica plated onto an SC plate lacking leucine and incubated at the same temperature as was the master YEPD plate.

These results demonstrate that *TPD1* is conditionally essential: it is required for growth of *S. cerevisiae* at 37°C but not at 23°C.

***tpd1*::*LEU2* strains accumulate unspliced tRNA precursors.** As noted above, a strain containing the *tpd1*::*LEU2* disruption exhibits a temperature-sensitive phenotype, similar to that observed for the *tpd1-1* mutation. Since the *tpd1-1* mutation causes accumulation of intervening sequence (IVS)-containing tRNA precursors, we examined the accumulation of the IVS-containing $tRNA^{Ser}_{UCG}$ precursor in a *tpd1*::*LEU2-1* disruption strain. A time course was performed on cultures of two sister spore clones, one carrying *TPD1* and the other carrying *tpd1*::*LEU2-1*. Figure 6 shows the accumulation of the IVS-containing $tRNA^{Ser}_{UCG}$ precursor, normalized to the level of the mature $tRNA^{Ser}_{UCG}$ species. As evident in Fig. 6, the *tpd1*::*LEU2* strain exhibited a four- to five-times-higher accumulation of the IVS-containing $tRNA^{Ser}_{UCG}$ precursor even at 23°C compared with the *TPD1* strain. The level of accumulation of the unspliced intermediate in the *tpd1*::*LEU2* strain increased to approximately eight times that in the *TPD1* wild-type strain after 2 h at 37°C. In contrast, the *tpd1-1* mutant strain showed accumulation of IVS-containing tRNA precursors only at 37°C and after a significantly longer time at the nonpermissive temperature (74). Thus, complete inactivation of *TPD1* results in an effect on tRNA precursor processing more dramatic than that exhibited by the original *tpd1-1* allele.

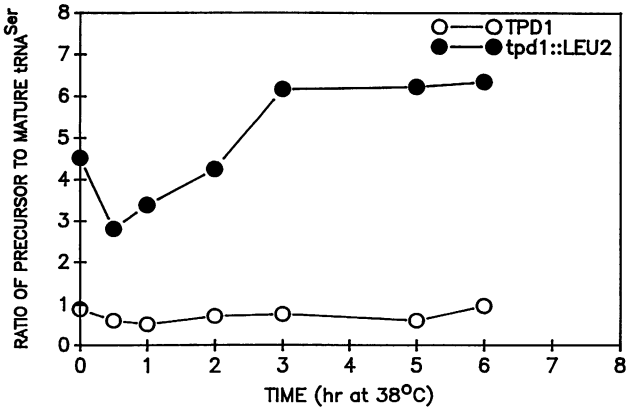

FIG. 6. Time course of $tRNA^{Ser}_{UCG}$ precursor accumulation in *TPD1* and *tpd1*::*LEU2* strains. Strains Y1784 (*tpd1*::*LEU2-1*) and Y1783 (*TPD1*) were grown at 23°C to 10^7 cells per ml, and the cultures were then shifted to 37°C. Samples were removed at the indicated intervals, RNA was extracted from the cells, and Northern analysis was performed as described in Materials and Methods, probing with an oligonucleotide specific for $tRNA^{Ser}_{UCG}$. An autoradiograph of the Northern blot was prepared and scanned with a densitometer. The ratios of the levels of IVS-containing $tRNA^{Ser}_{UCG}$ precursor to mature $tRNA^{Ser}_{UCG}$ are plotted as a function of time following a shift of the strains from 23 to 37°C. Ratios are given in arbitrary units.

Removal of the intron from pre-tRNA occurs in three steps in *S. cerevisiae*. An endonuclease cuts the pre-tRNA, removing the intron (22, 52, 56), tRNA ligase joins the two half molecules together (17, 54, 55), and the splice junction 2′ PO_4 is transferred to NAD to form ADP-ribose 1″-2″ cyclic phosphate (10, 40, 41). One explanation for the function of Tpd1p in the splicing pathway is that it dephosphorylates endonuclease to make it more active. However, we find that the endonuclease activity in extracts of strain SC475 (*tpd1*Δ/*tpd1*Δ) is identical to that in extracts of strain SC472 (*TPD1*/*TPD1*) (data not shown). Similarly, the tRNA ligase activity and 2′-phosphotransferase activity are identical in extracts of the two strains (data not shown). Thus, we have not been able to assign a specific biochemical defect in tRNA splicing to strains lacking Tpd1p. This suggests that the effects on tRNA maturation resulting from inactivation of *TPD1* are likely to be indirect.

***TPD1* is required for sporulation and for growth on certain nonfermentable carbon sources.** Two sporulation defects are associated with strains lacking a full complement of Tpd1p. First, homozygous *tpd1*::*LEU2* strains do not sporulate efficiently. In some genetic backgrounds, strains sporulate with less than 0.1% efficiency whereas the otherwise isogenic homozygous wild-type and heterozygous control diploids sporulate at 80% efficiency. This sporulation defect is observed on plates and in liquid and cannot be relieved by increased time of incubation. However, the effect is strain dependent, since other genetic backgrounds allow homozygous *tpd1*::*LEU2* strains to sporulate, albeit always at a much lower efficiency than the isogenic heterozygote. Second, strains heterozygous for *tpd1*::*LEU2*, although they sporulate with the same efficiency as wild-type strains, have a large percentage, typically between 10 and 25%, of asci with an extra appendage on the ascus wall. This extra appendage appears to be an unbudded cell, although it has not been fully characterized; however, it does not appear to affect sporulation or germination, since spores have normal viability when dissected (data not shown). Both sporulation phenotypes, the inability to sporulate in homozygous *tpd1*::*LEU2* strains and the presence of the extra appendage in

TABLE 4. Growth of *tpd1* strains on various carbon sources[a]

Carbon source	Growth			
	TPD1/TPD1	*TPD1/tpd1*	*tpd1/TPD1*	*tpd1/tpd1*
Glucose	++++	++++	++++	++++
Lactate	++++	++++	++++	++
Ethanol	+++	+++	+++	+ (+++)
Glycerol	++++	++++	++++	− (−)
Pyruvate	++	++	++	− (−)
Acetate	++	++	++	− (−)

[a] The indicated strains were patched on YP plates containing the indicated carbon sources at 2%. Growth was determined after incubation for 1 to 5 days at 28°C. ++++, full growth after 1 day; +++, full growth after 2 days; ++ full growth after 3 days; +, full growth after 4 days; −, no growth after 5 days. Symbols in parentheses refer to growth on the indicated media supplemented with 1 M sorbitol. Diploid strains were obtained by pairwise crosses of strains SC350, SC441, SC465, and SC466.

heterozygous strains, can be complemented by introducing *TPD1*, either on a multicopy plasmid or under control of the GAL promoter, into the cells prior to sporulation. Therefore, the lack of the *TPD1*-encoded protein in mutant cells is somehow responsible for the sporulation effects.

To pinpoint the defect in sporulation, we examined several properties of *tpd1/tpd1* strains, including their proficiency to grow on nonfermentable carbon sources. As noted in Table 4, *tpd1/tpd1* strains, but not heterozygous diploids or homozygous *TPD1* strains, fail to grow on a variety of nonfermentable compounds as sole sources of carbon. The *tpd1/tpd1* strains are not simply respiratory deficient, since they can grow almost normally on lactate and since the inability to use ethanol (but not other nonfermentable carbon sources) can be remediated by addition of 1 M sorbitol to the plates. This inability to utilize nonfermentable carbon sources likely accounts for the sporulation defect of these strains: sporulation competence of the strains can be restored to some extent by incubation in the presence of 1 M sorbitol. Thus, strains lacking a functional *TPD1* gene are defective in some aspect of carbon catabolism, and this defect prevents such strains from undergoing normal sporulation.

***TPD1* is required for cell separation.** We observed that incubation of a homozygous *tpd1*::*LEU2* diploid strain (SC475) at 37°C led to accumulation of multibudded cells. By 8 h after transfer to the nonpermissive temperature, more than 80% of the cells of such a culture possessed three or more buds, whereas an isogenic *TPD1* strain accumulated fewer than 1% multibudded cells (Fig. 7). In most cases, these novel multibudded structures consisted of a central (presumed mother) cell to which multiple single cells were attached. By DAPI staining, all of the buds emergent from the central mother cell appeared to carry a single nucleus, as did the central mother cell. These observations suggest (i) that mother cells undergo repeated rounds of cell division after the shift to the nonpermissive temperature, (ii) that daughter cells that emerge after the temperature shift do not undergo subsequent cell divisions, and (iii) that the daughter cells fail to complete cytokinesis or cell separation at the end of each cycle.

To test the growth pattern more directly, we examined the budding pattern of individual *tpd1*::*LEU2* cells over several generations by time-lapse photography. Several such pedigrees are shown in Fig. 8. As evident, single cells present at the time of the shift to 37°C bud several times after the temperature shift, but none of the daughter cells that emerged after the shift undergo a second cell division. (Occasionally, a daughter cell present at the time of the shift will undergo a cell division after the shift.) Thus, the mother cell continues to undergo normal cell division for several generations, but the daughter cells are not capable of further cell divisions. Mutant cells incubated at the permissive temperature (Fig. 8) or wild-type cells incubated at the elevated temperature (data not shown) show normal budding patterns.

To determine whether the multibudded phenotype exhibited by these strains resulted from a defect in cytokinesis or in cell separation, cells from a homozygous *tpd1*::*LEU2* diploid incubated at the nonpermissive temperature were treated with zymolyase. The results of this analysis, shown in Fig. 9, demonstrated clearly that treatment of the culture with zymolyase converted all of the multibudded cells to single cells. By contrast, these cells were largely resistant to sonication. The number of single cells present in the culture after treatment with zymolyase was essentially equivalent to the total number of mother and daughter cells prior to the treatment. Thus, treatment with zymolyase led simply to separation of the formerly attached cells rather than to lysis of the multibudded complexes. These results demonstrate that cytokinesis had been completed in the *tpd1*::*LEU2* strains at the nonpermissive temperature but that cell separation was incomplete under these conditions.

DISCUSSION

***TPD1* encodes a PP2C.** We have presented evidence suggesting that *TPD1*, a gene previously identified in a screen for mutants defective in tRNA biosynthesis, encodes a protein phosphatase with properties similar to those of PP2C of higher eukaryotes. First, *TPD1* encodes a protein that is substantially homologous to PP2C of larger eukaryotes (Fig. 2). Second, Tpd1p expressed in *E. coli* exhibits readily detectable protein phosphatase activity. Third, this activity is dependent on Mg^{2+} or Mn^{2+} as a metal cation and is resistant to EGTA and okadaic acid in the presence of either metal. These are the primary distinguishing enzymatic characteristics of mammalian PP2C. *TPD1* was recently identified and cloned by Maeda et al. (36) on the basis of synthetic lethality with *ptp2*, a yeast gene encoding a protein homologous to mammalian protein tyrosine phosphatases. This group also demonstrated that the gene encoded a protein with characteristics of PP2C and designated it *PTC1*.

While Tpd1p appears to be a member of the PP2C family, it is only distantly related to the predominant mammalian PP2C. All known mammalian PP2C proteins have almost identical amino acid sequences, while Tpd1p shares only 34% identity with this family. No other PP2C members are known, so the divergence among PP2C members over a broader phylogenetic base is not known. In addition, Tpd1p protein phosphatase activity differs from PP2C activity of other organisms in that Mn^{2+} stimulates protein phosphatase activity to a larger degree than does Mg^{2+} (Fig. 3); PP2C from other organisms is only 20 to 70% as active when Mn^{2+} is substituted for Mg^{2+} (21, 47, 52). Thus, Tpd1p appears to be a unique member of the PP2C family.

We find that Tpd1p comprises a small fraction of total PP2C activity extracted from cells: PP2C activity does not differ significantly in extracts made from *tpd1*::*LEU2* strains, wild-type strains, and strains carrying *TPD1* on a multicopy plasmid. Nonetheless, deletion of PP2C results in a distinct panoply of phenotypes, indicating that Tpd1p must play a specific role in the cell, for which the predominant PP2C in the cell cannot readily compensate. This is a situation analogous to that observed with *SIT4* (1), a unique member of the PP1/PP2A/PP2B superfamily.

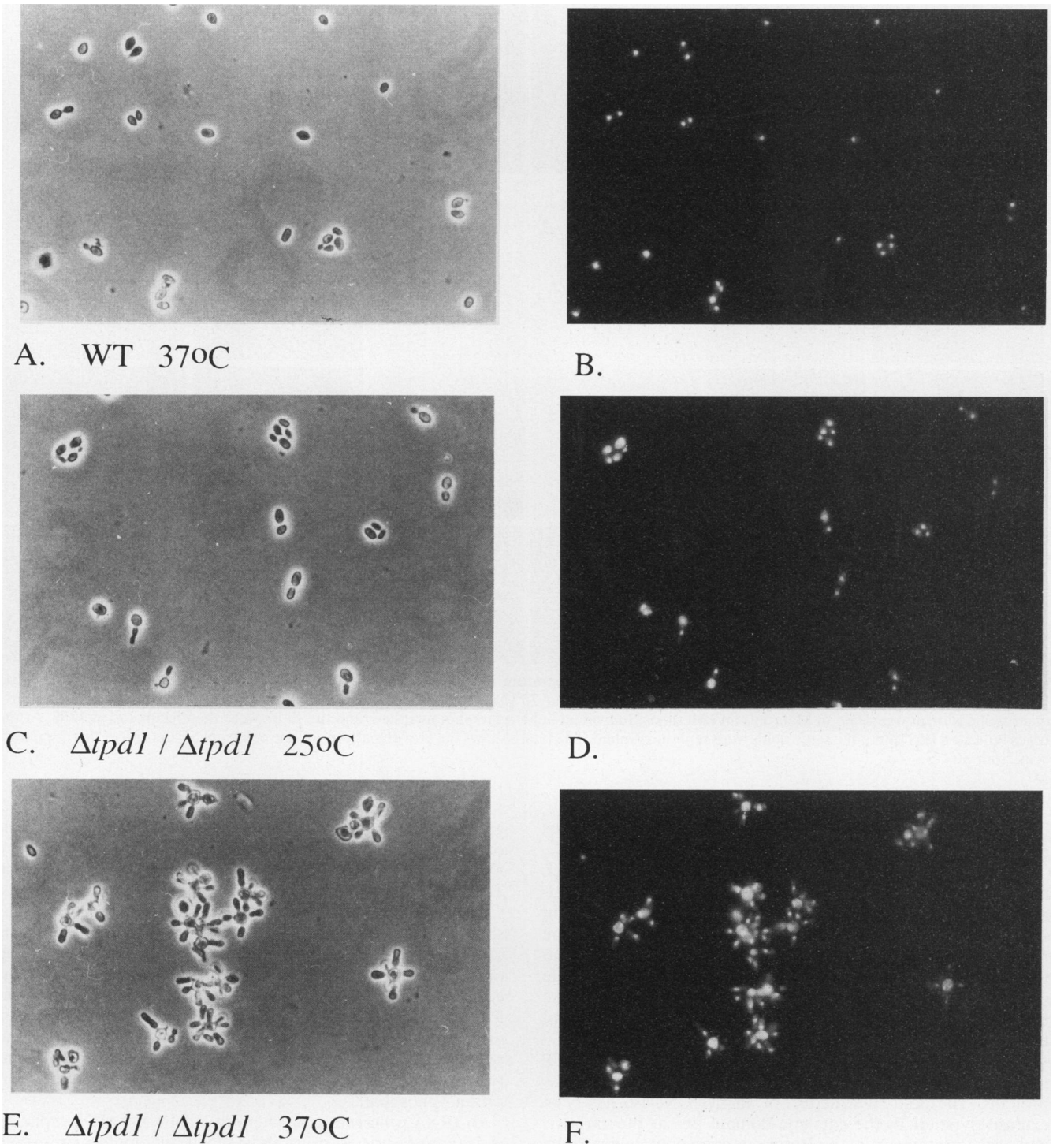

FIG. 7. *tpd1*::*LEU2* diploid strains accumulate multiple buds at the elevated temperature. Cells were grown at 25°C in rich medium to 5×10^6 cells per ml, shifted to 25 or 37°C overnight as indicated, stained with DAPI, and photographed as described in Materials and Methods. (A, C, and E) Phase contrast; (B, D, and F) fluorescence; (A and B) wild-type [WT] diploids (SC472) at 37°C; (C and D) *tpd1*::*LEU2*/*tpd1*::*LEU2* diploids (SC475) at 25°C; (E and F) *tpd1*::*LEU2*/*tpd1*::*LEU2* diploids (SC475) at 37°C.

Although we have measured Tpd1p as being a minor fraction of the total PP2C activity in the cell, this may not be the case. If Tpd1p is a unique species of PP2C with its own set of preferred substrates, then activity with respect to ^{32}P-casein may not be an accurate indicator of Tpd1p's activity relative to that of other PP2Cs. Alternatively, the level of Tpd1p in extracts may not represent the level of Tpd1p in the cell. This could occur if extraction is incomplete or if *TPD1* expression is regulated in the cell. There is evidence that mammalian PP2C may be regulated; its mRNA levels have been shown to

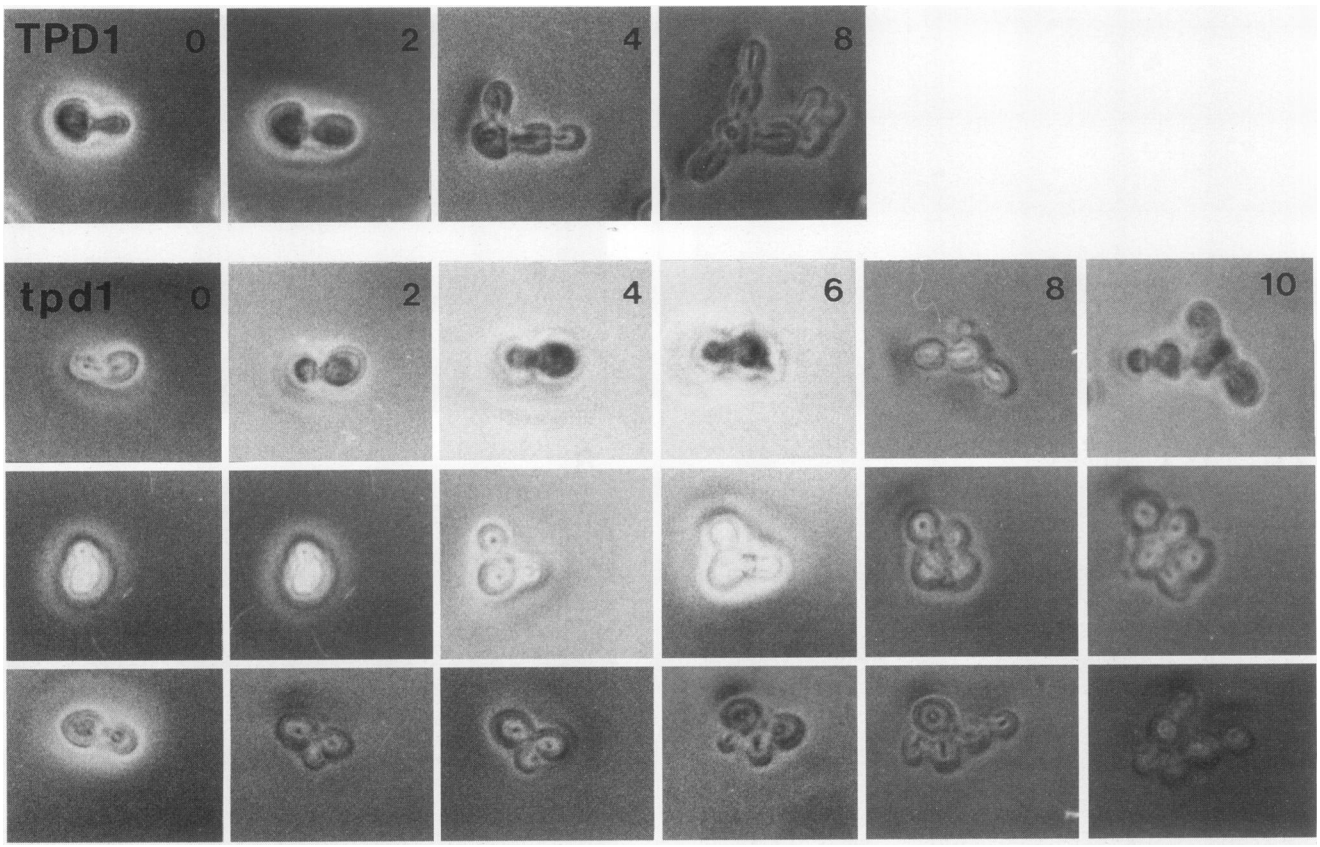

FIG. 8. *tpd1* cells continue to grow at the nonpermissive temperature but produce nonviable daughter cells. Strains SC462 (*TPD1/TPD1* [TPD1]) and SC475 (*tpd1*::*LEU2/tpd1*::*LEU2* [tpd1]) were grown at 25°C in YEPD to 5×10^6 cells per ml and plated on YEPD agar on a microscope slide as described in Materials and Methods. Individual fields were photographed, and the slides were then incubated at 37°C. At the times indicated (in hours), the same fields were rephotographed. The figure shows the growth patterns of one cell from SC462 and of three separate cells from SC475.

increase during the differentiation of stem cells into myoblasts and subsequent differentiation into myotubes (51).

Is Tpd1p related to adenylyl cyclase? Our homology search indicated that Tpd1p exhibits substantial homology over almost its entire length to a contiguous stretch of amino acids in adenylyl cyclase from *S. cerevisiae*. Not surprisingly, this same region of adenylyl cyclase also exhibits extensive homology to mammalian PP2C, as has been previously noted (69). The degree of homology between this region of adenylyl cyclase and either Tpd1p or mammalian PP2C is essentially equivalent to that between Tpd1p and PP2C.

Adenylyl cyclase in *S. cerevisiae* catalyzes conversion of ATP to cAMP in response to the activity state of the yeast Ras proteins. The domain structure of adenylyl cyclase has been extensively studied: the catalytic domain lies at the carboxyl end of the protein, while the Ras-responsive domain lies in the central half of the protein, encompassing a region consisting of multiple repeats of a leucine-rich motif. The region of homology to Tpd1p lies between these two domains, in a portion of the protein that has not been associated with any function (9, 67). An intriguing and testable hypothesis suggested by this homology is that adenylyl cyclase actually possesses protein phosphatase activity in *S. cerevisiae*, and we are in the process of testing this possibility.

Tpd1p is involved in multiple essential processes in the cell. *tpd1* strains are temperature sensitive for growth, even when the gene is completely inactivated. Thus, Tpd1p is required for growth only at elevated temperatures, suggesting either that other components in the cell compensate for the absence of Tpd1p at lower temperatures or that the processes mediated by Tpd1p become rate limiting only at the elevated temperatures. Consistent with the former interpretation, Maeda et al. (36) showed that *tpd1* (designated *ptc1* in their report) mutations exhibit synthetic lethality with *ptp2*, a gene encoding a homolog of mammal protein tyrosine phosphatases. This finding suggests that Ptp2p can compensate for the absence of Tpd1p at lower temperatures. Nonetheless, at elevated temperatures, *tpd1* strains exhibit a number of phenotypes even in the presence of Ptp2p and at least one other PP2C. These phenotypes yield some insight into the biological role of the Tpd1p protein phosphatase.

(i) tRNA processing. Biochemical analysis of tRNA splicing in *S. cerevisiae* has precisely defined the molecular events associated with removal of the IVS from the tRNA. Direct measurement of the processing endonuclease in *tpd1* and *TPD1* indicates that *tpd1* strains are not diminished in this activity, nor are such strains deficient in either of the two other activities directly responsible for tRNA splicing: tRNA ligase and 2′-tRNA phosphotransferase. Thus, Tpd1p likely affects the splicing pathway indirectly. Mutations in five other genes, *PTA1* (50), *LOS1* (25, 29), *SEN1* (12, 77), *RNA1* (24, 71), and *STP1* (75), all affect tRNA splicing at the endonuclease step in vivo, for reasons that are as yet unclear. None of these genes encode a protein with a known catalytic role in tRNA splicing;

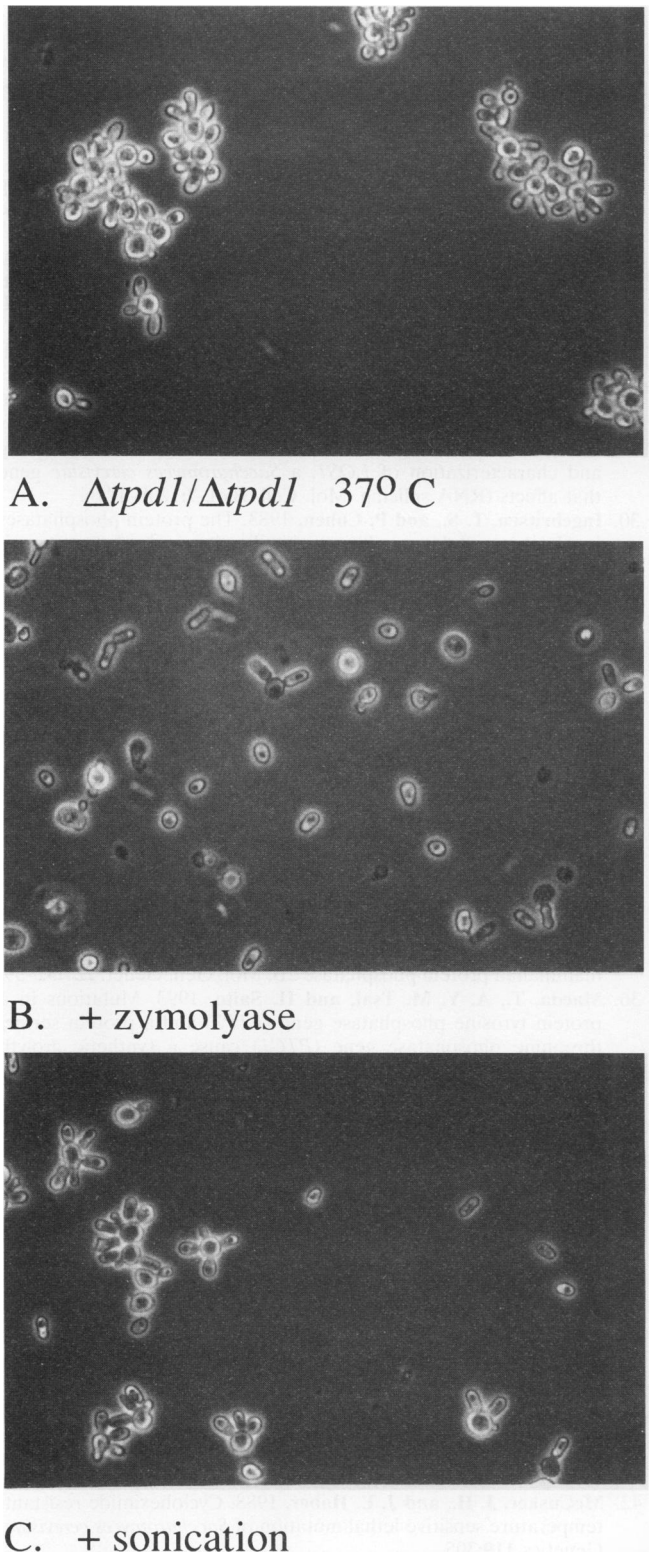

FIG. 9. *tpd1*::*LEU2* diploids are defective in cell separation. *tpd1*::*LEU2*/*tpd1*::*LEU2* diploid (SC475) were grown at 25°C in rich medium to 5×10^6 cells per ml, shifted to 37°C overnight, fixed, treated as indicated, and photographed as described in Materials and Methods. (A) No treatment; (B) zymolyase treatment; (C) probe sonication for 3 s.

indeed, the *RNA1* gene product is known to be cytoplasmic, whereas the tRNA splicing machinery is nuclear (26). Tpd1p might in principle dephosphorylate and activate any of these gene products to increase endonuclease efficiency in vivo.

(ii) Sporulation. Diploid cells homozygous for *tpd1* fail to sporulate or sporulate significantly less well than isogenic strains heterozygous or homozygous for *TPD1*. This defect is apparently due to an inability of such strains to use a variety of nonfermentable carbon sources. *tpd1* strains are not simply respiratory deficient, since they can grow on a subset of nonfermentable substrates and growth at least on ethanol is remediated by addition of 1 M sorbitol to the growth medium. Since the sporulation defect is also remediable to some extent by inclusion of 1 M sorbitol, we conclude that the sporulation defect results predominantly from the carbon source utilization defect.

The role of sorbitol in reversal of the carbon utilization defect is not clear. *tpd1* cells are not osmotically sensitive when grown on ethanol. Further, since the *tpd1* allele in these strains is a deletion, sorbitol does not act as an information suppressor through translational misreading. Thus, this unusual pattern of carbon source use and the role of sorbitol in it requires additional investigation.

(iii) Cell separation. *tpd1* strains exhibit two cell cycle defects. The first is an apparent asymmetric loss of viability. Following transfer to the nonpermissive temperature, preexistent cells continue to produce new daughters, while almost all of the daughters born at the high temperature fail to undergo a new round of cell division. Since the daughters all have nuclei, the phenotype is not like that of *cdc4*, in which the budding cycle is disengaged from the nuclear cycle. A strikingly similar phenotype was reported for alleles of *pma1*, which encodes the major plasma membrane ATPase (43). One possibility suggested by this correlation is that the plasma membrane ATPase is a substrate for the Tpd1p phosphatase and that its phosphorylation state affects the ability of cells to initiate a new cell cycle.

The second cell cycle defect exhibited by *tpd1* strains is the failure to undergo cell separation at the nonpermissive temperature. A number of genes have been shown to be required for cell separation. Mutants defective in chitinase or in a gene designated *ESS1* yield a cell separation phenotype similar to that of *tpd1* strains (18, 33). Thus, either of these proteins could be a substrate of Tpd1p. In addition, activation of PP2A or inactivation of yeast casein kinase II results in cytokinesis and cell separation defects (58, 59, 73), although in this case the cell separation defect may be only indirectly related to altered phosphorylation properties of these strains.

Tpd1p is the first PP2C species to be isolated and cloned in *S. cerevisiae*. Further genetic and biochemical analysis should allow us to determine the cellular roles of Tpd1p. Comparisons of other protein phosphatases found both in *S. cerevisiae* and higher eukaryotes have shown a great deal of functional conservation. Therefore, data obtained on Tpd1p may give us insight into the functions of PP2Cs in higher eukaryotes.

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