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ORIGINAL PAPER

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Suppressors of the temperature sensitivity of DNA polymerase α mutations in *Saccharomyces cerevisiae*

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Abstract We have isolated two high copy, allele-specific suppressors of the temperature sensitivity of mutations in *POL1*, the gene that encodes the catalytic subunit of DNA polymerase α in the yeast *Saccharomyces cerevisiae*. Both genes, *PSP1* and *PSP2*, also partially suppressed a mutation in *POL3* which encodes DNA polymerase δ, and both also affected a mutation in *CDC6*, which acts in initiation of DNA replication. Suppression was not general, since ts mutations in several genes unrelated to replication were not affected. *PSP1* was partially effective on low-copy-number vectors, while *PSP2* required high copy numbers. The presence of suppressing plasmids did not alter the steady-state level of Pol1 protein, so suppression does not appear to be due to an increase in production or stability of Pol1p. Deletion of either *PSP* gene or both in combination resulted in apparently normal viable cells. While neither gene is homologous to genes with known functions, *PSP1* and *PSP2* both have unusual amino acid compositions: *PSP1* is rich in asparagine and glutamine, while *PSP2* is rich in asparagine and contains "RGG" motifs that have been associated with RNA-binding proteins. We also describe a transposon-mediated strategy that should be generally effective for rapid characterization of multicopy suppressors.

Key words Multicopy suppression · Transposon mutagenesis · DNA polymerase · POL1 · POL3

Introduction

The yeast *Saccharomyces cerevisiae* has three essential DNA polymerases that are needed for DNA replication: polymerases α, δ and ε (Lucchini et al. 1988; Boulet et al. 1989; Sitney et al. 1989; Morrison et al. 1990; Campbell and Newlon 1991; Budd and Campbell 1993; Newlon 1996). DNA polymerase α is associated with subunits of a primase activity, but has low processivity and little or no proofreading exonuclease, while polymerases δ and ε are both processive in the presence of appropriate accessory factors and have proofreading ability (Campbell and Newlon 1991; Linn 1991; Wang 1991; Newlon 1996). Studies of the human homologs of these polymerases using the in vitro SV40 replication system have suggested that Polα and Polδ act at the leading and lagging strands of the replication fork, respectively (Wold et al. 1989; Tsurimoto et al. 1990; Weinberg et al. 1990; Eki et al. 1992; Kornberg and Baker 1992; Stillman 1994; Waga and Stillman 1994; Waga et al. 1994). Other studies suggest critical roles for Polε in replication, repair, and monitoring of DNA damage (Araki et al. 1992; Wang et al. 1993; Navas et al. 1995). The specific tasks performed by the three polymerases during chromosomal replication therefore remain controversial.

The catalytic subunits of DNA polymerases α and δ in *S. cerevisiae* are encoded by the genes *POL1* and *POL3*, respectively (Johnson et al. 1985; Lucchini et al. 1988; Boulet et al. 1989; Sitney et al. 1989; Morrison et al. 1990; Campbell and Newlon 1991; Newlon 1996). These genes were also found in a screen for mutations that blocked progression of the cell cycle at elevated temperatures (Pringle and Hartwell 1981); *POL1* is allelic to *CDC17* and *POL3* to *CDC2* (Carson 1987; Sitney et al. 1989). At 36° C, cells with the *cdc17-1* mutation in *POL1* (G904D; Lucchini et al. 1990) arrest uniformly as uni-nucleated, large-budded cells with approximately 2C DNA contents, but at 37–38° C replication is rapidly blocked (Pringle and Hartwell 1981; Carson 1987; Lucchini et al. 1990). *POL1* is therefore needed for replication fork elongation, but the cdc17-1 product is functional enough at 36° C to allow the bulk of S phase to be completed, even though mitosis is still blocked.

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Assays for multicopy suppression have been used successfully to identify interacting components of protein complexes (Rine 1991). For example, mutations in the *CDC28* gene, which encodes the *S. cerevisiae* cyclin-dependent kinase, are phenotypically suppressed by increases in the dosage of yeast cyclin genes (Hadwiger et al. 1989), and mutations in the protein kinase required for initiation of replication that is encoded by *CDC7* are suppressed by high levels of the *DBF4* gene, which encodes a regulatory subunit of the kinase complex (Kitada et al. 1992; Jackson et al. 1993). High levels of an interacting protein could drive marginally stable mutant proteins into complexes that are less susceptible to thermal denaturation, or high levels of an activator might induce normal activity from a partially active protein. A suppressor might also share, or induce a gene that shares, activity with the mutated gene, thereby reducing the need for the mutant gene product. Suppression could also be due to an elevated rate of production or decreased rate of destruction of the mutant gene product, leading to higher steady-state protein levels.

We previously found that mutations in two proteins that bind to Pol1 enhance *pol1* mutations, leading to a decrease in the maximal permissive temperature (Miles and Formosa 1992; Wittmeyer and Formosa 1997). Since decreases in the level of known binding partners enhanced *pol1* mutations, we sought high-copy suppressors of these mutations since they might identify proteins that are either members of the DNA replication complex or that regulate the expression or stability of such proteins. We describe here two genes, *PSP1* and *PSP2* (polymerase **s**uppressors), that partially suppressed the thermosensitivity of *POL1* mutations and were then also found to act on a DNA polymerase δ mutation.

Materials and methods

Strains and media

Yeast strains (Table 1) were either obtained from L. Hartwell and B. Garvik (University of Washington, Seattle) or derived from crosses among such strains; all were congenic with A364a. Media were prepared as described (Hartwell 1967; Rose et al. 1990).

Table 1 Strains used

7236	*MAT***a**/*MAT*α *his7*/+ *his3*/+ *leu2*/*leu2 trp1*/*trp1 ura3*/*ura3 can1*/+ *cyh2*/+
7311-2-4	*MAT***a** *his7 leu2 ura3 cdc17-1 pep4 prb1 can1*
7364-4-1	*MAT***a** *his7 leu2 trp1 ura3 cdc2-3*
7382-3-4	*MAT***a** *his7 leu2 trp1 ura3 can1 pep4 prb1*
7389-5-3	*MAT***a** *his3 leu2 trp1 ura3 cyh2 cdc17-1*
7391-1-1	*MAT***a** *his3 leu2 trp1 ura3 cdc17-2*
7417-2-3	*MAT*α *his3 leu2 trp1 ura3 cyh2*
7604-6-2	*MAT***a** *his7 leu2 trp1 ura3 cdc6-1*
7614-9-2	*MAT***a** *his7 leu2 trp1 ura3 cdc6-1*
7652-10-3	*MAT***a** *his7 leu2 trp1 ura3 cdc6-1 psp2-*Δ*1* (::*URA3*)

Plasmids

Yeast genomic libraries in the high-copy-number vectors YEp24 (M. Carlson and D. Botstein) and pTF63 (Miles and Formosa 1992) were used. pAD1 (containing *PSP1*) and pJB8 (containing *PSP2*) were isolated from the YEp24 and pTF63 libraries, respectively. The insert in pJB8 was removed as a *Cla*I-*Xba*I fragment and inserted into pHSS6 (Hoekstra et al. 1991) to produce pTF86. *Bam*HI-*Sal*I fragments containing *PSP1* or *PSP2* (see Figs 3 and 4; the *Sal*I site for *PSP2* clones was contributed by a transposon) were inserted into YEpFAT10 (Wellinger et al. 1993) to produce pTF100 and pTF101, respectively, which contain both *TRP1* and *leu2-d* markers for high or very high copy number selection. The same fragments were inserted into YCplac33 (Gietz and Sugino 1988) to form the low-copy-number plasmids pTF108 and pTF109.

To delete *PSP1*, the 316-bp *Bam*HI-*Dra*I fragment from the 5′ end of the gene and the 1295-bp *Eco*RI-*Kpn*I fragment from the 3′ end were cloned into YIplac128 (Gietz and Sugino 1988; Sikorski and Hieter 1989) to form pTF70. After digestion with *BamH*I this was transformed into a diploid strain (7236) using the lithium acetate method (Rose et al. 1990). Correct transplacement was verified by hybridization analysis (Sambrook et al. 1989) and disrupts the *PSP1* open reading frame (ORF) at codon 18 and removes 84% of the coding region. To delete *PSP2*, a 564-bp *Hin*dIII-*Sal*I fragment from the 5′ end of the gene and a 938-bp *Bam*HI fragment (some sites were derived from transposons) from the 3′ end were inserted into pRS406 (Christianson et al. 1992) to produce pTF82. Digestion of this plasmid with *Eco*RI and subsequent transplacement results in removal of 92% of the ORF and truncates the *PSP2* product at residue 21.

For transformation by electroporation, strains were grown to saturation in YM-1, collected by centrifugation, washed several times with 10% glycerol, suspended in 10% glycerol at about 2×10^9 cells/ml, then DNA was added to 60-μl aliquots and electroporated with a 5 ms pulse at 300 V (BTX Transfector 100).

Transposon mutagenesis

pTn-URA3 (Hoekstra et al. 1991) was digested with *Eco*RI, and the ends were filled in and ligated (Sambrook et al. 1989). The resulting plasmid was linearized with *Bam*HI and the 1576-bp *Sau*3A fragment from Yep24 containing the 2-μm origin of replication was inserted (Gietz and Sugino 1988). This mTn3-URA3, 2-μm element was transposed to the F′ element pOX38 (Hoekstra et al. 1991) and the resulting factor was introduced by conjugation into *Escherichia coli* strain DH1. pLB101 (Hoekstra et al. 1991), a plasmid containing the Tn*3* transposase, was transformed into this strain to produce BTF35. BTF35 was transformed with target plasmids, and these were then transferred by conjugation to strain NS2114 (Sm, λcre) for resolution of cointegrates (Hoekstra et al. 1991). The resulting plasmids carried the transposon with the *URA3* gene and the 2-μm origin of replication at random locations. The transposon was localized in a collection of isolates of pTF86 by restriction digestion, then tested for suppression activity.

Nucleotide sequences were determined using Sequenase as described by the manufacturer (US Biochemicals), with appropriate oligonucleotide primers for standard vectors or for the transposons constructed above (Hoekstra et al. 1991).

Electrophoresis and immunodetection

2×10^6 cells growing at log-phase in selective medium were suspended in SDS sample buffer, boiled for 5 min, then electrophoresed through 7.5% polyacrylamide (SDS-PAGE, Sambrook et al. 1989). Proteins were transferred to nitrocellulose and detected using a polyclonal rabbit antiserum generated against purified intact Pol1 protein (Miles and Formosa 1992) and alkaline phosphatase conjugate detection of the primary antibody using a colorigenic substrate (Harlow and Lane 1988).

Results

Isolation of multicopy suppressors of the *cdc17-1* phenotype

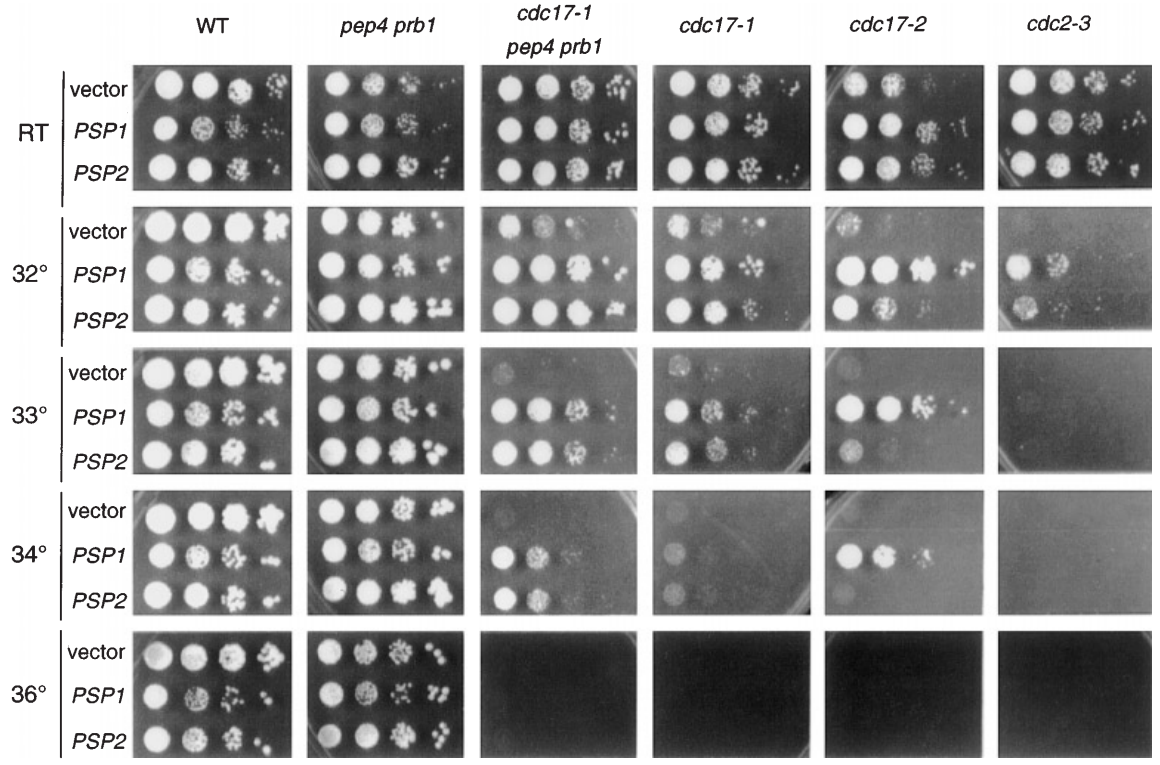
Libraries of yeast genomic sequences in high-copy-number (2-μm-derived) vectors were transformed into strain 7311-2-4 containing the *cdc17-1* allele of the *POL1* gene. About 43 000 transformants representing about 25 genome equivalents of insert DNA were screened for the ability to grow at temperatures from 34° C to 36° C; the maximal permissive temperature (MPT) for the *cdc17-1* strains used was 31–32° (see Fig. 1; suppression in strain 7311-2-4 by plasmids carrying *PSP1* or *PSP2* is most readily seen at 33° to 34° C). Clones capable of supporting growth at 35° C and higher were found to contain the wild-type *POL1* gene (not shown). Seventeen plasmids that allowed growth at 34° C upon recovery and retransformation were identified. Restriction digestion patterns and nucleic acid hybridization were used to place the plasmids in nine groups. Plasmids containing *PSP1* and *PSP2* were isolated three times each, and *PSP2* was also isolated as a suppressor of *cdc2-3* (an allele of *POL3*, which encodes the catalytic subunit of Pol δ) in a separate screen.

Fig. 1 Suppression of polymerase mutations by *PSP1* and *PSP2*. Strains 7417-2-3 (WT), 7382-3-4 (*pep4 prb1*), 7311-2-4 (*cdc17-1 pep4 prb1*), 7389-5-3 (*cdc17-1*), 7391-1-1 (*cdc17-2*), and 7364-4-1 (*cdc2-3*) were transformed with pTF63 (vector), pAD1 (*PSP1*), and pJB8 (*PSP2*), and grown to saturation in medium lacking uracil. Tenfold dilutions were prepared and aliquots spotted on plates lacking uracil; these plates were then incubated at the temperatures indicated

Suppression of *cdc17-1* was more effective in a strain lacking the vacuolar proteases encoded by *PEP4* and *PRB1* (Fig. 1; Jones 1990). In addition to its effect on *cdc17-1*, *PSP1* also efficiently suppressed the *cdc17-2* mutation of *POL1*, while *PSP2* had only a weak effect on this allele (Fig. 1; the MPT for *cdc17-2* was 30–31° C). *PSP2*, but not *PSP1*, partially suppressed the *pol1-17* phenotype (not shown). Both genes partially suppressed the *cdc2-3* mutation (MPT 29–30°; Fig. 1), indicating that they are effective with mutations in two distinct polymerases.

Specificity of interactions with *PSP* genes

To test whether *PSP* genes act in general to alleviate temperature sensitivity, we transformed high-copy-number plasmids (pTF100 and pTF101) containing *PSP1* or *PSP2* into strains with other ts mutations, including *cdc2-1*, *cdc6-1*, *cdc7-1*, *cdc8-1*, *cdc14-1*, *cdc15-2*, *cdc16-1*, and *cdc28-1*. The maximal permissive temperature was determined under *TRP1* selection (50 copies per cell) and under *leu2-d* selection (200 copies/cell; Wellinger et al. 1993). The temperature sensitivity of strains with *cdc2-1* (not shown, but see Fig. 1 for *cdc2-3*) and *cdc6-1* (Fig. 2) was suppressed by *PSP1* but none of the other strains were affected, and none of the strains were affected by the *PSP2* plasmid. This result shows that while *PSP* genes suppress multiple alleles of two DNA polymerase genes and another gene required for the function of DNA replication origins (Newlon 1996; Stillman 1996), they are not general ts suppressors since they do not affect other ts mutations in genes that act elsewhere in the cell cycle.



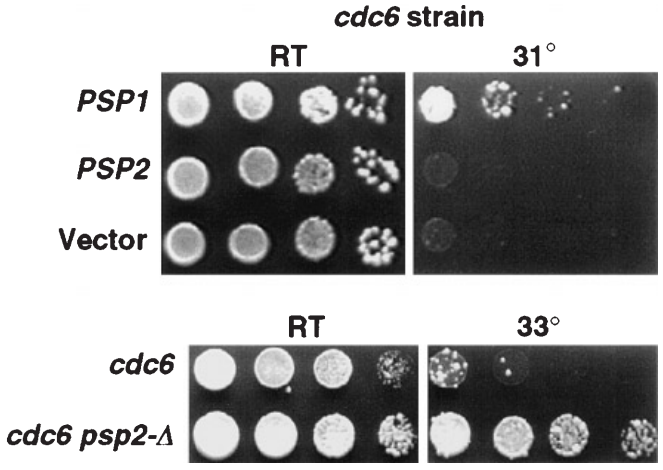


Fig. 2 A *cdc6-1* mutation is suppressed by increased *PSP1* dosage or by deletion of *PSP2*. Strain 7604-6-2 was transformed with pTF100 (*PSP1*), pTF101 (*PSP2*), and YEpFAT10 (vector). Strains 7614-9-2 (*cdc6*) and 7652-10-3 (*cdc6 psp2*-Δ) were constructed by standard genetic methods. Dilutions of saturated cultures were tested for growth as in Fig. 1 on selective medium (lacking tryptophan, *top panel*) or rich medium (*bottom panel*). The maximal permissive growth temperatures for *cdc6-1* are different on the two types of media

Table 2 Amino acid composition of PSP1 and PSP2

Protein	Molar % of residue (percentile ranking) [a]			
	N	Q	G	ILV
PSP1	13.7	6.9	4.3	18.1
	(99.4)	**(95.0)**	(37.2)	(16.5)
YLR177w	8.9	7.2	4.3	20.5
	(92.6)	**(95.5)**	(62.2)	(34.9)
PSP2	11.4	3.5	10.4	11.2
	(98.4)	(45.1)	**(97.8)**	**(0.8)**
ASM4	15.0	6.3	5.7	15.2
	(99.7)	(92.2)	(64.1)	(5.1)
Average	5.74	3.86	5.17	21.9
S.D.	2.2	1.9	2.2	4.2

[a] The YPD database (Garrels et al. 1994; version 7.17a, 6032 entries) was used to determine the relative abundance of various amino acid residues in *S. cerevisiae* proteins [average and standard deviation (S.D.) as shown]. The molar percentage and the percentile ranking (*in parentheses*) are given for PSP1 and its homologue YLR177w, PSP2, and ASM4, which blocks suppression of *pol3* by *sdp1* mutations (Giot et al. 1995). Notable values are indicated by *bold type*

Characterization of *PSP1*

Subclones of the insert containing *PSP1* were tested for suppression and a 4.5-kb fragment with activity was identified (Fig. 3). This was inserted into a high-copy-number yeast shuttle vector and deletions were generated using the exonuclease III method (Henikoff 1987). *PSP1* suppressor activity was found to coincide with a 2520-bp ORF (YDR505c) capable of encoding a 95 534-Da protein composed of unusually high levels of N and Q residues, with an asparagine content greater than those of 99.4% of the 6032 known yeast proteins (Table 2, also see Fig. 5). A gene with significant homology to *PSP1* (YLR177w) is found in the yeast genome, but no other similar genes have been identified and the function of YLR177w is not known. *PSP1* is also identified in the Saccharomyces Genome Database as *GIN5*, indicating that high-copy-number plasmids bearing this gene cause growth inhibition, but we do not see any evidence of this phenotype (see Fig. 1).

A deletion of the *PSP1* ORF was constructed as shown (Fig. 3). Strains with the deletion were found to be viable and to have no obvious phenotype: growth rates were normal at elevated temperatures, cells show a normal morphological distribution, and normal stability of plasmids, a chromosome III fragment, and an intact chromosome V (not shown, assays described in Wittmeyer and Formosa 1997). Strains with *cdc2-3*, *cdc4-1*, *cdc6-1*, *cdc8-1*, *cdc9-1*, *cdc15-2*, *cdc16-1*, *cdc17-1*, *cdc17-2*, or *cdc23-1* mutations all displayed the same maximal permissive temperature regardless of whether *PSP1* was intact or deleted (not shown). Therefore, while increased levels of *PSP1* suppressed mutations in two polymerase genes, decreased levels did not appear to enhance these same mutations or any others.

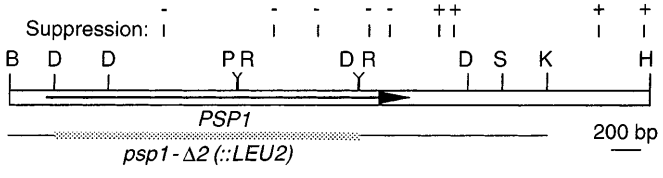


Fig. 3 The *PSP1* region. A 4.5-kb *Bam*HI-*Hin*dIII fragment of pAD1 was subcloned into pTF63 and found to contain full suppression activity. Deletions were generated using the exonuclease III method (Henikoff 1987) and tested for suppression of *cdc17-1*. All deletions from the *Bam*HI side failed to suppress, while deletions from the *Hin*dIII side to the sites indicated retained activity as noted. These and other deletions were used to determine the nucleotide sequence of this region (GenBank Accession Number U33115). Integration of pTF70 as shown creates a deletion of most of *PSP1*. Restriction sites shown are: B, *Bam*HI; D, *Dra*I; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I

Characterization of *PSP2*

The 9.6-kb insert from the original *PSP2* clone pJB8 was inserted into pHSS6 (Hoekstra et al. 1991). The mini-Tn*3* system developed by Heffron and co-workers (Hoekstra et al. 1991) was modified by inserting the origin of replication from the endogenous yeast 2-μm plasmid into mTn3-URA3 (see Materials and methods). The resulting transposon carries a yeast selectable marker and a high-copy replication origin, and so converts pHSS6-based plasmids into high-copy-number yeast shuttle vectors. Since the transposon integrates randomly, derivatives can be easily obtained that have a disruption in different locations and can be tested directly for suppression. The same derivatives can also be used for nucleotide sequencing using primer sites in the

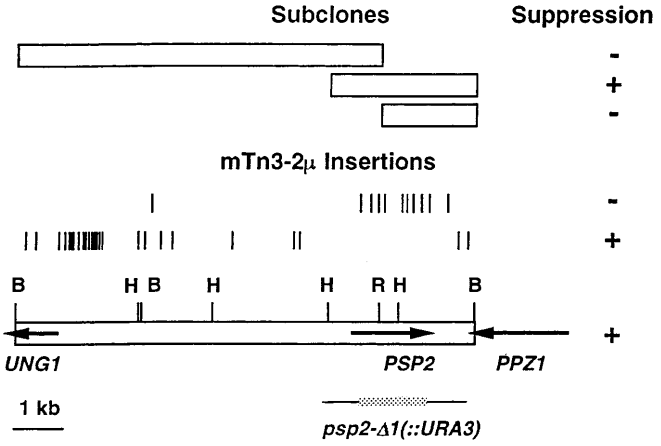


Fig. 4 Mapping of the *PSP2* region. pTF86 was mutagenized with the mTn3-URA3, 2-μm element (see Materials and methods), the locations of insertions of the transposon were determined by restriction endonuclease digestion, and the suppression of a *cdc17-1* strain was assayed as in Fig. 1. Derivatives that failed to suppress were found to disrupt the *PSP2* ORF (the single exception could be due to a mutation in this clone unrelated to the transposon insertion). A subclone containing only the *PSP2* ORF retained suppression activity, confirming the results of mTn3 mapping. The nucleotide sequence of a 3092-bp *Hin*dIII-*Bam*HI fragment was determined using primers complementary to the transposon (GenBank Accession Number U33116, YML017w). *PSP2* was deleted as shown (pTF82, see Materials and methods). Sites are as in Fig 3

transposon. Using this strategy, we were able to identify *PSP2* as the suppressor gene and determine its nucleotide sequence (Fig. 4).

The region required for suppression coincided with a 1737-bp ORF (YML017w) capable of encoding a 63 981 Da protein. This ORF had no homologs in available databases, but had an unusual composition, including high levels of N and G, and very low levels of the aliphatic side-chain residues I, L, and V (Table 2). While both *PSP1* and *PSP2* have high levels of N, this residue is dispersed throughout *PSP1* but clustered in *PSP2* (Fig. 5). *PSP2* also contains four copies of an "RGG" motif that has been associated with RNA-binding proteins (Burd and Dreyfuss 1994). A deletion of *PSP2* was constructed and again the resulting strain was found to be viable and the deletion had no effect on growth rate, morphology, plasmid stability, or chromosome stability, either alone or together with a deletion of *PSP1* (not shown). Strains with *cdc2-3*, *cdc4-1*, *cdc8-1*, *cdc9-1*, *cdc15-2*, *cdc16-1*, *cdc17-1*, *cdc17-2*, or *cdc23-1* mutations all displayed the same maximal permissive growth temperature with intact *PSP2* or a deletion of this gene (not shown), but the deletion suppressed the temperature sensitivity of a *cdc6-1* mutation in isolates from two independent and congenic crosses (Fig. 2). As observed with *PSP1*, decreased levels of *PSP2* did not diminish the stability of polymerase or other mutations, but the one gene found to be affected by its deletion was also a gene needed for DNA replication, in this case initiation (Stillman 1996).

Psp1

MDLPTVNSTTSISDNVDLKNYYEDLLFKNNSGKSLSDLPRKLNDNS
NNSGSDTVDPLAGLNNLRNSIKSAGNGMENRRTFDDIDFMGRFPYL
PPVPNQQQQPFSHQNGFIQEHPSSNLTSFQMSSSNSEPMSAPPISS
NNNNLNSTQMGNYQAQQRSFPQFNGNSFHSNGNDLMGNRMDSDYMR
LMNKTNIGFTSNSGSNFAAPSHSAGNPSSMNNQQVPSFNWQQPSHP
ESTIRRSSYISDTLINHQMPDARQKQTSQVQQQHAQGFNLFNSRFN
YDNLNSTHLTAKGVPEFGNGVQPPYPYDNEPNNASISNSNNNNNSH
NMVPMQQFRRNTQPVASFNPSLPTFQQQQQQPQQPQQPRNVNVPTS
FNGERVDDVQLVQLQRSSSVPSSTNSHNLQNENSNEGNVSLDNGLV
LIQGKHLTSSKTLHDLYSDCGSGYFASSAVFEFTDNIKKMLKLHDS
NESYDAKNMGLIDEEGNTYQSLLNFLDILRSCNMNYVNDPESNNGI
VSNNGGNKNRRKGSFTTELSCRNANNSFLPYTPLVLVALKNGKLEL
LSTPQATNLLLKRGDLVIIDGDRGRDLVLVVEPSVDLNLALFINFL
KKKIHFDSLITSESQHYRNDEFIQMLIDSKNGQKKKLNPKLYDVVE
LTELIIPSKQVLRFATPWEVTTNLHNKFEDELKALHIAQSKLQALN
DNSKSQNTNDSSSNNFTNAATYSKPKLNIKILNAEFQFDRKELTFY
YVCEERNDFRDLIKELFKYYKTRIWLCAIPNNLSIDSKYYDKQQKE
LKLYQNIVKNYNAEDLMNVNEFSQNRGNNRVNFAPPLNEIELDNFQ
IAVYEELVHELFH

Psp2

MSLEEFLGNDTLGESVWDEEDINLDAISNTTNIDILKQTKAGEHQR
DGHQQHPHGGHGPMNRSRFSNAGPFGGGSMGDFANHHHPLQHQQGP
PYIVKFSDLPPRFSNFDIEDLFQAKFTKFIKFKLFWEINKNPSIST
LKSGSIFDQNFKRDSKVAFVELYTSRDMDKILNYWTTPLKEIYHIT
TAPAEFEDFKDYSTKVKLLTDPKDDAGKPFITKTQRSKSNPFGSAK
PVDTQSKILDIEEKMENLHVEDTTTLRASLIPSSDSMATTATGSKI
TILKKQTPTEEESHSATPTPKPLSYSEVVERSVVNETSKKGTPLSK
LDSPALELQSKPDKSDEFKGGDEQGFEKGGDDKAQLDVSNDKDKGS
ETDVDKQFTFKNVEREHSMSRTKYNGNHNNNNGNFRGSNRYRGGPN
GSSYKGGHNNRGNRGGYRGGSSYNNNNNNTNDNNNNNNNSSSNNNN
GSRYHDRQNNEEGLTSDSSLDASGNKKNDFTNSTSNTQQYSIFKPA
SGFLGQGNNDSIRNNGRGNYNSSGMNGGSRGRGFGRGRGFGRGAYN
NRGSRGGRGSSGNYSNYNNRTTDMPL

Fig. 5 Features of the primary sequences of *PSP1* and *PSP2*. The amino acid sequences of *PSP1* and *PSP2* were inferred from their nucleotide sequences. N and Q residues are shown in *bold type*. For *PSP2*, four repeats of the "RGG" putative RNA-binding motif are shaded (Burd and Dreyfuss 1994). Three repeats of the chemically similar motif "KGG" are also found

Copy number effects

PSP1 and *PSP2* were isolated from libraries constructed in high-copy number vectors. To test whether high copy numbers are needed for suppression, each gene was cloned into a vector containing a centromere, which is expected to reduce the copy number to near 1 per cell (Campbell and Newlon 1991). As shown in Fig. 6, *PSP1* retained most of its effectiveness on a low-copy-number

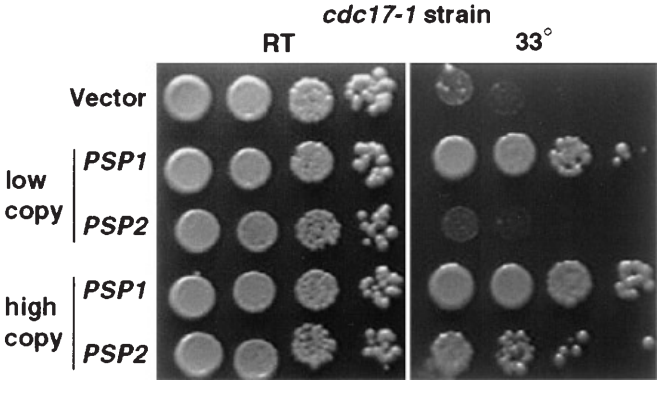


Fig. 6 Suppression is affected by plasmid copy number. Strain 7311-2-4 was transformed with vector (YCplac33), low-copy-number *PSP1* (pTF108) or *PSP2* (pTF109) plasmids, or high-copy-number *PSP1* (pAD1) or *PSP2* (pJB8) plasmids, and dilutions were tested for growth at RT and 33° C as in Fig. 1

vector, while *PSP2* suppressed only in a high-copy-number context. Apparently, one or a few extra copies of *PSP1* on a plasmid are sufficient to suppress a *cdc17-1* mutation, but *PSP2* expression must be more significantly elevated to produce an effect.

Suppression does not appear to involve elevated levels of Pol1 protein

High-copy-number suppression could be due to stabilization of the mutant polymerase protein, either by reducing its turnover or increasing its production. We measured the level of intact Pol1 protein in strains containing the suppressor plasmids by immunodetection after SDS-PAGE. As shown in Fig. 7, the levels of intact, wild-type Pol1 protein were similar in cells carrying either a suppressor plasmid or the empty vector when grown under suppressing conditions (34° C). However, we were unable reproducibly to detect intact Pol1 protein in extracts from cells with *cdc17-1* or *cdc17-2* mutations with or without suppressor plasmids, even when more sensitive chemiluminescent detection methods were used. It is therefore possible that the mutant Pol1 proteins produced by *cdc17* alleles are stabilized by the suppressors even though the wild-type Pol1 is not. The mutant proteins are not stabilized enough, however, to allow detection in this assay.

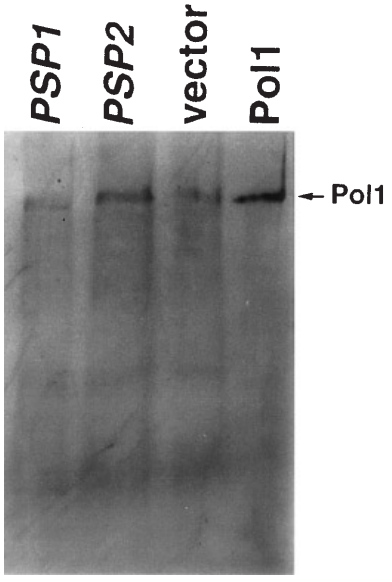


Fig. 7 Suppression conditions do not alter the level of Pol1 protein. Strain 7382-3-4 was transformed with pTF63 (vector), pAD1 (*PSP1*), and pJB8 (*PSP2*). Proteins extracted from 2×10^6 cells were loaded into each lane, and Pol1 protein was detected after SDS-PAGE as described in Materials and methods. Purified Pol1 protein was included as a standard as indicated. Identical samples electrophoresed and stained with Coomassie Blue dye indicated that uniform amounts of total protein were present in each lane containing whole cell extracts (not shown)

Discussion

We have isolated high-copy-number suppressors of DNA polymerase α mutations in *S. cerevisiae* in an attempt to further characterize the yeast DNA replication complex. We have analyzed two of these suppressors, called *PSP1* and *PSP2*. High copy numbers of either gene suppressed the temperature sensitivity of some mutations in *POL1*, and also in the gene for another DNA polymerase, *POL3*. Deletion of either gene or both simultaneously produced no obvious phenotype and did not alter the growth properties of cells with mutant DNA polymerases or most other *CDC* mutations tested, except that deletion of *PSP2* reproducibly suppressed a *cdc6-1* mutation. The concentration of intact, wild-type Pol1 protein was not changed by the suppressors, although the concentrations of the mutant proteins were too low to measure.

Both *PSP1* and *PSP2* suppressed more efficiently in strains that carried mutations in genes for the major vacuolar proteases, although these mutations did not appear to affect the maximal permissive growth temperature of the *pol1* strains themselves (Fig. 1). We had previously found that intact wild-type Pol1 protein was more stable in extracts prepared from *pep4 prb1* mutant strains (Miles and Formosa 1992), but it is not clear why these mutations would have an effect in vivo, since these proteases appear to be confined to the vacuole (Jones 1990) and should only indirectly affect the concentration of a nuclear protein. Presumably, either the level of suppressor proteins or some physiological effect that they cause are enhanced by protease mutations.

The suppression caused by *PSP1*, but not *PSP2*, was evident even when the gene was present on a low-copy-number vector, although the effect was greater with higher copy numbers. Suppression therefore shows a dose response and can be observed with even small changes in the number of copies of *PSP1* available.

Both *PSP1* and *PSP2* have unusual amino acid compositions, including high levels of asparagine. Giot et al. (1995) have isolated extragenic mutations that suppress the temperature sensitivity of defects in *POL3*, and while attempting to clone the normal copy of one called *sdp1* identified instead the unlinked gene *ASM4*. High levels of *ASM4* therefore appear to block the suppressor activity of *sdp1*. The *ASM4* gene product is also rich in N and Q residues (Table 2), indicating another link between N + Q-rich proteins and DNA replication, although in this case an increase in dosage of the *ASM4* gene blocked suppression of a polymerase mutation by another mutant gene. Glutamine-rich regions have been associated with transcriptional activators (although in at least one case tested, the Q-rich activation domain of human Sp1 was found to be unable to stimulate transcription in *S. cerevisiae*; Ponticelli et al. 1995). The *PSP2* product also contains "RGG" motifs, which is consistent with a role in binding RNA (Burd and Dreyfuss 1994). Together, these observations sug-

gest that suppression might be due to enhanced transcription or mRNA stabilization. However, in these cases we would expect to see an elevated level of Pol1 protein under suppressing conditions. Since we do not, we are unable to interpret the significance of the unusual compositions of these proteins.

Hovland et al. (1997) used a strategy similar to the one described here to identify genes that could simultaneously suppress mutations in both *POL1* and *POL3*, although the suppressor identified, *PAK1*, suppressed only *pol1* mutants. This gene encodes a protein kinase believed to stabilize Pol1 by modifying it or an interacting protein. The initial screens used differed in that we selected for suppression of only a *cdc17-1* mutation in a protease-deficient strain, but it is not clear why *PAK1* was not among the genes isolated in our screen. While *PSP1* and *PSP2* were each identified multiple times, other suppressors were represented only once, suggesting that additional suppressors, such as *PAK1*, may have been missed.

We developed a transposon strategy that should be of general use for characterizing high-copy-number suppressors in yeast. In this scheme, a large genomic insert was mutagenized with a transposon carrying a selectable marker and the origin of replication from the endogenous high-copy-number yeast 2-μm plasmid. This approach has several advantages. First, transposition is quite random and is very rapid and efficient compared to in vitro methods. Second, the isolates can be directly screened for activity since they are converted to high-copy-number yeast vectors by the transposon. Finally, construction of deletions of the suppressor gene is facilitated by the availability of transposon disruptions, since the transposon carries restriction sites near one end. Since this scheme is based on Tn*3*, it can not be used with vectors containing the β-lactamase gene, since these typically contain a 38-bp repeat from Tn*3*, and are therefore immune to transposition. Use of this strategy therefore requires subcloning of the initial insert into a suitable vector such as pHSS6. A similar scheme using Tn*1000* has also been described (Sedgwick and Morgan 1994, and personal communication).

The sequence of the yeast genome revealed that a homologue of *PSP1* can be found on chromosome XII. Investigation of this gene may illuminate the mechanism of suppression of polymerase mutations by *PSP* genes.

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