Genetic and Molecular Analysis of the *SOE1* Gene: A $tRNA_3^{Glu}$ Missense Suppressor of Yeast *cdc8* Mutations

Jin-Yuan Su, Lisa Belmont[1] and Robert A. Sclafani

Department of Biochemistry, Biophysics and Genetics and The Program in Molecular Biology, University of Colorado Health Sciences Center, Denver, Colorado 80262

Manuscript received December 2, 1988
Accepted for publication November 18, 1989

ABSTRACT

The *CDC8* gene of *Saccharomyces cerevisiae* encodes deoxythymidylate (dTMP) kinase and is required for nuclear and mitochondrial DNA replication in both the mitotic and meiotic cell cycles. All *cdc8* temperature-sensitive mutants are partially defective in meiotic and mitochondrial functions at the permissive temperature. In a study of revertants of temperature-sensitive *cdc8* mutants, the *SOE201* and *SOE1* mutants were isolated. The *SOE201* mutant is a disome of chromosome *X* to which the *cdc8* gene maps. Using the chromosome *X* aneuploids to vary *cdc8* gene dosage, we demonstrate that different levels of dTMP kinase activity are required for mitotic, meiotic or mitochondrial DNA replication. The *SOE1* mutant contains a dominant suppressor that suppresses five different *cdc8* alleles but does not suppress a complete *cdc8* deletion. The *SOE1* gene is located <1.5 cM from the *CYH2* gene on chromosome VII and is adjacent to the *TSM437-CYH2* region, with the gene order being *SOE1-TSM437-CYH2*. *SOE1* is an inefficient suppressor that can neither suppress the *cdc8* hypomorphic phenotype nor restore dTMP kinase activity *in vitro*. *SOE1* is a single C to T mutation in the anticodon of a $tRNA_3^{Glu}$ gene and thereby, produces a missense suppressor tRNA capable of recognizing AAA lysine codons. We propose that the resultant lysine to glutamate change stabilizes thermo-labile dTMP kinase molecules in the cell.

COORDINATION between the processes of DNA replication and biosynthesis of nucleotide precursors was first observed in prokaryotes. Proteins important for both processes may form multienzyme complexes or functional compartments (for a review see MATHEWS and ALLEN 1983). Several groups have also provided biochemical evidence for the existence of such multienzyme complexes in mammalian cells (REDDY and PARDEE 1980; WICKREMASINGHE, YAXLEY and HOFFBRAND 1983; for a review see MATHEWS and SLABAUGH 1986). We (SCLAFANI and FANGMAN 1984) and others (JONG, KUO and CAMPBELL 1984) have suggested that the yeast deoxythymidylate or dTMP kinase encoded by the *CDC8* gene may be an integral part of such complexes. The analysis of mutations in genes important for nucleotide biosynthesis and for DNA replication can be a powerful tool for investigating this compartmentation hypothesis. For example, in bacteriophage T4, mutations in the gene for an enzyme important for precursor biosynthesis, dCMP-hydroxymethylase, can be suppressed by mutations in an enzyme important for DNA synthesis, DNA polymerase (CHAO, LEACH and KARAM 1977). Similarly, T4 mutations that confer resistance to a folate analogue lie in the two genes that encode the subunits of DNA primase (MACDONALD and HALL 1984). We have isolated revertants of *cdc8* temperature-sensitive mutants in an effort to identify other gene products that are important for either nucleotide biosynthesis or DNA replication (J.-Y. SU and R. A. SCLAFANI, unpublished results). We refer to these suppressors as *SOE* (*s*uppressor *o*f cdc *e*ight) genes. However, one of these suppressors, *SOE1*, proved to be a novel, missense suppressor tRNA. Another suppressor, *SOE201*, was not a mutation but the result of increased *cdc8* gene dosage by disomy. In this report, we describe a genetic and molecular characterization of the *SOE1* suppressor and an analysis of the effect of *cdc8* gene dosage on mitotic, meiotic and mitochondrial chromosomal replication.

MATERIALS AND METHODS

Strains, plasmids and media: All yeast strains listed in Table 1 are *Saccharomyces cerevisiae*. The *Escherichia coli* bacterial strain used for transformation was DH5 *recA end1 gyrA96 thi-1 hsdR17 supE44*. Plasmids YRp7 *SOC8-1* (KUO and CAMPBELL 1983) and pYe (CEN11)12 (FITZGERALD-HAYES, BUHLER and COOPER 1982) were obtained from AMBROSE JONG and JOHN CARBON, respectively. Plasmid p511-1 Δ*CEN11* was produced from plasmid p511-1 (Figures 1 and 2) by deletion of the 1.6-kb *Sal*I *CEN11* fragment by digestion with *Sal*1 and ligation at a [DNA] of one μg/ml to favor intramolecular ligation. DNA restriction fragments were subcloned into either plasmid pYe (CEN11)12

[1] Present address: Department of Biochemistry, University of California School of Medicine, San Francisco, California 94143.

The publication costs of this article were partly defrayed by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Reference
131	*MAT***a** *trp1-289 lys2 his7 leu2-3,112 can1 cyh2 ade1,2*	1
126	*MATα trp1-289 ura3-52 leu2-3,112 can1 cyh2 ade1,2*	1
199	*MATα trp1-289 ura3-52 leu2-3,112 can1 cdc8-1*	1
504-4C	*MAT***a** *trp1-289 ura3-52 his7 can1 cdc8-1*	This work
227	*MAT***a** *ade2 cyh2 cdc8-1*	This work
206	199 *SOE1-1*	This work
348-8C	*MAT***a** *trp1-289 ura3-52 his7 leu2-3,112 can1 ade2 cdc8-1 SOE1-1*	1, 2
336	*MAT***a** *trp1-289 leu2-3,112 ade2 cdc8-1 SOE1-1*	This work
238	199 *SOE201* = *cdc8-1/cdc8-1* chromosome *X* disome	This work
226	*MATα ura3-52 leu2-3,112 cdc8-2 can1 cyh2 ade1,2*	1, 2
348	*MATα trp1-289 leu2-3,112 cdc8-3 can1 cyh2 ade1,2*	1, 2
346	*MATα leu2-3,112 his7 lys2 can1 cdc8-4 ura3-52 ade1,2*	1, 2
203	*MATα ura3-52 leu2-3,112 can1 cdc8-5 cyh2 ade1,2*	1, 2
258	*MAT***a** *arg4 aro2 lys5 his7 met4 ura2 ade1*	5503-9-2 of D. JENNESS
JY118	*MAT***a** *trp1 trp5 ura3-52 leu2-3,112 leu1 ade3 ade5 lys5 cyh2 cdc8-1*	This work
130	*MAT***a** *leu2-3,112 trp1-289 his7 lys2 ade1,2 cdc7-3*	3, 4
139	*MAT***a** *leu2-3,112 ade1,2 cdc7-1 cyh2 can1*	3, 4
274	*MATα ade1 ade2 can1 ura3-52 cdc21-1 cyh2 rho*$^-$	2, this work
372-11B	*MAT***a** *leu2-3,112 his7 cdc8Δ::LEU2 trp1-289* (pRC4-TK$^+$ = *TRP1 HIS3 TK*)	1, this work
JY121	206 *SOE1*::p511-1 Δ*CEN11* (*TRP1*)	This work
JY130	348-8C *SOC8*:: YRp7 *SOC8-1* (*TRP1*)	This work

All strains except 258 (5503-9-2) are congenic with A364a (HARTWELL 1967). References are: 1 = SCLAFANI and FANGMAN 1984; 2 = HARTWELL 1973; 3 = HARTWELL *et al.* 1973; 4 = PATTERSON *et al.* 1986).

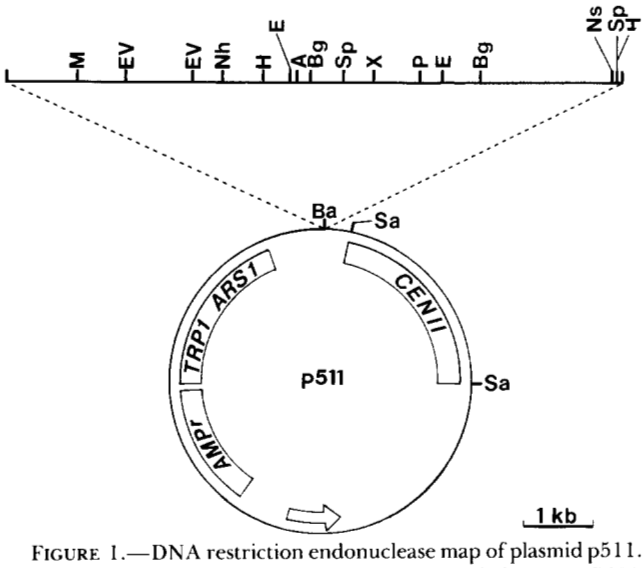

FIGURE 1.—DNA restriction endonuclease map of plasmid p511. Vector pYe (CEN11)12 is drawn as a circle and the yeast DNA insert as a line. The insert is a partial 9-kb *Sau*3A fragment ligated into the *Bam*HI site of the vector. DNA restriction endonuclease sites are abbreviated as follows: A = *Aat*II, M = *Mlu*I, Nh = *Nhe*I, Ns = *Nsi*I, H = *Hin*dIII, EV = *Eco*RV, E = *Eco*RI, Bg = *Bgl*II, Sp = *Sph*I, X = *Xho*I, P = *Pvu*II, and Sa = *Sal*I. The location of the yeast *TRP1*, *CEN11* and *ARS1* DNA sequences are shown as boxes.

or YCp50 (ROSE *et al.* 1987). Yeast and bacterial media have been described (SCLAFANI and FANGMAN 1984, 1986). Cell counts were determined with a model Zf Coulter counter with a 100 μm orifice. DNA transformations employed the sphaeroplast method (HINNEN, HICKS and FINK 1978) except that 1.5% low melting temperature agarose replaced 3% agar in the overlay for regeneration.

DNA manipulations: Plasmid isolation from bacteria and yeast, DNA restriction enzyme digestions, DNA ligations, nucleic acid agarose electrophoresis and DNA blot hybridizations followed previous procedures (SCLAFANI and FANGMAN 1984; PATTERSON *et al.* 1986). Yeast genomic DNA was isolated by the guanidine method (HOLM *et al.* 1986).

Yeast *SOE1* genomic library: DNA from the *SOE1 cdc8-1* strain (206) was digested partially with enzyme *Sau*3A to yield 10–15-kb restriction fragments that were isolated from a 0.5% preparative agarose gel using an ELUTRAP as described by the manufacturer (Schleicher & Schuell). The fragments were ligated into a *Bam*HI digested vector pYe (CEN11)12 (FITZGERALD-HAYES, BUHLER and COOPER 1982) that had been treated with calf intestinal phosphatase (CARLSON and BOTSTEIN 1982). About 4000 bacterial Amp^R transformants were obtained, 85% of which contained yeast genomic inserts. The colonies were pooled and used to inoculate a one liter culture from which plasmid DNA was isolated.

Assay of mitochondrial and meiotic function: Respiratory defective colonies or petites (rho$^-$) were detected by an inability to grow on YEPG agar with glycerol as carbon source. All rho$^-$ colonies examined in this work were rho^0 because they lack mitochondrial DNA. The presence of mitochondrial DNA was detected by observing the cells with a Leitz fluorescence microscope at 1500× total magnification using the DNA-specific fluorescent dye, 4′,6-diamidino-2-phenylindole or DAPI (WILLIAMSON and FENNEL 1975). Control rho$^+$ cells displayed both nuclear and cytoplasmic fluorescence, while control rho^0 cells that were produced by growth in the presence of ethidium bromide at 50 μg/ml (SLONIMSKI, PERRODIN and CROFT 1968; DUJON 1981) displayed only nuclear fluorescence as previously documented (WILLIAMSON and FENNEL 1975). To effect sporulation, stationary phase YEPD-grown diploid cultures were washed with 0.3% potassium acetate (KAC) and resuspended in the KAC medium at 10^7 cells per ml. Appropriate supplements were added to auxotrophic diploids. Sporulating cultures were aerated by vigorous shaking in Erlenmeyer flasks. Cultures were split and incubated at different temperatures

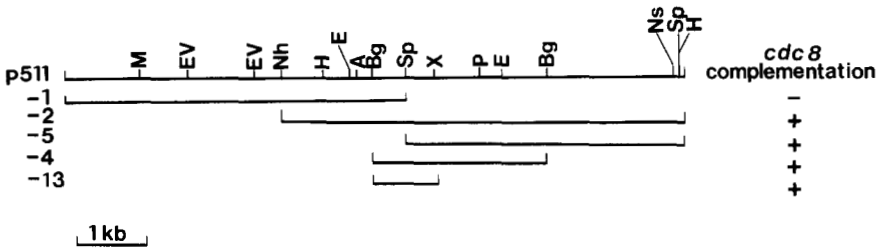

FIGURE 2.—Physical location of the *SOE1* gene on plasmid p511 by deletion mapping. DNA restriction fragments from the 9-kb insert of plasmid p511 (Figure 1) were subcloned into plasmid pYe (CEN11)12 (p511-1, -2, -4 and -5) or YCp50 (p511-13). All were tested for the presence of the *SOE1* suppressor by complementation (+ or −) of a *cdc8-1* mutation using strain 199. DNA restriction endonuclease sites are abbreviated as in Figure 1.

for 3 days. Percentage sporulation was determined by counting asci using phase contrast microscopy at 400× total magnification (SIMCHEN 1974; SCHILD and BYERS 1978).

Genetic analysis and plasmid loss detection: Diploid Cyh^R mitotic recombinants were selected on YEPD plates containing cycloheximide at 10 μg/ml. The recombinants were detected at a frequency of 1–0.5 × 10^{-4}. Loss of the *SOE1* suppressor was detected by replica-plating these colonies to YEPD plates at 36°. Matings, tetrad dissections and analysis of progeny spores followed standard protocols (MORTIMER and HAWTHORNE 1975). The majority (>80%) of the asci produced in these crosses were four-spored and only these asci were dissected. Genetic distances were calculated using the formula of PERKINS (1949). Sporulation of *cdc8/cdc8* homozygous diploids was at 17° to improve asci production (SCHILD and BYERS 1978) for genetic analysis.

Colonies that had lost a plasmid were detected by plating cells on nonselective YEPD solid medium and replica-plating the resultant colonies to selective medium, for example, −Trp drop-out medium was used to detect the presence of plasmid pRC4-TK$^+$ or plasmid p511. Trisomes or diploids containing a *cdc8*Δ::*LEU2* deletion were produced by mating strain 372-11B with a *cdc8-1 trp1* strain, for example, strain 238 or 199, and then isolating Trp$^-$ Tsm$^-$ colonies that had lost the *cdc8*-complementing plasmid, pRC4-TK$^+$ (SCLAFANI and FANGMAN 1984).

Pulse-field analysis: Electrophoretically separated yeast chromosomes were transferred to nylon membranes as described (GARDINER, LAAS and PATTERSON 1986).

Yeast plasmid integrations: Targeted integrations (ORR-WEAVER, SZOSTACK and ROTHSTEIN 1983) were performed by DNA restriction digests of the plasmid at a single-site in the yeast DNA insert, followed by transformation and selection for the plasmid marker. Both YRP7-*SOC8* and p511-1 Δ*CEN11* were integrated by using 1 μg of *Xho*I and *Mlu*I digested plasmid, respectively. About 500–1000 Trp$^+$ transformants were obtained. Five transformants were purified and the integration confirmed by a genomic DNA blot and subsequent DNA hybridization.

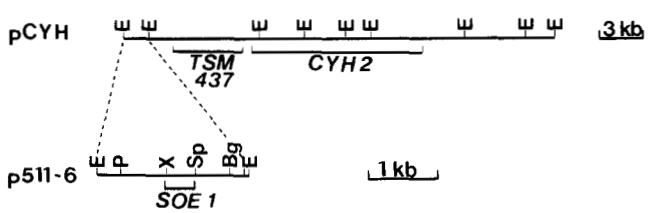

FIGURE 3.—Physical location of the *SOE1* gene in the *CYH2* region of yeast chromosome *VII*. A DNA restriction endonuclease map of the inserts from plasmid p511-6, a subclone containing the 2.2-kb *Eco*RI fragment of p511 (Figure 1) and plasmid pCYH (31 kb insert) are depicted to indicate overlapping homologies. The position of the *CYH2*, *TSM437* and *SOE1* genes are shown by the bracketed line below the DNA restriction map. DNA restriction endonuclease sites are abbreviated as in Figure 1.

dTMP kinase assay: The dTMP kinase assay using crude extracts was performed as described (SCLAFANI and FANGMAN 1984).

DNA sequencing and data bank analysis: Dideoxy-DNA sequencing employed the modified T7 DNA polymerase (Sequenase) as described by the manufacturer (US Biochemicals). The *SOE1* 0.75-kb *Xho*I-*Bgl*II DNA fragment (Figure 3) cloned into the *Sal*I-*Bam*HI sites of M13mp18 and M13mp19 was used as a template with the universal DNA primer #1212 (New England Biolabs). DNA sequences were analyzed with the MICROGENIE (Beckman Inst.) software program on a NCR PC8 computer. The nucleic acid sequences are from 3/88 update version 54 from GENBANK.

RESULTS

Isolation of dominant suppressors of a *cdc8-1* mutation: In order to identify suppressors of *cdc8-1*, colonies of strain 199 (*cdc8-1*) were replica-plated to YEPD plates and incubated for 2 days at the restrictive temperature of 30° or 36°. Spontaneously papillating colonies were isolated at a frequency of 1–4 × 10^{-6} and purified under permissive conditions at 22°. Two phenotypic classes of temperature-resistant (Tsm$^+$) revertants were obtained: those that could form colonies at 30° but not 36° (12 colonies-class I) and those that could form colonies at either temperature (nine colonies-class II). When suppressed strains were crossed to strain 227 (*cdc8-1*), the resultant diploids were Tsm$^+$, indicating that mutations of both classes were dominant. One colony from each class, *SOE201* from class I and *SOE1* from class II, was chosen for further study.

***SOE1* is an extragenic suppressor and *SOE201* is disomic for chromosome X.** These strains (206 and 238) were mated with *CDC8* strain 131, diploids sporulated and asci dissected. For both heterozygotes, the majority of asci produced tetrads in which the Tsm$^+$:Tsm$^-$ phenotype segregated predominantly 3:1 with 2:2 and 4:0 segregations also being detected (Table 2). In contrast, a cross with the unsuppressed parent strain 199 yielded only 2:2 segregations. These data are indicative of the segregation of two unlinked loci, presumably the extragenic suppressor and the *cdc8-1* gene. We confirmed that the suppressor mutations segregated as single Mendelian genes by sporulating homozygous *cdc8/cdc8* diploids that were heterozygous for the suppressor (Table 2). Only 2:2 asci were detected for the Tsm$^+$:Tsm$^-$ phenotype with both suppressors. By using the *TRP1* gene as a cen-

TABLE 2

Tetrad analysis of *SOE1* and *SOE201* strains

Cross[a]	Relevant genotype		No. of tetrads Tsm$^+$:Tsm$^-$		
	cdc8[b]	*SOE*[b]	4:0	3:1	2:2
131 × 206	*cdc8/+*	*SOE1/+*	7	25	5
131 × 238	*cdc8/+*	*SOE201/+*	4	13	2
131 × 199	*cdc8/+*	*+/+*	0	0	19
227 × 206	*cdc8/cdc8*	*SOE1/+*	0	0	26
227 × 238	*cdc8/cdc8*	*SOE201/+*	0	0	20

[a] Diploids made by crossing the strains listed were sporulated and tetrads were dissected.

[b] The relevant genotype at the *cdc8* and *SOE1* or *SOE201* loci is listed.

TABLE 3

Tetrad analysis of *Ura2/ura2* strains

Cross[a]	*SOE*[b]	No. of tetrads *URA2*$^+$:*ura2*$^-$[c]		
		4:0	3:1	2:2
238 × 258	*SOE201/+*	2	9	0
206 × 258	*SOE1/+*	0	0	11
199 × 258	*+/+*	0	0	11

[a] Diploids made by crossing the strains listed were sporulated and tetrads were dissected. All diploids are heterozygous for the *URA2* locus on chromosome *X*.

[b] The relevant genotype at the *SOE1* and *SOE201* loci is listed.

[c] The *ura2* and *ura3* mutations were detected by a complementation test using *ura2* and *ura3* tester strains of both mating types.

tromere linked marker in the latter cross, we determined that the *SOE1* gene is not linked to its centromere (17 T asci of 26 total) but *SOE201* behaved as though it were tightly centromere linked (no T asci of 20 total).

We hypothesized that the *SOE201*-associated phenotype, that is, partial temperature resistance, might be the result of doubling the copy number of the mutant *cdc8* locus by disomy. A similar situation has been shown to occur for the yeast *rna1-1* allele (ATKINSON and HOPPER, 1987) and for a disruption of the yeast *TUB1* gene (SCHATZ, SOLOMON and BOTSTEIN 1986). If the *SOE201*-bearing strains were disomic for chromosome *X*, the map location of the *CDC8* gene, we would expect tight centromere linkage (see above) and aberrant segregation for any other marker on this chromosome, because two copies of any gene on this chromosome would be present. Therefore, we mated *SOE201*-bearing strain 238 to *ura2*-bearing strain 258 to produce the potential trisomy for chromosome *X* (Table 3). In 11 tetrads *URA2* segregated predominantly 3:1 (9 tetrads) with two 4:0 and no 2:2 asci. In contrast, *SOE1* strain 206 or the parent *soe1* strain 199 when crossed to strain 258, produced all 2:2 asci for *URA2*. In addition, markers on other chromosomes, including *MAT* on chromosome *III* and *URA3* on chromosome *V* segregated 2:2, as expected. We conclude that the *SOE201* partial suppressor phenotype is due to disomy for the *cdc8-1* bearing chromosome.

***SOE1* is gene-specific but is neither allele-specific nor a bypass suppressor.** We tested the allele specificity of the *SOE1* mutation by producing and sporulating *SOE1/soe1*$^+$ *cdc8-1/cdc8-2*, cdc8-3, *cdc8-4* and *cdc8-5* heteroallelic diploids (strain 336 crossed with strains 226, 348, 346, and 203, respectively). In all cases, the Tsm$^+$ phenotype segregated 2:2 in ten asci for each of the four diploids. Therefore, *SOE1* was capable of suppressing all five alleles of *cdc8*. This lack of allele specificity suggested that the *SOE1* suppressor might be bypassing the requirement for *CDC8* activity. To test this hypothesis, we constructed diploids of genotype *cdc8Δ::LEU2/cdc8-1 leu2/leu2 trp1/trp1 SOE1/soe1*$^+$ containing plasmid pRC4-TK, which complements *cdc8* mutations (SCLAFANI and FANGMAN 1984) by mating strain 372-11B and strain 206. If the *SOE1* mutation suppressed the *cdc8* deletion, among meitoic progeny we would expect to find *cdc8Δ::LEU2* cells that could lose the TK plasmid under nonselective conditions and therefore become Leu$^+$ Trp$^-$ (MATERIALS AND METHODS). The plasmid contains a 2-μm plasmid origin and will segregate frequently 4:0 in a non-Mendelian manner (LIVINGSTON 1977). In 14 of 16 tetrads in which the plasmid segregated 4:0, Leu$^+$ segregated 2:2. All Leu$^+$ *cdc8Δ::LEU2* segregants were Trp$^+$ and did not yield plasmidless Trp$^-$ colonies even after two rounds of propagation for single colonies on YEPD plates (<0.1% Trp$^-$ or no Trp$^-$ colonies detected in 10^3 colonies examined). In contrast, *cdc8*-1 Leu$^-$ segregants easily gave rise to Trp$^-$ colonies (30% Trp$^-$). These results are similar to those obtained in the absence of *SOE1* (SCLAFANI and FANGMAN 1984). Therefore, we conclude that the *SOE1* suppressor cannot bypass the *CDC8* requirement during mitotic growth.

We have tested whether the *SOE1* gene can suppress a number of other mutations. Results were negative with either of two different *cdc7* mutations, *cdc7-1* and *cdc7-3* (PATTERSON *et al.* 1986) and with the *cdc21-1* mutation (HARTWELL *et al.* 1973), all of which are Tsm$^-$. Results were also negative with the suppressible amber nonsense mutation, *trp1-289* (MIOZZARI, NEIDERBERGER and HUTTER 1978) and all the mutations listed in Table 1. By these criteria, the *SOE1* suppressor is specific for *cdc8* Tsm$^-$ mutations only.

Effects of the *SOE1* suppressor on mitotic, meiotic and mitochondrial chromosomal replication: Because all *cdc8* Tsm$^-$ mutants are partially defective in meiotic and mitochondrial functions at the permissive temperature (SIMCHEN 1974; SCHILD and BYERS 1978; NEWLON, LUDESCHER and WALTER 1979; SCLAFANI and FANGMAN 1986), we tested for suppression

TABLE 4

Mitotic, mitochondrial and meiotic phenotypes of *cdc8* mutants

Genotype[a] *cdc8*	*soe1*	Growth[b] Mitotic 22	Mitotic 30	Mitotic 36	Mitochondrial rho	Meiotic Spo
cdc8-1/cdc8Δ	+/+	+	−	−	−	NA
cdc8-1/cdc8-1	+/+	+	−	−	+	−(<1%)
cdc8-1/cdc8-1/cdc8-1	+/+	+	+	−	+	+(40%)
cdc8-1/cdc8Δ	*SOE1*/+	+	+	+	−	NA
cdc8-1/cdc8-1	*SOE1*/+	+	+	+	+	−(5%)
cdc8-1/cdc8-1/cdc8-1	*SOE1*/+	+	+	+	+	+(40%)

[a] Genotype at the *cdc8* and *soe1* loci is listed. NA = not applicable because rho$^-$ cells do not sporulate.

[b] Mitotic growth was tested at 22°, 30°, and 36° by assaying colony forming ability (+) or inability (−) on YEPD plates. Mitochondrial growth was tested by assaying colony forming ability (rho$^+$) or inability (rho$^-$) at 22° on YEPG plates. Meiotic growth was tested by the ability (Spo$^+$) or inability (Spo$^-$) to sporulate in KAC medium at 22°; (%) = percentage of asci formed.

of these phenotypes by *SOE1*. By using the chromosome X aneuploids isolated in a *SOE201* strain and the *cdc8Δ::LEU2* deletion, we produced a series of diploid and trisomic strains that contain different copy numbers of the *cdc8-1* gene and/or the *SOE1* suppressor (Tables 1 and 4). These strains contain one, two or three copies of the *cdc8-1* gene. All the strains are diploid except those with three copies that are trisomes. They were assayed for mitotic growth at different temperatures, for mitochondrial function and the ability to sporulate at 22° (Table 4). By comparing the effects of gene dosage to that of the *SOE1* suppressor, we can measure the degree of suppression.

Strain 199/372-11B Trp$^-$, which has one *cdc8-1* copy, is rho^0 and lacks detectable mitochondrial DNA (MATERIALS AND METHODS). Strain 199/227, which has two *cdc8-1* copies, is rho$^+$ but Spo$^-$. Strain 238/227, which has three *cdc8-1* copies, is rho$^+$ Spo$^+$ and, in addition, can also grow at 30°. The presence of the *SOE1* suppressor allows strains of this type to grow at 36° but does little to suppress the other phenotypes (see strains 206/372-11B Trp$^-$, 206/227 and 238/336 in Table 4). In fact, the effect of the *SOE1* suppressor on these phenotypes is not even equivalent to an additional copy of the *cdc8-1* gene (compare strains 206/372-11B Trp$^-$ and 199/227).

The *SOE1* suppressor does not stabilize *cdc8-1* mutant dTMP kinase *in vitro*. Previously, we and others (SCLAFANI and FANGMAN 1984; JONG, JUO and CAMPBELL 1984) have shown that crude extracts prepared from *cdc8* mutants that were grown at 22° and assayed at 15°, 23° or 37° do not contain detectable levels of dTMP kinase activity (less than 0.1 unit per mg protein or 0.1% of *CDC8*$^+$ extracts). Therefore, we tested the effect of the *SOE1* suppressor on this phenotype. No effect could be detected in extracts of strain 206.

The *SOE1* suppressor is not allelic with the *SOC8* suppressor: The *SOC8-1* gene was identified as a cloned wild-type DNA sequence that only complemented a *cdc8-1* mutation when present in high copy number (KUO and CAMPBELL 1983). It is possible that the *SOE1* suppressor is the result of a *SOC8-1* mutation that increases *SOC8-1* expression, yielding the same dominant phenotype. We integrated the YRp7 *SOC8-1* (*TRP1*) plasmid (KUO and CAMPBELL 1983) into *SOE1 cdc8-1 trp1* strain 348-8C to produce strain JY130 and verified that the plasmid integrated at the *SOC8-1* locus by genomic DNA hybridization (data not shown). The strain was mated with *cdc8-1 trp1* strain 199. Tetrad dissection of the asci produced by sporulation of this diploid (Table 5) indicated that the Trp$^+$ phenotype associated with the *SOC8-1* locus was unlinked to the Tsm$^+$ phenotype produced by the *SOE1* suppressor. As expected, the *SOC8* gene in a single copy does not suppress the *cdc8-1* mutation and only asci in which Tsm$^+$:Tsm$^-$ segregated 2:2 due to the presence of the *SOE1* gene were recovered. Therefore, the *SOE1* and *SOC8* loci are unlinked.

TABLE 5

Tetrad analysis of *SOC8* and *SOE1* integrants

Cross[a]	Relevant genotype[b]	Ascus type (No.) PD	NPD	T
JY130 × 199	*SOE1 SOC8::TRP1* × *soe1*$^+$	5	5	20
JY121 × 504-4C	*SOE1::TRP1* × *soe1*$^+$	35	0	0

[a] Diploids made by crossing the strains listed were sporulated and tetrads were dissected. Strains JY130 and JY121 have plasmids YRp7 *SOC8-1* or p511-1 Δ*CEN11*, each carrying *TRP1*, integrated at the *SOC8* and *SOE1* loci, respectively.

[b] Both strains produced are *trp/trp1 cdc8/cdc8* homozygous diploids. Both integrated plasmid DNAs are marked with the *TRP1* gene and are detected by the Trp$^+$ phenotype. *SOE1* is detected by the Tsm$^+$ phenotype.

Molecular cloning of the *SOE1* suppressor: Because the *SOE1* suppressor is dominant, we have cloned this mutant allele from a genomic library constructed with the DNA of *SOE1 cdc8-1* strain 206. Mutant *trp1 cdc8-1* strain 199 was transformed with the library DNA and Trp$^+$ plasmid-bearing colonies were selected. Because both donor and recipient

DNAs contain the *cdc8-1* allele only the *SOE1* suppressor on the plasmid should confer Tsm$^+$ phenotype. Therefore, we screened 80,000 transformants by replica-plating to YEPD plates and incubating at the restrictive temperature of 36°. Approximately 50 clones were obtained. Subsequent colony purification and examination of Trp$^-$ plasmidless cells isolated under permissive conditions demonstrated 100% linkage of the Tsm$^+$ and Trp$^+$ characters. The plasmid was isolated from a number of the clones by transformation of *E. coli* cells to Amp^R with genomic DNA from the transformants. All clones contained the same plasmid, p511 (Figure 1). Retransformation of strain 199 by Trp$^+$ selection with purified plasmid p511 DNA yielded all Tsm$^+$ colonies (200/200). The insert size was estimated at 9 kb and subsequent subcloning indicated the *cdc8-1* complementing activity to be located in the 0.50-kb *Sph*I-*Xho*I fragment (Figures 2 and 3). The plasmid complements all five *cdc8* alleles but not the *cdc7* alleles, as expected. Genomic DNA hybridization indicated that the cloned 9-kb DNA sequence is a single copy sequence with the correct DNA restriction map (data not shown).

The cloned *SOE1* suppressor integrates by homologous recombination at the *SOE1* locus: In order to verify that the cloned DNA contained the *SOE1* suppressor, we targeted a nonsuppressing subclone, plasmid p511-1 Δ*CEN11* (Figure 2; MATERIALS AND METHODS) to integrate by homologous recombination by digesting at the unique *Mlu*1 site. We then employed the same type of genetic and molecular analysis using this integrant as for the *SOC8* integrant. Strain JY121, a Trp$^+$ integrant of *SOE1 cdc8-1 trp1* strain 206, was made and mated to *cdc8-1 trp1* strain 504-4C. The diploid was sporulated and asci were analyzed by tetrad dissection (Table 5). In contrast to the *SOC8* results, 100% linkage was seen between the plasmid marker, Trp$^+$ and the chromosomal *SOE1* locus both of which segregated 2:2. The absence of recombinant spores indicates that the *SOE1* suppressor and cloned DNA are closely linked (<1.4 cM). Most likely, the original cloned DNA contains the *SOE1* suppressor.

The *SOE1* suppressor is located <1.5 cM from the *CYH2* gene on chromosome *VII*: We have used the cloned DNA to map the chromosomal location of the *SOE1* suppressor by DNA blot hybridization to a pulse-field gel of yeast DNA (GARDINER, LAAS and PATTERSON 1986). Using radiolabeled plasmid p511 as a probe, hybridization was detected to a doublet containing both chromosomes *VII* and *XV*, in addition to chromosomes *IV* (*TRP1 ARS1*) and *XI* (*CEN11*) to which the vector DNA sequences hybridize (data not shown). We then tested if the *SOE1* gene mapped to chromosome *VII* by mitotic recombination methods. Homozygosis of both *cyh2^r* and *soe1$^+$* was obtained by selecting spontaneous Cyh^r isolates of diploid strain *CYH2^s/cyh2^r SOE1/soe1$^+$ cdc8-1/cdc8-1* that was produced by mating strain 206 and 227. Of 100 Cyh^r mitoic recombinants selected, 40% had lost the *SOE1* mutant allele and become Tsm$^-$. These data indicate that the *SOE1* suppressor may map to the left arm of chromosome *VII* linked to the *CYH2* gene (MORTIMER and SCHILD 1985). We performed meiotic mapping experiments by sporulating a diploid strain produced by mating strains JY118 and 206 (Table 6). In this cross, the *SOE1* suppressor, *cyh2* and *lys5* loci on chromosome *VII* are segregating. In 33 tetrads, no recombinants were detected between *cyh2* and *SOE1*. Therefore, the two loci are less than 1.5 cM apart. In addition, a linkage of 42 cM was detected between the *SOE1* and *lys5* loci. Two genes have been identified and cloned in this region, *TSM437* and *CYH2* that are 1.5 cM apart (FRIED and WARNER 1982). We have shown by DNA blot hybridization and restriction mapping that the left end of plasmid pCYH2 (FRIED and WARNER 1982) overlaps the *SOE1* cloned DNA (Figures 1 and 3). By comparison of the DNA restriction maps, the 0.50-kb *Sph*I-*Xho*I restriction fragment that contains the *SOE1* gene was shown to overlap neither the *TSM437* nor *CYH2* genes. Therefore, the *SOE1* suppressor cannot be allelic to either of these two genes. The order of the three genes is *SOE1 TSM437 CYH2*.

TABLE 6

Meiotic genetic mapping of *SOE1*

Loci	Ascus type (No.) PD	NPD	T	Map distance (cM)[a]
SOE1-cyh2	33	0	0	<1.5
SOE1-lys5	10	1	21	42

The diploid made by crossing strains JY118 and 206 was sporulated and tetrads were dissected.

[a] Map distances were calculated according to PERKINS (1949).

***SOE1* is a single C to T transition mutation producing a tRNA$_3^{Glu}$ that recognizes AAA lysine codons:** Molecular subcloning experiments indicated that only 500 bp of DNA is required for *SOE1* suppression (Figure 2). Because of the small size, we hypothesized that *SOE1* might be an informational missense suppressor tRNA. RNA blot analysis indicated that the 500 bp *Sph*I-*Xho*I DNA restriction fragment hybridized to yeast tRNA and not to poly(A$^+$) mRNA (data not shown). The entire 500-bp fragment was sequenced (Figure 4) and compared to the data bank of DNA sequences to test the hypothesis (see MATERIALS AND METHODS). Homology was detected to several DNA sequences. Homology (70–85%) to the repeated delta sequences found at the ends of Ty transposons (CLARE and FARABAUGH 1985) and near many tRNA genes (TSCHUMPER and CARBON 1982; HAUBER *et al.* 1988) is present near the *Xho*I site, also conserved

```
        10          20          30          40          50
CTCGAGAAGA ACTTGTAGTA ACATAATATT ATAGTCTTTA TCAACAACGC
XhoI
        60          70          80          90          100
AATCTCAACA ATTATCAAGA TTCACCTAAC ATTCAATATT ATATATGATG

        110         120         130         140         150
TAAAAATATG ATATAAGGAT TGCGAAATAT ATTTCAGATT TAATGAAGGT

        160         170         180         190         200
GATATGCAAG TATTGATCAT TTAGAAGAAT AACTAGGGAA TGAAAAAAGA

        210         220         230         240         250
GCTAATACTA TTATTATATA AAATACCGAT TGCCCTTTTG CGATTACTCT

        260         270         280         290         300
ATCATGAGAG ATACTAGTAT ATCTGACTGA TTATACACTA TACGTTATAA

        310         320         330         340         350
TGAGTTCCAA AACTCTCATA TGGCCTAATT TCTGCGATAT GATGTATACG

        360         370         380         390         400
TGTAAAGTTT CCATATAGTA ATTTACCTTT GTAATGAATT TCCAACAATT

        410         420         430         440         450
ATTGTCCGAT ATAGTGTAAC GGCTATCACA TCACGCTTTT ACCGTGGAGA
                                            C
        460         470         480         490         500
CCGGGGTTCG ACTCCCCGTA TCGGAGTTTT TTGTCGTAAG TTGAGAAAGT

        510         520         530         540         550
TTTTTCCAGT TAAGTTATTA TGACGCATGC ACATAGAAGG ATCACCGAAC
                        SphI
        560         570         580         590         600
AAAGTTGCAA TTATTAGGCA CCGAATGAAG AAAATGAGAA CCTAATTTCG

        610         620         630         640         650
CTTGGCAATA GTAAAATGTT TTATCTGCAT CGAAAAAGTT ATATAGATTT

        660         670         680         690         700
ATGCCTCATA TATAAAATAG AATTTTAGAA TAAGATAATC CTTATTTATT

        710
TTGCA...
    BglII
```

FIGURE 4.—Nucleotide sequence of the *SOE1* $tRNA_3^{Glu}$ gene and flanking regions. The 705-nucleotide DNA sequence from the *Xho*I site towards the *Bgl*II site (Figure 3) is shown. The $tRNA_3^{Glu}$ encoding DNA sequence is boxed. The C to T *SOE1-1* mutational change in the anticodon is indicated at nucleotide 440. Transcription of the $tRNA_3^{Glu}$ gene is from left to right as shown. Regions homologous to delta sequences are underlined. The location of the *Xho*I, *Sph*I and *Bgl*II restriction sites is also shown.

in many deltas. About 150 bp from the delta sequence, there is an exact match to the 72 bp yeast $tRNA_3^{Glu}$ sequence from nucleotide 405 to 476 (KOBAYASHI *et al.* 1974; EIGEN and FELDMANN 1982) except for a single C to T change at position 440. This change is located in the anticodon of the tRNA. UUC is the anticodon in wild-type $tRNA_3^{Glu}$ (KOBAYASHI *et al.* 1974), while UUU is the anticodon in the *SOE1* suppressor $tRNA_3^{Glu}$. Because AAA is a lysine codon, *SOE1* is a missense suppressor $tRNA_3^{Glu}$ that substitutes glutamate for lysine at AAA codons. Normal recognition of GAA codons would still occur because there are at least twelve other $tRNA_3^{Glu}$ genes (EIGEN and FELDMANN 1982; HAUBER *et al.* 1988). This also explains why haploid strains containing a deletion of the 750-bp *Xho*I-*Bgl*II *SOE1* fragment (Figure 3) are viable (data not shown). In contrast to nine $tRNA_3^{Glu}$ DNA sequences that are nonhomologous in their flanking regions, the *SOE1* region has extensive homology to the DNA sequence of plasmid pYH5 (HAUBER *et al.* 1988). The highest homology is in the sequence 3′ to the tRNA, in which 97% of 230 bp are identical. The lowest homology is in the 5′ sequence in the delta region, as expected for the repeated element (CAMERON, LOH and DAVIS 1979; HAUBER *et al.* 1988). Most likely, *SOE1* and pYH5 DNAs are from the same region of chromosome *VII*. HAUBER *et al.* (1988) did not map pYH5, but they showed that either chromosome *VII* or *XV* contain a $tRNA_3^{Glu}$ gene.

Although both delta and $tRNA_3^{Glu}$ sequences are repeated throughout the genome (CAMERON, LOH and DAVIS 1979; HAUBER *et al.* 1988), only unique DNA was detected in genomic Southern hybridization using the 9-kb insert as a probe (see above). Under the high stringency used for the hybridizations, the partial homology that various deltas share with each other would not have been detected (Figure 4; CAMERON, LOH and DAVIS 1979; HAUBER *et al.* 1988). In addition, both types of repeats are small in size (72 bp for the tRNA and about 330 bp for the delta) relative to the unique flanking DNA of the probe used (9-kb fragment of plasmid p511), and therefore, would represent only about 4% of the radiolabeled probe.

DISCUSSION

The *CDC8* gene encodes the enzyme dTMP kinase that is required for all cellular DNA molecules to replicate in both meiosis and mitosis. The *CDC8* protein may be part of a multienzyme complex that supplies nucleotides efficiently at the nuclear DNA replication fork (SCLAFANI and FANGMAN 1984; JONG, KUO and CAMPBELL 1984). This study demonstrates that hypomorphic *cdc8* mutations affect mitotic, meiotic and mitochondrial DNA replication differently (Table 3). Similar results have been reported in previous analyses of the effects of *cdc8* (dTMP kinase), *cdc21* or *tmp1* (thymidylate synthase), and *rnr2* (ribonucleotide reductase) mutations on both mitochondrial DNA (BARCLAY and LITTLE 1978; LITTLE and HAYNES 1979; NEWLON, LUDESCHER and WALTER 1979; MCNEIL and FREISEN 1981; SCLAFANI and FANGMAN 1986; ELLEDGE and DAVIS 1987) and premeiotic DNA syntheses (SIMCHEN 1974; BISSON and THORNER 1977; SCHILD and BYERS 1978). We hypothesize that these results may reflect the efficiency with which each genre of DNA replication utilizes thymidylate precursors produced by this complex. According to this hypothesis, the nuclear mitotic complex would be the most efficient. Mitochondria do not contain the *CDC8* protein (JONG and CAMPBELL 1984) and would have to obtain thymidylate exogenously. Therefore, mitochondrial DNA replication would be less efficient. The nuclear meitoic system may not have the complex and would be the least efficient.

In a screen of extragenic suppressors of a *cdc8-1* mutation, the *SOE1* suppressor was isolated as a dom-

inant mutation. We demonstrate that the *SOE1* suppressor is a $tRNA_3^{Glu}$ that recognizes AAA lysine codons. Therefore, *SOE1* is a classic missense suppressor tRNA, as first shown for *E. coli trpA* mutations (CARBON, BERG and YANOFSKY 1986). Both *E. coli* and yeast missense suppressors of tryptophan synthetase mutations are inefficient and produce undetectable levels of enzymatic activity *in vitro*, yet allow for Trp^+ growth (CARBON, BERG and YANOFSKY 1966; SINGH and MANNEY 1974). The *SOE1* suppressor does not suppress as well as an increase in *cdc8-1* gene dosage (Table 2) and is unable to produce detectable levels of dTMP kinase activity *in vitro*. We would expect that an efficient missense suppressor would be lethal. *SOE1* is not deleterious to cell growth even when present on a high copy number 2-μm vector. Therefore, the *SOE1* suppressor is also very inefficient.

Unlike the previously characterized missense suppressors in yeast and *E. coli* (CARBON, BERG and YANOFSKY 1966; GORMAN and GORMAN 1971; SINGH and MANNEY 1974), *SOE1* is not allele-specific. All the five *cdc8* alleles (HARTWELL 1967) used in this study are indeed different mutations because Cdc^+ recombinants can be obtained from heteroallelic diploids following UV-irradiation (ZUK, BARANOWSKA and ZABROWOSKA 1988; J.-Y. SU and R. A. SCLAFANI, unpublished results). The *cdc8* mutations may be all glutamate to lysine changes at different regions in dTMP kinase, because these changes might frequently produce a temperature-sensitive protein. There are eleven GAA codons out of 216 total codons in the *CDC8* gene (BIRKENMEYER, HILL and DUMAS 1984; JONG, KUO and CAMPBELL 1984). Any of these codons could be mutated to AAA by a single G to A transition. *SOE1* could result in the substitution of glutamate for lysine during translation at AAA codons, thereby suppressing the defect. Another possibility is that different mutational changes are stabilized by lysine to glutamate changes at other regions of dTMP kinase. Insertion of glutamate(s) at some of the twelve AAA lysine codons would produce an effect similar to the action of a second-site intragenic suppressor. DNA sequencing of the *cdc8* mutant alleles, currently underway, will help to resolve these possibilities.

A number of suppressors have been proposed to be missense suppressor tRNAs in *S. cerevisiae* (GORMAN and GORMAN 1971; SINGH and MANNEY 1974; for a review, see SHERMAN 1982), but none have been sequenced or shown to be tRNA genes. This publication demonstrates that missense informational suppression by a mutant tRNA does occur in *S. cerevisiae* and that *cdc8* temperature-sensitive mutants are especially useful in the isolation of missense suppressors.

This paper is dedicated to the memory of HERSCHEL ROMAN. This work was supported by U. S. Public Health Service Grant GM35078 awarded to R.S. We thank AMBROSE JONG for supplying YRp7 *SOC8-1*, JOHN CARBON for pYe (CEN11)12 and HOWARD FRIED for pCYH. We thank GARY STETLER for a critical reading of the manuscript.

Notes added in proof: As predicted, the *SOE1* suppressor can suppress the *cdc7-7* Tsm^- allele that we have shown to be a GAA (glutamate) to AAA (lysine) mutation (R. HOLLINGSWORTH and R. SCLAFANI, unpublished results).

These sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X17270.

LITERATURE CITED

ATKINSON, N. S., and A. K. HOPPER, 1987 Chromosome specificity of polysomy promotion by disruptions of the *Saccharomyces cerevisiae* RNA1 gene. Genetics **116:** 371–375.

BARCLAY, B. J., and J. G. LITTLE, 1978 Genetic damage during thymidylate starvation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **160:** 33–40.

BIRKENMEYER, L. C., HILL, J. C. and L. B. DUMAS, 1984 *Saccharomyces cerevisiae CDC8* gene and its product. Mol. Cell. Biol. **4:** 583–590.

BISSON, L., and J. THORNER, 1977 Thymidine 5′-monophosphate-requiring mutants of *Saccharomyces cerevisiae* are deficient in thymidylate synthetase. J. Bacteriol. **132:** 44–50.

CARLSON, M., and D. BOTSTEIN, 1982 Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:** 145–154.

CAMERON, J. R., E. Y. LOH and R. W. DAVIS, 1979 Evidence for transposition of dispersed repetitive DNA families in yeast. Cell **16:** 739–751.

CARBON, J., P. BERG and C. YANOFSKY, 1966 Studies of missense suppression of the tryptophan synthetase A-protein mutant A36. Proc. Natl. Acad. Sci. USA **56:** 764–771.

CHAO, J. M., M. LEACH and J. KARAM, 1977 In vivo functional interaction between DNA polymerase and dCMP-hydroxymethylase of bacteriophage T4. J. Virol. **24:** 557–563.

CLARE, J., and P. FARABAUGH, 1985 Nucleotide sequence of a yeast Ty element: evidence for an unusual mechanism of gene expression. Proc. Natl. Acad. Sci. USA **82:** 2829–2833.

DUJON, B., 1981 Mitochondrial genetics and function, pp. 505–635, in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, edited by J. N. STRATHERN, E. W. JONES and J. E. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

EIGEN, A., and H. FELDMANN, 1982 Ty1 and delta elements occur adjacent to several tRNA genes in yeast. EMBO J. **1:** 1245–1250.

ELLEDGE, S. J., and R. W. DAVIS, 1987 Identification and isolation of the gene encoding the small subunit of ribonucleotide reductase from *Saccharomyces cerevisiae*: DNA damage-inducible gene required for mitotic viability. Mol. Cell. Biol. **7:** 2783–2793.

FITZGERALD-HAYES, M., J. M. BUHLER, T. G. COOPER and J. CARBON, 1981 Isolation and subcloning analysis of functional centromere DNA (CENII) from *Saccharomyces cerevisiae* chromosome XI. Mol. Cell. Biol. **2:** 82–87.

FRIED, H. M., and J. R. WARNER, 1982 Molecular cloning and analysis of the yeast gene for cycloheximide resistance and ribosomal protein L29. Nucleic Acids Res. **10:** 3133–3148.

GARDINER, K., W. LAAS and D. PATTERSON, 1986 Fractionation of large mammalian DNA restriction fragments using vertical pulsed-field gradient gel electrophoresis. Somatic Cell Mol. Genet. **12:** 185–195.

GORMAN, J. A., and J. GORMAN, 1971 Genetic analysis of a gene required for the expression of allele-specific missense suppression in *Saccharomyces cerevisiae*. Genetics **67:** 337–352.

HARTWELL, L. H., 1967 Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93:** 1662–1670.

HARTWELL, L. H., 1973 Three additional genes required for deoxyribonucleic acid synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **115:** 966–974.

HARTWELL, L. H., R. K. MORTIMER, J. CULOTTI and M. CULOTTI, 1973 Genetic control of the cell cycle in yeast. V. Genetic analysis of *cdc* mutants. Genetics **74:** 267–286.

HAUBER, J., R. STUCKA, R KRIEG and H. FELDMANN, 1988 Analysis of yeast chromosomal regions carrying members of the glutamic acid tRNA gene family: various transposable elements are associated with them. Nucleic Acids Res. **16:** 10623–10634.

HINNEN, A. H., J. B. HICKS and G. R. FINK, 1978 Transformation of yeast. Proc. Natl. Acad. Sci. USA **75:** 1929–1933.

HOLM, C., D. W. MEEKS-WAGNER, W. L. FANGMAN and D. BOTSTEIN, 1986 A rapid, efficient method for isolating DNA from yeast. Gene **42:** 169–173.

JONG, A. Y. S., and J. L. CAMPBELL, 1984 Characterization of *Saccharomyces cerevisiae* thymidylate kinase, the *CDC8* gene product. J. Biol. Chem. **259:** 14394–14398.

JONG, A. Y. S., C. L. KUO and J. L. CAMPBELL, 1984 The *CDC8* gene of yeast encodes thymidylate kinase. J. Biol. Chem. **259:** 11052–11059.

KOBAYASHI, T., I. TSUNEMASA, M. YOSHIDA, K. TAKEISHI and T. UKITA, 1974 The primary structure of yeast glutamic acid tRNA specific to the GAA codon. Biochim. Biophys. Acta **366:** 168–181.

KUO, C. L., and J. L. CAMPBELL, 1983 Cloning of *Saccharomyces cerevisiae* DNA replication genes: isolation of the *CDC8* gene and two genes that compensate for the *cdc8-1* mutation. Mol. Cell. Biol. **3:** 1730–1737.

LITTLE, J. G., and R. H. HAYNES, 1979 Isolation and characterization of yeast mutants auxotrophic for 2′-deoxythymidine 5′-monophosphate. Mol. Gen. Genet. **168:** 141–151.

LIVINGSTON, D. M., 1977 Inheritance of the 2-micron DNA plasmid from *Saccharomyces*. Genetics **86:** 73–84.

MACDONALD, P. M., and D. H. HALL, 1984 Genetic evidence for physical interactions between enzymes of nucleotide synthesis and proteins involved in DNA replication in bacteriophage T4. Genetics **107:** 343–353.

MATHEWS, C. K., and J. R. ALLEN, 1983 DNA precursor biosynthesis, pp. 59–70 in *The Bacteriophage T4*, edited by C. K. MATHEWS, E. M. KUTTER, G. MOSIG and P. B. BERGET. ASM Press, Washington, D.C.

MATHEWS, C. K., and M. B. SLABAUGH, 1986 Eukaryotic DNA metabolism: are deoxyribonucleotides channeled to replication sites? Exp. Cell Res. **162:** 285–295.

MCNEIL, J. B., and J. D. FRIESEN, 1981 Expression of the Herpes simplex virus thymidine kinase gene in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **184:** 386–393.

MIOZZARI, G., P. NEIDERBERGER and R. HUTTER, 1978 Tryptophan biosynthesis in *Saccharomyces cerevisiae*: control of the flux through the pathway. J. Bacteriol. **134:** 48–75.

MORTIMER, R. K., and D. C. HAWTHORNE, 1975 Genetic mapping in yeast. Methods Cell Biol. **121:** 211–233.

MORTIMER, R. K., and D. SCHILD, 1985 Genetic map of *Saccharomyces cerevisiae*, edition 9. Microbiol. Rev. **49:** 181–212.

NEWLON, C. S., R. D. LUDESCHER and S. K. WALTER, 1979 Production of petites by cell cycle mutants of *Saccharomyces cerevisiae* defective in DNA synthesis. Mol. Gen. Genet. **169:** 189–194.

ORR-WEAVER, T., J. SZOSTACK and R. ROTHSTEIN, 1983 Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101:** 228–245.

PATTERSON, M., R. A. SCLAFANI, W. L. FANGMAN and J. ROSAMOND, 1986 Molecular characterization of the cell cycle gene *CDC7* from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:** 1590–1598.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

REDDY, G. P. V., and A. B. PARDEE, 1980 Multienzyme complex for metabolic channeling in mammalian DNA replication. Proc. Natl. Acad. Sci. USA **77:** 3312–3316.

ROSE, M. D., P. NOVICK, J. H. THOMAS, D. BOTSTEIN and G. R. FINK, 1987 A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:** 237–243.

SCHATZ, P. J., F. SOLOMON and D. BOTSTEIN, 1986 Genetically essential and nonessential alpha-tubulin genes specify functionally interchangeable proteins. Mol. Cell. Biol. **6:** 3722–3733.

SCHILD, D., and B. BYERS, 1978 Meiotic effects of DNA-defective cell division cycle mutations of *Saccharomyces cerevisiae*. Chromosoma **70:** 109–130.

SCLAFANI, R. A., and W. L. FANGMAN, 1984 Yeast gene *CDC8* encodes thymidylate kinase and is complemented by herpes thymidine kinase gene TK. Proc. Natl. Acad. Sci. USA **81:** 5821–5825.

SCLAFANI, R. A., and W. L. FANGMAN, 1986 Thymidine utilization by *tut* mutants and facile cloning of mutant alleles by plasmid conversion in *S. cerevisiae*. Genetics **114:** 753–767.

SHERMAN, F., 1982 Suppression in the yeast *Saccharomyces cerevisiae*, pp. 463–486 in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, edited by J. N. STRATHERN, E. W. JONES and J. E. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SIMCHEN, G., 1974 Are mitotic functions required in meiosis? Genetics **76:** 745–753.

SINGH, A., and T. R. MANNEY, 1974 Suppression of two missense alleles of the TRP5 locus of *Saccharomyces cerevisiae*. Genetics **77:** 661–670.

SLONIMSKI, P. P., G. PERRODIN and J. H. CROFT, 1968 Ethidium bromide induced mutation of yeast mitochondria: complete transformation of cells into respiratory deficient nonchromosomal "petites." Biochem. Biophys. Res. Commun. **30:** 232–239.

TSCHUMPER, G., and J. CARBON, 1982 Delta sequences and double symmetry in a yeast chromosomal replicator region. J. Mol. Biol. **156:** 293–307.

WICKREMASINGHE, R. G., J. C. YAXLEY and A. V. HOFFBRAND, 1983 Gel filtration of DNA polymerase and DNA precursor-synthesizing enzymes from a human lymphoblastoid cell line. Biochim. Biophys. Acta **740:** 243–248.

WILLIAMSON, D. H., and D. J. FENNEL, 1975 The use of fluorescent DNA binding agent for detecting and separating yeast mitochondrial DNA. Methods Cell Biol. **12:** 335–351.

ZUK, J., H. BARANOWSKA and D. ZABOROWSKA, 1988 Evidence that the single *CDC8* gene which encodes thymidylate kinase is involved in DNA replication, recombination and mutation in yeast. Curr. Genet. **14:** 303–309.

Communicating editor: E. W. JONES