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Selection by Genetic Transformation of a *Saccharomyces cerevisiae* Mutant Defective for the Nuclear Uracil-DNA-Glycosylase

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A coliphage M13 chimer containing the *Saccharomyces cerevisiae TRP1* gene and *ARS1* replication origin (mPY2) was grown on an *ung⁻ dut⁻* strain of *Escherichia coli*. The resulting single-stranded phage DNA had 13% of thymine residues substituted by uracil. This DNA failed to transform a Δ*trp1* yeast strain to prototrophy. However, when a mutagenized yeast stock was transformed with uracil-containing single-stranded mPY2 DNA, unstable transformants were obtained. After plasmid segregation, about half of these were retransformed at a high frequency by uracil-containing single-stranded mPY2 DNA. In vitro, these mutants were defective for uracil-DNA-glycosylase activity. They were designated *ung1*. Strains containing the *ung1* mutation have an increased sensitivity to sodium bisulfite and sodium nitrite but a wild-type sensitivity to methyl methanesulfonate, UV light, and drugs that cause depletion of the thymidylate pool. They have a moderate mutator phenotype for nuclear but not for mitochondrial genes. A low mitochondrial uracil-DNA-glycosylase activity was demonstrated in the mutant strains.

Uracil is introduced into DNA either during DNA replication by incorporation of dUMP instead of dTMP or by spontaneous or induced deamination of cytosine to uracil (10, 19, 28, 30, 33, 39). From both of these modifications, although different in nature (i.e., a dA · dU base pair as a result of misincorporation versus a dG · dU base pair through deamination) uracil is efficiently excised by the enzyme uracil-DNA-glycosylase. This enzyme has been isolated from a wide variety of procaryotic and eucaryotic organisms (16). It is typically a monomeric enzyme with a molecular weight of 20,000 to 30,000, equally active on single-stranded (ss) and double-stranded (ds) DNAs (16). Unlike nucleases, which require Mg^{2+} for activity, uracil-DNA-glycosylase is fully active in the presence of EDTA. This facilitates activity measurements in crude extracts containing nucleases.

Escherichia coli mutants (*ung*) deficient for uracil-DNA-glycosylase have been useful in the study of the mechanism of DNA replication (24, 39) and in delineating excision repair pathways (36). Similarly, such a mutant in the yeast *Saccharomyces cerevisiae* could help in the study of analogous problems. To our knowledge all uracil-DNA-glycosylase mutants have been obtained by enzymatic screening of extracts from large numbers of colonies after mutagenesis. This approach, however, tends to generate predominantly leaky mutants, as indeed has been observed for *E. coli*, *Bacillus subtilis*, and the smut fungus *Ustilago maydis* (13, 29, 42). In addition, in vivo enzymatic activities may be different from those measured in vitro.

The observation by Duncan et al. (13), however, that uracil-containing T4 phage will plate efficiently only on *E. coli ung* mutants with an in vitro enzymatic activity of <1% of that of the wild type led us to devise a genetic scheme to isolate uracil-DNA-glycosylase mutants in *S. cerevisiae*. Using a genetic transformation procedure, we selected for survival of the transforming DNA only if the cell were defective for uracil-DNA-glycosylase. This paper describes the isolation and characterization of such mutants. In addition, evidence is presented for a separate mitochondrial uracil-DNA-glycosylase.

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MATERIALS AND METHODS

Strains and plasmids. *E. coli* strains used were HB101, K37 (Hfr *supD*), and BW313 (*dut-1 ung-1 thi-1 relA spoT1 F1 lysA*) (32). Yeast strains used were A364a (**a** *ade1 ade2 ura1 his7 lys2 tyr1 gal1*); HP147 (**a** or α, *his1-7 lys1-1′ ade2-1 hom3-10 trp5-48 mut1-1*); HP153 (as HP147, but *mut6-1*) and HP155 (as HP147, but *mut8-1*) from P. J. Hastings (University of Alberta, Edmonton, Canada); YM599 (**a** *ura3-52 ade2-101 lys2-801 trp1* [Δ(*trp1-ars1*) *Eco*RI fragment]) and YM600 (as YM599, but α) from M. Johnston (Washington University, St. Louis, Mo.); and LL20 (α *his3-11,15 leu2-3,112 can1*) from J. Szostak (Harvard University, Cambridge, Mass.). YM599 has a relatively low and LL20 a high transformation frequency. PY2 {α *ura3-52 leu2-3,112 trp1* [Δ(*trp1-ars1*)] *can1*} was selected from a cross between YM599 and LL20, and the resulting spores were screened for high transformation frequency. PY11 {α *ura3-52 leu2-3,112 trp1* [Δ(*trp1-ars1*)] *ung1-1 can1*}, PY15 {α *ura3-52 leu2-3,112 trp1* [Δ(*trp1-ars1*)] *ung1-5 can1*}, and PY16 {**a** *trp1* [Δ(*trp1-ars1*)] *his7 ung1-1 can1*} were isolated in this study. Homozygous diploids were made by transformation of haploids with a plasmid containing the wild-type HO gene (YCp50-HO) to URA^+ (21). The plasmid was then segregated and diploidy confirmed by failure to mate with **a** and α strains.

The recombinant M13 phage mPY2 was made with standard recombinant DNA techniques by insertion of the *TRP1-ARS1 Eco*RI fragment from YRp7 into the *Eco*RI site of the ds form of the M13 vector mWB2344 (a gift of W. Barnes, Washington University) (5, 38). Phages were grown on strain K37 in L broth to obtain wild-type ss and ds replicative-form DNAs. The protocol of Sagher and Strauss was followed to obtain uracil-substituted and 5-[^{3}H] uracil-substituted ss and replicative-form mPY2 DNAs (32). Phage mPY2 was passed three times serially through strain BW313 to ensure that negligible unsubstituted phage was left. Phages were purified

by polyethylene glycol precipitation and CsCl banding (41). To obtain ss DNA, phages at ca. 10^{13} PFU/ml were incubated in 10 mM Tris hydrochloride (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.1% sodium dodecyl sulfate, and 0.1 mg of proteinase K per ml at 37°C for 2 h. The medium was then incubated 15 min at 55°C and phenol extracted, and the DNA was precipitated in ethanol. Plasmid DNA and phage replicative-form DNA were prepared using the alkali procedure (6). Poly(dA-[^{3}H]dU) was synthesized by the method of Yamamoto and Holliday (42). Protein was determined by the method of Bradford (7), with bovine serum albumin as a standard.

Yeast transformation procedure. The procedure of Sherman et al. (35) was followed with the following modifications. Spheroplast formation with lyticase (34) and transformations were performed at 5- to 10-fold lower cell densities. A total of 10 μg of DNA (plasmid plus *E. coli* carrier DNA) was used per transformation.

Mutagenesis and mutant selection. PY2 cells were grown in 5 ml of medium to 5×10^7 cells per ml and mutagenized with 3% (vol/vol) ethyl methanesulfonate for 30 min at 30°C as described by Sherman et al. (35). Survival was 20%. Cells were then split into two portions, and each was grown in 5 ml of yeast extract-peptone-dextrose (YPD) medium for 5 h. A 50-μl sample was then diluted into 50 ml of YPD medium, grown to 2×10^7 to 3×10^7 cells per ml, and spheroplasted for transformation as described above. Each transformation tube contained 5 μg of uracil-substituted ss mPY2 DNA (ss mPY2 *ura*). Three transformations were done per independent culture. Control transformations contained no DNA (no tranformants) or 5 μg of wild-type ss mPY2 DNA ($>10^5$ transformants per plate). A total of nine colonies appeared on the six plates. They were purified by restreaking on SD medium (0.67% [wt/vol] yeast nitrogen base without amino acids, 2% [wt/vol] glucose, 2% [wt/vol] agar) containing uracil and leucine. Plasmids were segregated by overnight growth in YPD broth and plating on YPD medium. Colonies that failed to grow on selective medium (without tryptophan) were again transformed with ss mPY2 *ura* DNA. Five (*ungl-1* through *ungl-5*) of the nine original transformants were transformed by ss mPY2 *ura* DNA at high frequency.

Two independent clones (*ungl-1* and *ungl-5*) were crossed with strain YM599. The diploids were sporulated, and spores were screened for in vitro uracil-DNA-glycosylase activity and sensitivity to nitrous acid (see below). For the *ung-1* mutant 11 of 20 spores screened were deficient for the enzyme and all of those were more sensitive to nitrous acid than the spores containing enzymatic activity or the parental strain PY2. One of these isolates was designated PY11{α *ungl-1 ura3-52 leu2-3,112 trpl* [Δ(*trpl-arsl*)] *canl*}. The *ungl-5* mutant clearly contained multiple mutations. The original isolate grew more slowly than did the wild-type strain, was less sensitive than the wild-type strain to UV and chemicals, and was abnormally sensitive to the action of aminopterin. Examination of the spores from a YM599X *ungl-5* cross showed that the aminopterin sensitivity did not segregate with the *ung* mutation. One spore that had lost the abnormal aminopterin sensitivity was designated PY15 {**a** *ungl-5 ura3-52 leu2-3,112 trpl* [Δ(*trpl-drsl*)] *canl*}. The other phenotypes have not yet been resolved.

Preparation of cell extracts for screening of enzymatic activity. Cells were grown in 2 ml of YPD medium to 5×10^7 cells per ml. They were then harvested and suspended in 1 ml of a medium containing 1 M sorbitol, 0.1 M sodium citrate [pH 5.8], 10 mM EDTA, and 30 mM 2-mercaptoethanol. After suspension, cells were transferred to a Microfuge tube and made into spheroplasts with lyticase. The spheroplasts were pelleted by a few short pulses in the Microfuge and resuspended in 0.5 ml of a solution of 50 mM Tris hydrochloride (pH 7.5), 10% (vol/vol) glycerol, 5 mM 2-mercaptoethanol, 400 mM ammonium sulfate, and 0.2% (vol/vol) Triton X-100. After a freeze/thaw cycle that used dry ice, the lysates were centrifuged in a Microfuge for 10 min at 4°C, and 1 μl of the supernatants containing about 1 to 3 μg of protein was used for enzyme assays.

Subcellular fractionation. Cells were grown in 1 liter of YPD medium to early saturation. The cells were harvested in a GS-3 rotor for 5 min at 4,000 rpm, washed once with 500 ml of water and 100 ml of 1 M sorbitol, and suspended in 50 ml of 1 M sorbitol–0.1 M sodium citrate–10 mM EDTA–30 mM 2-mercaptoethanol. They were then spheroplasted with 10,000 U of lyticase at 30°C for 1 h. The spheroplasts were harvested at 1,000 × *g* for 10 min at 0°C. The remaining procedures were all carried out at 0 to 4°C. The spheroplasts were resuspended in 50 ml of 1 M sorbitol and divided into three portions. These were (i) whole-cell extract, (ii) nuclear extract, and (iii) mitochondrial extract.

(i) Whole-cell extract. Spheroplasts (5 ml) were centrifuged for 10 min at 1,000 × *g*, and the pellet was resuspended in 5 ml of lysis buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid]-KOH [pH 7.5], 10% [vol/vol] glycerol, 1 mM EDTA, 5 mM 2-mercaptoethanol, 0.2% Triton X-100, 400 mM ammonium sulfate, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 2 μM pepstatin A) and frozen in dry ice. After thawing, the lysate was centrifuged at 35,000 × *g* for 20 min, and the protein was precipitated from the supernatant by addition of 0.31 g of solid ammonium sulfate per ml. After 1 h at 0°C the precipitate was collected for 20 min at 35,000 × *g* and resuspended in 1 ml of buffer C (20 mM Tris hydrochloride [pH 7.5], 10% glycerol, 5 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM benzamidine, 2 μM pepstatin A).

(ii) Nuclear extract. Nuclei were prepared from 25 ml of spheroplasts with a French press and differential centrifugation (23). The crude preparation of nuclei obtained by this method was resuspended in 1 ml of lysis buffer and treated as described above for the whole-cell extract except that the final ammonium sulfate precipitate was dissolved in 200 μl of buffer C.

(iii) Mitochondrial extract. Spheroplasts (20 ml) were pelleted, resuspended in 40 ml of buffer M (20 mM Tris hydrochloride [pH 7.5], 0.6 M sorbitol, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 1 μM pepstatin A) plus 2 mg of bovine serum albumin per ml, and passed through a French press at 4,000 to 5,000 lb/in^2. After a 10-min spin at 800 × *g* to pellet whole cells, spheroplasts, and cell wall debris, the mitochondria were pelleted at 12,000 × *g* for 10 min. The pellet was resuspended in 40 ml of buffer M, and the mitochondria pelleted from the supernatant of a 10-min spin at 800 × *g* by centrifuging at 12,000 × *g* for 10 min. This procedure was repeated once more. The final mitochondrial pellet was resuspended in 1 ml of lysis buffer and treated as described above for the whole-cell extract, except that the final ammonium sulfate precipitate was dissolved in 200 μl of buffer C.

Uracil-DNA-glycosylase assay. The 200-μl assay mixture contained 20 mM Tris hydrochloride (pH 7.8), 5 mM dithiothreitol, 4% glycerol, 0.1 mg of bovine serum albumin per ml, 2 mM EDTA, 40 mM NaCl, DNA, and enzyme. The DNA was either ss M13 [^{3}H]*ura* DNA (2 μg, 10,000 cpm), or poly(dA-[^{3}H]dU) (15 ng, 20,000 cpm), together with 250 ng of poly(dA-dT) carrier DNA. After 1 h at 37°C the reactions

were returned to ice, and 5 μg of calf thymus carrier DNA and 150 μl of 20% trichloroacetic acid were added. The tubes were left on ice for 10 min and centrifuged in a Microfuge for 10 min; 300 μl of the supernatant was then added to 4 ml of Biofluor (New England Nuclear Corp.) and counted in a liquid scintillation counter. One unit of enzyme activity solubilizes 1 nmol of uracil per h at 37°C.

For screening purposes poly(dA-[^{3}H]dU) was used as the template with 1 μl of the cleared lysate. Strains mutant for uracil-DNA-glycosylase invariably gave <500 cpm, whereas wild-type strains gave about 15,000 cpm (total conversion of substrate) of acid-soluble radioactivity.

For chromatographic analysis, part of the reaction mixture (50 μl) was applied to a Silica Gel F254 plate (E. Merck AG), with nonradioactive uracil and deoxyuridine used as markers. The mixture was developed twice in 12% (vol/vol) $CH_3OH/CHCl_3$ containing 0.1 N HCl. The uracil and deoxyuridine spots were scraped off, dissolved in 150 μl of 0.1 N NaOH, and counted after addition of 4 ml of Biofluor.

Mutagenic treatments. Cells were grown on YPD plates for 2 days, after which individual colonies were grown in YPD broth for 3 more days to stationary phase. Cells were then harvested, washed once with water, and resuspended at 10^6 to 10^7 cells per ml in the appropriate buffer for treatment: 5 to 20 mM sodium bisulfite in 0.1 M sodium citrate (pH 3.6) for 90 min at 30°C (11); 7.5 to 30 mM sodium nitrite in 0.1 M sodium citrate (pH 4.5) for 45 min at 23°C (43); or 2 to 8 mg of methyl methanesulfonate per ml in 0.1 M sodium phosphate (pH 7.2) for 60 min at 23°C. Treatment was stopped by at least 100-fold dilution in 0.1 M Tris hydrochloride, pH 7.5. Cells were then further diluted and plated on YPD plates to determine survival. For treatment with UV light, stationary-phase cells were diluted appropriately, plated on YPD plates, and irradiated immediately at 254 nm. Colonies were counted after a 3-day incubation at 30°C.

Measurement of thymineless death. Log-phase cultures of PY2 and PY11 were diluted to 10^6 cells per ml in YPD medium, and sulfanilamide and aminopterin were added to 5 mg/ml and 100 μg/ml, respectively (3). Samples were withdrawn periodically, diluted at least 1,000-fold, sonicated for 15 s at a setting of 3 on a Branson 185 sonifier (Branson Sonic Power Co.) to disperse clumps of cells, and plated on YPD plates. Survivors were counted after 3 days at 30°C.

Determination of spontaneous mutation frequency. Strains PY2 and PY11 were plated to obtain single colonies on YPD plates. From each plate 10 colonies were grown further in 5 ml of YPD medium for 2 days to saturation. Half of the cells were pelleted in a clinical centrifuge, washed once with water, and resuspended in water to ca. 10^8 cells per ml. The other half was incubated in a roller drum for another 28 days at 30°C. After appropriate dilutions the cells were plated on the following media: YPD to determine total cell number; YPD containing 20 mM of caffeine or 1 μg of cycloheximide per ml to determine the number of caffeine- and cycloheximide-resistant cells, respectively (4, 40); SD plates containing uracil, leucine, and tryptophan (each 20 μg/ml) and 1 μM 5-fluorocytosine to determine fluorocytosine-resistant cells (22); aminoadipic acid plates to determine the number of *lys2* auxotrophs (35); and YPGE plates (2% Bacto-Peptone, 1% yeast extract, 3% glycerol, 2% ethanol) containing 0.6 mg of erythromycin per ml or 2 mg of chloramphenicol per ml to determine erythromycin- or chloramphenicol-resistant cells, respectively. Chloramphenicol-resistant colonies were replated on YPGE plates containing tetracycline (2 mg/ml) to screen for mitochondrial or nuclear origin of the mutants (15).

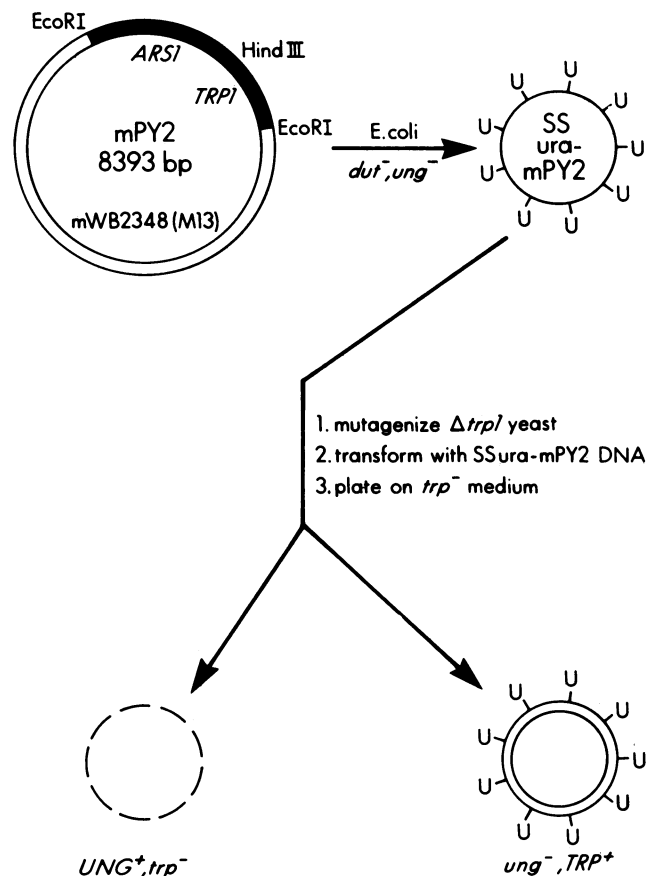

FIG. 1. Selection scheme for the uracil-DNA-glycosylase mutant.

RESULTS

Transformations with uracil-containing DNA. The structure of the recombinant M13 phage containing the *S. cerevisiae TRP1* gene and the putative replication origin *ARS1* is shown in Fig. 1. Both ss and ds mPY2 DNAs transformed a *trp1* strain (PY2) with high frequency, in agreement with previous results (Table 1) (37). For our experiments, a yeast strain was chosen that was deleted for the same *TRP1-ARS1* fragment used in the construction of the mPY2 phage. This eliminates the possibility of complications due to plasmid integration by homologous recombination. In addition this measure eliminates the appearance of Trp$^+$ colonies due to ethyl methanesulfonate-induced reversion of a *trp1* point mutation.

Growth of M13 phages on an *E. coli* strain deficient for both dUTPase and uracil-DNA-glycosylase produces phage DNA with substantial substitution of thymine residues by uracil (32). To efficiently dilute out the unsubstituted phage, progeny phages were used to infect the same strain again. Progeny phages from this infection were used for a third infection. The original phage should now have been present at a ratio of less than 10^{-6}. The ss and ds mPY2 DNA obtained after growth of the phage in this strain were digested to deoxynucleosides with DNase I, snake venom phosphodiesterase, and alkaline phosphatase (26). High-pressure liquid chromatography of the deoxynucleosides indicated 13 and 12% substitution of thymine by uracil respectively (26).

TABLE 1. Transformation frequencies of *UNG* and *ung1* strains

Strain	Relevant genotype	Transformants/μg[a]			
		ss mPY2	ss mPY2 *ura*	ds mPY2	ds mPY2 *ura*
PY2	α *trp1 UNG*	3×10^6	0[b]	1.0×10^6	10^2[b]
YM599	**a** *trp1 UNG*	5×10^5	0[b]	ND[c]	ND
PY11	α *trp1 ung1-1*	4×10^6	3.5×10^6	1.2×10^6	1.5×10^6
PY11/PY11	**a** *trp1 ung1-1* / α *trp1 ung1-1*	2.2×10^6	1.7×10^6	ND	ND
PY15	α *trp1 ung1-5*	2×10^6	6×10^5	ND	ND
PY15/PY15	**a** *trp1 ung1-5* / α *trp1 ung1-5*	1.8×10^6	5×10^5	ND	ND
PY16/PY15	**a** *trp1 ung1-1* / α *trp1 ung1-5*	1.2×10^6	6×10^5	ND	ND
PY2/YM599	**a** *trp1 UNG* / α *trp1 UNG*	2×10^5	0[b]	ND	ND
PY11/YM599	**a** *trp1 UNG* / α *trp1 ung1-1*	5×10^5	0[b]	ND	ND

[a] 1 ng of phage DNA was added to 10 μg of *E coli* DNA. At least three independent transformations were performed for each strain and for each plasmid. The most representative result is shown.
[b] For each transformation, 10 μg of phage DNA was used.
[c] ND, Not determined.

The ds form of the uracil-substituted phage transformed wild-type yeast very inefficiently, and the ss form did not transform it at all (Table 1). For the ss DNA this failure to transform was presumably due to uracil excision upon introducing the DNA into the spheroplasts, resulting in loss of genetic information. For the ds form, where uracil excision should in principle not be fatal, the extensive endonucleolytic and exonucleolytic action following the initial excision event may diminish survival greatly.

Isolation of *ung1* mutants. The absence of Trp^+ colonies upon transformation of strain PY2 with ss mPY2 *ura* DNA formed the basis for selection of an uracil-DNA-glycosylase mutant, as shown in Fig. 1. This selection method was highly successful. Of the nine Trp^+ colonies that appeared after transformation of mutagenized yeast with ss mPY2 *ura* DNA, five were retransformed by ss mPY2 *ura* DNA with various but high frequencies (10^5 to 10^7 transformants per μg of DNA) and were deficient in an in vitro assay for uracil-DNA-glycosylase activity. The mutants *ung1-1* and *ung1-5* were of independent origin. Of the other mutants (*ung1-2*, *ung1-3*, and *ung1-4*) that derived from the same transformation plate as the *ung1-1* mutant, two were petite and one grew very slowly. The *ung1-1* isolate was crossed with strain A364a, and strain PY16 (**a** *ung1-1*), which resulted from sporulation of the diploid, was mated with the five original mutants (*ung1-1* through *ung1-5*). All five diploids were defective for uracil-DNA-glycosylase activity (results not shown). After thus having established that all mutants fell into the same complementation group, only *ung1-1* and *ung1-5* were further studied. The strains were backcrossed with YM599, and diploids were sporulated to segregate (in the case of the *ung1-5* isolate) secondary mutations (see Materials and Methods). The mutants resulting from these crosses were designated PY11 (*ung1-1*) and PY15 (*ung1-5*), respectively.

Characterization of the *ung1* mutants. Whole-cell extracts from strains PY11 (*ung1-1*) and PY15 (*ung1-5*) were deficient for uracil-DNA-glycosylase activity in in vitro assays. The assay with ss M13 [^{3}H]*ura* DNA was such that an activity of 1% or less of wild-type genetic activity would not have been

TABLE 2. Uracil-DNA-glycosylase activities in various strains

Strain	Relevant genotype	Uracil-DNA-glycosylase activity[a] with:	
		ss M13 [^{3}H]ura	poly (dA-[^{3}H]dU)
PY2	α *UNG*	0.18	9.4
YM599	**a** *UNG*	0.13	+[c]
PY11	α *ung1-1*		0.0041
PY15	α *ung1-5*		0.0053
YM599/PY11	**a** *UNG* / α *ung1-1*	ND[b]	+
PY16	**a** *ung1-1*	ND	0[d]
PY16/PY15	**a** *ung1-1* / α *ung1-5*	ND	0
HP147	*mut1-1*	ND	+
HP153	*mut6-1*	ND	+
HP155	*mut8-1*	ND	+

[a] Expressed as nanomole of uracil solubilized per milligram of protein per hour (with partialy purified extracts).
[b] ND, Not determined.
[c] +, Activity positive with crude lysates.
[d] 0, Activity negative with crude lysates.

detected (Table 2). More sensitive assays with the ds substrate poly(dA-[^{3}H]dU) showed a small amount of uracil-DNA-glycosylase activity in extracts from strains PY11 and PY15 (0.05% and 0.06% of wild-type activity, respectively). As described below, this activity is probably of mitochondrial origin, and the nuclear or cytoplasmic activity is either nil or extremely low in these mutant strains.

The genetic transformation data were, in general, in agreement with the enzymatic assays. Thus, ss mPY2 DNA and ss mPY2 *ura* DNA were equally efficient in transforming PY11 (*ungl-1*) to Trp$^+$, and the same applied to ds mPY2 and ds mPY2 *ura* DNA, indicating that in vivo no uracil excision occurred in either ss or ds DNA (Table 1). Strain PY15 (*ungl-5*), however, consistently gave a two- to four-fold-lower transformation frequency with ss mPY2 *ura* DNA than with the wild-type ss mPY2 DNA. Although this strain still contained secondary mutations (see Materials and Methods), this difference was observed consistently with various strains containing the *ungl-5* allele, as well as with a diploid containing the *ungl-1* and *ungl-5* alleles, but not with the homozygous *ungl-1*/*ungl-1* diploid (Table 1). For these reasons we assume that the *ungl-5* allele is slightly leaky, although this leakiness is not measurable in in vitro assays (see Tables 2 and 4). Mating of strain PY11 with strain YM599 produced a diploid homozygous for Δ*trpl* and heterozygous for the *UNG1* gene. As expected, the mutant allele was recessive to wild-type strains. Wild-type levels of uracil-DNA-glycosylase activity were found in cleared lysates from these cells. Moreover, transformation of the diploid with ss mPY2 *ura* DNA failed to give Trp$^+$ transformants (Table 1). Diploids homozygous for the *ungl-1* or *ungl-5* allele or a heterozygous *ungl-1*/*ungl-5* diploid were transformed efficiently by uracil containing ss mPY2 DNA (Table 1).

Wild-type strain PY2 and the isogenic mutant PY11 were subjected to the DNA-damaging agents, sodium bisulfite, sodium nitrite, methyl methanesulfonate, and UV light. Both bisulfite and nitrite damage DNA primarily through deamination of cytosine to uracil (19, 33), and the mutant was expected to be more sensitive than wild-type strains to treatment with these reagents. Methyl methanesulfonate and UV light are general DNA-damaging agents acting predominantly through base methylation and pyrimidine dimer formation, respectively. The enzyme uracil-DNA-glycosylase from *E. coli* does not recognize this type of damage (27). Because of the similarity between the yeast and the *E. coli* enzymes (9), we did not expect the yeast enzyme to act on methylated bases or pyrimidine dimers. Thus, the mutant strain was not expected to be more sensitive to the action of these agents. Representative dose-response survival curves for wild-type and mutant strains are shown in Fig. 2. Sensitivities depended significantly on the growth history of the cells. Reproducible results were obtained with cells that were first grown on solid media before transfer to liquid media and growth to stationary phase (see Material and Methods). PY11 (*ungl-1*) cells were much more sensitive to bisulfite and nitrite than were PY2 cells (*UNG1*), whereas sensitivities to methyl methanesulfonate and UV light were indistinguishable from the wild type (Fig. 2).

Depletion of the thymidylic acid pool through the action of the antifolate drugs aminopterin and sulfanilamide is lethal to yeasts (8). The primary cause for thymineless death has been attributed to excessive strand breakage as a result of extensive repair following the excision of uracil, which is incorporated in increased amounts from dUTP in the absence of dTTP (17, 29). For this reason one would expect mutant strain PY11, deficient for the first step in the uracil excision repair pathway, to be less sensitive to thymidylic acid deprivation. However, our results showed that PY2 and PY11 were equally sensitive to the action of aminopterin and sulfanilamide (Fig. 3).

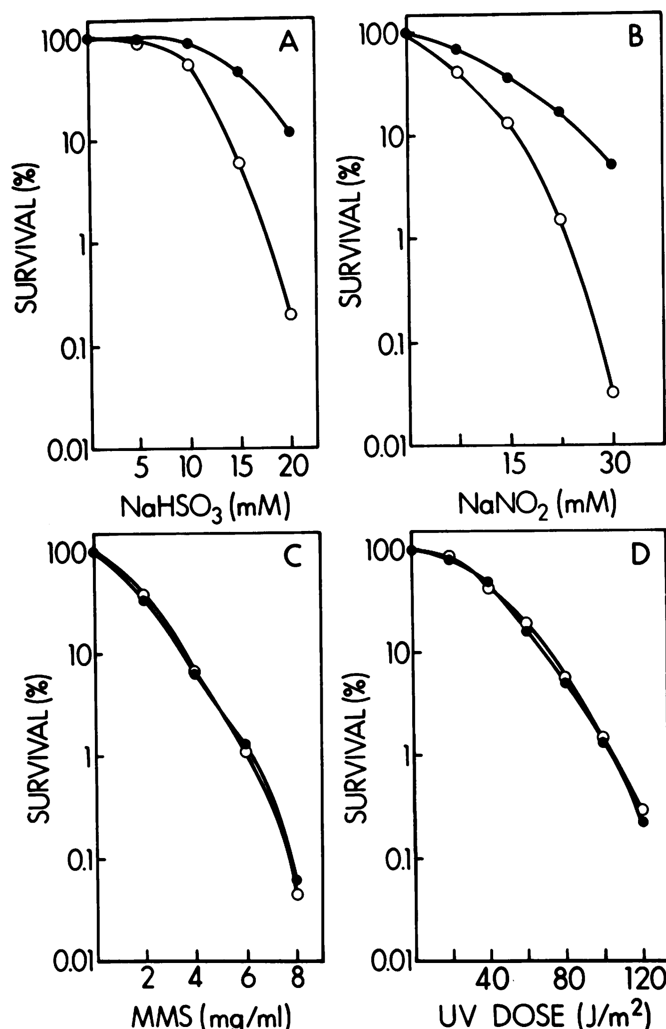

FIG. 2. Dose-response survival curves for PY2 (*UNG1*, ●) and PY11 (*ungl-1*, ○) after treatment with sodium bisulfite (A), sodium nitrite (B), methyl methanesulfonate (C) and UV light (D). For experimental details see the text.

Mutator phenotypes of mutants lacking uracil-DNA-glycosylase. The forward mutation rate of nuclear genes to give resistance to one of several drugs (caffeine, fluorocytosine, and cycloheximide) and to *lys2* auxotrophy was measured for strains PY2 and PY11. The spontaneous mutation rates of the mutant were 4 to 40 times that of the wild-type strain (Table 3).

If deamination of deoxycytidine to deoxyuridine is the primary cause for the induction of mutations, one would expect a stationary-phase culture of the repair-deficient strain to accumulate mutations if left for a long time at 30°C. In an attempt to quantitate this, cultures were left in the spent YPD medium for 1 month and then plated on the various drug-containing plates. No clear increase in mutation frequency was observed that could be attributed to the absence of uracil-DNA-glycosylase activity (Table 3). After 3 months of incubation of the stationary-phase cells survival

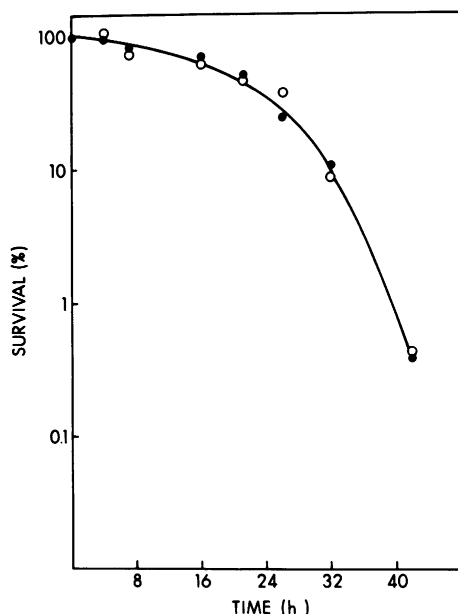

FIG. 3. Survival curves for PY2 (*UNG1*, ●) and PY11 (*ung1-1*, ○) under thymidylateless death conditions. For experimental details see the text.

was too low (0.7% for PY2 and 0.5% for PY11) to carry out the mutation rate experiment.

Resistance to erythromycin, which selectively inhibits mitochondrial protein synthesis, is conferred by mutations in the mitochondrial 21S rRNA gene (12). Spontaneous mutation rates to *ERY*^{r} are not increased in strain PY11. Similarly mutations in the mitochondrial 21S rRNA gene can confer resistance to chloramphenicol. Chloramphenicol resistance, however, can also originate through mutations in nuclear genes. The latter are pleiotropic and also confer resistance to tetracycline (15). To test for nuclear or mitochondrial origin of chloramphenicol resistance, 100 resistant colonies derived from strain PY2 were replated on tetracycline-containing plates (see Materials and Methods). Five colonies grew on tetracycline plates, indicating their nuclear origin. Of 100 chloramphenicol-resistant colonies derived from strain PY11, on the other hand, 86 grew on tetracycline-containing plates, indicating that only 14% of the resistant colonies were of mitochondrial origin. Thus, although the mutation rate to chloramphenicol resistance was much larger for the mutant strain PY11 than for the isogenic wild-type strain PY2, the contribution from mitochondrial mutations was very similar (Table 3).

Mutator mutants nondefective for uracil-DNA-glycosylase. Hastings et al. have isolated a large number of *S. cerevisiae* mutants with an increased spontaneous mutation phenotype (*mut1* through *mut10*) (18). Most of these mutator mutants also show an increased sensitivity to X rays, UV light, and a variety of chemicals (20). Three mutants, *mut1*, *mut6*, and *mut8*, however, have wild-type sensitivity to UV and methyl methanesulfonate; this sensitivity is phenotypically very similar to that of the *ung* mutant. Enzyme assays with crude-cell extracts from these three mutator mutants show wild-type uracil-DNA-glycosylase activity, indicating that these mutator mutants are not deficient for this enzyme.

Detection of a mitochondrially located uracil-DNA-glycosylase. Subcellular fractionation of whole-cell extract showed that in wild-type (PY2) the enzyme uracil-DNA-glycosylase is primarily located in the nucleus and cytoplasm (Fig. 4A; Table 4). The enzyme was much less active in mitochondrial extracts, and the activity measured could be accounted for by contamination of the mitochondria by cytoplasm, as monitored by the cytoplasmic marker glyceraldehyde-3-phosphate dehydrogenase (Table 4). However, from these measurements with wild-type cells the possible existence of a minor mitochondrially located uracil-DNA-glycosylase could not be excluded.

The presence of a minor, mitochondrially located enzyme is, in fact, borne out by subcellular fractionation of *ung1* cells. Thus, in contrast with the results with wild-type cells, there was more uracil-DNA-glycosylase in mitochondrial extracts from the mutant PY11 (*ung1-1*) than in nuclear or whole-cell extracts (Fig. 4B; Table 4). The activity in the nuclear extract (14% of the mitochondrial specific activity) was probably due to contamination of the nuclei by mitochondria. This was evident from the presence of considerable activity of fumarase (a marker for the mitochondrial matrix) in the nuclear extract (Table 4). In addition, microscopic observation of the isolated nuclei showed the presence of some mitochondria. The mitochondria, on the other hand, were not contaminated by nuclei. Similarly, mitochondrial extracts from PY15 (*ung1-5*) were also enriched for the enzyme (Table 4). Mixing the extracts (wild-type with mutant extracts and mitochondrial with nuclear or whole-cell extracts) showed that lack of activity in certain extracts was not due to inhibition of enzymatic activity.

TABLE 3. Spontaneous mutation rates of *UNG* and *ung1* strains[a]

Resistance[a]	Mutation rate (10^{-7}) after following no. of days[b]:			
	PY2		PY11	
	2 (100)	28 (81)	2 (100)	28 (76)
CAF^R	0.3 ± .2	0.5 ± .2	9.9 ± 2	19 ± 5
CYH^R	22 ± 3	10 ± 3	91 ± 15	40 ± 8
FCY^R	23 ± 5	38 ± 10	200 ± 40	240 ± 30
lys2	180 ± 30	145 ± 30	880 ± 180	1,410 ± 280
ERY^R	5 ± 1.2	3.4 ± .5	5.7 ± 1.5	4.5 ± 1.2
CAP^R				
Nuclear	0.7 ± 0.4	ND[c]	110 ± 30	ND
Mitochondrial	14 ± 4	ND	20 ± 7	ND

[a] Resistances to caffeine (*CAF*^{R}), cycloheximide (*CYH*^{R}), fluorocytosine (*FCY*^{R}), erythromycin (*ERY*^{R}), chloramphenicol (*CAP*^{R}), and *lys2* auxotrophy were measured.

[b] Percent survival is shown within parentheses.

[c] ND, Not determined.

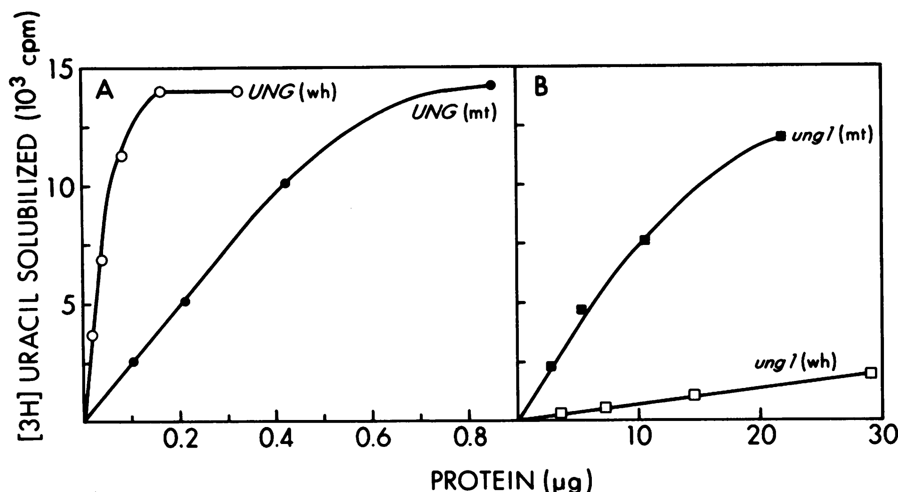

FIG. 4. Uracil-DNA-glycosylase activities in whole-cell (wh) and mitochondrial (mt) extracts from strain PY2 (A), and wh and mt extracts from strain PY11 (B). Note the 50-fold difference in the abcissa scales between A and B.

Thin-layer chromatographic analysis showed that all acid-soluble radioactivity produced by whole-cell or mitochondrial extracts from PY2 cells was in the form of uracil. A total of 80 percent of the acid-soluble radioactivity produced by the whole-cell extract from PY11 cells was in the form of uracil, and 20% was in the form of deoxyuridine. In the mitochondrial extract from PY11 cells 95% of the acid-soluble radioactivity was in the form of uracil, and 5% was in the form of deoxyuridine. In addition, when identical assays were carried out with poly(dA-[^{3}H]dT) as substrate prepared in the same way and with the same specific radioactivity as poly(dA-[^{3}H]dU), about 15% of the radioactivity in comparison with the uracil-containing substrate was solubilized by the whole-cell extract from PY11. With the mitochondrial extract from PY11 no significant radioactivity was solubilized from poly(dA-[^{3}H]dT).

The nuclear and cytoplasmic enzyme from PY2 cells was compared with the mitochondrial enzyme from PY11 cells. The inhibition by salt was the same: 50% inhibition at 140 mM NaCl for both enzymes. Product inhibition by uracil was also similar for both enzymes. The nuclear and cytoplasmic enzyme was inhibited by 50% with 0.75 mM uracil, and the mitochondrial enzyme was inhibited by 50% with 1.2 mM uracil. Because of the very low activity of the mitochondrial enzyme, no attempt was made to purify it from mitochondrial extracts. The activity (measured as specific activity) did not change more than twofold in extracts prepared from log phase cells (PY11) grown in YPD or in YPG medium or from stationary-phase cells (results not shown). The activity of the mitochondrial enzyme was not increased by addition of cofactors (Mg^{2+} and ATP).

TABLE 4. Uracil-DNA-glycosylase activity in various cell extracts

Strain	Extract	Enzyme activity (U/mg of protein)[a]		
		GDH	Fumarase	UNG
PY2	Whole cell	0.19 (100)	0.91 (12)	9.4 (97)
	Nuclear	0.024 (13)	1.15 (15)	9.7 (100)
	Mitochondrial	0.021 (11)	7.8 (100)	1.25 (13)
PY11	Whole cell	0.21 (100)	1.11 (16)	0.0041 (9)
	Nuclear	0.022 (10)	1.7 (24)	0.0061 (14)
	Mitochondrial	0.016 (8)	7.1 (100)	0.045 (100)
PY15	Whole cell	0.17 (100)	1.42 (17)	0.0053 (16)
	Mitochondrial	0.020 (12)	8.3 (100)	0.032 (100)

[a] Enzyme activities were determined by the methods of Krebs (25) for glyceraldehyde-3-phosphate dehydrogenase (GDH) (1 U = 1 μmol of NADH reduced per min at 25°C) and Racker (31) for fumarase (1 U = 1 μmol of fumarate formed per min at 25°C), except that spectrophotometric readings were at 250 nm; see the text for uracil-DNA-glycosylase (UNG). Percentages are given in parentheses.

DISCUSSION

A major problem in the study of DNA repair mechanisms in the yeast *S. cerevisiae* is that the ease of generating mutants in DNA metabolism is offset by the difficulty of their biochemical characterization. Thus, for none of the about 100 independent DNA repair mutants, isolated on the basis of increased sensitivity to DNA-damaging agents, is it known what exact enzymatic activity is affected (20). This is largely because of the complex pleiotropic phenotype of many of these mutants as well as the difficulty in setting up reliable in vitro assays in crude yeast extracts.

An alternative way to isolate repair mutants is to start from a well-characterized repair enzyme and obtain mutants either by screening for loss of enzyme activity after mutagenesis or by a specific selection procedure. Uracil-DNA-glycosylase is an ideal target for such an approach because the enzyme is well characterized biochemically and its activity can be easily assayed in crude extracts. Rather than try to isolate an uracil-DNA-glycosylase mutant by mass screening of individual colonies after mutagenesis, a procedure which tends to give leaky mutants, we settled on a genetic selection procedure (Fig. 1). A total of only five mutants, all in the same complementation group, was obtained from this selection procedure. None of these, however, had measurable enzyme activity in an assay with crude lysates. The simplest explanation for these observations is that all five mutants were in the structural gene (*ung1*) for the enzyme uracil-DNA-glycosylase.

The *ung1* mutation does not confer a serious disadvantage upon *S. cerevisiae*. The wild-type strain PY2 and the isogenic mutant PY11 are microscopically identical, and they have identical growth rates. They also survive extended periods in stationary phase to the same extent. Preliminary

investigations show that yeast has an extremely efficient dUTPase activity (data not shown). Our preliminary measurements of deoxyuridine levels in yeast DNA were in accord with this observation. Both in strain PY2 and in the mutant PY11 these levels were well below 0.1% (data not shown). In contrast with all this are the *U. maydis ung* mutants, which have a pleiotropic phenotype (42). Some of these mutant strains, however, contain secondary mutations, which could explain their pleiotropic phenotypes.

Two of the mutants studied in more detail, PY11 and PY15, showed a very low uracil-DNA-glycosylase activity, about 0.05% of wild-type activity in whole-cell extracts. Most of this activity is of mitochondrial origin. One of the mutants (PY15), however, was transformed less efficiently by uracil-containing DNA than by wild-type DNA (Table 1). This probably reflects the extreme sensitivity of this type of transformation procedure towards detecting low levels of the enzyme, although we cannot exclude the possibility that the enzyme is weakly active in vivo in this mutant but loses all activity upon the preparation of extracts. Subcellular fractionation identified the low residual activity in the mutants as a mitochondrial enzyme (Table 4). A rough calculation shows that there is about 1,000-fold more of the nuclear and cytoplasmic activity per wild-type cell than of the mitochondrial activity per mutant cell. Since about 10% of the cellular DNA is mitochondrial, this equates to 100-fold less of the mitochondrial enzyme on a nucleotide basis. It has been proposed that the high A+T content of yeast mitochondrial DNA (82%) is caused by the absence of a mitochondrial uracil-DNA-glycosylase (12). In the absence of such an activity cytosine deamination in positions that would not impair mitochondrial function may lead to the accumulation of T · A base pairs. In agreement with this hypothesis is the codon usage of mitochondrial genes, which is 90 to 95% A or T in position 3, where C or G also codes for the same amino acid (12). Our finding that yeast has a mitochondrial uracil-DNA-glycosylase activity does not necessarily refute the hypothesis. The mitochondrial activity is so low that some evolutionary drift to A+T richness through incomplete uracil excision may be possible. Anderson and Friedberg have reported on a mitochondrial uracil-DNA-glycosylase activity from human KB cells (1). In these cells, however, the mitochondrial enzyme is present at much higher levels, about 5% of the nuclear activity.

The spontaneous mutation data are in accord with the enzymatic observations. Ung$^-$ strain PY11 is a mutator for nuclear, but not for mitochondrial genes (Table 3). It should be emphasized that the mutation frequencies given in Table 3 are not absolute mutation rates. They have not been corrected for residual growth after plating of the cells on the drug-containing plates. Substantial residual growth occurred after smearing plates containing fluorocytosine and aminoadipic acid (*lys2* selection), and the absolute mutation rates were lower for these selections. The mutation rate ratio of wild-type to mutant strains, however, is not affected by this. The mutator phenotype of *ung* mutants is the result of G · C → A · T transitions caused by spontaneous deamination of cytosine residues to uracil (14). The resulting G · U base pair is nonspecifically repaired by excision repair mechanisms or, if not repaired, will lead to a A · T base pair at that particular position in one of the two daughter chromosomes after the next DNA replication cycle. Spontaneous cytosine deamination is most rapid for ss DNA, i.e, at the replication fork. Deamination of cytosines in ds DNA occurs about 100-fold more slowly (28). Since DNA is present in ss form for only a very short time, during which cytosine deamination occurs (measurable by mutation rates), one should also be able to measure cytosine deamination in ds DNA if the cells are left long enough in stationary phase. However, even after 1 month at 30°C we did not see an increase in mutation frequency in the mutant strain (Table 3). Possibly cytosine deamination in ds DNA is much slower than estimated. Alternatively, a very low level of uracil-DNA-glycosylase activity in the mutant may be sufficient for the repair of these sporadic events in nonreplicating cells. To answer these and related questions we are currently cloning the *UNG1* gene so that we can construct a deletion strain.

The sensitivity of mutant PY11 to bisulfite is most apparent at the low pH of 3.6 (Fig. 2A). At a pH of 6.0 a similar degree of killing was only obtained with 1 M bisulfite, and both wild-type (PY2) and mutant (PY11) strains were killed at similar rates (results not shown). This indicates that bisulfite is most reactive in and specific for DNA deamination at very low pH. It has been shown previously that the mutagenic action of bisulfite is also most apparent at pH 3.6 (11). Similarly, the mutant strain is much more sensitive than the wild-type strain to exposure to sodium nitrite at a low pH (Fig. 2B). Cytosine deamination by nitrous acid clearly is the predominant cause of mutation induction (43) and loss of viability in yeast.

Contrary to our expectations, the mutant strain was as sensitive to aminopterin and sulfanilamide as the wild-type strain. These drugs deplete the pool of the folate cofactor necessary for conversion of dUMP to dTMP and thus cause depletion of the dTTP pool. Models for thymidylateless death in yeast assign a pivotal role to the uracil excision repair pathway (2). Under these conditions increased incorporation of dUTP instead of dTTP occurs. The ensuing excision repair after uracil excision causes so many nicks and gaps and eventual double strand breaks that the cell dies. This excision repair pathway is not operative in an *ung1* strain; as a result, survival of the mutant is enhanced. This has been observed in *B. subtilis ung* mutants (29). Our results, however, indicate that increased strand incision is not the primary cause for cell death under thymidylate starvation conditions. Either the absence of dTTP at the replication fork and its subsequent break-up, or the morphological changes that the cell undergoes when exposed to certain drugs (enlargment of the cell and loss of storage bodies through a weakened cell wall), cause cell death.

Finally, the *ung1* mutant should be very useful in delineating the excision repair pathways and the study of the replication fork in yeasts.

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LITERATURE CITED

1. **Anderson, C. T. M., and E. C. Friedberg.** 1980. The presence of nuclear and mitochondrial uracil-DNA-glycosylase in extracts of human KB cells. Nucleic Acids Res. **8**:875–888.
2. **Barclay, B. J., B. A. Kunz, J. G. Little, and R. H. Haynes.** 1982. Genetic and biochemical consequences of thymidylate stress. Can. J. Biochem. **60**:172–194.
3. **Barclay, B. J., and J. G. Little.** 1978. Genetic damage during thymidylate starvation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **160**:33–40.
4. **Bard, M., J. L. Neuhauser, and N. D. Lees.** 1980. Caffeine resistance of *Saccharomyces cerevisiae*. J. Bacteriol. **141:**

999–1003.

5. **Barnes, W. M., and M. Bevan.** 1983. Kilo-sequencing: an ordered strategy for rapid DNA sequence data acquisition. Nucleic Acids Res. **11**:349–368.
6. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
8. **Brendel, M., and U. C. Langjar.** 1974. "Thymineless death" in a strain of *Saccharomyces cerevisiae* auxotrophic for deoxythymidine-5′-monophosphate. Mol. Gen. Genet. **131**:351–358.
9. **Crosby, B., L. Prakash, H. Davis, and D. C. Hinkle.** 1981. Purification and characterization of an uracil-DNA-glycosylase from the yeast *Saccharomyces cerevisiae*. Nucleic Acids Res. **9**:5797–5809.
10. **Da Roza, R., E. C. Friedberg, B. K. Duncan, and H. R. Warner.** 1977. Repair of nitrous acid damage to DNA in *Escherichia coli*. Biochemistry **16**:4934–4939.
11. **Dorange, J. L., and P. Dupuy.** 1972. Mise en evidence d'une action mutagene du sulfite de sodium sur la levure. C. R. Acad. Sci. Ser. D **274**:2798–2800.
12. **Dujon, B.** 1981. Mitochondrial genetics and functions, p. 505–635. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Duncan, B. K., P. A. Rockstroh, and H. R. Warner.** 1978. *Escherichia coli* K-12 mutants deficient in uracil-DNA-glycosylase. J. Bacteriol. **134**:1039–1045.
14. **Duncan, B. K., and B. Weiss.** 1982. Specific mutator effects of *ung* (uracil-DNA-glycosylase) mutations in *Escherichia coli*. J. Bacteriol. **151**:750–755.
15. **Forster, J. L., and R. A. Kleese.** 1975. The segregation of mitochondrial genes in yeast. II. Analysis of zygote pedigrees of drug resistant X drug sensitive crosses. Molec. Gen. Genet. **139**:341–355.
16. **Friedberg, E. C.** 1985. DNA repair, p. 154–160. W. H. Freeman & Co., New York.
17. **Goulian, M., B. Bleile, and B. Y. Tseng.** 1980. Methotrexate-induced misincorporation of uracil into DNA. Proc. Natl. Acad. Sci. USA **77**:1956–1960.
18. **Hastings, P. J., S. K. Quah, and R. C. von Borstel.** 1976. Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA. Nature (London) **264**:719.
19. **Hayatsu, H.** 1976. Bisulfite modification of nucleic acids and their constituents. Prog. Nucleic Acids Res. Mol. Biol. **16**:75–124.
20. **Haynes, R. H., and B. A. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Jensen, R., G. F. Sprague, and I. Herskowitz.** 1983. Regulation of yeast mating type inter-conversion: feedback control of HO gene expression by the mating type locus. Proc. Natl. Acad. Sci. USA **80**:3035–3039.
22. **Jund, R., and F. Lacroute.** 1970. Genetic and physiological aspects of resistance to 5-fluoropyrimidines in *Saccharomyces cerevisiae*. J. Bacteriol. **102**:607–615.
23. **Kamimura, K., E. Tsuchiya, T. Mayakawa, S. Fuki, and A. Hirata.** 1981. Isolation and characterization of the nuclei from *Saccharomyces cerevisiae*. Curr. Microbiol. **6**:175–180.
24. **Kornberg, A.** 1980. DNA replication, p. 347. W. H. Freeman & Co., New York.
25. **Krebs, E. G.** 1955. Glyceraldehyde-3-phosphate dehydrogenase from yeast. Methods Enzymol. **1**:407–409.
26. **Kuo, K. C., R. A. McCune, and C. W. Gehrke.** 1980. Quantitative reversed-phase high performance liquid chromatographic determination of major and modified deoxyribonucleosides in DNA. Nucleic Acids Res. **8**:4763–4776.
27. **Lindahl, T., S. Ljungquist, W. Siegert, B. Nyberg, and B. Sperens.** 1977. Properties of uracil-DNA-glycosidase from *Escherichia coli*. J. Biol. Chem. **252**:3286–3294.
28. **Lindahl, T., and B. Nyberg.** 1974. Heat-induced deamination of cytosine residues in deoxyribonucleic acid. Biochemistry **13**:3405–3410.
29. **Makino, F., and N. Munakata.** 1978. Deoxyuridine residues in DNA of thymine-requiring *Bacillus subtilis* strains with defective *N*-glycosidase activity for uracil-containing DNA. J. Bacteriol. **134**:24–29.
30. **Olivera, B. M.** 1978. DNA intermediates at the *Escherichia coli* replication fork: effect of dUTP. Proc. Natl. Acad. Sci. USA **75**:238–242.
31. **Racker, E.** 1950. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim. Biophys. Acta **4**:211–213.
32. **Sagher, D., and B. Strauss.** 1983. Insertion of nucleotides opposite apurinic/apyrimidinic sites in deoxyribonucleic acid during *in vitro* synthesis: uniqueness of adenine nucleotides. Biochemistry **22**:4518–4526.
33. **Schuster, H.** 1960. The reaction of nitrous acids with deoxyribonucleic acid. Biochem. Biophys. Res. Commun. **2**:320–323.
34. **Scott, J. H., and R. Schekman.** 1980. Lyticase: endoglucanase and protease activities that act together in yeast cell lysis. J. Bacteriol. **142**:414–423.
35. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Simmons, R. R., and E. C. Friedberg.** 1979. Enzymatic degradation of uracil-containing deoxyribonucleic acid. J. Bacteriol. **137**:1243–1252.
37. **Singh, H., J. J. Bieker, and L. B. Dumas.** 1982. Genetic transformation of *Saccharomyces cerevisiae* with single-stranded circular DNA vectors. Gene **20**:441–449.
38. **Stinchcomb, D. T., K. Struhl, and R. W. Davis.** 1979. Isolation and characterization of a yeast chromosomal replicator. Nature (London) **282**:39–43.
39. **Tye, B., J. Chien, I. R. Lehman, B. K. Duncan, and H. R. Warner.** 1978. Uracil incorporation: a source of pulse-labeled DNA fragments in the replication of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. USA **75**:233–237.
40. **Wilkie, D., and B. K. Lee.** 1965. Genet. Res. **6**:130.
41. **Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber.** 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology **40**:734–744.
42. **Yamamoto, Y., and R. Holliday.** 1983. Uracil-DNA-glycosylase defective mutants of *Ustilago maydis*. Current Genetics **7**:289–298.
43. **Zimmerman, F. K., R. Schwaier, and U. von Laer.** 1965. The influence of pH on the mutagenicity in yeast of N-methylnitrosamides and nitrous acid. Z. Vererbungsl. **97**:68–71.