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Specific Mutator Effects of *ung* (Uracil-DNA Glycosylase) Mutations in *Escherichia coli*

BRUCE K. DUNCAN[1]* AND BERNARD WEISS[2]

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111,[1] and Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205[2]

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Studies of *trpA* reversions revealed that G:C→A:T transitions were stimulated about 30-fold in *E. coli ung* mutants, whereas other base substitutions were not affected. A dUTPase (*dut*) mutation, which increases the incorporation of uracil into DNA in place of thymine, had no significant effect on the rate of G:C→A:T transitions. The results support the proposal that the glycosylase functions to reduce the mutation rate in wild-type cells by acting in the repair of DNA cytosine residues that have undergone spontaneous deamination to uracil. Further support was provided by the finding that when λ bacteriophages were treated with bisulfite, an agent known to produce cytosine deamination, the frequency of clear-plaque mutants was increased an additional 20-fold by growth on an *ung* host. Bisulfite-induced mutations of the cellular chromosome, however, were about equal in *ung*$^+$ and *ung* strains; it was found that during the treatment of *ung*$^+$ cells with bisulfite, the glycosylase was inactivated.

Uracil-DNA glycosylase releases uracil from DNA by hydrolyzing the bond between the base and a deoxyribose residue. In *Escherichia coli* and in many other organisms, the enzyme catalyzes the first step in a repair pathway for uracil-containing DNA (13). The presence of uracil in DNA is a consequence of the incorporation of dUTP or of the hydrolytic deamination of DNA cytosine residues. The occasional incorporation of uracil in place of thymine is likely because dUTP is an intermediate in the biosynthesis of thymidine in *E. coli* (20). The DNA of bacterial mutants lacking both dUTPase and uracil-DNA glycosylase can have as much as 20% of its thymine residues replaced by uracil (23). DNA phages grown on these mutants remain viable despite the substitution of as much as 30% of their thymine by uracil (22, 24). Because these A:U base pairs in DNA appear to be relatively harmless, uracil-DNA glycosylase probably did not evolve to correct them, but rather to correct G:U base pairs. A:U base pairs formed by dUTP incorporation are not mutagenic, but G:U base pairs are. The latter may arise from the spontaneous deamination of DNA cytosine (G:C→G:U) or from the misincorporation of dUTP opposite DNA guanine. Subsequent replication of G:U base pairs should result in G:C→A:T transitions.

Lindahl and Nyberg originally observed that the spontaneous deamination of DNA cytosine in vitro was rapid enough to suggest the necessity for uracil-DNA repair pathways, and Lindahl (13) suggested that uracil-DNA glycosylase was suited for this role. In confirmation of this hypothesis, we have shown that uracil-DNA glycosylase (*ung*) mutants have an increased rate of spontaneous G:C→A:T mutation. We present evidence that these spontaneous mutations are mostly the result of C deaminations and that *ung* mutations, under some conditions, enhance the mutagenicity of bisulfite, an agent that promotes cytosine deamination. Some of these results were described in an earlier communication (6).

MATERIALS AND METHODS

Strains. The *E. coli* K-12 strains used in this study are listed mainly in Table 1; additional *trpA* mutants of KD1107 and their *ung-1* derivatives are described in Table 2. The *ung-1* mutation was scored via the sensitivity of mutants to uracil-containing dUTPase-deficient T5 phages (T5 *dut*) (24). The *dut-1* mutation was scored via uracil sensitivity (10) and T5 *dut* resistance (24). *dut-1* mutants, which are thermosensitive thymidine auxotrophs, were propagated at 30°C in tryptone-yeast extract (TY) broth supplemented with 125 μg of thymidine per ml. To minimize genetic drift due to growth during storage, bacterial strains were stored in 20% glycerol at −70°C or in 50% glycerol at −20°C. The bacteriophages used were T5 *dut* (24), λ$^+$, λ *vir*, and λ *b*221 *c*I857 *c*III::Tn*10* *O*am (12).

Media. Minimal medium A has been described by Miller (14). TY medium was the nutrient medium of Adelberg et al. (1). Agar media supplemented with tetracycline contained 25 μg of tetracycline per ml and 30 mM sodium citrate.

Spontaneous mutation frequencies. Modified fluctua-

TABLE 1. Bacterial strains used

Strain	Genotype[a]	Source or reference[b]
BD10	W3110 *thyA36 deoC ung-1*	(5)
BD1101	*ung-1 tyrA2 thi-1 lacY1 gal-6 tonA2 tsx-70 rpsL supE*	(5)
BD1102	BD1101 *tyrA*$^+$ *ung*$^+$ *zfe-208*::Tn*10*[c]	P1(KL16::Tn*10*)[d] × BD1101 → Tyr$^+$ Tet^r
BD1208	KL16 *ung-1*	P1(BD10) × KA169 → Phe$^+$
BD1219	W3899-nam11 *zfe-208*::Tn*10*	P1(BD1102) × W3899-nam11 → Tet^r
BD1349	KD1105 *nadB7 ung-1 zfe-208*::Tn*10*	P1(BW280) × KD1105 → Tet^r
BD1375	KD1105 *ung-1*	P1(P90C) × BD1349 → Nic$^+$
BD1376	Same as KD1105	P1(P90C) × KD1349 → Nic$^+$
BD1383	BD1375[e] *pyrE zia-207*::Tn*10*	P1(BW228) × BD1375 → Tet^r
BD1384	BD1376 *pyrE zia-207*::Tn*10*	P1(BW228) × BD1376 → Tet^r
BD1385	Same as KD1105	P1(BW3101) × BD1384 → Ura$^+$
BD1388	KD1105 *dut-1*	P1(BW3101) × BD1384 → Ura$^+$
BD1391	KD1105 *ung-1*	P1(BW3101) × BD1383 → Ura$^+$
BD1394	KD1105 *ung-1 dut-1*	P1(BW3101) × BD1383 → Ura$^+$
BW187	KS468 *pyrE*$^+$ *zia-207*::Tn*10*	P1(KL16::Tn*10*) × KS468 → Ura$^+$ Tet^r
BW210	KS468 *gltS10*	P1(CS5) × KS468 → Glt^C
BW228	BW210 *zia-207*::Tn*10*	P1(BW187) × BW210 → Tet^r
BW280	KL16 *ung-1 nadB7 zfe-208*::Tn*10*	P1(BD1219) × BD1208 → Tet^r
BW3101	KS468 *dut-1 pyrE*$^+$	(10)
CH904	*argH ilvD130 lysA* Δ(*trp-tonB*)	C. Hill
CS5	Hfr Cavalli (PO2A) *metB1 gltS10 relA1 tonA22* T2^r	CGSC
ES927	W3110 *trpE9777*	E. C. Siegel
KA169	KL16 *pheA97*	CGSC
KD1105	KD1107 *trpA446*	E. C. Cox (11)
KD1107	F$^-$ *trp*$^+$ *his arg leu thr rpsE*	E. C. Cox
KL16	Hfr (PO-45) *thi-1 relA1 spoT1*	CGSC
KS468	*metB pyrE thi-1 rpsL lacMS286* (φ80dIII*lacBK1*)	(10)
P90C	F$^-$ Δ(*lac-proB*)$_{XIII}$ *ara*	J. H. Miller
RH21	*leu lacZL32 trp his argA lysA ilv rpsL*	R. Hoess (11)
W3899-nam11	*pncA1 nadB7*	CGSC

[a] For gene symbols, see Bachmann and Low (2). The nomenclature of the Tn*10* insertions follows the system used by Kleckner et al. (12).

[b] P1 transductions are described as follows: P1 (donor strain) × recipient → selected phenotype. CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn. Phenotypic symbols are derived from gene symbols except as follows: Nic$^+$, nicotinate independence, Tet^r, tetracycline resistance; Ura$^+$, uracil independence; Glt^C, glutamate utilization (specified by *gltS10*).

[c] *zfe-208*::Tn*10* is an insertion between the *glyA* and *guaB* genes. It cotransduces *nadB7* and *ung-1* with frequencies of 8 and 4%, respectively.

[d] KL16::Tn*10* is a pool of Tn*10* insertions into the chromosome obtained by infecting KL16 with λ *b*221 *c*I857 cIII::Tn*10* *O*am as described by Kleckner et al. (12).

[e] *zia-207*::Tn*10* is an insertion between the *xyl* and *dut* genes. It cotransduces with *pyrE* and *dut* at a frequency of 6%.

tion tests were carried out as described by Hoess and Herman (11). Single colonies were picked and grown to saturation at 25°C. The cultures were diluted to 100 cells per ml, and each was divided into 24 to 30 2-ml subcultures, which were regrown to stationary phase at 37°C in 18 h. The cultures were centrifuged, washed in medium A (minus tryptophan), and suspended in 1 ml of medium A. A 0.1-ml amount of each culture was spread on minimal medium plates (minus tryptophan) and incubated for 37°C for 3 days to test for revertants. In experiments with the leaky *trpA446* allele, 0.23 μg of 5-methyl tryptophan per ml was included in the selection plates to inhibit subsequent background growth and reversion. True revertants were distinguished by the 5-methyl tryptophan test described by Persing et al., which employs 80 μg of 5-methyl tryptophan on a filter disk to measure the zone of growth inhibition (16). In working with the *trpA446* allele, we found that the G:C→A:T revertants could be observed by incubating selective plates for 40 h and counting only the colonies that were about 1 mm or more in diameter. The viable cell count was determined by pooling the cultures and plating cells on TY plates. Mutation (reversion) frequencies were presented as the median rather than the number average to avoid the bias introduced by mutant clones occurring early in an experiment, but both sets of values were approximately equal.

Bisulfite mutagenesis. Bacterial cultures were grown to saturation in TY broth containing 0.2% maltose and 10 mM $MgSO_4$ at 25°C. The cells were washed by centrifugation and suspended in 0.25 volume of 0.2 M sodium acetate plus either 1 M NaCl or 1 M $NaHSO_3$, pH 5.2 (15). After incubation at 37°C, the cells were diluted 20-fold into medium A, adjusted to pH 7.0 to 7.4 with 1.0 M Tris, and centrifuged. After two additional washes in medium A, the cells were suspended in 10 mM $MgSO_4$.

TABLE 2. Base pair specificity of the *ung* mutator effect

Parental strain[a]	*trpA* allele	Reversion pathway	*trpA* reversion frequency[b] per 10^9 cells		Mutagenic enhancement[c] by *ung*
			ung$^+$	*ung*	
KD1089	A3	A:T → T:A	1.6	1.5	0.9
KD1088	A11	G:C → C:G	<0.5	<0.5	—
KD1092	A58[d]	A:T → G:C, C:G	5.6	5.1	0.9
K1094	A223	A:T → G:C, C:G	4.0	5.1	1.3
K1090	A23	A:T → G:C, C:G, T:A G:C → T:A, C:G	7.3	4.6	0.6
K1105	A446	G:C → A:T	0.7	12.4	18

[a] These *trpA* mutants of KD1107 were obtained from E. C. Cox. For the reversion tests, congenic pairs of *ung*$^+$ and *ung-1* derivatives were prepared by P1 transduction as described for BD1349 (Table 1). Strain BW280 was the donor, and the transductants were picked from the Tet^r Nic$^-$ (*nadB7*) recombinants.

[b] The reversion frequencies, listed for *ung*$^+$ and *ung* derivatives of the parent strains, are the fractions of Trp$^+$ cells in the cultures after 18 h of growth at 37°C.

[c] The mutagenic enhancement is the ratio of the reversion frequency of a *trpA* mutation in an *ung* strain to that in the corresponding *ung*$^+$ strain.

[d] After prolonged growth on minimal media, the *ung trpA58* strain, but not its *ung*$^+$ counterpart, gave rise to additional colonies that were not recorded in the table because they were due to extragenic suppression. All of 25 such strains tested retained the mutant *trpA* allele; it was cotransduced with *trpB*$^+$ (indole utilization) into strain CH904. The suppression may have been due to G:C → A:T mutations in the *glyV* gene (16).

CsCl-purified phage λ$^+$ were mutagenized (9) by diluting 10-fold into 4 M $NaHSO_3$-40 mM mercaptoethanol (pH 5.8) and incubating at 37°C. After 120 min, the phages were dialyzed at 4°C against 2,000 volumes of 20 mM Tris-hydrochloride buffer (pH 8.0)–20 mM $MgSO_4$–1 mM mercaptoethanol; three buffer changes were made in 24 h. Clear-plaque mutants were observed by plating 1×10^4 to 3×10^4 PFU/plate on either *ung* or *ung*$^+$ cells, followed by incubation for 15 h at 37°C. Mutation frequencies are reported as the fraction of mutants per viable organism.

Other methods. The Dowex-1 assay specific for uracil-DNA glycosylase in crude extracts has been described elsewhere (5). Cells transduced to tetracycline resistance were grown for 60 min at 25°C in TY plus 30 mM citrate before being spread on tetracycline plates.

RESULTS

Specificity of *ung*-induced mutations. *ung* mutants are base substitution mutators (5). To find the precise base changes enhanced by the uracil-DNA glycosylase deficiency, we used a series of well-characterized *trpA* mutants that revert to Trp$^+$ by known single-base-pair substitutions that have been discerned from amino acid sequence analysis. This *trpA* reversion system has been used extensively in mutagenesis specificity studies (11, 16). Each of the mutant *trpA* alleles used can revert to yield a fully active tryptophan synthetase A protein by only one amino acid substitution. Other substitutions or missense suppressor mutations result in no more than a partial restoration of activity. Such partial revertants are readily distinguished from the full revertants because the former remain sensitive to 5-methyl tryptophan and accumulate indoleglycerol in the medium (16). Table 2 lists the possible base pair changes that will generate a Trp$^+$ phenotype for each of the *trpA* mutant alleles used; all possible types of transitions and transversions are represented by this battery of test strains.

To measure the effect of the *ung* mutation on the frequency of various spontaneous base substitution mutations, we examined congenic *ung*$^+$ and *ung* derivatives of each *trpA* mutant (Table 2). *ung-1* markedly altered the reversion rate of only the *trpA446* allele, increasing it about 18-fold. In additional experiments, the reversion frequency of *trpA446* was remeasured, using larger numbers of cells, 2×10^{11} *ung*$^+$ cells and 5×10^{10} *ung* cells. The *trpA446* reversion frequency increased 15-fold from 9×10^{-10} to 1.3×10^{-8}. Over 95% (32/33) of the Trp$^+$ revertants of the *ung-1 trpA446* strain were resistant to 5-methyl tryptophan and therefore full revertants (G:C→A:T), whereas only 38% (24/63) of the Trp$^+$ revertants of the *ung*$^+$ strain were full revertants. Thus, the actual *ung*-induced increase in the G:C→A:T mutation frequency at *trpA446* was about 30-fold (i.e., from 5×10^{-10} to 1.31×10^{-8}). Similar studies with the frameshift indicator strains RH21 and ES927 showed no significant effect (less than twofold) of *ung-1* on the incidence of frameshift mutations.

Effect of dUTP incorporation. Whereas dUTP is only occasionally incorporated into DNA in wild-type cells, dUTPase-deficient (*dut*) mutants incorporate dUTP at a significantly higher rate, and the uracil-containing DNA remains largely unrepaired if an *ung* mutation is simultaneously present (19, 23). It was possible, therefore, that in *ung* mutants, normally occurring dUTP incorporation would lead to persistent G:U or A:U base pairs that would enhance G:C→A:T transitions; A:U base pairs might, for example, hinder the faithful replication or editing of the DNA. To test this possibility, we constructed a congenic set of *ung* and *dut* derivatives of the G:C→A:T tester strain. The *dut* mutation had no effect on the frequency of G:C→A:T mutations in either the *ung* or *ung*$^+$ cells. Thus, the mutator phenotype of *ung* strains cannot be attributed to the

TABLE 3. Effect of $NaHSO_3$ treatment on intracellular uracil-DNA glycosylase activity and on the plating efficiency of uracil-containing λ phages

Incubation time[a] (min)	% Enzyme activity remaining[b]		% Relative plating efficiency[c]			
			λ(T)		λ(U)	
	NaCl	$NaHSO_3$	NaCl	$NaHSO_3$	NaCl	$NaHSO_3$
0.2	100	100	87	52	0.075	.037
5	74	50	69	50	0.059	.032
10	59	18	58	50	0.087	.076
15	36	8	63	53	0.16	0.15
30	13	2	68	59	0.34	0.27
40[d]	11	1	61	46	0.32	0.23

[a] BD1385 was treated with NaCl or $NaHSO_3$, pH 5.2. At the indicated times, samples were assayed for enzyme activity and phage plating efficiency.

[b] The specific enzyme activity of untreated and 0.2-min crude extracts was 2.5 nmol min^{-1} mg^{-1} (100%).

[c] The plating efficiencies are reported relative to the plating efficiency of λ(T) and λ(U) on untreated BD1391 (*ung-1*). λ(T) and λ(U) are λ phages grown in strains BD1385 and BD1394 (*dut ung*), respectively. λ(T) plated with an efficiency of 100% on the overnight culture of BD1385 and 107% on BD1385 washed before treatment with $NaHSO_3$. λ(U) plated with an efficiency of 0.15% on untreated BD1385 and 0.12% on washed cells.

[d] The frequency of $NaHSO_3$-induced Trp^+ revertants was 3.4×10^{-8} in BD1385. BD1391 was mutagenized at the same time. The induced mutation frequency in BD1391 was 3.5×10^{-8}.

persistence in their DNA of uracil that was incorporated in place of cytosine or thymine. It is more likely that the hypermutability results from the unrepaired deaminated cytosine residues in DNA.

Effects of bisulfite. Simmons and Friedberg (18) and Hayakawa et al. (7) have observed that *E. coli ung* mutants are slightly hypersensitive to the lethal effects of sodium bisulfite. $NaHSO_3$ reacts with cytosine compounds to form an unstable cytosine-HSO_3 adduct that is hydrolyzed to uracil under acidic conditions (8). Thus, $NaHSO_3$ deaminates DNA cytosines, and it has been used as a specific G:C→A:T mutagen both in vivo and in vitro (8, 17). To test the hypothesis that the *ung* mutator phenotype is due to events subsequent to cytosine deamination, the bacteria were mutagenized with $NaHSO_3$. The repair-deficient *ung* mutants were expected to show a large increase in the frequency of *trpA446* reversion. This was not the case. Although the efficiency of mutagenesis varied from experiment to experiment (the $NaHSO_3$-induced Trp^+ mutation frequency ranged from 3×10^{-8} to 8×10^{-7}), the ratio of induced reversions was always approximately equal in ung^+ and *ung-1* cells (the ratio of frequencies, ung/ung^+, ranged from 0.9 to 2.7 in eight experiments). The results of a control experiment (Table 3) provided an explanation for this result; apparently cellular uracil-DNA glycosylase was inactivated by the low pH or high salt concentration or both during the bisulfite treatment. After 30 min at pH 5.2, the ung^+ cells retained only 2% of their original glycosylase activity when incubated with $NaHSO_3$ and 13% when incubated with NaCl. This inactivation may have made the ung^+ strain behave like a Ung^- strain during bisulfite mutagenesis.

To test for phenotypic conversion of ung^+ cells to Ung^- under the conditions of bisulfite treatment, we used a biological assay. Phages that contain large amounts of uracil in their DNA (i.e., those propagated on *dut ung* mutants) have a very low efficiency of plating on ung^+ as compared with *ung* hosts (22, 24). In ung^+ hosts their DNA is extensively degraded by excision-repair pathways that require the glycosylase in their first step (24). Accordingly, we first grew bacteriophage λ *vir* on a *dut ung* mutant under conditions that led to a 10% substitution of its thymine by uracil (23). We then compared the plating efficiency of these phages, designated λ(U), with normal λ *vir*, λ(T), on an ung^+ strain after treatment of the cells with bisulfite or with NaCl at pH 5.2 (Table 3). The decline in enzyme activity during these treatments was accompanied by a four- to sevenfold increase in the plating efficiency of λ(U). The plating efficiency of λ(T) decreased less than 20%. These differences indicate a partial phenotypic conversion of the cells to Ung^- and confirm that the enzyme was inactivated in vivo and not merely upon extraction.

Because bisulfite-treated bacteria could not be used to test the uracil-DNA repair hypothesis, bacteriophage λ was used. λ^+ phages were mutagenized in vitro with $NaHSO_3$ to deaminate cytosine residues in their DNA. Phage mutations were then measured by determining the titers of phages on ung^+ and *ung* bacteria that had not been treated with $NaHSO_3$ (Table 4). Bisulfite treatment increased the frequency of clear-plaque mutants about 13-fold in ung^+ cells

TABLE 4. Enhancement of yield of clear-plaque mutants in $NaHSO_3$-treated phages in an *ung* host

Phage treatment[a]	Host genotype	Titer (ml^{-1})	Mutants (ml^{-1})	Mutation frequency
None	ung^+	9.0×10^8	4.0×10^4	4.5×10^{-5}
	ung-1	9.0×10^8	4.0×10^4	4.5×10^{-5}
$NaHSO_3$	ung^+	1.1×10^7	6.4×10^3	5.8×10^{-4}
	ung-1	3.9×10^6	4.7×10^4	1.2×10^{-2}

[a] λ^+ was grown on BD1385 (ung^+) and mutagenized with $NaHSO_3$ as described in the text.

but about 270-fold in *ung* cells. The survival of the bisulfite-treated phages was also lower in the *ung* mutants. These were the results expected if DNA cytosine residues deaminated by $NaHSO_3$ are efficiently repaired only in cells containing uracil-DNA glycosylase.

DISCUSSION

The data indicate that a deficiency of uracil-DNA glycosylase enhances the occurrence of spontaneous G:C→A:T base substitution mutations. This result is consistent with Lindahl's proposal (13) that mutagenic deamination of DNA cytosine occurs spontaneously at a rate in excess of the observed mutation rate and that the glycosylase plays a key role in the excision-repair of the resulting DNA uracil. From the estimated rate of deamination (13), we calculate that unrepaired cytosine deaminations could have yielded a *trpA446* reversion frequency of at least 2×10^{-8} in our experiments. The *ung-1* mutant displayed a frequency of about 1×10^{-8} to 2×10^{-8} (Table 2). Therefore, the spontaneous deamination of DNA cytosine is rapid enough to account for the mutation rate of our *ung* mutants.

Coulondre et al. (3) observed that 5-methylcytosine residues in the *lacI* gene are more susceptible to transition mutations than are unmodified cytosines, although the two pyrimidine bases are deaminated at about the same rate (13). This finding was explained by the inability of the deaminated methylcytosine residues (i.e., thymine) to be recognized and removed by uracil DNA glycosylase. In *ung* mutants, however, G:C→A:T transitions were elevated to the point where they equaled G:MC→A:T transitions (4). Those results further support the idea that the hypermutability of *ung* mutants is due to their inefficient repair of deaminated cytosine residues in DNA.

The low pH and high ionic strength used for the bisulfite treatment of whole cells enhanced mutagenesis, at least in part, by inactivating uracil-DNA glycosylase. When λ phages were treated in vitro with bisulfite and then propagated on untreated cells, a *ung* host yielded about 20 times as many phage mutants as an ung^+ host. Bisulfite is now being widely used for the mutagenesis of DNA in vitro (17). Ung^- host cells may prove to be quite useful in increasing the yield of mutants after bisulfite treatment of DNA phages, of transducing particles, or of transforming DNA.

Although bisulfite mutagenesis is widely believed to be primarily due to cytosine deamination, we do not know for certain whether this is always the case. Why, for example, does cellular glycosylase fail to completely protect phage λ from bisulfite mutagenesis? When the bisulfite-treated phages were plated on ung^+ cells, their frequency of induced clear-plaque mutations was still 5% that of *ung* cells. These mutations could be due to (i) a failure of the cells to excise all of the uracil before replication of G:U base pairs, (ii) uracil-HSO_3 adducts remaining in the DNA and protecting it from base excision, (iii) bisulfite-catalyzed deamination of 5-methylcytosine, or (iv) bisulfite mutagenesis by a mechanism unrelated to cytosine deamination. At the present time we cannot distinguish among these possibilities.

Cytosine deamination is but one of several spontaneous hydrolytic reactions affecting the cellular genome. Because such deamination is an intrinsic property of DNA in solution, cells must have a uracil-DNA (and hypoxanthine-DNA) repair pathway to reduce spontaneous base substitution mutations to the observed low frequencies. Because the uracil-DNA glycosylase attacks G:U base pairs, A:U base pairs, and even uracil in single-stranded DNA (13), its existence precludes uracil being a normal constituent of DNA, and the incorporation of dUTP into DNA is largely prevented by dUTPase (23).

Uracil-DNA glycosylase effects a type of base mismatch repair in which the incorrect base (i.e., uracil) is selectively excised. Although there may be other base mismatch repair mechanisms in *E. coli* (21), they are apparently unable to substitute efficiently for the glycosylase and to suppress the high mutability of the *ung* mutants. It is possible that other enzymes might not recognize G:U base pairs, or they may be limited by their specificity for the primer ends of growing chains or for undermethylated nascent strands. What is unique about the glycosylase pathway is that the repair, like the damage, can theoretically occur in nonreplicating chromosomes, whereas other postulated mismatch repair systems are supposedly geared to correct errors in replication and need to distinguish the template from the nascent strand during replication to excise the incorrect member of a base pair.

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