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Synthesis and Metabolism of Uracil-Containing Deoxyribonucleic Acid in *Escherichia coli*†

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Significant amounts of uracil were found in the deoxyribonucleic acids (DNAs) of *Escherichia coli* mutants deficient in both uracil-DNA glycosylase (*ung*) and deoxyuridine 5'-triphosphate nucleotidohydrolase (*dut*) activities, whereas little uracil was found in the DNAs of wild-type cells and cells deficient in only one of these two activities. The amounts of uracil found in the DNAs of *dut ung* mutants were directly related to the growth temperature of the cultures, apparently because the deoxyuridine 5'-triphosphate nucleotidohydrolase synthesized by *dut* mutants was temperature sensitive. The *dut* mutant used failed to grow exponentially, became filamentous at temperatures above 25°C, and exhibited a hyperrec phenotype; however, the *ung* mutation suppressed all of these effects. Although the *dut ung* mutants grew exponentially at all temperatures, their growth rates were always slower than the growth rate of the wild type. Since pool size measurements indicated that both deoxyuridine triphosphate and deoxythymidine triphosphate pools were markedly elevated in *dut* mutants, the reduced growth rate of *dut ung* cells apparently was due to the actual presence of uracil in the DNA, rather than to a deficiency of deoxyuridine triphosphate and deoxyribosylthymine triphosphate for DNA synthesis. The presence of uracil in *E. coli* donor DNA also markedly reduced the recombination frequency when the recipient cells were *ung*$^+$, indicating that DNA repair commenced before the entering DNA could be replicated.

Although DNA in all living cells contains thymine, recently it has become clear that a small amount of dUTP may be normally incorporated into DNA and that uracil is subsequently removed from the DNA by uracil-DNA glycosylase (19, 20). Cells apparently synthesize large amounts of dUTP, a precursor of dTTP, but also contain a very active deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) activity to prevent significant incorporation of this dUTP into DNA. *Escherichia coli* mutants deficient in dUTPase activity (*dut* mutants) have been isolated (9), but these mutants are all leaky. These mutants are identical to a class of hyperrec mutants isolated previously and named *sof* mutants, because they accumulate short Okazaki fragments (12). These fragments were originally thought to be intermediates in DNA synthesis, but are now known to result from the incorporation of uracil into DNA and its subsequent removal by uracil-DNA glycosylase (21).

E. coli mutants deficient in uracil-DNA glycosylase activity (*ung* mutants) have also been isolated (3). Their only easily recognizable phenotype is their ability to serve as hosts for uracil-containing phage.

In this paper we report the synthesis of uracil-containing DNA in *E. coli dut ung* mutants and describe some of the properties of this DNA in vivo.

(A preliminary report of some of these results has appeared [23].)

MATERIALS AND METHODS

Bacterial strains and growth of bacteria. The *E. coli* K-12 strains used in this study are listed in Table 1. These *E. coli* strains were grown in a shaking water bath in various media. The minimal medium described by Davis and Mingioli (2) was supplemented with 0.5% Casamino Acids (Difco Laboratories), 2 μg of thiamine per ml, 2 μg of nicotinic acid per ml, and, when necessary, thymidine or thymine and is referred to below as Davis medium. The medium described by Fraser and Jerrel (6) was modified by adding 0.1% yeast extract (Difco) and reducing the Casamino Acids to 0.5% and is referred to as modified Fraser medium. A rich nutrient medium containing 1% tryptone

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TABLE 1. *E. coli* strains used

Strain	Genotype	Source and/or reference
BD1137	*thi-1 argH1 nadB4 purI66 pyrE41 lacY1 malA1 xyl-7 rha-6 ara-13 gal-7 rpsL9 tonA2* or *tonA22 supE44* T2^r *rel-1*	(3)
BD1154	BD1137 *nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction (25)
BD1153	BD1137 *nadB*$^+$ *purI*$^+$ *pyrE*$^+$ *ung-1*	Transduction (25)
BD1156	BD1137 *nadB*$^+$ *purI*$^+$ *pyrE*$^+$ *dut-1*	Transduction (25)
BD1157	BD1137 *nadB*$^+$ *purI*$^+$ *pyrE*$^+$ *dut-1 ung-1*	Transduction (25)
W3110	*thyA36 deoC2*	J. Fuchs (1)
BW212	W3110 *dut-11 ung-1*	B. Weiss
BD1212	W3110 Tn*10*::(*glyA-purB*)	Transduction (25)
BD1284	W3110 *nadB dut-11* Tn*10*::(*glyA-purB*)	Transduction
KL16	Hfr (PO45) *relA1*	CGSC[a]
BW272	KL16 *dut-1 ung-1*	Transduction
RH2130	*leu trp his argA lysA ilv lacZL32 str thyA*	R. Hoess (10)
BD1301	RH2130 *dut-1 ung-1*	Transduction
BD1240	RH2130 *ung-1*	Transduction
KS468	*metB thi pyrE lacMS286* (φ80 dII *lacBK*1) *rpsL* F$^-$	B. Konrad (12)
BD1361	KS468 *pyrE*$^+$	Transduction
BD1362	BD1631 *dut-1*	Transduction
BD1363	BD1361 *ung-1*	Transduction
BD1364	BD1361 *dut-1 ung-1*	Transduction

[a] CGSC, Coli Genetic Stock Center, Yale University School of Medicine, New Haven, Conn.

(Difco), 0.5% yeast extract, 1% sodium chloride, and 50 μg of thymine per ml is referred to as TYT-medium. TYTG medium was TYT medium plus 0.5% glucose. Lactose tetrazolium plates were prepared as described by Miller (13). To label nucleotide pools, we used a Tris minimal medium (4) with the phosphate content reduced to 0.3 mM, which was supplemented with 0.2% glucose, 0.2% Norite-treated Casamino Acids, and 2 μg of thiamine per ml (03P medium). The addition of Casamino Acids increased the phosphate concentration of this medium to 0.5 mM.

Materials. [6-^{3}H]uridine was purchased from Schwarz/Mann and had a specific activity of 19 mCi/μmol. All commercial enzymes were purchased from Worthington Biochemicals Corp. Carrier-free $^{32}P_i$, in 0.01 N HCl was obtained from Atomenergikommisionens Forsøgsanlaeg, Risø, Denmark.

Incorporation of [6-^{3}H]uridine into *E. coli* DNA. *E. coli* BD1153, BD1154, BD1156, and BD1157 were grown in Davis medium at 37°C to a concentration of 2×10^8 to 3×10^8 cells per ml, and [6-^{3}H]-uridine was added to 5 ml of each culture to give a final concentration of 2.5 μCi/ml (0.5 nmol/ml). After the cultures were shaken for 40 min, the nucleic acids were precipitated with trichloroacetic acid, the RNA was removed by alkaline hydrolysis, and the distribution of radioactivity in the pyrimidine bases was determined as described previously (25).

To determine the stability of incorporated uracil in DNA, *E. coli* BW212 was grown at 37°C in modified Fraser medium supplemented with 20 μg of thymidine per ml and 400 μg of deoxyadenosine per ml to a concentration of 4×10^8 to 5×10^8 cells per ml. The cells were harvested, washed with modified Fraser medium, and resuspended in modified Fraser medium. [6-^{3}H]uridine was added to give a final concentration of 4.1 μCi/ml (0.8 nmol/ml). Samples were removed 10, 20, and 30 min later, the nucleic acids were precipitated with trichloroacetic acid, and the DNAs were isolated and analyzed for the amounts of radioactivity incorporated into their pyrimidine bases (25). The cells labeled for 30 min were also harvested, washed, and suspended in modified Fraser medium containing 0.1 μmol of unlabeled uridine per ml. Growth was continued, samples were removed at varying times, and the DNA was analyzed for the amount of radioactivity remaining in its pyrimidine bases.

Determination of base composition of DNA. Cultures (100 ml) of *E. coli* were grown in Davis medium containing either 0.1 or 0.2% glucose and supplemented with varying concentrations of thymine or thymidine (see Table 3). The cells were harvested by centrifugation, washed with 20 ml of 0.9% KCl, and suspended in 10 ml of 1× SSC (0.15 M sodium chloride plus 0.015 M sodium citrate). Sodium dodecyl sulfate was added (final concentration, 0.5%), and the mixture was stirred for 1 h at room temperature. Pronase was added (final concentration, 0.5 mg/ml), and after incubation for 15 min at 37°C, the mixture was extracted twice with phenol. Two volumes of cold ethanol was added to the deproteinized aqueous layer, and the nucleic acids were collected by centrifugation and then dissolved in 5 ml of 0.1× SSC. After 80 μg of RNase was added, this solution was incubated for 15 min at room temperature to hydrolyze the RNA; 0.5 ml of 3 M sodium acetate (pH 7) containing 0.001 M EDTA was added, and then 3 ml of isopropanol was added dropwise. The DNA was "spooled" to separate it from oligoribonucleotides, washed with 70% ethanol, and dissolved in 1 ml of 0.05 M Tris-chloride (pH 8).

To this DNA were added 20 μmol of $MgCl_2$, 0.5 mg of bovine serum albumin, 0.1 mg of pancreatic DNase, and 0.2 U of venom phosphodiesterase in a final volume of 1.08 ml. This solution was incubated for 20 min at 37°C to completely digest the DNA to 5′-deoxyribonucleotides and then for 2 min at 100°C to inactivate the enzymes. The reaction products were fractionated on a DEAE-Sephadex A-25 column (1.1 by 20 cm) by

using a linear gradient of ammonium formate (0.05 to 0.2 M, pH 3.7; 175 ml of each). The dUMP and dTMP eluted together, but separate from the other deoxyribonucleotides. The relative amounts of dUMP and dTMP were then determined by high-performance liquid chromatography on a Waters Bondapack C_{18} column eluted with 5 mM tetrabutyl ammonium hydrogen sulfate–5 mM potassium phosphate (pH 7.0) in 10% methanol. dTMP and dUMP were resolved clearly on this column.

Effect of temperature on growth. *E. coli* BD1153, BD1154, BD1156, and BD1157 were grown overnight at 30°C without shaking in Davis medium containing 0.1% glucose. The cultures were then diluted into fresh Davis medium containing 0.2% glucose, and growth was continued with shaking at different temperatures. Samples were removed at different times and titrated for viable cells. Some experiments were done in the presence of 100 μg of thymidine per ml or 100 μg of deoxyuridine per ml.

Effect of temperature on incorporation of uracil into DNA. *E. coli* BD1157 was grown in Davis medium at 30°C to a concentration of 2×10^8 to 3×10^8 cells per ml. Each culture was split into four portions, and these were grown at 25, 30, 38, and 42°C for 30 min. [6-^{3}H]uridine was added to each portion to give a final concentration of 2.5 μCi/ml (0.5 nmol/ml), and growth was continued for 30 min. The nucleic acids were then precipitated with trichloroacetic acid, and the DNA was isolated and analyzed for the amount of radioactivity in its pyrimidine bases.

Measurement of hyper-rec phenotype. *E. coli* BD1361, BD1362, BD1363, and BD1364 were spread on to lactose tetrazolium plates. After 3 days at 30°C, single colonies were examined at ×5 magnification for the number of *lac*$^+$ papillae present (11).

Effect of uracil on recombination frequency. Exponentially growing streptomycin-sensitive donor cells (*E. coli* BW272) and streptomycin-resistant recipient cells (*E. coli* RH2130 and BD1240) were combined in TYT medium to give final concentrations of about 1×10^7 and 3×10^8 cells per ml, respectively. After 65 min at 37°C, mating was interrupted, and cultures were spread onto selective plates. The reversion frequencies of all markers were less than 10^{-8} in this experiment.

Determination of nucleotide pool sizes. *E. coli* cells were grown in 03P medium for two generations in the presence of $^{32}P_i$ (35 μCi/ml; 0.5 μmol/ml) at 30 or 37°C; 0.5-ml samples of the labeled cultures were added to 0.1-ml portions of 2 M formic acid at 0°C; after 30 min the extracts were centrifuged, and the nucleotides in the supernatant fractions were analyzed by two-dimensional chromatography on polyethyleneimine-cellulose thin-layer plates. This was followed by one-dimensional chromatography of the dTTP-dUTP spots on the same plates, as described by Neuhard et al. (14).

RESULTS

An isogenic set of *E. coli* strains whose genotypes differed only in the *dut* and *ung* genes were grown for 40 min at 37°C in the presence of [6-^{3}H]uridine. The DNAs of these strains were separated from the RNAs, and the distributions of radioactivity in the pyrimidines were determined (Table 2). A significant amount of uracil was found only in the DNA of the *dut ung* strain. A small amount of cytosine (0.5 to 1%) was deaminated to uracil during formic acid hydrolysis, so it was not possible to determine the exact upper limit of uracil content in the other strains in this experiment. The relative incorporation of uridine into cytosine and into thymine plus uracil in these strains varied from 1.14 to 1.23, indicating that the cytosine content was slightly overestimated (accepted ratio would be 1.0). This could have been due to the presence of [5-^{3}H]uridine in the [6-^{3}H]uridine used to label the DNA since any ^{3}H in the 5 position of uracil would have been lost when dUMP was converted to dTMP. Using these uncorrected values, we estimated that the replacement of thymine by uracil in the *dut ung* strain was about 14%, whereas the replacement in the *dut* and *ung* strains was 0.5% or less.

Because of the possibility that our radioisotopic analysis may have reflected the composition of only the DNA made during the labeling period, DNA was isolated from growing cultures so the uracil content of the total DNA could be compared with the uracil content of the newly made DNA (Table 3). Both *thy*$^+$ and *thy* strains were included in this study, and the concentrations of thymine and thymidine in the medium were varied to determine how this affected the base composition of the DNA. In most cases the amount of uracil in the total DNA in the *dut*$^+$ *ung*$^+$ strains was less than the limit of detection of the experimental procedure. In general, the values for the uracil content of the total DNA and the uracil content of the radioactive DNA were in agreement. The most notable exceptions occurred in the low-thymine-requiring *dut ung* strain BD1284, in which the radioisotopic analysis overestimated the uracil content severalfold. Values between 10 and 20% replacement of thymine by uracil occurred in the *dut ung thy*$^+$ strain BD1157, which was viable under the con-

TABLE 2. *Incorporation of [6-^{3}H]uridine into E. coli DNA at 37°C*

Strain	Genotype	Base composition of DNA			
		% of radioactivity in the following pyrimidines:			Ratio of uracil to uracil plus thymine
		Cytosine	Uracil	Thymine	
BD1154	*dut*$^+$ *ung*$^+$	53.9	0.6	45.5	0.013
BD1153	*dut*$^+$ *ung-1*	52.8	0.8	46.4	0.017
BD1156	*dut-1 ung*$^+$	54.8	0.8	44.4	0.018
BD1157	*dut-1 ung-1*	54.6	6.8	38.7	0.149

TABLE 3. *Uracil contents of DNAs isolated from E. coli dut ung mutants*

Strain	Genotype			Concn (μg/ml) of: [b]		Ratio of uracil to uracil plus thymine in bulk DNA (×100)	Ratio of [^{3}H]-uracil to [^{3}H]-cytosine in labeled DNA (×100)[c]
	thy[a]	*dut*	*ung*	Thymine	Thymidine		
BD1154	+	+	+			<0.1, <0.1[d]	1, 1.5[d]
BD1157	+	−	−			13, 18[d]	19, 19[d]
BD1212	−(L)	+	+	0			3.5
				2		<0.1	
				20		<0.1	1
BD1284	−(L)	−	−	0			25
				0.2			19
				2		3.5	9
				20		0.6	4
RH2130	−(H)	+	+		0		1
					5	<0.1[d]	1
					50	<0.5[d]	1
BD1301	−(H)	−	−		0		55
					5	19[d]	12
					50	7[d]	7

[a] L, Low thymine requirements of *thyA* cells; H, high thymine requirement of *thyA* cells.

[b] Concentration in the growth medium.

[c] The radioactivity data were expressed in terms of the ratio of uracil to cytosine rather than the ratio of uracil to uracil plus thymine because no [^{3}H]uracil was incorporated into thymine in DNA in *thy* mutants; the theoretical ratio of cytosine to thymine plus uracil in *E. coli* DNA is 1.0, although the data in Table 2 indicate that the incorporation is biased slightly toward cytosine.

[d] These cultures were grown overnight to stationary phase by limiting glucose to 1 g/liter and were then harvested for analysis of bulk DNA. In one experiment with BD1154 and BD1157 portions of these stationary-phase cultures were diluted into fresh medium for an analysis of the incorporation of [6-^{3}H]uridine into DNA.

ditions of this experiment. Uracil substitutions of more than 20% occurred only in the cells starved for thymine, and these cells presumably were dying. However, it was not clear whether the cells were dying because of the high uracil content of the DNA or because the combined pools of dUTP and dTTP were too low to support normal DNA synthesis.

To determine whether uracil incorporated into the DNA in the *dut ung* strain remained in the DNA or was turned over rapidly, we studied the incorporation of [6-^{3}H]uridine into cytosine and uracil for 30 min (Fig. 1A) and then followed the fate of radioactivity in these two bases during an 80-min chase in a medium containing unlabeled uridine (Fig. 1B). We found that (i) uridine was continuously incorporated into the DNA, (ii) the ratio of radioactivity in cytosine and uracil was constant during this incorporation, and (iii) uracil was excised from the DNA very slowly during the chase. This indicated that uracil-DNA glycosylase is the major enzyme responsible for the removal of uracil from DNA and that the activity of endonuclease V, which is somewhat uracil specific (7), must be minimal on uracil-containing DNA in vivo. The slight decrease in uracil content observed during the chase compared with the cytosine content may have been due to the residual uracil-DNA glycosylase activity (less than 1%) remaining in the *ung-1* mutants (16).

We observed that when *E. coli* BD1156 (*dut-1*) was grown at 37°C, more than 50% of the cells became filamentous. A similar observation has been reported by Taylor and Weiss (18). This filamentation can be reduced either by growing the cells at a lower temperature (e.g., 30°C), by adding 100 μg of thymidine per ml to the medium, or by the presence of the *ung* mutation. Hochhauser and Weiss (9) reported that the residual dUTPase activity in *dut-1* cells may be heat sensitive in vitro. These observations suggest that at 37°C *E. coli* BD1156 is at least partially thymine starved due to a deficiency of dUMP, the product of the dUTPase reaction. Since growth of *E. coli* BD1156 at different temperatures probably affects the amount of dUTPase activity and the amount of dUTP in the cells, the uracil content of newly synthesized DNA may increase with increasing temperature. In *ung*$^+$ cells, such as *E. coli* BD1156, this uracil presumably is removed continuously by uracil-DNA glycosylase, leading to excessive demands on the DNA repair systems. To test directly the effect of temperature on the incorporation of uracil into DNA, we grew *E. coli* BD1157 (*dut-1 ung-1*) at different temperatures and labeled the DNA of these cells with [6-^{3}H]uridine. The

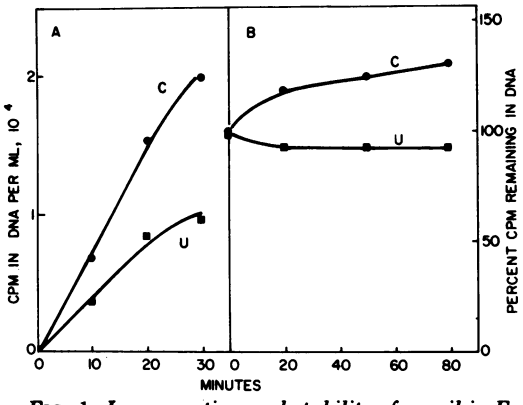

FIG. 1. *Incorporation and stability of uracil in E. coli DNA. (A) At various times after [6-^{3}H]uridine was added to a culture of E. coli BW212 (dut-11 ung-1) growing at 37°C, samples were removed, and the DNA was isolated and analyzed for the amount of radioactivity in its pyrimidine bases. (B) E. coli BW212 was labeled with [6-^{3}H]uridine for 30 min; then the cells were harvested and washed with nonradioactive medium, and growth was continued in medium containing 0.1 μmol of uridine per ml. At varying times the DNA was isolated and analyzed for the amount of radioactivity remaining in its pyrimidine bases. C, Cytosine; U, uracil.*

replacement of thymine by uracil in the DNA increased from about 4% at 25°C to 18% at 42°C (Fig. 2). These results confirm that increased incorporation of dUTP into DNA did occur in *E. coli dut-1* cells as the temperature was increased.

The increased need for uracil-specific DNA repair in *E. coli dut-1* cells as the growth temperature increases might be expected to affect the growth of these cells adversely. To determine whether cells which were incorporating significant amounts of uracil into DNA could replicate as well as cells with uracil-free DNA, we studied the increase in viable cell numbers as a function of time and temperature (Fig. 3). Our results clearly indicated that strain BD1156 grew exponentially only at temperatures below 30°C. As the temperature increased, strain BD1156 became increasingly less able to sustain exponential growth, and at 42°C the cells became nonviable. In contrast, strain BD1157 (*dut-1 ung-1*) grew exponentially over the entire temperature range, although in all cases this strain had a longer generation time than the wild-type strain (strain BD1154). Thus, when the uracil excision repair system was blocked by the *ung* mutation, the *dut* mutation had much less effect on cell viability. Furthermore, microscopic examinations of these cultures indicated that strain BD1157 cells were much less filamentous than strain BD1156 cells at all temperatures. The generation times calculated from Fig. 3 are summarized in Table 4. Strain BD1153 grew as well as strain BD1154 at 30 and 37°C and was not included in further experiments.

We also found that cells containing as much as 18% replacement of thymine by uracil were

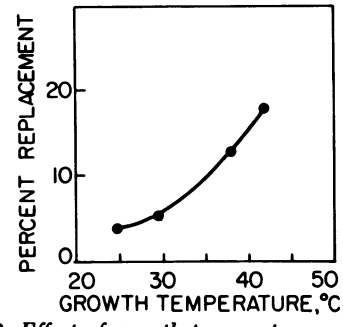

FIG. 2. *Effect of growth temperature on incorporation of uracil into E. coli DNA. E. coli BD1157 (dut-1 ung-1) was grown at 30°C; then portions of the culture were shifted to different temperatures and labeled for 30 min with [6-^{3}H]uridine. The DNA was then isolated and analyzed for the amount of radioactivity in its pyrimidine bases. The percent replacement of thymine by uracil was calculated as the amount of radioactivity in uracil divided by the total amount of radioactivity in thymine and uracil.*

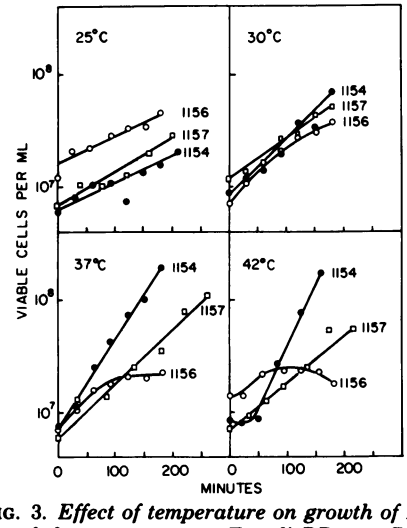

FIG. 3. *Effect of temperature on growth of E. coli dut and dut ung mutants. E. coli BD1154, BD1156, and BD1157 were grown overnight at 30°C without shaking in Davis medium containing only 0.1% glucose. At the beginning of the experiment cultures were diluted into fresh medium containing 0.2% glucose, grown for several hours at 30°C with shaking, and then rediluted and grown at different temperatures. At varying times samples were removed and titrated for viable cells.*

TABLE 4. *Effect of temperature on growth rate*

Strain	Genotype	Generation time (min) at:			
		25°C	30°C	37°C	42°C
BD1154	*dut*$^+$ *ung*$^+$	121	59	39	30
BD1153	*dut*$^+$ *ung-1*		57	40	
BD1156	*dut-1 ung*$^+$	120	-[a]	-	-
BD1157	*dut-1 ung-1*	100	84	65	72

[a] -, Growth was not exponential.

capable of exponential growth. However, it was not clear whether the slower growth rate of strain BD1157 compared with strain BD1154 was due to the actual presence of uracil in the DNA or to limited availability of nucleotide precursors able to base pair with adenine during DNA synthesis. When strain BD1157 was grown in the presence of 100 μg of deoxyuridine per ml or 100 μg of thymidine per ml, the generation times were lowered nearly to those of wild-type cells at 37 and 42°C (data not shown). Thymidine would be expected to increase the dTTP pool, whereas deoxyuridine might increase both the dTTP and the dUTP pools. In either case the availability of precursors for DNA synthesis should increase, so the results did not distinguish between the two possible explanations for the slow growth of strain BD1157.

Because a dUTPase deficiency would be expected to both increase the dUTP pool and decrease the amount of dUMP available for dTTP synthesis, we determined the sizes of the dUTP and dTTP pools (Table 5). We found that the presence of the *dut-1* mutation increased the concentration of dUTP in the cells by more than 10-fold at 37°C, as expected. Very surprisingly, however, the *dut-1* mutation did not reduce the dTTP pool size in the cells, but rather increased it severalfold. These results suggested very strongly that the slower growth rate of *dut ung* cells compared with wild-type cells was not due to limiting pools of dUTP plus dTTP. The concentration of dUTP in *dut ung* cells decreased as the temperature decreased from 37 to 30°C, as expected if the residual dUTPase activity in *dut-1* cells was heat sensitive; however, the concentration of dTTP was not affected by this temperature shift.

The above-described results confirm that uracil-containing DNA is synthesized in *dut ung* mutants and that this DNA is reasonably functional. Furthermore, it is apparent that uracil-DNA glycosylase plays a major role in the repair of uracil-containing DNA in vivo. Therefore, we studied the effect of an active uracil-DNA glycosylase on the metabolism of uracil-containing DNA. *E. coli dut* mutants were isolated originally by screening for a hyper-rec phenotype, as described by Konrad (11). To test for an effect of uracil-DNA glycosylase deficiency on recombination, we constructed an isogenic series of strains in the partial *lac* diploid strain KS468 (12). These strains were tested for the hyper-rec phenotype, as indicated by the number of *lac*$^+$ papillae per colony which appeared on lactose tetrazolium plates after 3 days at 30°C. The *dut* strains exhibited the hyper-rec phenotype with 39 papillae per colony (average for five colonies), compared with only 8 papillae per colony in *dut*$^+$ *ung*$^+$ strains. The *ung* mutation suppressed the *dut* hyper-rec phenotype and lowered the average number of papillae per colony to five per colony in *dut ung* cells. This apparent hypo-rec effect may have been due to the slower growth rate of *dut ung* cells compared with *dut*$^+$ *ung*$^+$ cells. There was no effect of the *ung* mutation alone on recombination as measured by this assay, since we observed 10 papillae per colony with *ung* cells. This result suggests that occasional, low-level incorporation and excision of uracil by *dut*$^+$ *ung*$^+$ cells may not contribute significantly to the observed recombination in *dut*$^+$ *ung*$^+$ cells. If it did, the *ung* strain would be hypo-rec.

To test the recombination and mating proficiency of uracil-containing DNA, we used an Hfr derivative, strain BW272 (KL16 *dut-1 ung-1*), as a donor into isogenic *ung*$^+$ and *ung* recipients (Table 6). Efficient mating occurred in the *ung* recipient, but recombination in the *ung*$^+$ recipient was decreased about 10^3-fold for all of the markers tested. An analysis of 25 *trp*$^+$ and 15 *his*$^+$ recombinants revealed that no recombinants of nonselected genetic loci were recovered. Only when the closely linked genes *lysA*, *thyA*, and *argA* were analyzed did we observe any linkage of nonselected markers. Among 25 *arg*$^+$ recombinants, 7 were *thy*$^+$ and 2 were *lys*$^+$. The

TABLE 5. *Pool sizes of dUTP and dTTP in E. coli*[a]

Strain	Genotype	Temp (°C)	Amt (nmol/g, dry wt) of:		Ratio of dUTP to dTTP
			dUTP	dTTP	
BD1153	*dut*$^+$ *ung-1*	37	<10[b]	530	<0.019
BD1154	*dut*$^+$ *ung*$^+$	37	<10[b]	620	<0.016
BD1156	*dut-1 ung*$^+$	37	120	1,840	0.065
BD1157	*dut-1 ung-1*	37	130	2,000	0.065
BD1157	*dut-1 ung-1*	30	40	2,000	0.020

[a] These data are the averages of two independent determinations.

[b] These are maximum values. The amount of radioactivity in the dUTP spots was about twice the amount in the background. The actual values may have been considerably less, since the autoradiograms of the chromatograms showed no visible labeling in the dUTP spots, even after several days of exposure.

TABLE 6. *Effect of uracil-DNA glycosylase on recombination frequency of markers in uracil-containing DNA*

Marker transferred from BW272	No. of recombinants per ml with:	
	BD1240 (*ung-1*)	RH2130 (*ung*$^+$)
thyA$^+$	1.3×10^5	60
argA$^+$	1.4×10^5	50
his$^+$	1.4×10^5	20
trp$^+$	1.7×10^4	40
lac$^+$	6.7×10^2	0

very low efficiency of marker transfer and the unlinking of genetic markers in the *ung*$^+$ strain were consistent with the idea that uracil-DNA glycosylase efficiently degraded incoming uracil-containing DNA before the genetic information could be conserved in the recipient by replication (24, 25). These results further emphasize the restriction enzyme-like activity of uracil-DNA glycosylase on uracil-containing DNA.

DISCUSSION

The results described above indicate that uracil can be incorporated into DNA in *E. coli* if the cells are deficient in dUTPase activity, but that the uracil only remains in the DNA if the cells are also deficient in uracil-DNA glycosylase activity. We observed as much as 15 to 20% replacement of thymine by uracil in cells which are growing exponentially and contain normal levels of thymidylate synthetase activity. Higher substitutions can be obtained in *thyA* cells, particularly in high-thymine-requiring cells in the absence of exogenous thymine or thymidine, but these cells are no longer viable due to thymine starvation.

Our results suggest that the incorporation of large amounts of uracil into DNA slows down the growth rate, even in the virtual absence of uracil-DNA glycosylase activity. Presumably, glycosylase-initiated DNA repair cannot account for the decreased growth rate of *dut ung* cells, so other processes must be involved. Growth inhibition may be due to the presence of uracil residues in the DNA, which alter replication or transcription processes in the cells through changes in protein-DNA interactions. For example, the binding of repressor to *lac* operator DNA is weakened by the replacement of a single methylcytosine or thymine residue by cytosine or uracil, respectively (5, 8). Thus, thymine methyl groups in DNA may be important determinants in the recognition of DNA sequences by regulatory proteins. In contrast, the presence of uracil in viral DNA appears to affect DNA functioning very little. Both T4 phage and T5 phage, which contain uracil in their DNAs replicate normally in *ung-1* cells, although they are restricted in *ung*$^+$ cells (24, 25). If uracil does affect T4 or T5 viral DNA function, the functions affected are not rate limiting.

The amount of replacement of thymine by uracil in cellular DNA can be compared with the relative pool sizes of dTTP and dUTP in cells to estimate the in vivo selectivity of the DNA polymerases for dUTP and dTTP. Our results indicate that at 37°C there is about 12% replacement of thymine by uracil in the DNA, whereas the pool size data predict that only a 6 to 7% replacement should occur. A similar discrepancy occurs at 30°C. This suggests that the DNA polymerases responsible for DNA replication may possess a 2-fold preference for dUTP over dTTP. This conclusion is not in agreement with the results which others have obtained in vitro. Shlomai and Kornberg showed that polymerase III could use dUTP almost as well as dTTP for DNA synthesis in vitro (17), whereas Olivera et al. found a 2.5-fold discrimination against dUTP when they measured the ratio of dUTP to dTTP incorporated into DNA by lysates on cellophane disks (15). However, this latter experiment was carried out at 27 to 28°C, so residual dUTPase activity may have reduced the actual dUTP concentration in the experiment. Another possibility is that the expanded dTTP pool in *dut* mutants is not available for DNA synthesis in vivo due to some form of compartmentalization. Although the source of the discrepancy between the in vivo and in vitro results is not clear from our data, all of the results taken together indicate that DNA polymerase III lacks the ability to prevent incorporation of dUTP into *E. coli* DNA if significant pools of dUTP are present in the cells. An important role for dUTPase is to reduce the pool size of dUTP in the cells to a level such that only one dUTP is incorporated into DNA per 1,000 to 3,000 nucleotides (19). Assuming that there is no discrimination against dUTP by DNA polymerase III, the pool size shown in Table 5 would permit a maximum frequency of incorporation of dUTP into DNA of one uracil per 250 bases in the wild-type strain. It could be much less than this because it was not possible to measure accurately the dUTP pool sizes in *dut*$^+$ strains.

Figures 2 and 3 show that as the amount of uracil incorporated into DNA in *dut-1* cells rises above 5% replacement of thymine by uracil, the excessive DNA repair initiated by uracil-DNA glycosylase slows down growth and eventually leads to a loss of viability. This loss of viability could result from double-strand breaks which occur during repair when uracil molecules are incorporated nearly opposite each other in the

two strands of the DNA. The presence of the *ung* mutation reverses this lethal effect, presumably by eliminating the repair initiated by excision of uracil. These data also suggest that the dUTPase synthesized in *dut-1* mutants is a heat-sensitive enzyme, although the amount of residual activity detectable in extracts is too low to permit confirmation of this by in vitro assays (B. Weiss, personal communication). If this is so, the *dut-1* mutation must occur in the structural gene for the enzyme. This loss of viability is also accompanied by filamentous growth of the cells. The nucleotide pool sizes indicate that filamentous growth does not occur in response to a low level of dTTP in the cells. Some other lesion, such as excessive DNA degradation, must be responsible for the filamentous growth of *dut* mutants.

Even though *dut* mutants are hyper-rec and the hyper-rec phenotype is reversed by *ung* mutations, *ung* mutants are not hypo-rec. This indicates that the occasional incorporation of uracil into DNA (one molecule per 1,000 to 3,000 nucleotides) does not contribute significantly to the rate of recombination in normal cells. The apparent hypo-rec phenotype of *dut ung* mutants is probably caused by the slower growth of these mutants due to uracil in their DNAs.

The decreased recombination frequencies of *dut ung* mutants due to the failure to excise uracil from their DNAs indicate that the actual recombinogenic lesion responsible for the hyper-rec phenotype of *dut* mutants is an intermediate in the repair of sites generated by uracil-DNA glycosylase. These sites are known as apyrimidinic sites (AP sites) and also result from depurination of DNA, which generates apurinic sites. The repair of AP sites is generally assumed to involve the participation of exonuclease III, DNA polymerase I, and DNA ligase. Deficiencies of these enzymes cause a hyper-rec phenotype in partial *lac* diploid strains (26), suggesting that a low repair efficiency leads to increased recombination frequencies due to persistence of DNA chain breaks. Warner et al. (22) suggest that endonuclease III could also play a role in repair of DNA containing AP sites. This endonuclease incises the DNA on the 3′ side of the AP site, and the 3′-terminal AP site must be removed before the missing nucleotide can be replaced by nick translation with DNA polymerase I. Whereas exonuclease III can remove 3′-terminal AP sites efficiently, exonuclease III-deficient mutants may be defective in this process, and these nicks may lead to a high recombination frequency. The hyper-rec phenotype of *dut* mutants could also result from slow repair of nicks containing 3′-terminal AP sites, if the amount of exonuclease III in the cells is insufficient to both nick the AP sites being generated by uracil-DNA glycosylase and remove any 3′-terminal AP sites generated if endonuclease III nicks some AP sites.

Although uracil incorporation causes recombinogenic DNA repair in *ung*$^+$ cells, as observed here with the partial *lac* diploid bacteria and with a tandem duplication assay in bacteriophage λ (J. S. Hays, B. K. Duncan, and S. Boehmer, unpublished data), this is not always the case. When uracil-containing DNA is injected into *ung*$^+$ recipients during Hfr matings (Table 6) or phage experiments (24, 25), the DNA is subjected to defective DNA repair in the sense that the repair intermediates are apparently degraded instead of being repaired. As a result, recombinant formation and phage viability are decreased. In these cases uracil-containing DNA can only be expressed in *ung* bacteria.

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