Excision Repair of Uracil Incorporated in DNA as a Result of a Defect in dUTPase

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Mutants of Escherichia coli that are severely defective in the enzyme dUTPase (dut) accumulate short (4 to 5 S) Okazaki fragments following brief pulses with [3H]thymidine. The transient appearance of DNA fragments in these mutants is plausibly explained by the misincorporation of uracil in DNA as a result of an increase in available dUTP, followed by its rapid excision and repair. The evidence in support of this interpretation is the following: (1) accumulation of short DNA fragments can be partially suppressed by a mutation in dCTP deaminase, presumably by decreasing the intracellular level of dUTP relative to dTTP; (2) accumulation of the short DNA fragments can be almost completely suppressed by a mutation in uracil N-glycosidase, probably by preventing the introduction of nicks at the sites of uracil incorporation; (3) introduction of DNA polymerase I or DNA ligase mutations into dUTPase-defective strains results in the persistence of the 4 to 5 S fragments and rapid cessation of DNA synthesis. Uracil N-glycosidase, DNA polymerase I and DNA ligase must therefore be involved in the excision repair of uracil-containing DNA.

1. Introduction

When mutants of Escherichia coli defective in the enzyme dUTPase (dut) are exposed to brief pulses with [3H]thymidine, labeled DNA fragments appear that are rapidly incorporated into high molecular weight DNA, and hence resemble the now classic Okazaki fragments. The size of the labeled fragments and the extent to which they persist depend on the severity of the dUTPase defect (Tye et al., 1977). In the experiments reported here, we have examined the effects of a defect in DNA polymerase I and in DNA ligase on the behavior of a dut mutant in which the labeled fragments are abnormally small, 4 to 5 S (the Sof phenotype (Konrad & Lehman, 1975)). We have found that introduction of a non-lethal polA or lig mutation into this strain results in (1) temperature-sensitive conditional lethality, (2) inability of the small pulse-labeled fragments to be joined, and (3) rapid shut-off of DNA replication. DNA polymerase I and DNA ligase must therefore be involved in the repair of uracil-induced damage to DNA.

We have also observed that introduction of a mutation in uracil N-glycosidase (ung) into the dut− strain completely suppresses the abnormal accumulation of the

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small fragments. Hence, this enzyme is also very likely involved in the post-replication excision repair of uracil-induced damage to DNA. Finally, we have found that elimination of dCTP deaminase, an enzyme that catalyzes the deamination of dCTP to generate dUTP, diminishes the extent to which small pulse-labeled fragments accumulate. These findings provide strong support for the idea suggested earlier (Tye et al., 1977), that a decrease in dUTPase results in an increased incorporation of uracil into DNA; rapid removal of the uracil by an excision repair process involving uracil N-glycosidase, DNA polymerase I and DNA ligase then leads to the transient appearance of the small DNA fragments.

2. Materials and Methods

(a) Media

The liquid media used were M63 minimal salts medium, TY medium, and Tryptone broth prepared as described by Miller (1972). Solid media were: M63 medium containing 1.5% agar, TY medium containing 1.5% agar, Tryptone containing 1.5% agar, and TY-MMS agar, which is composed of TY medium containing 1.5% agar and 0.04% methylmethane sulfonate. H-top agar was prepared according to Gottesman & Beckwith (1969).

(b) Strains and bacteriological methods

The E. coli K12 strains used are given in Table 1.

We have adopted the designations dut-11, dut-12 and dut-13 for the sof-1, sof-2 and sof-3 mutations, respectively, in conformity with accepted nomenclature for the dUTPase genetic locus (Tye et al., 1977).

Bacterial matings were carried out according to Miller et al. (1968), and transductions with Plvir phage were performed as described by Signer (1966), except that transductants were selected on plates supplemented with 5 mM-sodium citrate.

Plvir stocks were made by the following method. A culture was grown to a titer of about 2 x 10^9/ml, made 5 mM in CaCl2, and incubated for 5 min at 37°C. A portion (1 ml) of culture was mixed with 10^8 Plvir phage, incubated for 20 min, then plated in 2.5 ml of H-top agar. Phage were harvested after 12 h.

his- deletion mutants were isolated by P2 prophage eduction as described by Miller (1972). Phage P2 lyec was a gift from Dr Richard Calendar.

(c) Isolation of pulse-labeled DNA

The following procedure, modified from Okazaki et al. (1968), was used for isolation of DNA pulse-labeled with [3H]thymidine. Cultures were grown to an A595 of about 0.5 at 30°C in M63 medium supplemented with 0.2% glucose, 0.5% Casamino acids, 10 μg thiamine/ml, and 2 μg thymine/ml. A portion (6 ml) of culture was removed and shaken for 5 min at the temperature (30°C or 44°C) at which it was to be pulsed. Then 100 μl of [3H]thymidine (20 Ci/mmol 1 mCi/ml; New England Nuclear) was added. The pulse was terminated by addition of an equal volume of 75% ethanol, 2 mM-EDTA. The resulting suspension was centrifuged and the pellet resuspended in 0.6 ml of 0.2 M-NaOH, 10 mM-EDTA. 32P-labeled φX174 (a gift of Dr Schlomo Eisenberg of this Department) was added as a sedimentation marker, and the mixture was incubated at 37°C for 1 h.

(d) Alkaline sucrose density-gradient centrifugation

Alkaline sucrose gradients (5% to 20%) were run in a Beckman L265B ultracentrifuge using an SW41 Ti rotor for 14 h at 40,000 revs/min at 5°C in solutions containing 0.8 M-NaCl, 0.2 M-NaOH and 2 mM-EDTA in addition to the sucrose. At the end of the run, 0.4-ml fractions were collected from the bottom of the tube. Salmon sperm DNA (0.1 ml of 0.4 mM-nucleotide) was added to each fraction, followed by 0.6 ml of cold 10% trichloroacetic acid. Precipitates were collected on Whatman GF/C glass filters and washed with 4 portions (5 ml each) of cold 1 M-HCl and 2 portions (3 ml each) of ethanol.
Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference or construction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM383</td>
<td>W3110 (E. coli C strain) polA12 rha'- thy' - lac' str+</td>
<td>Monk &amp; Kinross (1972)</td>
</tr>
<tr>
<td>K2483</td>
<td>Hfr KL16 thi' pts-140</td>
<td>Strain CHEp40 from W. Epstein</td>
</tr>
<tr>
<td>814</td>
<td>' strA lop-11</td>
<td>M. Gellert</td>
</tr>
<tr>
<td>58M4</td>
<td>Hfr Cavis arbutin+ pyrE- met- str++</td>
<td>Konrad &amp; Lehman (1975)</td>
</tr>
<tr>
<td>RS474</td>
<td>F- melB thi'- lac MS826 $\phi$80dII lac BK1 str+</td>
<td>Konrad &amp; Lehman (1975)</td>
</tr>
<tr>
<td>RS5087</td>
<td>F- melB thi'- lac MS826 $\phi$80dII lac BK1 dut-11 str+</td>
<td>Mating between KS243 and RS5087 from E. B. Konrad</td>
</tr>
<tr>
<td>RS5090</td>
<td>F- lac MS826 $\phi$80dII lac BK1 dut-11 pts-140 str+</td>
<td>RS5090 transduced with P1vir grown on 814; this work</td>
</tr>
<tr>
<td>BKT102</td>
<td>F- lac MS826 $\phi$80dII lac BK1 dut-11 lop-11 str+</td>
<td>BKT102 transduced with P1vir grown on RS5087; this work</td>
</tr>
<tr>
<td>BKT103</td>
<td>F- polA12 rha' strthyA'- lac Y14pyrE'- arbutin+</td>
<td>Mating between 58M4 and MM383; this work</td>
</tr>
<tr>
<td>BKT106</td>
<td>F- lac MS826 $\phi$80dII lac BK1 dut-11 lop-4 str+</td>
<td>RS5090 transduced with P1vir grown on N1626; this work</td>
</tr>
<tr>
<td>BKT108</td>
<td>F- lac Y'- strthyA'- rha'- dut-11 polA12</td>
<td>BKT103 transduced with P1vir grown on RS5087; this work</td>
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<tr>
<td>N1626</td>
<td>F- str' lop-4 pts+</td>
<td>M. Gellert</td>
</tr>
<tr>
<td>BD1153</td>
<td>F- thi-1 argH1 lacY1 gal-6 malA1 xyl-7 rha-6 ara-13 strA9 supE-44 $\lambda^b$ ung-1</td>
<td>H. Warner &amp; B. Duncan</td>
</tr>
<tr>
<td>BD1154</td>
<td>F- thi-1 argH1 lacY1 gal-6 malA1 xyl-7 rha-6 ara-13 strA9 supE-44 $\lambda^b$</td>
<td>H. Warner &amp; B. Duncan</td>
</tr>
<tr>
<td>BD1156</td>
<td>F- thi-1 argH1 lacY1 gal-6 malA1 xyl-7 rha-6 ara-13 strA9 supE-44 $\lambda^b$ dut-1</td>
<td>H. Warner &amp; B. Duncan</td>
</tr>
<tr>
<td>BD1157</td>
<td>F- thi-1 argH1 lacY1 gal-6 malA1 xyl-7 rha-6 ara-13 strA9 supE-44 $\lambda^b$ dut-1 ung-1</td>
<td>H. Warner &amp; B. Duncan</td>
</tr>
</tbody>
</table>

(c) Assay of dCTP deaminase

Cultures (400 ml) were grown in M63 media supplemented with 0.2% glucose, 0.5% Casamino acids, 10 $\mu$g thiamine/ml and 2 $\mu$g thymine/ml to A$_{555}$ = 0.75. They were then harvested, washed with 0.15 M NaCl, resuspended in 1 ml of 50 mM-potassium phosphate (pH 6.8), 2 mM-EDTA, 1 mM-dithiothreitol, and sonicated for 1 min. The extracts were centrifuged, and the supernatant fluid treated with 0.25 vol. 15% streptomycin sulfate at 0°C. After 30 min, the suspensions were centrifuged. The supernatant fluid was dialyzed for 2 to 4 h against 500 vol. of the sonication buffer and assayed directly (Beck et al., 1977).

The reaction mixture contained in 20 ml, 50 mM-potassium phosphate (pH 6.8), 1 mM-dithiothreitol, 1 mM-[3H]dCTP (spec. act. 24-1 Ci/mmol), 5 mM-MgCl$_2$ and extract. Incubation was at 37°C for 20 min, then 1 ml samples were withdrawn and applied directly to PEI-cellulose thin-layer plates together with 10 nmol each of dCTP, dCDP, dCMP, dUTP, dUDP and dUMP as markers. The plates were washed for 10 min in absolute methanol, dried, and developed stepwise in 1 dimension. The solvents used were the following: (1) absolute methanol to the starting line; (2) 1 M-formic acid to 1 cm above the starting line; (3) 1 M-formic acid, 0.25 M-LiCl to 3 cm above the starting line; (4) 1 M-formic acid, 1-25 M-LiCl to 12 cm above the starting line. After drying with hot air, the spots corresponding to dCTP, dCDP, dCMP, dUTP, dUDP and dUMP were identified under ultraviolet light, cut out, and placed in counting vials together with 0.2 ml of 1 m-NH$_4$HCO$_3$ to determine the nucleotides from the PEI-cellulose. After 20 min at room temperature, 8 ml of a Triton X100/toluene scintillation fluid was added to each vial and the radioactivity determined in a Beckman LS-230 scintillation spectrometer.
From the radioactivity in each of the nucleotides, the amount of conversion of dCTP to dUTP and dUMP (the latter resulting from the action of dUTPase) was calculated. The assay was linear in the range of 0-1 to 1-0 nmol of dCTP converted to dUTP (+dUMP).

(f) Histidine complementation tests

The following strains of Salmonella typhimurium (gifts from Dr John Roth and John Scott) were used in his complementation tests: (1) derivatives of LT2 trpA8 purE801 his612 (BHAfIE deletion) carrying E. coli F' hisbD2377, F' hisabD2382; (2) derivatives of LT2 ser821 arg501 his721 (DCBHAfIE deletion) carrying E. coli F' his+, F' his C2385, F' hisa(b)cdB2405, F' hisa2406, F' hisa(b)E2414, F' hisa(b)cdB2415; (3) LT2 F'his DG. (The lower case letters denote deficiencies of the mutant with respect to intragenic complementation groups.)

his" auxotrophs carrying point mutations should be complemented by Salmonella strains carrying F' factors that are mutant in any of the his structural genes other than that in which the point mutation occurs. In contrast, deletions that extend through several genes are not complemented by F' factors that carry his" mutations in any of the his structural genes included in the deletion.

Complementation tests of his" auxotrophs by Salmonella carrying E. coli F' his episomes involved streaking out the test strain and Salmonella F' his donor strains on minimal plates and scoring for growth where the two strains overlap. Neither the donor nor recipient strain are able to grow on minimal medium, since the donor is auxotrophic for several nutritional requirements and the recipient is auxotrophic for histidine.

3. Results

(a) The dut-11 polA12 double mutant

The labeling patterns observed after subjecting strains with either the polA12 or dut-11 mutation to ten-second pulses with [3H]thymidine at 44°C are shown in Figure 1. The fragments in the dut-11 strain were smaller than those in the polA12 mutant (4.4 S versus 8.8 S). In other dut mutants, with levels of dUTPase higher than that in dut-11 (4% of wild type), the pulse-labeled fragments had average sedimentation coefficients close to that seen in the polA mutant (Tye et al., 1977).

Under all conditions examined, survival of the double mutant E. coli dut-11 polA12 at 43°C was significantly less than the dut-11 or polA12 strains from which it was constructed (Table 2). In addition, the double mutant, unlike either parent, was abnormally sensitive to methylmethane sulfonate even at 30°C.

Upon shift of a culture of the double mutant from 30°C to 44°C, DNA synthesis ceased within ten minutes (Fig. 2). Thus, combination of the dut-11 and polA12 mutations results in the inability of the double mutant to grow at 44°C and blocks DNA synthesis at this temperature.

The defect in DNA replication in the dut-11 polA12 double mutant was further examined in pulse-labeling experiments. A culture of the double mutant was grown at 30°C, then shifted to 44°C and pulsed with [3H]thymidine for increasing period of time. In contrast to the dut-11 mutant, the 4 to 5 S fragments that appeared in the double mutant persisted for pulse periods ranging from ten seconds to five minutes (Fig. 3). Thus, at 44°C, the polA12 mutation appears to block conversion of the 4 to 5 S DNA fragments to high molecular weight DNA. In contrast, the sedimentation profile of DNA isolated from the dut-11 polA12 double mutant after pulsing with [3H]thymidine at 30°C was similar to that of the dut-11 mutant at this temperature, i.e. [3H]thymidine was incorporated into DNA of increasing molecular weight as length of the pulse increased.
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Fig. 1. Sedimentation profiles of DNA in alkaline sucrose density-gradients after [3H]thymidine pulse-labeling of wild type and polA12 and dut-11 mutants of E. coli.

Cultures (6 ml) were grown in supplemented M63 medium at 33°C to $A_{595} = 0.5$, then shifted to 44°C for 5 min and pulsed with 100 μl of [3H]thymidine (spec. act. 20 mCi/μmol, 1 mCi/ml) for 10 s. The cultures were lysed and the DNA sedimented in a 5% to 20% alkaline sucrose density-gradient as described in Materials and Methods. (a) Wild type (KS474); (b) polA12 (MM383); (c) dut-11 (RS5087).

Table 2

Effect of lig and polA mutations on the survival of the dut-11 mutant at 43°C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medium</th>
<th>Survival at 43°C (%)</th>
<th>Tryptone</th>
<th>M63</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TY-MMS</td>
<td>TY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lig4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>polA12</td>
<td>1.5 x 10^{-3}</td>
<td>1.0†</td>
<td>23</td>
<td>100‡</td>
</tr>
<tr>
<td>lig4 dut-11</td>
<td>10^{-2}</td>
<td>80</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>dut-11</td>
<td>100</td>
<td>57</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>dut-11 pts</td>
<td>68</td>
<td>100</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>polA12 dut-11</td>
<td>&lt;10^{-5}</td>
<td>1.4 x 10^{-3}</td>
<td>0.1</td>
<td>2‡</td>
</tr>
</tbody>
</table>

Cells were grown to $A_{595} = 0.5$ at 30°C in M63 medium supplemented with 0.2% glucose, 0.5% Casamino acids, 10 μg thiamine/ml and 2 μg thymine/ml and then plated as indicated at 30°C and 43°C.

† The polA12 mutant used in these experiments, in contrast to the strain isolated initially (Monk & Kinross, 1972), is somewhat temperature sensitive.
‡ The plating efficiency (number of colonies relative to $A_{595}$) was low even at 30°C.
Fig. 2. Rate of incorporation of [3H]thymidine in dut-11 polA12 and dut-11 lig-4 double mutants at 44°C.

Cultures (10 ml) were grown in supplemented M63 medium at 30°C to A_595 = 0.5, then shifted to 44°C. Portions (0.5 ml) were pulsed with 10 μl of [3H]thymidine (20 mCi/μmol, 1 mCi/ml) for 30 s, then 0.2 ml of ice-cold 17% trichloroacetic acid containing 2.5 mg unlabeled thymidine/ml was added. Acid-insoluble radioactivity was measured as described in Materials and Methods. (a) dut-11 polA12; (b) dut-11 lig-4.

Fig. 3. Sedimentation profiles of DNA in alkaline sucrose density-gradients after [3H]thymidine pulse labeling of the dut-11 polA12 double mutant.

Cultures were grown at 30°C in supplemented M63 medium to A_595 = 0.5, then (a) pulsed with [3H]thymidine at 30°C as described in the legend to Fig. 1, for 10 s (-- △ -- △ --), 30 s (-- ● -- ● --), 1 min (-- △ -- △ --), and 2 min (-- ○ -- ○ -- ○ --); (b) shifted to 44°C for 5 min before being pulsed with [3H]thymidine for 10 s (-- △ -- △ --), 30 s (-- ● -- ● -- ● --), 2 min (-- ○ -- ○ -- ○ -- ○ -- ○ -- ○ --) and 5 min (-- △ -- △ -- △ --). The cultures were lysed and the DNA sedimented in a 5% to 20% alkaline sucrose density-gradient as described in Materials and Methods.
To examine the fate of the 4 to 5 S fragments upon suppression of the polA12 mutation, pulse-chase experiments were done in which the dut-11 polA12 double mutant was pulsed with $[^3H]$thymidine for one minute at 44°C, shifted to 30°C and then treated with a large excess of cold thymidine (100 μg/ml) for 0.5, 1, 2 and 5 minutes. As shown in Figure 4, there was a significant increase in the sedimentation coefficient of the pulse-labeled DNA with increasing time of exposure to unlabeled thymidine. Furthermore, label appeared in 8 to 10 S material before being incorporated into large molecular weight DNA, suggesting that the 4 to 5 S fragments might be precursors of the 8 to 10 S Okazaki fragments.

![Figure 4. Sedimentation profiles of DNA in alkaline sucrose density-gradients following pulse-chase of the dut-11 polA12 double mutant.](image)

_Cultures were grown at 30°C in supplemented M63 medium to an A$_{600}$ = 0.5, then shifted to 44°C for 5 min, and pulsed with $[^3H]$thymidine for 1 min, as described in the legend to Fig. 1. The cultures were then cooled to 30°C and an excess (100 μg/ml) of unlabeled thymidine was added for 0 min (—Δ—Δ—), 1 min (—●—●—), 2 min (—○—○—), 5 min (—○——○—). The cultures were lysed and the DNA sedimented in a 5% to 20%, alkaline sucrose density-gradient as described in Materials and Methods._

(c) The dut-11 lig-4 double mutant

Like the dut-11 polA12 double mutant, there was a rapid cessation of DNA synthesis on shift of the dut-11 lig-4 mutant from 30°C to 44°C (Fig. 2(b)). Again, like the dut-11 polA12 double mutant, $[^3H]$thymidine was incorporated almost exclusively into 4 to 6 S DNA fragments, regardless of the length of the pulse period (Fig. 5).

(d) The dut-11 lop-11 double mutant

Inasmuch as DNA ligase appears to be required for the joining of the 4 to 5 S fragments, the effect of the mutation lop-11 (Gellert & Bullock, 1970), which leads to a fivefold increase in the level of this enzyme in extracts, was examined. Cultures of _E. coli_ dut-11 lop-11 were pulsed with $[^3H]$thymidine at 43°C for ten seconds. As shown in Figure 6, most of the labeled DNA sedimented at 4 to 5 S. However, unlike the
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FIG. 5. Sedimentation profiles of DNA in alkaline sucrose density gradients after \(^{3}H\)thymidine pulse-labeling of the dut-11 lig-4 double mutant.

Cultures were grown at 30°C in supplemented M63 medium to \(A_{660} = 0.5\), then (a) pulsed with \(^{3}H\)thymidine at 30°C as described in the legend to Fig. 1, for 10 s (\(\Delta\)), 30 s (\(\Delta\)), 1 min (\(\bullet\)), and 2 min (\(\bigcirc\)); (b) shifted to 44°C for 5 min before being pulsed with \(^{3}H\)thymidine for 10 s (\(\Delta\)), 30 s (\(\Delta\)), 2 min (\(\bullet\)), and 5 min (\(\bigcirc\)). The cultures were lysed and the DNA sedimented in a 5% to 20% alkaline sucrose density gradient as described in Materials and Methods.

dut-11 mutant, there was a shoulder with a sedimentation coefficient of 8 to 10 S. Thus, an increase in the level of DNA ligase can overcome partially the dut mutation, presumably by increasing the rate of conversion of 4 to 5 S to 8 to 10 S DNA fragments.

e) Suppression of Sso phenotype by a mutation in uracil N-glycosidase

Uracil N-glycosidase cleaves the N-glycosidic bond linking uracil to deoxyribose in DNA (Lindahl, 1974) and may, therefore, be involved in the removal of uracil residues in DNA. Mutants are known that are defective in the uracil N-glycosidase (ung) (Duncan & Warner, 1976); however, they have no obvious phenotype. To determine whether a defect in uracil N-glycosidase can decrease the level of 4 to 5 S fragments that appear in the dut-11 mutant, the double mutant dut-1 ung-1 was examined. When cultures of the double mutant were pulsed with \(^{3}H\)thymidine for ten seconds at 30°C, a sedimentation profile similar to that of the wild type was

Cultures were grown in supplemented M63 medium at 30°C to \(A_{600} = 0.5\), then shifted to 43°C for 5 min, and pulsed with [\(^3\)H]thymidine for 10 s as described in the legend to Fig. 1. \(^{32}\)P-labeled \(\Phi X174\) DNA served as a sedimentation marker. (a) lop-11; (b) dut-11 lop-11. The cultures were lysed and the DNA sedimented in a 5% to 20% alkaline sucrose density-gradient as described in Materials and Methods.

observed (Fig. 7). Thus, an ung\(^-\) mutation suppresses the Sof phenotype, presumably by preventing cleavage of phosphodiester bonds at apyrimidinic acid sites generated by the action of \(N\)-glycosidase\(^\dagger\).

(f) Partial suppression of the Sof phenotype by loss of dCTP deaminase activity

As noted above, the dut-11 polA12 double mutant, in contrast to either of the single mutants, is temperature sensitive for growth (Table 2). A group of temperature-resistant revertants selected at 43°C on TY plates remained sensitive to methylmethane sulfonate and thus retained the polA12 mutation. However, almost all (63 out of 64) of the revertants were auxotrophic for histidine. Mutants defective in DNA polymerase I are known to accumulate auxotrophs (Berg, 1971) and to generate deletions at a high frequency (Conkell & Yanofsky, 1970). Since the genetic locus for dCTP deaminase (dcd) is very near the his region, it was likely that the his\(^-\) revertants contained deletions in the his region extending through the dcd locus. Three of the

\(^\dagger\) Growth of the dut-1 ung-1 double mutant in the presence of [6-\(^3\)H]uridine results in substantial replacement of thymine by uracil in the DNA of these cells, but not in wild type or ung-1 cells. (H. B. Warner & B. Duncan, personal communication.)
Fig. 7. Sedimentation profiles of DNA in alkaline sucrose density gradients after \(^{3}H\)thymidine pulse-labeling of wild type strain, and \textit{ung-1}, \textit{dut-1} and \textit{dut-1 ung-1} mutants of \textit{E. coli}.

Cultures were grown in supplemented M63 medium at 30°C to \(A_{595} = 0.5\), then pulse-labeled with \(^{3}H\)thymidine at 30°C for 10 s as described in the legend to Fig. 1. (a) \textit{ung-1}; (b) wild type; (c) \textit{dut-1}; (d) \textit{dut-1 ung-1}.

**TABLE 3**

Complementation in \(\textit{his}^{-}\) revertants of \textit{BKT108} (\textit{dut-11 polA12}) with \textit{Salmonella} strains carrying an \textit{E. coli} \(\textit{F}^{\prime}\) \textit{his} factor

<table>
<thead>
<tr>
<th>LT-2 (\textit{F}^{\prime})(\textit{his}) (\Delta\textit{G})</th>
<th>BKT108R1</th>
<th>BKT108R2</th>
<th>BKT108R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\textit{F}^{\prime})(\textit{his}^{+})</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(\textit{F}^{\prime})(\textit{his}) D2377</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\textit{F}^{\prime})(\textit{his}) D2382</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
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<td>(\textit{F}^{\prime})(\textit{his}) C2385</td>
<td>+</td>
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<td>(\textit{F}^{\prime})(\textit{his}) B2405</td>
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<td>(\textit{F}^{\prime})(\textit{his}) A2406</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\textit{F}^{\prime})(\textit{his}) E2414</td>
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<td>-</td>
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<tr>
<td>(\textit{F}^{\prime})(\textit{his}) B2415</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(\textit{his}^{-}\) auxotrophs were tested for their ability to complement a series of \(\textit{F}^{\prime}\) factors (carrying heterologous \(\textit{his}\) genes from \textit{E. coli}), each of which was mutant in one of the \(\textit{his}\) structural genes (see Materials and Methods). A \textit{Salmonella} strain which has a \(\textit{his}\) deletion in the \(\textit{hisG}\) gene was used as a control.
Values are relative to that of wild type extracts (0.3 nmol of dCTP deaminated/min per mg protein). Enzyme assays were performed as described in Materials and Methods.

his- mutants were subjected to complementation tests after mating with Salmonella strains, each with an E. coli F' episome containing the his operon mutant in a different gene of the operon. All three of the mutants bore deletions of the his region (Table 3) and lacked dCTP deaminase activity (Table 4). Thus, introduction of a dCTP deaminase deletion restores the viability of the double mutant, dut-11 polA12, at 43°C. It also suppresses the Sox phenotype. The alkaline sucrose sedimentation profile of the pulse-labeled DNA of the triple mutant dut-11 polA12 ∨ dcd showed predominantly 8 to 10 S fragments, unlike the 4 to 5 S fragments seen in the dut-11 polA12 double mutant (Fig. 8). However, the Sox phenotype was only partially suppressed by the dcd deletion. When the dut-11 ∨ dcd double mutant was constructed, either by

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**Table 4**

*dCTP deaminase activity of E. coli dut-11 polA12 pseudorevertants*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>dCTP deaminase (% of wild type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>dut-11</td>
<td>100</td>
</tr>
<tr>
<td>RS5087</td>
<td>dut-11</td>
<td>94</td>
</tr>
<tr>
<td>BKT108</td>
<td>dut-11 polA12</td>
<td>106</td>
</tr>
<tr>
<td>BKT108R1</td>
<td>dut-11 polA12 ∨ his</td>
<td>9</td>
</tr>
<tr>
<td>BKT108R2</td>
<td>dut-11 polA12 ∨ his</td>
<td>4</td>
</tr>
<tr>
<td>BKT108R3</td>
<td>dut-11 polA12 ∨ his</td>
<td>2</td>
</tr>
</tbody>
</table>

---

**Fig. 8.** Sedimentation profiles of DNA in alkaline sucrose density-gradients after [3H]thymidine pulse-labeling of the dut-11 polA12 double mutant and dut-11 polA12 bearing a dCTP deaminase (dcd) deletion.

Cultures were grown in supplemented M63 medium at 30°C to A595 = 0.5 then shifted to 43°C for 5 min and pulsed with [3H]thymidine at 43°C for 30 s as described in the legend to Fig. 1. (a) dut-11 polA12 (BKT108); (b) dut-11 polA12 ∨ his ∨ dcd (BKT108R1).
reversion of the dut-11 polA12 V dcd strain to methylmethane sulfonate resistance, or by introduction of a dcd deletion into the dut-11 strain by education of phage P2, there was little or no suppression of the Sof phenotype as judged by alkaline sucrose sedimentation of the pulse-labeled DNA. However, the dut-11 V dcd P2eductant was no longer hyper rec (Konrad, 1977).

4. Discussion

DNA polymerase I, DNA ligase and uracil N-glycosidase are all required for the excision repair of uracil incorporated in DNA as a result of a defective dUTPase. A plausible sequence to account for this process is shown in Figure 9. Uracil N-glycosidase specifically cleaves the N-glycosidic bond linking uracil to deoxyribose. Endonuclease II (Friedberg et al., 1969; Weiss, 1976) or the apurinic endonuclease (Verley & Rassart, 1975) could then cleave the phosphodiester bond at the apyrimidinic acid site created by excision of uracil. The 5' → 3' exonuclease associated with DNA polymerase I may remove the deoxyribose phosphate moiety remaining after the excision, and the polymerase function could fill in the gap that remains. Finally, DNA ligase could seal the nick and complete the repair process.

The abrupt halt in DNA synthesis on shift of the dut-11 polA12 and lig-4 dut-11 double mutants to restrictive temperature suggests that persistence of uracil-induced nicks and/or gaps as a consequence of a defective DNA polymerase I or DNA ligase may very quickly destroy the replication fork.

Synthesis of dUTP occurs by two routes: deamination of dCTP by dCTP deaminase (Beck et al., 1975), and reduction of UDP to dUDP by ribonucleoside diphosphate reductase (Reichard, 1967) followed by its conversion to the triphosphate by nucleoside diphosphate kinase. If the transient accumulation of DNA fragments in the dut mutants is a consequence of a defect in dUTPase, leading to an increase in the intracellular level of dUTP, then a decrease in dUTP concentration, resulting from inhibition of one or both of the pathways, might alleviate the dUTPase defect. Indeed, since 75 to 80% of the dUTP synthesized in E. coli results from the deamination of dCTP (Karlström & Larsson, 1967), partial suppression of the dut mutation by a

![Diagram](https://example.com/diagram.png)

**Fig. 9.** Diagrammatic representation of the probable steps in the post-replication excision repair of uracil incorporated into DNA in dUTPase defective strains.
defect in the dcd locus alone might be anticipated. Deletion of the dCTP deaminase gene permits the dut-11 polA12 strain to grow at non-permissive temperatures and suppresses the hyper rec phenotype of the dut-11 strain.

Although the excision repair process requires the concerted action of at least four different enzymes, it proceeds with great efficiency. Thus, when the dut-11 strain is pulsed with [\textsuperscript{3}H]thymidine for five to ten seconds, labeled 4 to 5 S fragments appear; however, at 30 seconds, most of the label is associated with large molecular weight DNA (>30 S).

dUTPase-defective mutants accumulate DNA fragments that closely resemble Okazaki fragments in that they appear transiently and can be readily incorporated into high molecular weight DNA. Their joining is also retarded in polA- and lig- mutants. As judged by pulse-chase experiments, the fragments behave as though they are precursors to Okazaki fragments. It is therefore clear that simple pulse-chase experiments of this kind are not sufficient to define DNA replicative intermediates. Were uracil to be incorporated into DNA at the level of one or two molecules per 2000 nucleotides, a significant portion of the Okazaki fragments that appear in a pulse experiment could result from excision of the uracil and subsequent repair of the DNA.

Mutants of E. coli that are defective in DNA adenine methylase (dam) are also hyper rec (Marinus & Morris, 1974; Konrad, 1977). Thus, it is possible that other post-replication excision repair processes, in addition to that following uracil incorporation, might contribute to the pool of Okazaki fragments as observed in \textsuperscript{3}H]thymidine pulse experiments.

The finding that the ung-dut- double mutant is viable and lacks a discernible phenotype is surprising. One might have expected that a significant number of uracil residues incorporated in DNA as a result of a dUTPase defect would have an adverse effect on the replication and transcription of DNA. On the other hand, excision repair of uracil in the ung-dut- double mutant may occur relatively slowly, so that repair would not be detected in the short pulses (ten seconds) used in our experiments. In particular, the ung- mutation might be leaky, or endonuclease V, which preferentially attacks uracil-containing DNA (Gates & Linn, 1977) could substitute for uracil N-glycosidase, but at a reduced rate. Finally, it is even possible that incorporation, excision and repair of uracil in DNA may serve a specific function in DNA replication.

We are indebted to Drs Huber Warner and Bruce Duncan for their gift of the uracil N-glycosidase defective (ung-) mutants. We are also grateful to Drs C. Beck and B. Weiss for many helpful discussions. Janice Chien provided expert technical assistance in several aspects of this work.

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