Reduction of Postreplication DNA Repair in Two *Escherichia coli* Mutants with Temperature-Sensitive Polymerase III Activity: Implications for the Postreplication Repair Pathway†

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Daughter strand gaps are secondary lesions caused by interrupted DNA synthesis in the proximity of UV-induced pyrimidine dimers. The relative roles of DNA recombination and de novo DNA synthesis in filling such gaps have not been clarified, although both are required for complete closure. In this study, the *Escherichia coli* E486 and E511 dnaE(Ts) mutants, in which DNA polymerase I but not DNA polymerase III is active at 43°C, were examined. Both mutants demonstrated reduced gap closure in comparison with the progenitor strain at the nonpermissive temperature. These results and those of previous studies support the hypothesis that both DNA polymerase I and DNA polymerase III contribute to gap closure, suggesting a cooperative effort in the repair of each gap. Benzoylated, naphthoylated diethylaminoethyl-cellulose chromatography analysis for persistence of single-strand DNA in the absence of DNA polymerase III activity suggested that de novo DNA synthesis initiates the filling of daughter strand gaps.

DNA synthesized in *Escherichia coli* exposed to UV light contains daughter strand gaps which average 1,000 nucleotides in length (5). The *recA* gene product is essential for postreplication repair of gaps in daughter strand DNA (16). Other gene products involved in the closure of daughter strand gaps without low-dose UV preinduction include *dnaB* (6) and *dnaG* (7). In the *E. coli* strain BT1026-1, the products of the *dnaE* and *polA* genes have some role in postreplication repair, as demonstrated by Sedwick and Bridges (14). Postreplication repair ceases when both *dnaE* and *polA* gene products are inactive. A possible completely interchangeable role for *dnaE* and *polA* gene products in postreplication repair was suggested when the BT1026-1 strain closed gaps at the same rate at temperatures both permissive and nonpermissive for polymerase III activity. The BT1026-1 strain has a *dnaE* (Ts) genotype derived by methyl methane sulfonate selection of a resistant *dnaE* (Ts) *polA* parent (14). Sedwick and Bridges concluded that polymerase I alone could repair gaps at the same rate as polymerase III and polymerase I working simultaneously. These results were confirmed in my laboratory with the BT1026-1 strain (R. C. Johnson, unpublished data). However, observation of slow repair of daughter strand gaps with two DNA synthesis mutants, BT306 (*dnaG*) (7) and E279 (*dnaB*) (6), has led to a reexamination of *dnaE* gene mutants. The subjects of this paper are the *dnaE* mutants E386 and E511, their respective spontaneously derived revertants, and the parent strain CR34 from which E486 and E511 were derived. These two mutants, like strain BT1026-1, demonstrate temperature-sensitive DNA polymerase III activity. However, the BT1026-1 is derived from a different parent strain, and the mutation site within the *dnaE* gene probably is not the same as that in E486 and E511 (14, 18).

Benzoylated, naphthoylated diethylaminoethyl (BND)-cellulose chromatography was used to assay for single-stranded DNA in double-stranded DNA after attempted postreplication repair with and without polymerase III activity. BND-cellulose chromatography is useful for more complete characterization of postreplication repair processes than is afforded by DNA profiles from alkaline sucrose density gradient analysis. Thus, BND-cellulose column chromatography was used to determine whether postreplication repair in these mutants might be blocked at a stage as early as the initiation of gap filling.

MATERIALS AND METHODS

*E. coli* CR34 (*thr leu thi lac rpsL tonA*), E486 (*thr leu thi lac rpsL tonA met dnaE486), and
E511 (thr leu thi lac rpsL tonA dnaE511) were provided by J. A. Wechsler (18). Spontaneous dnaE to dnaE" revertants of E511 and E486 capable of colony formation at 43°C were selected in this laboratory.

Strains were grown in KB medium to about 3 x 10^8 cells per ml with or without [14C]thymidine at 2 μg and 0.06 μCi/ml as described in each experiment. The bacteria were exposed to 60 ergs of UV irradiation per mm², and the washed cells were pulse-labeled by addition of 20 μCi of [methyl-3H]thymidine per ml for 5 or 10 min at 30°C. Cells were then either placed on ice or washed and reincubated with nonradioactive thymidine at optimal (30°C) or nonpermissive (43°C) temperatures for DNA polymerase III activity. The pulse-labeled DNA was analyzed in alkaline sucrose density gradients immediately after the pulse-labeling and at intervals after resuspension in nonradioactive medium and reincubation at either 30 or 43°C. Procedures for growth, irradiation, lysis, and centrifugation were as previously published (6, 12).

The procedure for BND-cellulose chromatography was as published by Iyer and Rupp (5).

RESULTS

E. coli E486 and E511 are phenotypically DnaE" at 43°C and DnaE+ at 30°C. Alkaline sucrose density gradient sedimentation profiles of DNA synthesized in E. coli E486 after irradiation and after subsequent incubation at 30°C for 20 min demonstrated an increase in size to that of DNA synthesized in unirradiated E. coli (Fig. 1A and B). In contrast, the DNA profile from cells reincubated at 43°C for 20 min and otherwise treated the same demonstrated a persistence of low-molecular-weight DNA (Fig. 1C, closed circles). This result suggested that absence of polymerase III activity reduced postreplication repair. DNA profiles from cells exposed to 43°C alone were the same as those for unirradiated cells (data not shown).

The possibility was considered that the effect of 43°C on gap filling might not be due to the inactivity of DNA polymerases. E. coli CR34, the parent strain (18) of strains E486 and E511, is DnaE+ at 43°C. Sedimentation profiles of DNA synthesized by E. coli CR34 after UV irradiation demonstrated an increase in normal size after 20 min of reincubation at 43°C (Fig. 2A and B). Thus, postreplication repair in the parent was unaffected by incubation at 43°C, demonstrating the absence of any non-DNA-polymerase-specific inhibition of postreplication repair.

It is also possible that processes antagonistic to repair, as discussed by Tait et al. (17), could occur during incubation. Specifically, the slow or blocked repair observed in the E486 and E511 strains incubated at the nonpermissive temperature for DNA polymerase III activity might be

![Fig. 1. Alkaline sucrose density gradient sedimentation profiles of daughter strand DNA synthesized after exposure of E. coli E486 to UV. (A) Unirradiated control, 5 min of incubation with [3H]thymidine at 30°C; (B) 60 ergs of UV irradiation per mm² followed by 10 min of incubation with [3H]thymidine (○), followed by resuspension in nonradioactive medium and 20 min of reincubation at 30°C (○); (C) 60 ergs of UV irradiation per mm² followed by 10 min of incubation with [3H]thymidine, followed by resuspension in nonradioactive medium and 20 min of reincubation at 43°C (○), followed by an additional 60 min of incubation in nonradioactive medium at 43°C (80 min of total reincubation) (○).](https://journals.asm.org/journal/jb)
Fig. 2. Alkaline sucrose density gradient sedimentation profiles of DNA synthesized after exposure of E. coli CR34 to UV. (A) Unirradiated control, 5 min of incubation with \(^3H\)thymidine at 30°C; (B) 60 ergs of UV irradiation per mm² followed by 10 min of incubation with \(^3H\)thymidine at 30°C (○), followed by resuspension in nonradioactive medium and 20 min of reincubation at 43°C (○).

the result of DNA degradation subsequent to the conditional loss of DNA polymerase activity. Inhibition by preferential degradation of DNA synthesized after UV exposure at daughter strand gap sites seems to be suggested by the finding that the total amount of acid-precipitable DNA as determined by \(^3H\)thymidine counts per minute recovered from the alkaline sucrose gradient was less after 80 min of post-UV incubation than after 20 min of incubation (Fig. 1C). However, a study of degradation kinetics demonstrated that DNA from cells exposed to UV and subsequently incubated for 40 min at 43°C was not significantly degraded (Fig. 3). Thus, after 40 min, which is twice the time needed for repair in wild-type cells, most if not all \(^3H\)thymidine-labeled DNA was recovered. Similar results were obtained for the 43°C, unir-
This time). From the cells reincubated from precipitable 3H-labeled radiated control (data not shown). More acid-
precipitable 3H-labeled DNA was recovered from cells reincubated at 30°C after UV than from the same cells before reincubation (zero time). This was probably due to continued incorporation of cellular pools of 3H]thymidine as a result of elongation of DNA by semiconservative synthesis at 30°C. These experiments demonstrated that inhibition of postreplication repair observed after 20 min of incubation at 43°C is not accompanied by a large irreversible loss of DNA by degradation.

Alternatively, because some dnaE mutant strains have elevated spontaneous mutation frequencies (15) which may reflect abnormalities in repair of DNA, the results observed at 43°C might be attributed to the additive or cooperative effect of some abnormality in repair and the conditional absence of DNA polymerase III activity. This possibility seems to be ruled out by control experiments in which strain E486 and its parental strain both completed postreplication repair after 20 min of reincubation at 30°C (Fig. 1B and Fig. 2). Furthermore, an additional dnaE mutant, strain E511, was also studied. Figure 4 demonstrates the increase in DNA size after 20 min of reincubation for strain E511 when phenotypically DnaE+ as compared with reincubation at the nonpermissive temperature. When the revertants (dnaE+) of both E486 and E511 were incubated for 20 min at 43°C, complete postreplication repair was observed. These results further suggest that the repair inhibition observed in the original E486 and E511 mutants was not the result of a background mutation but was associated with inactivity of DNA polymerase III at 43°C.

Even if inactivity of polymerase III prevented completion of gap closure, it is possible that alternate and cooperative pathways might initiate repair and partial gap filling. To answer this question, BND-cellulose chromatography was used to assay for partial filling of daughter strand gaps in the presence of normal polA, polB, recA, and lex gene products but deficient DNA polymerase III activity (Table 1). DNA was assayed for the persistence of single-stranded regions in double-stranded DNA from E. coli E486 reincubated at 43°C and unable to repair in 20 min as determined by DNA gradient profiles. Sheared DNA from cells exposed to 60 ergs of UV irradiation per mm² and labeled with [3H]thymidine for 10 min was placed on the BND-cellulose column. Step elution with 1.0 M NaCl-tris(hydroxymethyl)aminomethane (Tris)-ethylenediaminetetraacetic acid (EDTA) was used to remove double-stranded DNA, and subsequent elution with 2.0% caffeine in 1.0 M NaCl-Tris-EDTA was used to remove single-stranded DNA carrying single-stranded regions. The results in Table 1 represent the means of three to five experiments. Results of parallel experiments using S1 nuclease to detect single strandedness (to be published elsewhere) complemented the BND-cellulose data. Daughter strands synthesized after UV exposure included gaps (single-stranded regions in double-stranded DNA), as indicated by a retention of 40% of the 3H-labeled DNA fragments on the column after 1 M NaCl elution (Table 1; UV, no repair). The single-stranded regions persisted when the cells were reincubated at 43°C and polymerase III was inactive (Table 1, UV and reincubation at 43°C). Furthermore, batch elutions with increasing concentrations of caffeine (0.025% to 2%) demonstrated that most (80%) of the DNA con-

![Figure 3. Acid-precipitable DNA from E. coli E486 cells uniformly labeled with [14C]thymidine, irradiated with UV (80 ergs/mm²), labeled with [3H]thymidine for 10 min, and then reincubated with 2 µg of nonradioactive thymidine per ml for 40 min. Open symbols represent [3H]thymidine label (post-UV daughters strand DNA) and closed symbols represent [14C]thymidine label (parental DNA). Circles represent reincubation at 30°C and squares represent incubation at 43°C.](https://journals.asm.org/journal/jb)
taining daughter strand gaps eluted between 0.1 and 0.5% caffeine. A similar result for the same protocol was observed earlier with another strain by Iyer and Rupp (5). When the fourth protocol in Table 1 was followed (UV, DnaE- repair) prohibiting DNA polymerase III from participating in postreplication repair, the same elution profile between 0.1 and 0.5% caffeine was observed. Thus, gap filling does not appear to initiate and proceed without polymerase III activity.

**DISCUSSION**

Postreplication repair gap filling has been observed in strain E486 after reincubation in excess of 50 min at 43°C by Tait et al. (17). These results suggested that pathways other than DNA polymerase III might be available to complete gap filling. However, their study did not answer the question of which gene products are involved or preferred during repair in wild-type cells when all gene products are available. Because this question is of primary interest in this paper, comparisons of repair capacity were made for time frames relevant to wild-type repair. The data presented here demonstrate no gap filling after 20 min but do not rule out slower initiation of gap filling by residual DNA polymerase III activity and completion of gap filling by recombination after extended reincubation. Plotting of actual counts is also essential to obviate profile changes by preferential degradation, which is more likely with extended incubation.

Previous studies (14, 17) have failed to discriminate between DNA polymerase I and DNA polymerase III as the normal postreplication repair polymerase when both are present and potentially active. Data presented in this paper support the hypothesis that when both polymerases are present they act additively if not cooperatively in closing postreplication daughter strand gaps. The data supporting this hypothesis are from the following specific observations. Two dnaE mutants demonstrate reduced postreplication repair activity, as determined by analysis of alkaline sucrose density gradient profiles. Second, strain E486 retains postreplication daughter strand gaps when DNA polymerase III is inactive, as determined by BND-cellulose chromatography. Third, daughter strand gap repair is slower in cells deficient in DNA polymerase I activity than in wild-type cells, as observed by Sedgwick and Bridges (14) at the permissive temperature for DNA polymerase III activity with strain 1026, which is dnaE(Ts) polA, and with polA strains in my laboratory (data not shown). Results demonstrating reduced repair in either DNA polymerase I or DNA polymerase III mutants when respective activity is reduced support the hypothesis of cooperation between DNA polymerase I and III in the repair process for daughter strand gap filling when both polymerases are active. Although events per daughter strand gap cannot be distinguished in this study, the results presented may reflect a cooperative or additive mode of repair for each gap.

The possible sequence of events necessary for closure of postreplication daughter strand gaps has been discussed in reviews by Hanawalt (3) and Howard-Flanders (4). Cells deficient in recA gene product (16) or polA dnaE gene product activity (14, 17) are unable to complete closure. With wild-type cells, postreplication repair has been shown to be accompanied by recombination of DNA involving parental DNA (2, 13) and de novo DNA synthesis (10). The relationship of recombination and DNA synthesis is unclear. In this study BND-cellulose chromatography demonstrated the retention of postreplication daughter strand gaps in cells with a potentially active recombination capacity (RecA+ Lex- phenotype) and inactive polymerase III. These re-

**TABLE 1. BND-cellulose chromatography of DNA synthesized by E. coli E486 (dnaE) after UV irradiation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>UV (60 ergs/mm²)</th>
<th>[³H]dThd label</th>
<th>Reincubation in nonradioactive medium</th>
<th>Counts recovered (%) on elution with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No UV (control)</td>
<td>-</td>
<td>+</td>
<td>30°C</td>
<td>1 M NaCl</td>
</tr>
<tr>
<td>UV</td>
<td>+</td>
<td>+</td>
<td>43°C</td>
<td>1 M NaCl-2% caffeine</td>
</tr>
<tr>
<td>UV, 30°C DnaE+ repair</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UV, 43°C DnaE- repair</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Samples of sheared DNA from cells exposed to UV, incubated with [³H]thymidine for 10 min, and reincubated in nonradioactive medium for 20 min at 30 or 43°C were placed on BND-cellulose columns. Step elution with 1.0 M NaCl-Tris-EDTA was used to remove double-stranded DNA, and elution with the same buffer plus 2% caffeine was used to remove double-stranded DNA containing single-stranded regions. These data represent the mean of three to five experiments. Controls with no UV exposure but 20 min of incubation at 43°C demonstrated 11% caffeine elution or less.
results support the hypothesis that de novo DNA synthesis is the primary step in filling UV-induced postreplication daughter strand gaps. If de novo DNA synthesis and recombination of DNA cooperate in the closure of each gap, BND-cellulose chromatography would suggest that recombination follows the initiation of closure by de novo DNA synthesis (9).

If both DNA polymerase I and III participate in some additive mode but each polymerase may functionally substitute for the other role in gap closure, then the consequence of substitution might be expressed by altered levels of UV survival and frequency of UV-induced mutation. Previous studies demonstrate little if any lethality when the participation of DNA polymerase III (1, 8) is delayed or when DNA polymerase I (11) is deficient. The UV-induced mutation rate may be altered after a delay greater than 20 min in the participation of DNA polymerase III (1, 8). Protocols in these latter experiments (1, 8) involved higher doses of UV, which probably excluded inducible repair phenomena. Post-UV replication was allowed to form gaps before the polymerase activity was reduced. Further studies with additional mutagens and new DNA polymerase mutant strains are in progress in this laboratory.

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LITERATURE CITED


