DNA POSTREPLICATION REPAIR GAP FILLING IN TEMPERATURE-SENSITIVE dnaG, dnaC, dnaA MUTANTS OF Escherichia coli

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SUMMARY: Gaps in daughter-strand deoxyribonucleic acid (DNA) synthesized after exposure of wild-type Escherichia coli to ultraviolet light are filled during reincubation. In this study the dnaG, dnaC, and dnaA gene products have been examined for their role in postreplication repair. These gene products are unique in their specific control of certain types of DNA synthesis: initiation of rounds of replication and chain propagation. Initiation of rounds of replication is not essential to gap filling; however, chain propagation by short DNA piece initiation appears to be essential for gap filling.

INTRODUCTION

The closing of gaps in deoxyribonucleic acid (DNA) synthesized after exposure of bacteria to ultraviolet light (UV) can be studied with Escherichia coli strains possessing temperature-sensitive DNA polymerase activity. Current evidence supports the hypothesis that the E. coli exrA recA, dnaB, dnaE, and polA gene products have some role in postreplication repair gap filling in DNA synthesized after exposure of bacteria to UV (1-6). The potential role of specific genes in gap filling has been assessed by analysis of change in DNA size on alkaline sucrose gradients. The ability of a cell to increase the size of DNA synthesized after exposure to UV irradiation demonstrates postreplication repair proficiency. In this study I have continued to examine possible additional unique requirements for gap filling. DNA initiation and DNA chain propagation by short DNA piece initiation were studied for their potential roles. Both of these types of DNA synthesis mutants demonstrate a specific deficiency

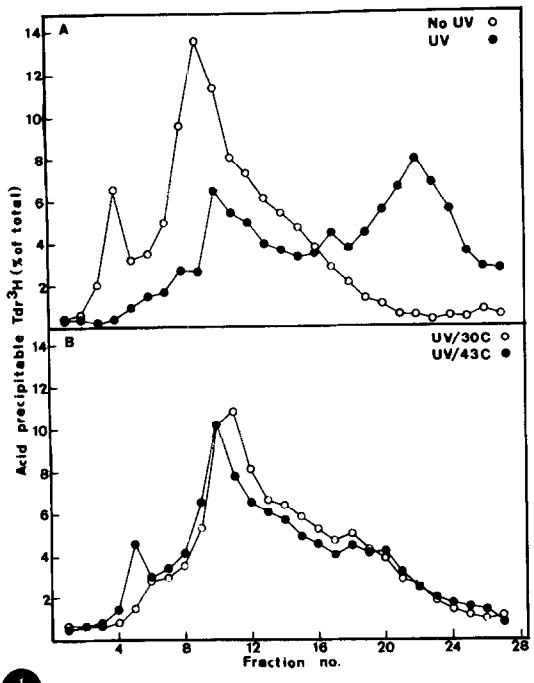
at a non-permissive temperature. Two DNA initiation mutants, CRT46, and PC7, synthesize amounts of DNA consistent with an ability to complete current rounds of DNA synthesis but an inability to initiate subsequent rounds when shifted to high temperatures (7). A third mutant, BT308, is probably unable to initiate nascent (Okazaki) fragments in replication of DNA (8). In this study I have attempted to establish if the dnaA, dnaC, or dnaG gene products have a role in postreplication repair gap filling.

MATERIALS AND METHODS

E. coli strains CRT46 (thr$^-$ leu$^-$ thi$^-$ thy$^-$ lac$^-$ str^r tonA ilv$^-$ dnaA46); PC7 (leu$^-$ thy$^-$ str^r dnaC7); and BT308 (thr$^-$ leu$^-$ thi$^-$ thy$^-$ lac$^-$ str^r tonA dnaG308) were provided by J. A. Wechsler (7). Strains were grown in KB media to about 3 x 10^8cells/ml. The bacteria were exposed to 60 J/mm^2 of UV, and then the washed cells were grown for a 10 or 5 minute pulse of [^{3}H] thymidine (20μCi). Cells were then either iced or reincubated with thymine at optimal or nonpermissive temperatures for DNA synthesis activity. Procedures for growth, irradiation, and alkaline sucrose centrifugation were the same as those published by Rupp and Howard-Flanders (9). Postreplication incubation was at 30°C or 43°C for 50 minutes. The shift from 30°C to 43°C took place in less than 15 seconds. Recovery from the gradient was between 75% and 95% with no correlation with particular strain or sample.

RESULTS

Previous studies have examined the possible essential roles of polymerases I and III in postreplication repair gap filling (1,2). The use of alkaline sucrose gradient zonal centrifugation for determination of rates of gap filling can be reviewed in several publications (4,10). Thus an exrA gene product deficiency was analyzed for rate of gap filling (4) with the conclusion that the cells with the exrA deficiency were capable



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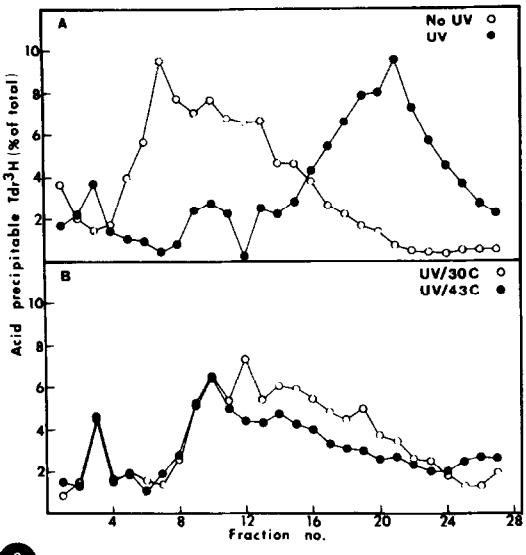

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Figure 1: Alkaline sucrose gradient sedimentation profiles of acid-precipitable DNA from E. coli mutant PC7 (dnaC), temperature sensitive for initiation. (A) No UV, 10 min. incubation with [^{3}H]thymidine (O); UV, 60 J/mm^2, 10 min. incubation with [^{3}H]thymidine (●). (B) UV, 60 J/mm^2, 10 min. incubation with [^{3}H]thymidine, followed by 50 min. reincubation in 2μg/ml of thymine at 30°C (O) or 43°C (●).

Figure 2: Alkaline sucrose gradient sedimentation profiles of acid-precipitable DNA from E. coli mutant CRT46 (dnaA). The procedure was as described in Fig. 1.

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of partial repair. E. coli PC7 and CRT46 are dnaC$^+$ and dnaA$^+$ when incubated at 30°C and dnaC$^-$ and dnaA$^-$ when incubated at 43°C. Sedimentation profiles of alkaline sucrose gradients of DNA synthesized on irradiated parental DNA showed that, for cells incubated at 30°C for 50 minutes, DNA was increased to its normal size (Figs. 1 and 2). When the cells were incubated at 43°C for 50 minutes, the DNA was able to increase to normal size at a rate comparable to the rate of repair seen at 30°C.

In contrast to the results for the dnaA and dnaC gene product, the dnaG gene product demonstrated an essential role in gap repair (Fig. 3). At 43°C the thermolabile gene product was dnaG$^-$. With a 30°C reincubation, the DNA reached normal size in 50 minutes, but at 43°C it did not.

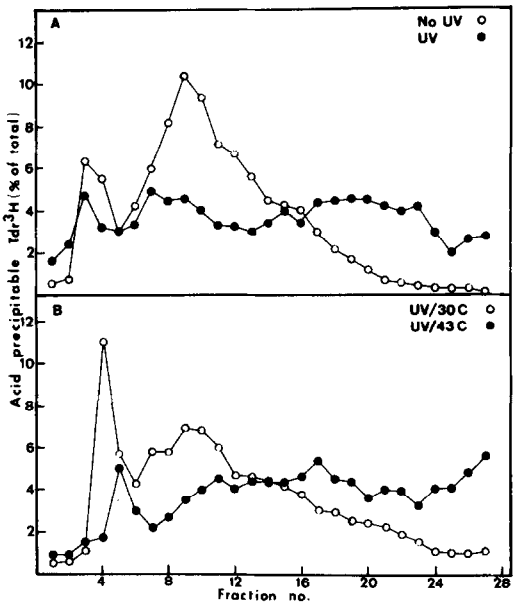

Figure 3: Alkaline sucrose gradient sedimentation profiles of acid-precipitable DNA from E. coli mutant BT308 (dnaG). The procedure was as described in Fig. 1.

DISCUSSION

The results of this study demonstrated no difference in the extent of completion of gap filling between cells that were dnaA$^-$ or dnaC$^-$ and cells incubated at 30°C and phenotypically dnaA$^+$ or dnaC$^+$. Thus the dnaA and dnaC gene products are not essential for post replication repair of gap structures. Some overlapping role, as proposed for polymerases I and III (1,2), might be discerned by study of a temperature-sensitive double mutant polAdnaA. Also, the batch cultures and limits inherent in the gradient technique did not allow analysis of the role of the gene products in filling of gaps that eclipsed the origin and/or terminus of replication. Enrichment for such molecular species relevant to the dnaA and dnaC gene products' functions might be possible using various cell synchrony methods.

The dnaG gene product was initially described as a DNA synthesis "immediate" or "quick stop" temperature-sensitive mutation (7). More re-

cently Bouché, Zechel, and Kornberg have reported that the dnaG gene product probably initiates nascent (Okazaki) fragments in E. coli chromosome replication. The dnaG gene product catalyzes the incorporation of the four ribonucleotide triphosphates into an oligoribonucleotide using single stranded DNA as a template. In this study it was of interest that early studies proposed discontinous replication only on one strand. This suggested a possible specificity of the dnaG gene product for initiation of DNA synthesis for UV-induced postreplication gaps only within DNA synthesized opposite the movement of the replication fork. This would involve 50% of the postreplication gaps. The apparent 100% absence of postreplication repair at the non-permissive temperature for the dnaG gene product activity supports the more recent conclusions that discontinuous replication occurs on both arms of DNA replication forks (11). The dnaG gene product is essential for the joining of bulk DNA synthesized after UV irradiation to fill the postreplication repair gaps on both DNA replicating forks. It should be noted that alkaline sucrose gradient studies measure only completed gap filling. Further analysis of the gaps remaining with the dnaG$^-$ phenotype generated at 43^{o}C for any partial filling of individual gaps is in progress.

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