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# DNA Repair in *Escherichia coli* Mutants Deficient in DNA Polymerases I, II, and/or III

**(*polA1*, *polB*, *dnaE* mutants/UV irradiation/1,β-D-arabinofuranosylcytosine/alkaline sucrose gradients)**

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**ABSTRACT** ***E. coli* mutants deficient in DNA polymerase I, in DNA polymerases I and II, or in DNA polymerase III, can efficiently and completely execute excision repair and post-replication repair of UV-damaged DNA at 43° when assayed by alkaline sucrose gradients. Repair by cells deficient in polymerase I and in polymerases I and II is inhibited by 1-β-D-arabinofuranosylcytosine at 43°, whereas that by cells deficient in polymerase III is insensitive to the inhibitor. When both DNA polymerases I and III are deficient, both excision repair and post-replication repair are greatly reduced at 43°, and the residual repair capability is inhibited by 1-β-D-arabinofuranosylcytosine. Very little dark repair is observed in cells deficient in DNA polymerases I, II, and III, and the DNA is extensively degraded. These results suggest that either DNA polymerase I or DNA polymerase III is required for complete and efficient repair, and that when both DNA polymerases I and III are deficient, DNA polymerase II mediates a limited, incomplete dark repair of UV-damaged DNA. DNA polymerases I and III thus appear to be important enzymes in both DNA replication and DNA dark repair.**

*Escherichia coli polA* mutants (1) are deficient in DNA polymerase I (PolI). From such mutants, further DNA polymerizing enzymes, DNA polymerases II and III, have been isolated and studied (2–5). *E. coli polB* mutants deficient in DNA polymerase II (PolII) are phenotypically similar to *E. coli polB*$^+$ cells (6, 7). Thermosensitive *E. coli* DNA replication mutants mapping in the *dnaE* region (8, 9) are deficient in DNA polymerase III (PolIII) activity *in vitro*, and PolIII isolated from *E. coli* BT1026 is thermosensitive (10, 11). DNA polymerase III is thus the product of the *dnaE1026* gene and is indispensable for DNA replication. *E. coli* cells possess at least two types of enzymatic systems for dark repair of ultraviolet (UV) radiation induced DNA damage: (*1*) excision repair systems, with excision of the photoproducts from the DNA and subsequent repair replication of the excised region; and (*2*) post-replication, or recombination, repair systems, with replication proceeding slowly past nonexcised photoproducts and subsequent recombination events yielding a bacterial chromosome containing no photoproducts (12, 13).

1-β-D-Arabinofuranosylcytosine (ara-C), a nucleoside with antiviral and cancer chemotherapeutic activity (14, 15), is a specific inhibitor of DNA replication. The 5′-triphosphate of ara-C (ara-CTP) specifically inhibits the ATP-dependent DNA synthesis *in vitro* using toluenized *E. coli* cells or *E. coli* cells lysed after immobilization in agar (16, 17). Further, with $Mg^{++}$, ara-CTP strongly inhibits PolII, inhibits PolIII to a lesser extent, and does not inhibit PolI *in vitro* (16).

Initial studies of the DNA repair capabilities of *E. coli* mutants deficient in one or more of the three known *E. coli* DNA polymerases are presented here. Effects of ara-C on repair capabilities at 43° are also presented.

## MATERIALS AND METHODS

*Bacterial Strains.* *E. coli* W3110 *thy*$^-$ *pol*$^+$ and p3478 *thy*$^-$ *polA1* were provided by Dr. J. Cairns, HMS83 *thy*$^-$ *endA*$^-$ *lac*$^-$ *polA1 polB*$^-$ by Dr. C. C. Richardson, BT1026 *thy*$^-$ *endA*$^-$ *polA1 dnaE1026* by Dr. F. Bonhoeffer, E486 *thr*$^-$ *leu*$^-$ *thi*$^-$ *thy*$^-$ *lac*$^-$ *str*$^r$ *tonA met*$^-$ *dnaE486* by Dr. J. A. Wechsler, E4862 *arg*$^-$ *his*$^-$ *str*$^r$ *xyl*$^-$ *mtl*$^-$ *polA1 dnaE486* by Dr. I. Scheffler, and H10265 *thy*$^-$ *endA*$^-$ *polA1 polB*$^-$ *dnaE1026* by Dr. M. Gefter. A thymine auxotroph of E4862 was obtained by trimethoprim selection (18).

*Materials.* Salts, media, sugars, enzymes, and buffers were purchased from CalBiochem, Sigma, and Difco Corps. [$^{14}$C]Thymidine, [$^{14}$C]thymine, and [$^3$H]thymidine were from New England Nuclear Corp. Ara-C was a generous gift from Dr. S. S. Hendler.

*Growth and Treatment of Cells.* Cells were grown in Tris–glucose–minimal salts ($TG_0$) medium (19) containing 0.1% casamino acids and [$^{14}$C]thymine (2 μCi/ml and 2 μg/ml) at 30° to 2 × $10^8$ cells per ml. Half of the culture was harvested by centrifugation (4°, 5 min, 10,000 × *g*), resuspended in an equal volume of $TG_0$ lacking glucose, and split two ways. Half was UV-irradiated in the dark at 22° with a dose of 105 ergs/mm$^2$, as described (20). The UV-irradiated and non-irradiated cell suspensions were split four ways. Two fractions of each were added to tubes containing supplement and ara-C, and two fractions to tubes containing only supplement. Supplement contained [$^3$H]thymidine, to yield 6 μCi/ml, and glucose, casamino acids, and thymine in amounts needed to yield $TG_0$ containing 0.1% casamino acids. The final concentration of ara-C was 2 mg/ml. After incubation at 30° with aeration in the dark for 10 min, one each of the tubes contain-

Abbreviations: ara-C, 1-β-D-arabinofuranosylcytosine; ara-CTP, 1-β-D-arabinofuranosylcytosine-5′-triphosphate; PolI, DNA polymerase I; PolII, DNA polymerase II; PolIII, DNA polymerase III.

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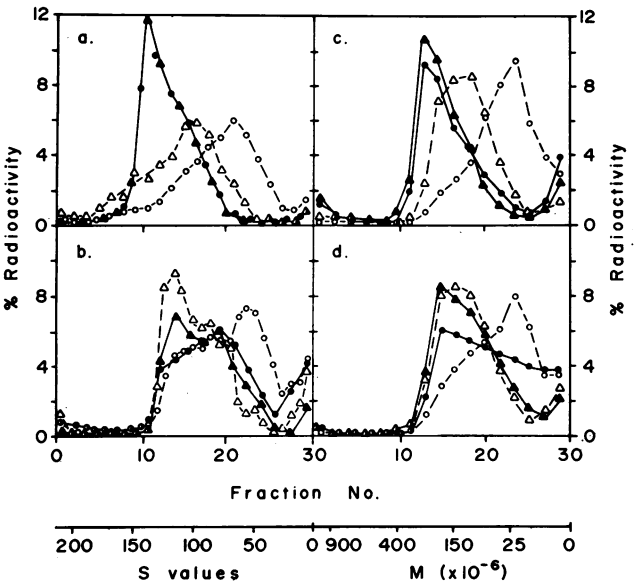


FIG. 1. *E. coli* p3478 and HMS83 cells were grown and treated, and the DNA was analyzed on alkaline sucrose gradients as described in *Methods*. Sedimentation coefficients were determined from the relation of McGrath and Williams (35), with T4 DNA as a standard; molecular weights were determined from sedimentation coefficients from the relation from Studier (36); our values agree with those of McGrath and Williams (35) for unirradiated [$^{14}$C]DNA. Molecular weights have been multiplied by $10^{-6}$. (*a*) p3478, −araC, 43°; (*b*) p3478, +araC, 43°; (*c*) HMS83, −araC, 43°; (*d*) HMS83, +araC, 43°. (Δ---Δ) 10-min [$^{14}$C]DNA; (O---O) 10-min [$^{3}$H]DNA; (▲——▲) 80-min [$^{14}$C]DNA; (●——●) 80-min [$^{3}$H]DNA.

ing nonirradiated or irradiated cells, growing in the presence or absence of ara-C, was rapidly frozen in dry ice–acetone, and nonradioactive thymidine to 50 μg/ml was added to the remaining four tubes. After incubation at 30° in the dark for a further 70 min, these tubes were frozen in dry ice–acetone. The remaining half of the original culture was shifted to 43° for 15 min, harvested by centrifugation (4°, 5 min, 10,000 × *g*), resuspended in an equal volume of $TG_0$ minus glucose at room temperature, and split two ways. Half was UV-irradiated as above. Each half was further divided four ways, added to supplement with or without ara-C at 43°, and permitted to grow with aeration in the dark for 10 min and 80 min at 43°, as described above. The final temperature of 43° was attained within 30 sec after the samples were placed at 43°.

*Spheroplast Formation and Alkaline Sucrose Gradients.* Frozen cell suspensions (1-ml) were thawed, harvested (15 min, 4°, 5000 × *g*), and resuspended in 0.36 ml of $TG_0$ minus glucose but containing 5% sucrose. 10% Lysozyme (0.04-ml) in 10 mM Tris·HCl, pH 8.1, and 0.10 ml of 32 mM EDTA, pH 8.1, were added. The suspension was incubated at 0° for 5–10 min until cells were visibly converted into spheroplasts. Fifty microliters ($5 \times 10^7$ cells) of this preparation were gently layered into 0.1 ml of 0.5 N NaOH over a 4.7-ml alkaline sucrose gradient (5%–20% linear gradient in 0.1 N NaOH). After centrifugation (30,000 rpm, 15°, 105 min, Spinco SW 50.1 rotor), fractions were collected directly onto Whatman 3 MM paper squares. These were dried, washed twice in cold 5% $Cl_3CCOOH$, twice in 95% ethanol, once in acetone, dried, and counted. Recovery from the gradient was routinely 75–95% for both [$^{14}$C]DNA and [$^{3}$H]DNA with no correlation with particular sample or strain. The data are presented as percentage of total recovered radioactivity.

## RESULTS

*DNA Polymerase I is not Essential for DNA Repair at 43°.* When isolated from p3478 cells grown for 80 min after UV irradiation, single-stranded irradiated [$^{14}$C]DNA and unirradiated [$^{3}$H]DNA sediment as rapidly as DNA isolated from nonirradiated cells (not shown), whereas similar DNA isolated 10 min after UV irradiation sediments slowly (Fig. 1*a*). These results are consistent with earlier observations of excision-repair and post-replication repair (21–23), and thus indirectly indicate that both types of repair are completed in 80 min at 43° in cells deficient in PolI. Similar results have been obtained for W3110 cells (data not shown), with analysis after shorter times of incubation after UV irradiation indicating that the rate of repair in W3110 cells is faster than in p3478, as observed (24–26).

*Ara-C Sensitivity of DNA Repair in p3478 Cells.* In the presence of ara-C, the amount of DNA synthesized after UV irradiation is greatly reduced. However, the molecular weight distribution of both unirradiated and irradiated DNA isolated from p3478 10 min after UV irradiation is rather similar to that observed in the absence of ara-C (Fig. 1*b*). During the subsequent 70-min incubation, some degradation of unirradiated DNA occurs (35% loss of trichloroacetic acid-precipitable $^{3}$H counts), and essentially no excision repair, and very little post-replication repair, takes place (Fig. 1*b*). Thus, the total dark repair capabilities of p3478 are sensitive to ara-C at 43°. However, after UV irradiation of W3110 cells, some excision repair, as well as post-replication repair, occurs during the 80-min incubation at 43° in the presence of ara-C, although the molecular weight distribution typical of DNA from nonirradiated cells is not attained during this time (data not shown). This residual repair may be mediated by DNA polymerase I, which is not inhibited by ara-CTP *in vitro* (16).

*DNA Polymerase II is not Essential for DNA Repair at 43°.* *E. coli* HMS83, a strain deficient in both PolI and PolII *in vitro* (6), is able to completely repair both unirradiated and irradiated DNA at 43° (Fig. 1*c*), since the DNA distributions after both 10 min and 80 min of repair are nearly identical with the analogous DNA distributions from p3478 cells (Fig. 1*a*). In the presence of ara-C (Fig. 1*d*), DNA synthesis during the first 10 min of repair after UV irradiation is reduced, and degradation of irradiated [$^{14}$C]DNA (35% loss of trichloroacetic acid-precipitable counts) and of unirradiated [$^{3}$H]DNA (55% loss of trichloroacetic acid-precipitable counts) occurs during the subsequent 70-min repair. The 10-min DNA profiles are similar to those observed in the absence of ara-C (Figs. 1*c* and *d*). The 80-min [$^{14}$C]DNA profile is nearly identical to the 10-min [$^{14}$C]DNA profile, indicating that very little excision repair has occurred. The 80-min [$^{3}$H]DNA profile is intermediate between that of the 10-min [$^{3}$H]DNA and that of unirradiated DNA, indicating that some, but incomplete, post-replication repair has occurred (compare Fig. 1*c*). Thus, the dark repair capabilities of HMS83 are sensitive to ara-C at 43°. The genotype of HMS83 suggests that DNA polymerase III can efficiently repair UV-induced damage in an ara-C sensitive process, or processes, at 43°.

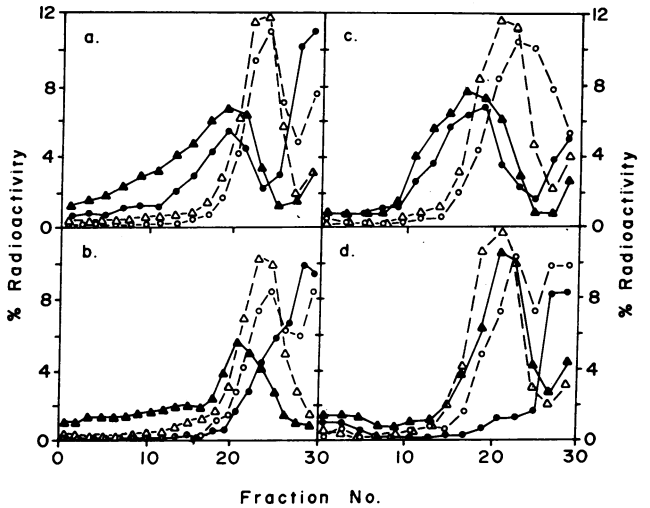


FIG. 2. *E. coli* E4862 and BT1026 cells were grown at 30° and treated, and the DNA was analyzed as described in *Methods*. Symbols are the same as in the legend of Fig. 1. (*a*) E4862, −araC, 43°; (*b*) E4862, +araC, 43°; (*c*) BT1026, −araC, 43°; (*d*) BT1026, +araC, 43°.

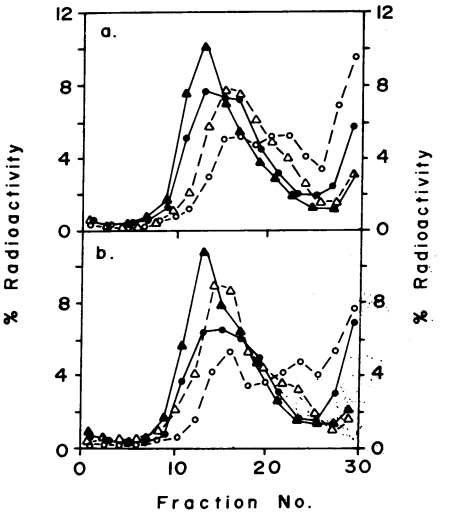


FIG. 3. *E. coli* E486 cells were grown at 30° and treated, and the DNA was analyzed as described in *Methods*. Symbols are the same as in the legend of Fig. 1. (*a*) −araC, 43°; (*b*) +araC, 43°.

*DNA Repair in the Absence of PolI and PolIII is Greatly Reduced. E. coli* E4862 (8, 10) and BT1026 (10, 11) are both deficient in PolI and thermosensitive for PolIII. In the absence of UV irradiation, DNA synthesis ceases within a few minutes at 43°. That DNA which is synthesized at 43° initially sediments slowly (about 20S) and is slowly converted in time into intermediate-sized 70S to 120S pieces (Tait and Smith, *Nature*, submitted). DNA synthesized at 30° continues to sediment rapidly (120S to 130S) after incubation at 43°. After UV irradiation, single-stranded unirradiated DNA and irradiated DNA, from both cell lines, is first rendered low in molecular weight (10-min profiles, Figs. 2*a* and *c*) and is then partially repaired (80-min profiles, Figs 2*a* and *c*), although a substantial fraction (25–50%) of both types of DNA becomes acid-soluble during this time. These results suggest that, in the absence of PolI and PolIII, the residual partial repair of UV-damaged DNA is executed by PolII, and that competing processes appear to degrade both the unirradiated and irradiated DNA before the dark repair processes can be completed. In the presence of ara-C, essentially no dark repair is observed (Figs. 2*b* and *d*), and extensive DNA degradation occurs (50–80%). Thus, the residual repair observed in these strains at 43° is ara-C-sensitive, whereas the degradative processes are not.

*DNA Polymerase III is not Essential for DNA Repair at 43°. E. coli* E486 (8, 10) is thermosensitive for PolIII, and ceases DNA synthesis in the absence of UV irradiation within a few minutes at 43°. When isolated 80 min after UV irradiation, single-stranded irradiated [$^{14}$C]DNA sediments as rapidly as nonirradiated DNA, whereas that isolated 10 min after UV irradiation sediments somewhat slowly (Fig. 3*a*). The sedimentation profiles of the unirradiated [$^{3}$H]DNA are very similar to those of the irradiated [$^{14}$C]DNA. However, some of the [$^{3}$H]DNA sediments very slowly (less than 20S), even after 80-min incubation at 43°. Further, the majority of the [$^{3}$H]-DNA isolated 80 min after UV irradiation sediments somewhat more slowly than the comparable [$^{14}$C]DNA. Both of these properties are found in DNA synthesized in nonirradiated E486 cells at 43° (Tait and Smith, *Nature*, submitted), and thus probably reflect properties of DNA replication by these cells at 43° rather than DNA repair properties. These results indicate that both excision repair and post-replication repair of UV-damaged DNA are completed within 80 min at 43° in cells deficient only in PolIII. The sedimentation profiles of DNA from cells incubated in the presence of ara-C are nearly identical to those of DNA from cells incubated in the absence of ara-C, indicating that the DNA repair observed in E486 is insensitive to ara-C treatment. PolI is not inhibited by ara-CTP *in vitro* (16), thus implicating PolI in these ara-C-insensitive repair processes.

*DNA Repair in H10265 at 30°. E. coli* H10265 is deficient in PolI and PolII and is thermosensitive for PolIII (7), permitting direct comparison with HMS83 at the permissive temperature (30°). After UV irradiation at 30°, essentially complete dark repair of both unirradiated and irradiated DNA occurs (Fig. 4*a*), as is also true with HMS83 cells (Fig. 1*c*; identical results are obtained at 30°). Repair of irradiated [$^{14}$C]DNA is insensitive to ara-C at 30° (Fig. 4*b*), whereas repair of unirradiated [$^{3}$H]DNA is somewhat inhibited by ara-C treatment. Ara-C-insensitive dark repair at 30° after UV irradiation has been observed in all *E. coli polA1* strains tested, including HMS83, indicating an apparent temperature dependence in the sensitivity of some dark repair systems to ara-C.

*DNA Repair in H10265 at 43°*. After UV irradiation at 43°, less repair is observed in H10265 cells (Fig. 4*c*) than in E4862 or in BT1026 cells (Fig. 2), and extensive DNA degradation occurs. This supports the hypothesis that the residual repair capabilities of E4862 and BT1026 at 43° are due to PolII in an ara-C-sensitive process. DNA profiles obtained from H10265 cells incubated at 43° in the presence of ara-C (data not shown) are similar to those of Fig. 4*c*, with even more extensive DNA degradation.

## DISCUSSION

Both excision repair and post-replication repair are completed within 80 min at 43° in *E. coli* cells deficient either in PolI or

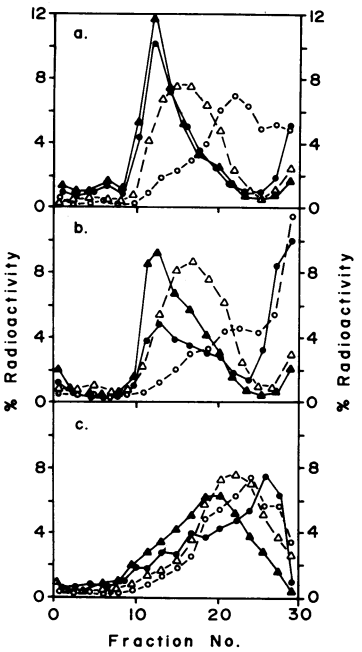


FIG. 4. *E. coli* H10265 cells were grown at 30° and treated, and the DNA was analyzed as described in *Methods*. Symbols are the same as in the legend of Fig. 1. (*a*) −araC, 30°; (*b*) +araC, 30°; (*c*) −araC, 43°.

in both PolI and PolII (Fig. 1). These repair processes are inhibited by 2 mg/ml of ara-C. Thus, neither PolI nor PolII is essential for these processes, and the residual activity at 43° in *polB*$^-$ *polA*$^-$ cells (HMS83), probably PolIII, efficiently catalyzes the required DNA synthesis steps. In the presence of ara-C at 43°, considerable DNA degradation occurs in these cells. A similar excision repair and post-replication repair is observed in H10265 cells at 30° in processes relatively insensitive to ara-C (Fig. 4). Since H10265 cells are deficient only in PolI and PolII at 30°, PolIII again probably catalyzes the required DNA synthesis steps.

When only PolIII is deficient, both excision and post-replication repair are completed within 80 min at 43° in processes insensitive to ara-C (Fig. 3). PolI-catalyzed reactions appear essential for the observed repair in these cells, since it is not observed in *polA*$^-$, *dnaE*$^-$ cells (Fig. 2). The ara-C insensitivity of both these repair processes and of PolI activity *in vitro* (16) further suggests PolI is the polymerase enzyme involved in repair when PolIII is deficient. Apparently neither PolI nor PolIII is required for excision repair or for post-replication repair.

In cells deficient in PolI and PolIII, both excision and post-replication repair are greatly reduced and some DNA degradation occurs at 43° (Fig. 2). Further, the residual repair capability is sensitive to ara-C. The simplest explanation is that PolII mediates the residual partial repair in an ara-C-sensitive process at 43°. Using toluenized cells, Masker *et al.* (27) have shown that UV-irradiated (300 ergs/mm$^2$) *E. coli* cells deficient in PolI and PolIII need PolII to execute repair replication, in agreement with our results. However, the alkaline sucrose gradient experiments of Youngs and Smith (28) indicate that no excision repair of UV-irradiated (25 ergs/mm$^2$) *E. coli* BT1026 cells occurs at 42°. Since the sedimentation profile observed by Youngs and Smith (28) following a 60-min incubation after UV irradiation is similar to that reported here following an 80-min incubation after UV irradiation (Fig. 2*c*), PolII-mediated repair in *polA*$^-$ *dnaE*$^-$ *E. coli* cells may be detectable with alkaline sucrose gradient analysis only after extensive UV irradiation. For example, a limited supply of exonuclease molecules might efficiently antagonize complete repair replication by PolII molecules of a limited number of repair regions. The ara-C sensitivity of the PolII-mediated repair might then be due either to ara-C inhibition of PolII or to ara-C stimulation of the putative exonuclease.

When all three DNA polymerase activities are deficient in the same organism (H10265 at 43°), very little repair is observed and the DNA is extensively degraded. Thus, the repair observed in cells deficient in PolI and PolII is most probably due to PolIII, and that observed in cells deficient in PolI and PolIII is most probably due to PolII.

Alkaline sucrose gradient analysis has been very useful in the study of DNA repair. Two excision repair steps are effectively monitored by this method: (*1*) the initial incision near a photoproduct, reducing the molecular weight of the single-stranded DNA, and (*2*) the final ligase joining step, restoring the initial high-molecular-weight. However, the technique as a measure of DNA repair is limited in several ways: (*1*) only an indirect measure of repair is obtained. The fate of the photoproducts is not assayed directly. (*2*) The sucrose gradient profiles measure only the final product of several biochemical reactions. Thus, the dynamics of antagonistic reactions of degradation and polymerization cannot be measured. (*3*) Only an average for the repair of all photoproducts is determined. Some photoproducts may be repaired more quickly than others. Also, the time required for complete repair of all photoproducts of a given type may vary. The partial repair observed in cells deficient in PolI and PolIII (Fig. 3) possibly reflects the average of a series of antagonistic enzymatic events. (*4*) If the excision, polymerization, and joining reactions occur very rapidly after the incision event, this method would not detect much repair. Our results indicate this is not the case, since the 10-min [$^{14}$C]DNA profiles exhibit reduced molecular weights compared with that of unirradiated DNA. Nevertheless, these 10-min profiles differ among the mutants examined, suggesting that the rate and/or extent of repair replication may vary with the DNA polymerase used. For example, DNA polymerase I apparently participates in a rapid excision repair process, involving excision of relatively few nucleotides per excised photoproduct (24). (*5*) The polymerization steps in dark repair are not assayed directly. Other methods yielding complementing information need to be combined with the alkaline sucrose gradient method for maximal information regarding repair in intact cells.

Several possibilities exist for the mechanism of inhibition of repair by ara-C. Since both PolII and PolIII are inhibited by ara-CTP *in vitro* (16), the most obvious possibility is a direct effect of the appropriate phosphorylated derivative of ara-C on the DNA polymerase in question. However, the apparent insensitivity of repair replication to ara-CTP in UV-irradiated toluenized *E. coli* BT1026 at 44° (27, 29) suggests that other possible inhibitory effects of ara-C need to be considered. One alternative is that ara-C affects the pools of the precursor molecules used by each DNA polymerase during repair, for example, through ara-CDP inhibition of ribonucleoside diphosphate reductase (14). A second alternative is that ara-C stimulates a deoxyribonuclease activity, perhaps one or more of those associated with the three DNA polymerases. Ara-C

treatment of hamster fibroblasts leads to chromosomal breaks (30), possibly due to an ara-C-stimulated nuclease. The ara-C sensitivity of the residual repair in *E. coli* E4862 at 43° (Fig. 2) might be explained by a competition between an ara-C-stimulated nuclease and an ara-C-insensitive PolII repair activity. Finally, ara-C may inhibit the final joining, or ligase, reactions that occur during excision and post-replication repair. Such inhibition would not be detected by the CsCl repair replication assay procedure (24, 27), but would be observed by the alkaline sucrose gradient assay used in these studies. However, insensitivity of repair in *E. coli* E486 (Fig. 3) to ara-C argues against this possibility. The inhibition by ara-C of these repair processes may be more complex than a simple inhibition of one DNA polymerase or another.

Out results demonstrate that all three known *E. coli* DNA polymerases can participate in dark repair. Whether entirely different sets of enzymes are involved in these repair processes or whether the three DNA polymerases can simply perform similar catalytic roles in the same enzymatic pathway is not known. The size of the excised region generated during excision repair involving PolI is possibly smaller than that generated during excision repair in cells deficient in PolI (24). This result suggests that the PolI-mediated excision repair system may include a different set of enzymes than other excision repair systems. Further experiments designed to distinguish between the repair involving PolII and that involving PolIII should resolve these questions.

Historically, because of their high degree of specificity, most enzymes have been considered to participate in only one reaction, or class of reactions, *in vivo*. Our results indicate that DNA polymerase III is an important enzyme for at least two cellular processes, DNA replication and repair of UV-damaged DNA, as is probably also true for DNA polymerase I (refs. 24 and 31–34; Tait and Smith, *Nature*, submitted). Whether the actual reaction catalyzed by PolI and PolIII in these two processes is similar or not remains unknown. Conversely, different enzymes appear capable of participating in similar or identical metabolic processes, as shown by the ability of all three DNA polymerases to catalyze reactions involved in excision repair pathways. Thus, *in vivo* studies of the *E. coli* DNA polymerases indicate two opposing features of these enzymes concerning their roles *in vivo*: (*1*) at least two of these enzymes, PolI and PolIII, appear to be important catalysts in more than one physiological process; and (*2*) more than one of the polymerases appear capable of participating in similar, or perhaps even the identical, processes. It will be of interest to delineate the precise *in vivo* catalytic activities of each of these enzymes, particularly in cells containing all three enzymes.

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