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DNA Polymerase I Modulates Inducible Stable DNA Replication in *Escherichia coli*

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Mutants of *Escherichia coli* lacking RNase HI activity and cells induced for the SOS response express modes of DNA replication independent of protein synthesis, called constitutive and induced stable DNA replication, respectively. We report here that mutants deleted for the *polA* gene express induced stable DNA replication at approximately 25-fold the rate of wild-type cells, whereas constitutive stable DNA replication is not enhanced.

The initiation of *Escherichia coli* DNA replication normally requires protein synthesis, but two alternatives which circumvent this requirement exist. The first, constitutive stable DNA replication (cSDR), occurs in cells lacking RNase HI, a 17.5-kDa protein encoded by the *rnh* gene (reviewed in reference 8). In these cells, replication initiates at several normally cryptic origins of replication, called *oriK*s (1, 5). Accordingly, *rnh* mutants can tolerate deletion of *oriC* and the *dnaA* gene, both of which are normally essential for viability (11). The physiological significance of cSDR for normal cells is not understood, as the levels of RNase H in normal cells appear to be relatively invariant (3). The second mode of DNA replication, induced stable DNA replication (iSDR), is elicited by the SOS response (reviewed in reference 9). It requires activated RecA and DnaA proteins, and it probably arises at *oriC* (3, 9, 10, 13). iSDR is more error prone and resistant to DNA damage than is normal DNA replication (10, 12). These properties and the requirement for activated RecA protein suggest that iSDR may be one pathway for SOS-induced mutagenesis (12, 16).

DNA polymerase I (Pol I) also has an RNase H activity, and this activity could play a role in either cSDR or iSDR. It has been reported that *polA1* mutant cells have normal levels of iSDR (9), but these cells have nearly normal levels of the 5′-to-3′ exonuclease RNase H activity of Pol I (15). Therefore, we have investigated the levels of SDR in cells carrying a deletion for the *polA* gene.

The *E. coli* strains used were AB1157 [F⁻ *thr-1 leu-B6 thi-1* Δ(*proA-gpt*)*62 hisG4 argE3 lacY1 galK2 ara-14 xyl-5 tsx-33 rpsL31 sup37 supE44*] (6); SC10 (as AB1157 but Δ*polA* Km^r; obtained by P1 transduction from CJ261 to AB1157 by S. M. Chin and S. Linn); BW543 (as AB1157 but *xth-1 zdh201*::Tn*10*; obtained from B. Weiss [4]); CM4722 [F$^+$ Δ(*gal-bio*) *thi-1 relA1 spoT1*; obtained from Catherine Joyce]; CM5409 (as CM4722 but *polA1* Tn*10*; obtained from C. Joyce); and CJ261 (as CM4722 but Δ*polA* Km^r; obtained from C. Joyce [7]).

Stable DNA was measured by using a modification of the procedure of Kogoma et al. (10). Cells were grown at 30°C to approximately 3×10^8 cells per ml in M9 medium (14) supplemented with appropriate amino acids at 40 μg/ml and vitamins at 1 μg/ml. An aliquot was diluted and plated to determine the viable cell count, and the rest of the culture was irradiated with UV light as indicated. Another aliquot was then diluted and plated to determine survival; then the remainder of the culture was incubated at 30°C for 70 min to allow for induction of the SOS response. [^{3}H]thymidine (10 μCi, 10 μg/ml), adenosine (to 1 mM), and chloramphenicol (to 150 μg/ml) were added, and then 0.1-ml samples were taken at various times and added to 1 ml of ice-cold trichloroacetic acid, filtered onto GF/C glass fiber filters, and washed with 5% trichloroacetic acid to determine the amount of DNA synthesis. At the same times, aliquots were also taken for plating to determine the viable cell count. The level of SDR is expressed as counts per minute incorporated per number of viable cells.

E. coli SC10, which carries a deletion of the *polA* gene, had the same ability to replicate DNA in the presence of chloramphenicol and the absence of any SOS-inducing treatment as did the wild-type parent, AB1157; neither expressed SDR (Fig. 1). (The level of iSDR was easily detectable in the parental strain in the same experiment [Fig. 1].) Parenthetically, strain BW543, which lacks exonuclease III and that enzyme's RNase H activity, also did not express cSDR (Fig. 1).

When SC10 cells were irradiated with UV light and the levels of iSDR were examined, however, preliminary data suggested that these levels were unusually high relative to the level of the wild-type parent, AB1157, when it was induced for the SOS response by irradiation either with an equal fluence of UV light or with a higher fluence of UV light so that the levels of survival for the two strains were equal. These observations were then pursued in another genetic background so that the *polA1* allele could be included in the analysis. (Strains derived from AB1157 suppress the *polA1* amber mutation.) Therefore, levels of iSDR after irradiation of the wild-type strain CM4722 at 40 and 60 J/m^2 were compared with the levels of iSDR in strains CM5409 (*polA1*) and CJ261 (Δ*polA*) after irradiation at 7 and 15 J/m^2 (Fig. 2). Different exposures were used in each case to achieve approximately equal killing (Table 1). Previous results for AB1157 (Fig. 1) had indicated that iSDR is higher in cells receiving irradiation dose of 40 J/m^2 (equal killing comparison) than in cells receiving 10 J/m^2 (approximately equal dose comparison). Thus, the equal killing comparison is the more stringent for estimating increased iSDR in *polA* mutants.

The Δ*polA* strain showed a markedly higher ability to carry out DNA synthesis via iSDR than did the wild-type strain (Fig. 2). The level of iSDR 210 min after addition of chloramphenicol and [^{3}H]thymidine was approximately 25-fold higher in the Δ*polA* mutant irradiated with 7 J/m^2 than in the wild-type CM4722 irradiated with 40 J/m^2. Similarly, for

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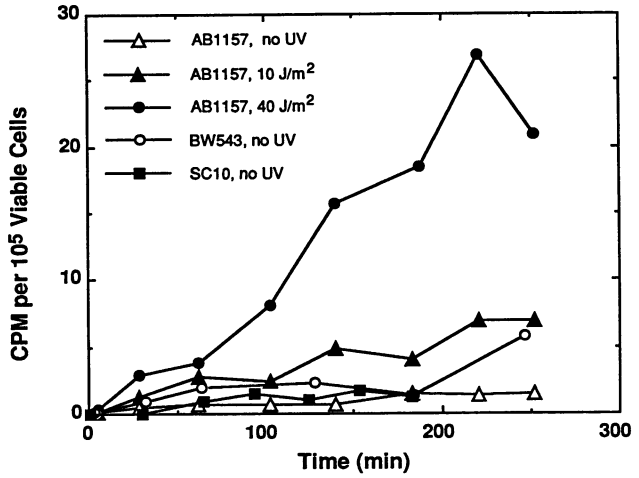

FIG. 1. cSDR. DNA synthesis in the presence of chloramphenicol was measured in strains SC10 (Δ*polA*), BW543 (*xth-1*), and AB1157 (wild type) without induction for the SOS response. As a positive control, AB1157 was irradiated with 10 or 40 J of UV light per m^2, and iSDR was observed.

the *polA1* strain, the amount of DNA synthesized at 200 min was approximately 10-fold higher at a dose of 7 or 15 J/m^2 than for the wild-type strain receiving a dose of 40 J/m^2 (Fig. 2). The amount of DNA synthesis via iSDR in the wild-type CM4722 was also investigated at 60 J/m^2 (Fig. 2) and found to be only about 1.5-fold higher than after a dose of 40 J/m^2, ensuring that induction of iSDR in the wild-type strain by irradiation with 40 J/m^2 was near maximal.

Since *E. coli* strains deficient in DNA Pol I or exonuclease III exhibited low levels of SDR in the absence of an inducing treatment, deficiencies in the RNase H activities of these enzymes do not derepress cSDR in the presence of a functional *rnh* gene, nor do they elicit iSDR in the absence of an SOS-inducing treatment. Apparently the level of RNase H activity provided by other enzymes suffices to suppress the initiation of DNA synthesis at the cryptic *oriK* origins.

Since iSDR was at least 25-fold higher in Δ*polA* cells than

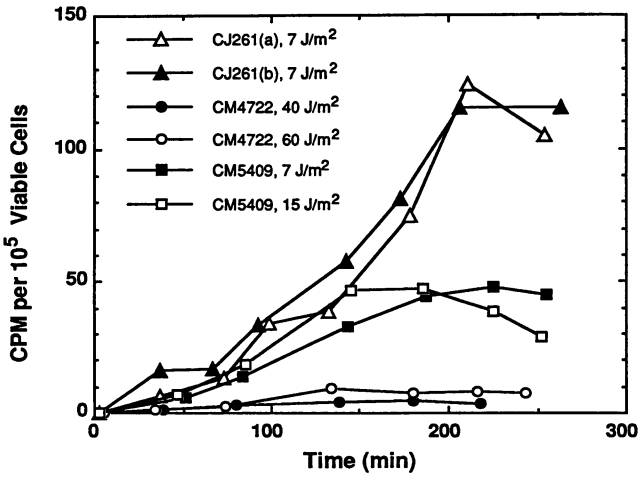

FIG. 2. iSDR. Strains CJ261 (Δ*polA*), CM5409 (*polA1*), and CM4722 (wild type) were irradiated with the indicated doses of UV light, and iSDR was measured. Survival levels are given in Table 1. Replicate cultures of CJ261 were used to assess reproducibility.

TABLE 1. Survival after UV irradiation to induce stable DNA replication

Strain	UV dose (J/m^2)	% Survival
CJ261	7	14, 16[a]
CM4722	40	10
	60	1.0
CM5409	7	27
	15	1.1

[a] Replicate cultures of CJ261 were subject to UV irradiation and measurements of iSDR (Fig. 2).

in cells with a functional *polA* gene, Pol I does appear to limit the extent of iSDR once the SOS regulon is activated. One might propose that the higher levels of iSDR in Δ*polA* and *polA1* mutants are due to a greater expression of SOS functions in these cells than in wild-type cells following UV irradiation. However, the levels of SOS mutagenesis in wild-type and Δ*polA* cells appear to be approximately equal (2). Therefore, it is more likely that Pol I is directly involved in modulating the level of iSDR. The most obvious scheme for Pol I involvement would utilize its RNase H activity to degrade RNA primers before they could take part in replication initiation via iSDR.

Cells with the *polA1* allele express nearly normal levels of Pol I 5′-to-3′ exonuclease activity and thus RNase H activity (when measured in the absence of deoxynucleoside triphosphates) but have only about 1% of the DNA Pol I activity. The *polA1* mutant was found to support an amount of iSDR substantially greater than that of wild-type cells though less than that of the Δ*polA* mutant. It is possible that the RNase H activity of the *polA1* protein is effectively reduced either by its inability to couple to polymerase (which stimulates the exonuclease 10-fold in vitro) or by loss of efficient targeting to the replication origins due to the truncated nature of the *polA1* polypeptide. On the other hand, it is possible that a function of Pol I other than RNase H is responsible for regulating iSDR and that the residual 1% of activity in the cell due to read-through of the *polA1* amber mutation is sufficient to reduce significantly the extent of iSDR in comparison with the level in strains with the Δ*polA* allele.

Since Pol I is a constitutively expressed enzyme, what might be the physiological significance of its ability to regulate iSDR in UV-irradiated cells? One possibility is that the amount of unutilized Pol I becomes scarce as DNA repair processes recruit Pol I. In such a case, the Pol I available to modulate iSDR would become dependent on the level of DNA damage, and thus, while the appearance of iSDR is dependent on induction of the SOS response, the diversion of Pol I to repair DNA damage would enhance its level.

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