DNA Polymerase II of *Escherichia coli* in the Bypass of Abasic Sites *in Vivo*

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ABSTRACT

The function of DNA polymerase II of *Escherichia coli* is an old question. Any phenotypic character that Pol II may confer upon the cell has escaped detection since the polymerase was discovered 24 yr ago. Although it has been shown that Pol II enables DNA synthesis to proceed past abasic sites *in vitro*, no role is known for it in the bypass of those lesions *in vivo*. From a study of phage S13 single-stranded DNA, we now report SOS conditions under which Pol II is needed for DNA synthesis to proceed past abasic sites with 100% efficiency *in vivo*. Overproduction of the $GroES^+L^+$ heat shock proteins, which are members of a ubiquitous family of molecular chaperones, eliminated this requirement for Pol II, which may explain why the role of Pol II in SOS repair had eluded discovery. Mutagenesis accompanied SOS bypass of abasic sites when the original occupant had been cytosine but not when it had been thymine; the quantitative difference is shown to imply that adenine was inserted opposite the abasic sites at least 99.7% of the time, which is an especially strict application of the A-rule. Most, but not all, spontaneous mutations from Rif^s to Rif^r, whether in a $recA^+$ or a *recA*(Prt^c) cell, require Pol II; while this suggests that cryptic abasic lesions are a likely source of spontaneous mutations, it also shows that such lesions cannot be the exclusive source.

POL I, the first DNA polymerase discovered (KORNBERG *et al.* 1956), was thought to play the major role in replication of the *Escherichia coli* chromosome. Thirteen years later this notion was dispelled by the isolation of a defective, but nevertheless viable, mutant of Pol I (DE LUCIA and CAIRNS 1969). That immediately led to the discovery of Pol II (KORNBERG and GEFTER 1970; KNIPPERS 1970; MOSES and RICHARDSON 1970), encoded by the *polB*, also called *dinA* (BONNER *et al.* 1990; IWASAKI *et al.* 1990), gene of *E. coli*. The cellular function of Pol II has been even more elusive than the function of Pol I. We know that Pol II enables DNA synthesis to proceed past abasic sites *in vitro* (BONNER *et al.* 1988), but no role is known for Pol II in the bypass of those lesions *in vivo* (KOW *et al.* 1993).

We now show that Pol II, which is induced sevenfold by the SOS response to DNA damage (BONNER *et al.* 1988), assists in the bypass of abasic sites in phage S13 single-stranded DNA under SOS conditions. Two distinct methods of preparing abasic sites were used, one where the original occupant had been cytosine, the other where it had been thymine. Contrasting mutagenic effects will enable us to conclude that adenine is faithfully polymerized opposite abasic sites. A preliminary report of this work has been presented (TESSMAN and KENNEDY 1993).

MATERIALS AND METHODS

Bacterial strains: The strains used are described in Table 1. The *recA1202*(Prt^c) gene produces a constitutively activated RecA protein that induces the SOS response without the need of UV irradiation of the cell (TESSMAN and PETERSON 1985). Strain AP1, an Hcr^- derivative of *E. coli* C, was used as bacterial indicator.

Transfection assays: Infectivity of phage S13 DNA was assayed by transfection of spheroplasts as described (TESSMAN *et al.* 1983). However, when the SOS system was activated by UV irradiation of the host cells, transfection was effected by electroporation. In that case the cells were grown to 4×10^8/ml in TB (13 g Bacto-tryptone and 7 g NaCl per liter). Two ml of cells were centrifuged and resuspended in the same volume of M9 salts (MILLER 1972) and irradiated with 50 J/m^2 of 254 nm light. The cells were then centrifuged and resuspended in the same volume of TB and incubated 30 min at 37°. To prepare the cells for electroporation they were centrifuged and resuspended three times in chilled H_2O, the last time in a volume of 50 μl and stored on ice until needed. For electroporation, 2 μl of phage DNA mixed with 20 μl of the prepared cells in a chilled cuvette were pulsed in a BRL Electro-porator set at 2.5 kV, 200 Ω and 2.5 μF. Following the voltage pulse, 0.5-ml SOC medium (2% Bacto-tryptone, 0.5% Bacto-yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) were added and the suspension was incubated 5 min at 37° before plating on indicator cells.

Abasic sites: We made abasic sites in infectious single-stranded DNA of phage S13 by two methods, the first at cytosine sites, the second at thymine sites. The methods complement each other in a critical way.

Method 1: This method is unusual (Figure 1). (i) Infectious single-stranded DNA of phage S13, at a concentration of approximately 35 μg/ml in 0.01 M Tris-HCl, 0.005 M EDTA, pH 7.5, was irradiated with 107 J/m^2 254 nm UV light from a germicidal lamp (G15T8), which produces primarily cyclobutane pyrimidine dimers, some of which contain cytosine, as in the case illustrated. The DNA was inactivated to a survival of 1.0×10^{-3} (± 10%) before repair

TABLE 1

Bacterial strains

Strain	Relevant genotype or phenotype	Source or reference
GW1000	*recA441 sulA11 ΔlacU169 rpsL31*	(Kenyon and Walker 1980)
GW1040	As GW1000, but *dinD1*::Mu d1(*lac* Ap) cts	(Kenyon and Walker 1980)
EST1130	AS GW1040, but Ts$^+$	(Tessman and Peterson 1985)
EST945	λ*recA*$^+$ Δ*recA306 srl*::Tn*10*	(Tessman and Peterson 1985)
EST1555	AS GW1000, but Δ*recA306 srl*::Tn*10*	(Tessman and Peterson 1985)
EST1926	As EST1555, but λ*recA*$^+$ ind	(Tessman and Peterson 1985)
IT1819	As EST1130, but St-1^r S13^s	This laboratory
EST2396	As IT1819, but Δ*recA306 srl*:: Tn*10*	P1 (EST945) × IT1819
EST2411	As EST2396, but Tet^s	This laboratory[a]
IT1993	As EST2411, but λ*recA1202* ind	This laboratory
BW12139	*dinA1*::Mu d1-1734(*lac* Kn)	B. Wanner[b]
IT3978	As EST1926, but *dinA1*::Mu d1-1734(*lac*) λ*recA1202* ind	This laboratory[c]
IT3979	As EST1926, but λ*recA1202 ind*	This laboratory
IT3980	AS IT3978, but λ*recA*$^+$ *ind*	This laboratory
IT3981	As IT3979, but λ*recA*$^+$ *ind*	This laboratory
EST1949	λ*recA1206*(Prt^c Rec$^-$) *lexA*(Def)*71 dinD1*::Mu d(*lac* Ap)	(Tessman *et al.* 1986)
IT3995	As IT3978, but p*groES*$^+$*L*$^+$	This laboratory[d]
IT4022	As IT3978, but pTG10	This laboratory

[a] Tet^s cells were selected by the method of Maloy and Nunn (1981).
[b] BW12139 is carried in our laboratory as IT2063. It was derived by B. Wanner from GW1010 (Kenyon and Walker 1980) by swapping Mu d1-1734(*lac* Kn) for Mu d1(*lac* Ap) by the method of (Metcalf *et al.* 1990).
[c] IT3978 was derived by P1 transduction of the *dinA1* allele of BW12139 into EST1926, followed by replacement of λ*recA*$^+$ with λ*recA1202*.
[d] Transformants containing the *E. coli groES*$^+$*L*$^+$ genes cloned into plasmid pTG10 (Goloubinoff *et al.* 1989) were selected by Cm^r.

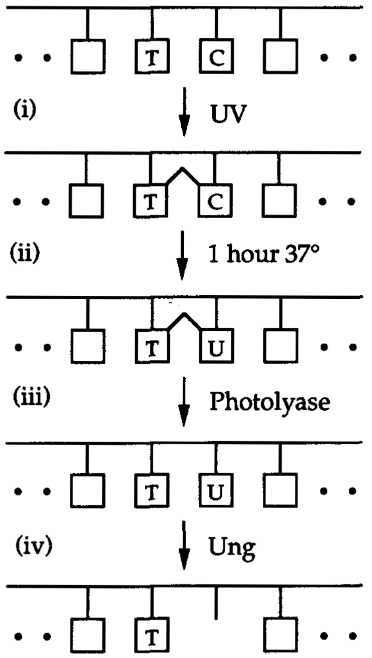

Figure 1.—Procedure for making abasic sites by treatment of UV-irradiated DNA with photolyase and uracil-*N*-glycosylase (Ung).

as assayed before and after irradiation on the Δ*recA* strain EST1555. (ii) The DNA was held at 37° for 1 hr to allow deamination of cytosines in pyrimidine dimers (Tessman *et al.* 1992; Tessman 1992; Tessman *et al.* 1994). (iii) The cyclobutane pyrimidine dimers were monomerized with photolyase (gift of A. Sancar) plus 0.01 m dithiothreitol as described (Sancar *et al.* 1987). The DNA suspensions were exposed for 20 min at 35° beneath a bank of six 15-W blacklight lamps (F15T8-BL) screened by 0.25-inch plate glass. This exotic method of getting uracil into DNA was used because it was an aspect of ongoing experiments to study the kinetics of deamination of cytosine in pyrimidine dimers (Tessman *et al.* 1994). It has the virtue that we can easily control the number of uracils per DNA molecule by choosing the appropriate number of UV hits that are lethal before photoreactivation. In addition, it will be seen that this method of preparing abasic sites, in sharp contrast to method 2, leads to mutations through a striking manifestation of the A-rule. (iv) Monomeric uracils were removed by treating with 2.0 or 4.0 units uracil-*N*-glycosylase (Ung, Boehringer Mannheim, Indianapolis) per ml of DNA for 30 min at 37° in accordance with the manufacturer's instructions, creating the abasic sites.

Method 2: A more conventional method for incorporating uracil into the DNA was also used: the DNA was allowed to replicate in the *dut ung* strain BW313 (Sagher and Strauss 1983). This strain is resistant to infection by the intact S13, which is a normal attribute of K12 strains. Usually mutants sensitive to S13 can be isolated by selecting cells resistant to phage St-1 (Tessman *et al.* 1986). This approach failed, however, because BW313 is already resistant to St-1. Instead, we transfected spheroplasts of BW313 with S13 DNA. The infected spheroplasts were aerated at 37° in a nutrient solution containing 1% Bacto nutrient broth, 1% Bacto casamino acids, 0.1% glucose. After 3–4 hr, the spheroplasts were lysed by vortexing with a few drops of $CHCl_3$. DNA was isolated from the progeny phage by phenol extraction and treated with Ung protein as in step (iv) of Figure 1. This treatment reduced the survival to approximately $e^{-8.6}$ in spheroplasts of the *recA*$^+$ host AP1, which is an Hcr$^-$ derivative of *E. coli* C. Assuming a Poisson distribution of incorporated uracils, there was an average of 8.6 uracils substituting for thymine among the 5386 bases of the DNA molecule. Despite the large reduction in survival when the DNA was Ung-treated *in vitro*, survival of the uracil-containing DNA was not reduced when assayed by infection of spheroplasts that are presumably Ung$^+$. We have suggested that this apparent ability to escape Ung activity *in vivo* may be due to the rapid replication of the DNA (TESSMAN and KENNEDY 1991).

Calculation of fraction of lesions repaired and specific mutation frequency: If the fraction of infectious DNA surviving UV irradiation is S, the number of lethal lesions is $-\ln S$ because inactivation of S13 DNA is exponential with UV dose. If S_b is the fraction surviving irradiation before repair treatment, and S_a is the fraction surviving after repair and subsequent treatment (such as with the Ung protein), then $-(\ln S_b - \ln S_a)$ equals the absolute number of lethal lesions repaired (a number we use below in calculating M_s). It follows that R, the fraction of the original lethal lesions that are repaired, is given by $R = 1 - (\log S_a)/(\log S_b)$, where any base for the logarithms can be used. R is sometimes called the repair sector.

S_b was determined from the survival of the irradiated phage DNA, with or without Ung treatment, relative to the titer of unirradiated DNA, as assayed in the dark on the Δ*recA* indicator strain EST1555. There are no known repair mechanisms operating in this strain in the dark. S_a is the survival of the irradiated DNA after photolyase treatment and with or without subsequent Ung treatment. The Ung treatment diminishes S_a unless the cell can bypass the resultant abasic sites. Thus, the value of S_a indicates whether abasic sites have been bypassed.

Temperature-sensitive phage mutants were scored by picking plaques developed at 35° and testing individually for ability of the phage to grow at 43°. A correction for multiple mutations in the same phage was generally made by applying the zero term of the Poisson distribution: $e^{-M} = 1 - m$, where M, the number sought, is the average mutation frequency, and m, the number observed, is the mutant frequency. When no mutants were found, an upper limit of 1.15 was assumed; this provides a 68% confidence limit, corresponding to one standard deviation used here in general to represent experimental errors. Inasmuch as it is the repair of a lesion that leads to a mutation, we calculate the mutation frequency per lesion repaired, which we call the specific mutation frequency (M_s): $M_s = M/(-R \ln S_b)$. It is more relevant for comparison purposes than the mutation frequency M (LIU and TESSMAN 1990a).

Spontaneous bacterial mutations (Rif^s → Rif^r): Each independent cell culture was grown overnight at 37° for approximately 24 generations to stationary phase from an inoculum of approximately 1000 cells in 20-ml tryptone broth (13-g tryptone and 7-g NaCl per liter). The cells were concentrated by centrifugation and overlaid on LB agar (10-g tryptone, 5-g yeast extract and 5-g NaCl per liter) containing rifampin at 25 µg/ml. Rif^r colonies were counted after overnight incubation at 35°.

RESULTS

SOS bypass of abasic sites prepared by method 1: The SOS system promotes translesion synthesis past abasic sites, which normally block DNA synthesis of the complementary strand (SCHAAPER and LOEB 1981). We elicited a constitutively activated SOS response by employing the *recA1202*(Prt^c) allele (TESSMAN and PETERSON 1985). By contrast, the SOS system is inactive in *recA*$^+$ cells without UV irradiation. To determine whether SOS-assisted DNA synthesis past an abasic site requires Pol II, we used the *dinA1*::Mu d1-1734(*lac* Kn) mutant, which produces a Pol II defect. For abasic sites made by the procedure outlined in Figure 1 (method 1), we determined R, the fraction of UV lesions repaired by the photolyase treatment, and compared that with the fraction of lesions that remained repaired after subsequent Ung treatment (Table 2).

In the *recA*$^+$ strains IT3981 and IT3980, where photorepair is the only known mechanism that can repair single-stranded DNA, the photolyase treatment repaired about 78% of the UV damages. In those strains there was a substantial reduction (by 45%) in the value of R after Ung treatment. This was expected because the abasic sites introduced by the removal of the uracils are lethal in a *recA*$^+$ cell. The Ung treatment also eliminated mutagenesis of the viruses in the *recA*$^+$ cells. In both *recA*$^+$ strains the specific mutation frequency, M_s, was reduced about 10-fold by the Ung treatment; that too was expected inasmuch as the mutations had been at sites where cytosine had been converted to uracil, and the Ung treatment eliminated the mutations by converting those sites into lethal blocks to DNA synthesis.

In the *recA1202* strains, we see that the SOS response promoted DNA synthesis past the abasic sites: IT3979 showed no reduction in R when the DNA was treated with Ung, indicating that the SOS system promoted translesion synthesis with approximately 100% efficiency. Significantly, the specific mutation frequency also showed no reduction, which provides additional evidence that approximately 100% of the abasic sites were bypassed. However, when the SOS response was elicited in a *dinA* mutant (IT3978), the Ung treatment produced a 56% decrease in R, indicating that the lack of Pol II activity caused the failure of translesion DNA synthesis. The large decrease in M_s that accompanied Ung treatment convincingly confirmed that there was little or no DNA synthesis past the abasic sites.

The Mu element *per se* was not responsible for the failure of the *dinA* mutant strains to overcome the block to DNA synthesis because with Mu inserted into the *dinD* gene (IT1993), the SOS-assisted DNA synthesis system bypassed the abasic site (+Ung) and produced mutations as efficiently as when uracil occupied the site (−Ung).

The next two table entries (IT3979 and IT3978) show that the mutant *dinA* allele did not affect SOS translesion synthesis at pyrimidine dimer sites. Photolyase treatment was omitted in those two cases, so that R describes only the effect of SOS bypass of the original dimers; SOS repair and mutagenesis were about the same in the *dinA1* and *dinA*$^+$ strains.

The split phenotype *recA1206*(Prt^c Rec$^-$) strain EST1949 is as defective in genetic recombination as a Δ*recA206* strain (TESSMAN *et al.* 1986). The ability of the Prt^c Rec$^-$ strain to bypass abasic sites with

TABLE 2

Effect of *dinA1*::Mu d(*lac* Kn) insertion on bypass of abasic sites produced by deamination of cytosine in single-stranded DNA

Strain and genotype[a]	Ung treatment	Fraction of lethal lesions repaired (R^b)	Mutants[c]	Specific mutation frequency ($M_s{}^d$)
		Spheroplast assay		
IT3981 λ*recA*⁺ *dinA*⁺	−	0.78 ± 0.04	16/96	0.034 ± 0.009
IT3981 λ*recA*⁺ *dinA*⁺	+	0.41 ± 0.02	0/96	<0.004
IT3980 λ*recA*⁺ *dinA1*	−	0.77 ± 0.04	16/96	0.035 ± 0.009
IT3980 λ*recA*⁺ *dinA1*	+	0.44 ± 0.02	1/96	0.004 ± 0.004
IT3979 λ*recA1202 dinA*⁺	−	0.87 ± 0.04	15/96	0.028 ± 0.007
IT3979 λ*recA1202 dinA*⁺	+[e]	0.87 ± 0.04	18/96	0.034 ± 0.007
IT3978 λ*recA1202 dinA1*	−	0.78 ± 0.04	15/96	0.031 ± 0.008
IT3978 λ*recA1202 dinA1*	+	0.34 ± 0.03	0/192	<0.003
IT1993 λ*recA1202 dinD1*	−	0.84 ± 0.04	16/96	0.034 ± 0.006
IT1993 λ*recA1202 dinD1*	+	0.85 ± 0.04	17/96	0.035 ± 0.007
IT3979 λ*recA1202 dinA*⁺	−	(0.57 ± 0.03)[f]	12/96	0.034 ± 0.010
IT3978 λ*recA1202 dinA1*	−	(0.56 ± 0.03)[f]	11/96	0.031 ± 0.009
EST1949 λ*recA1206* (Rec⁻)	−	0.67 ± 0.03	NT	
EST1949 λ*recA1206* (Rec⁻)	+	0.66 ± 0.03	NT	
IT3995 λ*recA1202 dinA1* p*groES*⁺*L*⁺	−	0.67 ± 0.03	13/96	0.032 ± 0.009
IT3995 λ*recA1202 dinA1* p*groES*⁺*L*⁺	+	0.68 ± 0.03	12/96	0.029 ± 0.008
IT4022 λ*recA1202 dinA1* pTG10	−	0.68 ± 0.03	NT	
IT4022 λ*recA1202 dinA1* pTG10	+	0.43 ± 0.03	NT	
		Electroporation assay[g]		
IT3981 λ*recA*⁺ *dinA*⁺ (UV)	−	0.61 ± 0.05	NT	
IT3981 λ*recA*⁺ *dinA*⁺ (UV)	+	0.69 ± 0.05	14/96	0.033 ± 0.010
IT3980 λ*recA*⁺ *dinA1* (UV)	−	0.71 ± 0.05	NT	
IT3980 λ*recA*⁺ *dinA1* (UV)	+	0.62 ± 0.05	11/96	0.028 ± 0.009

The standard deviation in M_s was obtained by assuming a binomial distribution of temperature-sensitive mutations among the phage tested.

[a] All strains presumably contain the *ung*⁺ allele.

[b] S_b, the survival of the UV-irradiated DNA in the absence of repair was close to 1.0×10^{-3} in every case. Since $R = 1 - (\log S_a)/(\log S_b)$, the values of S_a, the survival after repair, corresponding to the values of R in the table, ranged from $S_a = 1.0 \times 10^{-2}$ for $R = 0.34$ to $S_a = 4.1 \times 10^{-1}$ for $R = 0.87$. Each 10-fold increase of S_a over the value of S_b represents repair of 1/3 of the lethal lesions. More generally, the observed values of S_a that yield the calculated values of R were $S_a = 10^{-3(1-R)}$.

[c] Temperature-sensitive mutants/total number of phage tested. NT, not tested. The mutation frequency, M, is slightly larger than the mutant frequency, m, because multiple mutations in the same phage produce just one mutant. Thus, for example, the mutant frequency of 18/96 for IT3979 becomes a mutation frequency of 20/96 by the relation $e^{-M} = 1 - m$.

[d] Mutation frequency per lesion repaired. Calculation of the mutation frequency (correcting for multiple mutations) and number of lesions repaired is described in MATERIALS AND METHODS.

[e] 4.0 units per ml. All others, 2.0 units per ml.

[f] Not treated with photolyase. In these cases R measures only WEIGLE reactivation, that is, SOS translesion synthesis past the UV-induced lesions, which are primarily pyrimidine dimers. The SOS system is induced by the *recA1202* (Rec^c) allele.

[g] In these experiments the SOS system was activated by UV irradiation of the cells with a flux of 50 J/m².

approximately 100% efficiency shows that the bypass is independent of recombination.

Finally, when the *dinA* strain contained the p*groES*⁺*L*⁺ plasmid (IT3995), Pol II was no longer needed for SOS bypass of the abasic sites. Neither R nor M_s was affected by Ung treatment. By contrast, the parental plasmid pTG10, which lacks the *groES*⁺*L*⁺ genes, failed to substitute for the *dinA* gene (IT4022). We conclude, therefore, that overproduction of the GroES⁺L⁺ heat shock proteins eliminates the need for Pol II. The experiments with the *groES*⁺*L*⁺ plasmid was designed to explain why KOW *et al.* (1993) did not find that bypass of abasic sites depended on Pol II. They induced the SOS system by UV irradiation of the host cells, which is known to induce increased expression of the *groESL* operon (BALUCH *et al.* 1980; KRUEGER and WALKER 1984). When we also induced the SOS system by UV irradiation (Table 2), we confirmed their observations by finding that bypass was as effective in a *dinA1* strain (IT3980 + UV) as in a *dinA*⁺ strain (IT3981 + UV). Both strains also produced about the same frequency of mutation, as would be expected.

SOS bypass of abasic sites prepared by method 2: We repeated the critical experiments with uracil-containing DNA that had been prepared in a conventional way (method 2), namely, passage of S13 through the *dut ung* strain BW313 (Table 3). Ung treatment greatly reduced the DNA survival in *recA*⁺ cells whether they were *dinA*⁺ (IT3981) or *dinA1* (IT3980). When the cells contained an activated SOS system (IT3979), the treated DNA survived as well as the untreated DNA. But when these SOS active cells contained a defective Pol II enzyme (IT3978), they

TABLE 3

Effect of *dinA1*::Mu d(*lac* Kn) insertion on bypass of abasic sites produced by substitution of uracil for thymine in single-stranded DNA

Strain and genotype	Ung treatment[a]	Relative survival[b]	Mutants[c]	Specific mutation frequency (M_s[d])
		Spheroplast assay		
IT3981 λ*recA*$^+$ *dinA*$^+$	−	1.00	NT	
IT3981 λ*recA*$^+$ *dinA*$^+$	+	1.3×10^{-4}	NT	
IT3980 λ*recA*$^+$ *dinA1*	−	1.00	NT	
IT3980 λ*recA*$^+$ *dinA1*	+	1.3×10^{-4}	NT	
IT3979 λ*recA1202 dinA*$^+$	−	1.00	1/480	
IT3979 λ*recA1202 dinA*$^+$	+	1.06	0/480	<0.00028
IT3978 λ*recA1202 dinA1*	−	1.00	0/144	
IT3978 λ*recA1202 dinA1*	+	2.1×10^{-4}	0/69	
IT3995 λ*recA1202 dinA1 pgrosES*$^+$*L*$^+$	−	1.00	0/96	
IT3995 λ*recA1202 dinA1 pgroES*$^+$*L*$^+$	+	1.04	0/336	<0.00040
		Electroporation assay[e]		
IT3981 λ*recA*$^+$ *dinA*$^+$	−	1.00	NT	
IT3981 λ*recA*$^+$ *dinA*$^+$	+	3.3×10^{-4}	NT	
IT3981 λ*recA*$^+$ *dinA*$^+$ (UV)	+	1.0×10^{-1}	0/19	<0.009
IT3980 λ*recA*$^+$ *dinA1*	−	1.00	NT	
IT3980 λ*recA*$^+$ *dinA1*	+	3.1×10^{-4}	NT	
IT3980 λ*recA*$^+$ *dinA1* (UV)	+	2.3×10^{-1}	0/29	<0.006

Legend and footnotes to Table 2 apply here.

[a] 4.0 units per ml.

[b] For each bacterial strain, the absence of Ung treatment provided the reference value of 1.00.

[c] NT, not tested.

[d] Assumes 8.6 lethal lesions (abasic sites) repaired (IT3979 and IT3995), but only 0.77 of that number in UV-irradiated *recA*$^+$ strains (see footnote *e*).

[e] Where UV is indicated, the SOS system was activated by UV irradiation of the cells with a flux of 50 J/m^2 of 254 nm light. Under those conditions, the fraction of lethal lesions repaired (R) was 0.72 (IT3981) and 0.82 (IT3980).

no longer supported growth of the Ung-treated DNA. This confirmed that abasic sites can be bypassed by elements of the SOS system provided there is an active Pol II polymerase. When the p*groES*$^+$*L*$^+$ plasmid was added (IT3995), the need for Pol II was again eliminated. Irradiation of the host cell to induce the SOS system, as before, again eliminated the need for Pol II (IT3980 + UV compared to IT3981 + UV).

Mutations accompanying bypass of abasic sites: When abasic sites were bypassed in strain IT3979, distinctly different frequencies of temperature-sensitive mutations were observed depending on whether the abasic sites originally contained cytosine (method 1, Table 2) or thymine (method 2, Table 3); cytosine sites showed a high frequency of mutations, while thymine sites, in an extensive search, showed a complete absence of mutations. It will be shown in the DISCUSSION that this difference represents a remarkable demonstration of a practically exclusive choice of adenine for insertion by DNA polymerase opposite abasic sites.

Spontaneous bacterial mutations: The frequency of spontaneously occurring mutants (Rif^s → Rif^r) was measured in both *recA*$^+$ and *recA1202* hosts, each containing either a *dinA*$^+$ or a *dinA1*::Mu d(*lac* Kn) allele. Because we expected clonal fluctuations in the number of mutants in a culture, five (*recA*$^+$) and two (*recA1202*) independent determinations of the mutant frequency were made (Table 4). Despite the large fluctuations, the data show unambiguously that spontaneous mutation to Rif^r was overwhelmingly dependent on the presence of a functional Pol II polymerase. The spontaneous mutant frequency was particularly high in the *recA1202 dinA*$^+$ host IT3979, with a 150-fold reduction in the corresponding *dinA1* strain IT3978; by comparison, the *dinA1* allele produced a roughly 14-fold reduction in the *recA*$^+$ strain.

DISCUSSION

Requirement for Pol II in SOS bypass of abasic sites: At first glance our results might seem to be at odds with a recent demonstration by KOW *et al.* (1993) that SOS bypass of abasic sites is unaffected by mutations in the *dinA* gene. There is a significant difference, however, in the respective procedures for inducing the SOS response. They irradiated the host cell with UV, whereas we employed a *recA* mutant that has a constitutively activated RecA protein that induces the SOS response without the need for UV irradiation. We focused on this difference because irradiating the cell has the additional effect of increasing expression of the *groEL* heat shock gene (BALUCH *et al.* 1980; KRUEGER and WALKER 1984), and possibly *groES* too (KRUEGER and WALKER 1984). By introducing the p*groES*$^+$*L*$^+$ plasmid into strain IT3978 to make strain IT3995, we were able to confirm our suspicion that an increase in the GroES$^+$L$^+$ proteins might indeed explain our different results. In strain IT3995,

TABLE 4

Effect of the *dinA1* allele on spontaneous mutations (Rif^s → Rif^r) in *recA*$^+$ and *recA1202* strains of *E. coli*

Strain and genotype	Number of cells plated	Mutants[a]	Mutant frequency[b]
IT3981 λ*recA*$^+$ *dinA*$^+$	5.9×10^9	26, 30, 45, 46, 72	$(7.5 \pm 1.4) \times 10^{-9}$
IT3980 λ*recA*$^+$ *dinA1*	4.8×10^9	0, 0, 0, 4.5, 8	$(5.3 \pm 3.4) \times 10^{-10}$
			Ratio: 14 ± 9
IT3979 λ*recA1202 dinA*$^+$	2.0×10^7	140, 310	$(1.1 \pm 0.4) \times 10^{-5}$
IT3978 λ*recA1202 dinA1*	2.0×10^9	104, 188	$(7.3 \pm 2.1) \times 10^{-8}$
			Ratio: 150 ± 72

[a] Number of Rif^r colonies from independent overnight cultures. The numbers are averages of two (*recA*$^+$) or three (*recA1202*) assay plates.
[b] Error, standard error of the mean. The mutant frequency (m) can be converted to mutation frequency per cell formed per generation (μ) by the relation $\mu = m/g$, where g, the number of generations, is approximately 24.

the Ung-treated DNA was repaired as efficiently as the untreated DNA, showing that a functional *dinA* gene was no longer needed for SOS bypass of abasic sites and the accompanying mutagenesis. We verified that activation of the SOS system by UV irradiation eliminates the need for Pol II (Tables 2 and 3).

Why does overproduction of the GroES$^+$L$^+$ proteins compensate for a defective Pol II enzyme? These heat shock proteins are chaperones and are known to increase the stability of one of the Umu proteins needed for SOS repair, namely UmuC (Donnelly and Walker 1989), and might thereby increase the formation of functional complexes (Donnelly and Walker 1992). The increase in functional complexes promoted by the GroES$^+$L$^+$ proteins may eliminate the need for Pol II.

For the first time we recognize a cellular function for the Pol II enzyme. It was foreshadowed by evidence that Pol II can bypass abasic sites *in vitro* (Bonner *et al.* 1988). But proof that it is also needed *in vivo* may have eluded discovery because the UV irradiation that is commonly employed to induce the SOS response also induces the GroEL protein (and possibly GroES) in a process that is independent of the RecA/LexA regulatory system. If abasic sites are made under conditions that induce the SOS response but not the overexpression of the *groESL* genes, then Pol II would be essential for replication of the damaged DNA. For example, alkylation of DNA, which induces abasic sites (reviewed by Loeb and Preston 1986), and also induces the SOS response (Bagg *et al.* 1981; Quillardet *et al.* 1982), might provide those conditions.

Efficiency of bypass: We note that abasic sites formed by the deamination of cytosine and subsequent removal of the uracil (method 1) did not significantly alter the repair sector in the *recA1202 dinA*$^+$ strain (Table 2), indicating that translesion DNA synthesis past abasic sites by SOS repair was close to 100% efficient. When abasic sites were made by removal of the uracils that had substituted for thymines (method 2), we again saw that SOS activated cells containing a functional *dinA*$^+$ gene could bypass the sites with about 100% efficiency (Table 3). In our experiments, abasic sites were bypassed efficiently when the SOS system was induced whether by a *recA*(Prt^c) allele or by UV irradiation.

Other *in vivo* measurements show bypass of abasic sites with considerably lower efficiency. For φX174 containing apurinic sites, no increase at all in survival is observed in SOS-induced cells (Schaaper and Loeb 1981); only an increase in mutation frequency under SOS conditions is observed, and from that one can infer that about 1 in 77 lesions are bypassed. Other studies of apurinic sites in φX174 also show no significant increase in survival in cells SOS-induced by UV irradiation, implying that SOS bypass of apurinic sites is a rare event (Laspia and Wallace 1989; Kow *et al.* 1993). φX174 is almost identical to S13; both contain 5386 bases and differ in only 111 base substitutions, most of which are silent (Lau and Spencer 1985). We are inclined, therefore, to look for an explanation of the different results to the different methods of preparing abasic sites. Apurinic sites were introduced into φX174 by a combination of acid and heat treatment (pH 4–5, 70°). The inefficiency of bypass would be explained if lethal lesions, other than apurinic sites, are produced by the combined acid and heat treatments. Induction of the SOS response by UV irradiation may be just a little less effective than the use of a *recA*(Prt^c) mutant (Table 3), but that can hardly begin to explain the low efficiency of bypass observed in other laboratories.

In a different *in vivo* approach (Lawrence *et al.* 1990), an abasic lesion was introduced at one of two unique sites in the ssDNA of phage M13 mp7; the DNA was assayed in cells that are UV irradiated and then immediately made competent for transfection. The efficiency of bypass, though a substantial 5–7%, still falls far short of the 100% value that we observed.

Choice of base inserted opposite abasic sites: The fact that the specific mutation frequency, M_s, in Table 2 was not significantly altered upon Ung treatment (IT3979) indicates that the wild-type base G was not usually placed opposite an abasic site. On the other hand, when abasic sites were prepared by method 2 (Table 3), the bypass was nonmutagenic. In particular, strain IT3979 produced the value $M_s \leq 0.00028$ in Table 3, in sharp contrast to the value $M_s = 0.034$ in Table 2. This is especially revealing because the abasic sites had been occupied originally by T in the case of Table 3, and by C in the case of Table 2. It follows that the disparity in the values of M_s could be explained if A was always inserted opposite the abasic site.

The following calculation allows us to conclude that A was used exclusively at least 99.7% of the time. In the case of Table 2 there was an average of 6.9 [*i.e.*, $-\ln(1.0 \times 10^{-3})$] lethal UV lesions per DNA molecule. The fraction of these that became abasic sites and were bypassed successfully in IT3979 is obtained by the difference, 0.53, in R values for IT3979 (0.87) and IT3978 (0.34). Thus, the total number of abasic sites bypassed was 3.7 (6.9×0.53). Since the mutation frequency for IT3979 after Ung treatment was 20/96 (calculated from a mutant frequency of 18/96), we obtain $M_s = 0.056 \pm 0.013$ ($20/96 \div 3.7$) for the mutation frequency per abasic site bypassed. This case provides an estimated lower limit for what the frequency of temperature-sensitive mutations would be if the wrong base were inserted. In the case of Table 3 the lesions were all abasic sites. The value of $M_s \leq 0.00028$ for IT3979 is an upper limit to the frequency of temperature-sensitive mutations per abasic site bypassed; it is a measure of how frequently a base other than A is inserted opposite the abasic site. The ratio of the two M_s values, $\geq 200 \pm 46$ [$(0.056 \pm 0.013)/0.00028$], indicates that A was inserted opposite an abasic site at least 99.5% of the time when bypass occurred under SOS conditions. If we include in our calculations the lack of mutations in strain IT3995 (Table 3), the exclusiveness is raised to 99.7%.

Other measurements *in vivo* show a less exclusive preference for A under SOS conditions. Depurination treatment of ϕX174 at pH 5.0 and 70° leads to reversion of three amber mutants with the indication that A had been inserted opposite a depurinated site about 76% of the time (SCHAAPER *et al.* 1983). Similar depurination treatment of M13 mp2 viral DNA produces forward mutations in the phage *lac* gene following transfection of SOS-induced cells (KUNKEL 1984); A is the preferred residue incorporated opposite the mutant site 59% of the time, with T and G residues accounting for all but one of the remaining cases. When an abasic site is introduced at one of two unique sites in the ssDNA of phage M13 mp7 (LAWRENCE *et al.* 1990), A is inserted opposite the lesion 54% of the time in one case and 80% of the time in the other. In all of these examples the SOS system was induced by UV irradiation.

The A-rule: In its original form, the A-rule specified that when DNA synthesis is blocked at a UV-induced pyrimidine dimer, a DNA polymerase, under the influence of SOS proteins, overcomes the block by blindly inserting two adenines opposite the lesion (TESSMAN 1976). It was inspired by the remarkable fact that most mutations found after reactivation of UV-irradiated single-stranded DNA are C → T (HOWARD and TESSMAN 1964) even though the $\widehat{TT}$ dimer is the dominant UV lesion (SETLOW and CARRIER 1966). The studies on SOS bypass of abasic sites in ϕX174 (SCHAAPER *et al.* 1983) and M13 (KUNKEL 1984) also show a preference for adenine. The application of the A-rule to lesions consisting of abasic sites was stimulated by observations of a preferential (though by no means exclusive) insertion of adenine nucleotides opposite abasic sites during *in vitro* synthesis of DNA by various polymerases (BOITEUX and LAVAL 1982; STRAUSS *et al.* 1982; SAGHER and STRAUSS 1983; RANDALL *et al.* 1987). It is notable, however, that these latter experiments do not involve an SOS system.

When the replication block caused by abasic sites was overcome *in vivo*, we saw that it required the participation of the SOS system. It would seem as if a latent capacity of DNA polymerases to insert A has been exposed by the *in vitro* studies, but full expression of this capacity may require modification of a polymerase, as by some elements of the SOS system in *E. coli*. It should be noted that when the p*groES*$^+$*L*$^+$ plasmid is present, not only can abasic sites be efficiently bypassed when Pol II is absent (IT3995), but adenine is still the exclusive choice. The specific role of Pol II in the enforcement of the A-rule is not known. We also have not yet determined which elements of the SOS system are essential for bypass of abasic sites. Since *umuDC* is the only SOS operon that needs to be induced for SOS-dependent UV mutagenesis (SOMMER *et al.* 1993), one would naturally expect the UmuDC proteins to play an important role in bypass of abasic sites.

With the seemingly exclusive insertion of adenine opposite abasic sites that we observed here in our study of S13 (≥99.7% fidelity), a general A-rule now looks more rigorous than ever. Nevertheless, the A-rule, in its original form as a noninstructive mechanism for bypassing UV lesions, may seem to be superfluous in view of the fact that most of the accompanying SOS mutagenesis could simply be attributed to error-free bypass after the deamination of cytosine in cyclobutane pyrimidine dimers (TESSMAN *et al.* 1992). A noninstructive form of the A-rule may yet survive, however, by providing an intriguing explanation for why SOS bypass stalls at pyrimidine dimers for just the time needed for the cytosines to deaminate (TESSMAN *et al.* 1992).

This virtual monopoly by adenine encourages the thought that the A-rule may indeed reflect the ability of some SOS proteins, presumably in conjunction with an existing DNA polymerase, to help direct the insertion of A opposite a lesion, as originally proposed for pyrimidine dimers. However, we should keep in mind the counterargument that the abasic DNA template may largely determine the choice of adenine (LECLERC *et al.* 1991). The adenine nucleotide, in the normal *anti* intrahelical conformation, produces a stable structure opposite an abasic site (CUNIASSE *et al.* 1987). Guanine can do the same, though less readily than adenine at 37° (CUNIASSE *et al.* 1990). It is conceivable, therefore, that in the SOS bypass of abasic sites a different form of A-rule may be imposed by the DNA itself. On the other hand, while the SOS system incorporates A with remarkably high frequency in bypassing abasic sites in S13 DNA, a mammalian system, by contrast, fails to show a preferential insertion of A (NETO *et al.* 1992). It is thus possible that the abasic template stands ready to accommodate A, but the SOS system helps to encourage a practically exclusive choice. We then would have an A-rule that is partially (perhaps largely) noninstructive and partially instructive.

One might also argue that the choice of bases in the SOS bypass of pyrimidine dimers is completely determined by the template. An A opposite a dimer T may be acceptable despite the distortion, but a G opposite a dimer C might not; in the latter case replication would halt until the dimer C deaminated and then A would be acceptable opposite a dimer U. However, it would be a striking coincidence if some type of A-rule accompanied the SOS bypass of two such different lesions as abasic sites and pyrimidine dimers without in either case being imposed, at least in part, by an SOS protein.

Requirement for Pol II in spontaneous mutation: It is known that spontaneous mutagenesis is elevated in cells containing activated RecA protein (CASTELLAZZI *et al.* 1972; WITKIN 1976; MILLER and LOW 1984; TESSMAN and PETERSON 1985). We have seen here that spontaneous mutants arose at high frequency in a cell containing the constitutively activated RecA1202(Prt^c) protein; this frequency was approximately 150 times lower when Pol II was nonfunctional (Table 4). This is consistent with the suggestion that a large fraction of the spontaneous mutations are targeted by naturally occurring apurinic sites (MILLER and LOW 1984). Since we have shown that abasic sites are bypassed with essentially 100% efficiency in the *recA*(Prt^c) strain, and that A is placed opposite an abasic site, it follows that the SOS bypass of an apurinic site would always be mutagenic.

The spontaneous mutant frequency was approximately 15,000 times lower in the *recA*$^+$ strain IT3981 than in IT3979 (Table 4). Still, even most of this low mutation frequency was dependent on Pol II. This is consistent with the idea that even in a *recA*$^+$ cell there could be a low frequency of bypass of abasic sites (presumably mostly apurinic sites). The bypass might arise, for example, by a rare spontaneous activation of the RecA$^+$ protein so that SOS conditions might exist, though only in about 1/15,000 of the cells. Evidence for rare spontaneous activation has been inferred from the spontaneous induction of λ in lysogenic cells (LITTLE 1990).

Residual untargeted spontaneous mutations: The requirement for Pol II in most cases of spontaneous mutation suggests that the mutant sites are targeted by cryptic lesions such as abasic sites. But the residual mutations (Table 4), which are not dependent on Pol II (IT3980 and IT3978), may be another story; they may be the product of untargeted mutagenesis. Evidence for such an untargeted mechanism can be found in the mutagenic phenomenon seen in *E. coli* cells containing both a RecA(Prt^c) protein and a high copy number *umuD*$^+$*C*$^+$ plasmid, a phenomenon called proximal mutagenesis: the frequency of spontaneous mutation is exceptionally high in or near (proximal to) the *recA* gene (LIU and TESSMAN 1990b). The mutations are too frequent to be accounted for by known spontaneous lesions (S.-K. LIU and I. TESSMAN, unpublished data), thus suggesting that activated RecA protein may assist in the production of mutations by an untargeted mechanism, not only in strains exhibiting proximal mutagenesis, but also in the *recA*(Prt^c) strain IT3978 and even in the *recA*$^+$ strain IT3980. A different approach, which shows that spontaneous mutations arise by replication errors, also leads to the conclusion that an untargeted mechanism is an important source of spontaneous mutations (CAILLET-FAUQUET and MAENHAUT-MICHEL 1988).

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Note added in proof: The Pol II-dependent bypass of abasic sites requires functional UmuDC proteins. Abasic sites (generated by method 1) were not bypassed in a λ*recA1202*(Prt^c) *dinA*$^+$ strain when it contained a mutation in either the *umuD* or the *umuC* gene, which suggests that the mechanism of bypass is similar to that for bypass of UV lesions. There is no need for Pol II when the SOS response is induced by an alkylating agent. Cells treatedwith *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to induce the SOS response (BAGG *et al.* 1981) bypassed abasic sites (generated by method 2) with about 95% efficiency regardless of whether the cells cotained the *dinA*⁺ (IT3981) or the *dinA1* (IT3980) allele; this is essentially the same result obtained with UV induction.

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