Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 487-491, January 1983
Genetics

Infidelity of DNA synthesis associated with bypass of apurinic sites

(depurination/bacteriophage φX174/transversion mutagenesis/DNA polymerases/SOS repair)

ROELAND M. SCHAAPER*, THOMAS A. KUNKEL, AND LAWRENCE A. LOEB†

The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology SM-30, School of Medicine, University of Washington, Seattle, Washington 98195

Communicated by Daniel Mazia, October 12, 1982

ABSTRACT **The mutagenic potential of apurinic sites *in vivo* has been studied by transfection of depurinated φX174 DNA containing amber mutations into SOS-induced *Escherichia coli* spheroplasts. Mutagenicity is abolished by treatment of the depurinated DNA with an apurinic endonuclease from Hela cells, establishing the apurinic site as the mutagenic lesion. The frequency of copying apurinic sites *in vitro* was analyzed by measuring the extent of DNA synthesis using *E. coli* DNA polymerase I and avian myeloblastosis DNA polymerase. The inhibition of DNA synthesis by apurinic sites was less with avian myeloblastosis DNA polymerase, suggesting that this error-prone enzyme copies apurinic sites with greater frequency. Consistent with this conclusion is the observation that, upon transfection into (normal) spheroplasts, the reversion frequency of depurinated φX174 *am3* DNA copied with avian myeloblastosis virus DNA polymerase is much greater than that of the same DNA copied with *E. coli* DNA polymerase I. Sequence analysis of the DNA of 33 revertant phage produced by depurination indicates a preference for incorporation of deoxyadenosine opposite putative apurinic sites. The combined results indicate that mutagenesis resulting from apurinic sites is associated with bypass of these noncoding lesions during DNA synthesis.**

Depurination is the loss of purine bases from DNA through hydrolysis of the *N*-glycosylic bond that connects the base to the sugar-phosphate backbone. This process occurs spontaneously at significant rates. It has been estimated that 10,000 purines may be lost from the genome of a mammalian cell per 24-hr period (1). The presence of large amounts of apurinic endonuclease activity (2) and, possibly, insertase activity (3) in cells testifies to the potentially harmful effects of the loss of hereditary information through depurination. Apurinic sites also result from exposure of cells to various chemical carcinogens. Modification of bases, especially at positions N-3 and N-7 of purines (4) or position O-2 of pyrimidines (5) dramatically labilizes the *N*-glycosylic bond, and the total yield of apurinic sites in a cell upon treatment with chemical carcinogens might be increased many orders of magnitude (6, 7). Thus, apurinic sites may be an important intermediate in spontaneous mutagenesis as well as mutagenesis resulting from modification of DNA by chemical carcinogens.

We have studied the mutagenic potential of apurinic sites in various systems (8–10). Prokaryotic and eukaryotic DNA polymerases show increased misincorporation when copying synthetic polynucleotide templates containing apurinic sites (8). Depurination of φX174 *am3* DNA leads to enhanced mutagenesis when this DNA is copied *in vitro* by *Escherichia coli* DNA polymerase I (9). Finally, transfection of depurinated *am3* DNA into *E. coli* spheroplasts is highly mutagenic for the phage when the spheroplasts are prepared from bacteria previously exposed to UV light (10). Presumably, UV irradiation induces an SOS response in the bacteria (11) which persists in the spheroplasts.

Because mutagenesis in SOS-induced cells is thought to be associated with an error-prone process that permits bypass of otherwise blocking lesions, we have studied in detail the relationship between the ability of an enzyme to polymerize past apurinic sites and the mutation frequency of its product DNA. This was done by comparing these properties for DNA polymerases with different inherent accuracies: *E. coli* DNA polymerase I and avian myeloblastosis virus (AMV) DNA polymerase. The data support the concept that DNA polymerases can copy past apurinic sites and that copying past these sites *in vitro* leads to increased mutagenesis. DNA sequence analysis of *in vivo* mutagenesis also supports this idea. The data point to the usefulness of apurinic sites as model lesions in studying SOS-related mutagenesis.

MATERIALS AND METHODS

Bacteria and Bacteriophage. Bacterial strains *E. coli* HF4714 (*su-1*$^+$) and HF4704 (*su*$^-$) used for plating of φX174 phage and *E. coli* W6 and KT-1 for making spheroplasts have been described (12, 13). *E. coli* C520 (*su-1*$^+$) was obtained from I. Tessman (Purdue University). Bacteriophage φX174 *am3*, *am to8*, *am18*, and *am86* were obtained from J. M. Weisbeek (University of Utrecht). Phage *am3* was grown on HF4704 (12), ambers to8, 18, and 86 were grown on C520 with addition of 0.2 M $MgSO_4$ at 5 min after infection to prevent lysis (13). Revertant or wild-type phage were grown on HF4704 as described (13). Single-stranded (viral) DNA replicative form (RF) I were obtained as before (12) as were restriction endonuclease fragments after treatment of RF I DNA with *Hae* III or *Taq* I.

Preparation of Depurinated DNA, Spheroplasts, Transfection, and Plating. Depurination of φX174 single-stranded DNA was carried out by incubating in 30 mM KCl/10 mM Na citrate, pH 5.00, at 70.0°C. One apurinic site per circle is introduced every 5–7 min (10). Spheroplasts obtained from *E. coli* W6 or KT-1 were prepared by the lysozyme/EDTA method of Henner *et al.* (14) modified as described (12). Transfections were normally performed by adding an equal volume of spheroplasts to 20 mM Tris·HCl (pH 8.0) containing normal or depurinated viral DNA at a concentration of 0.1 μg/ml. The total amount of DNA was adjusted to obtain approximately 10^6–10^7 infective centers. After 12 min at 37°C, an equal volume of prewarmed PAM medium was added, followed by further incubation at 37°C for approximately 90 min. After freezing and thawing and addition of a few drops of chloroform, phage titers and reversion frequencies were determined by plating on HF4714 and HF4704 (12). SOS-induced spheroplasts were prepared by ir-

---

Abbreviations: RF, replicative form; AMV, avian myeloblastosis virus.

[*] Present address: NIEHS, P.O. Box 12233, Research Triangle Park, Durham, NC 27709.

[†] To whom reprint requests should be addressed.

radiation of exponentially growing bacteria with UV light (254 nm) at 80 J/m^2 followed by further incubation at 37°C for 40 min in fresh medium and conversion into spheroplasts (10). The mutagenic response with induced KT-1 spheroplasts is severalfold higher than that obtained with induced W6-spheroplasts.

DNA Sequence Analysis. The viral DNA of selected independent revertants was purified and subjected to sequence analysis by the chain-termination method of Sanger *et al.* (15). For *am3* revertants, *Hae* III restriction fragment Z5 was used as a primer (310 nucleotides long; 83 nucleotides removed from *am3* site). For *am86* revertants the primer was *Taq* I fragment number 2 (1,175 nucleotides long; 62 removed from *am86* site). For *am18* revertants, an unfractionated *Hpa* I digest of ϕX174 RF I (Bethesda Research Laboratories, Bethesda, MD) was used, and the product DNA was separated from the primer by digestion with *Taq* I. This yields a product strand starting between positions 56 and 57, 32 nucleotides away from the *am18* site. The ratio of dideoxy- to deoxyribonucleotides used was 100:1 for *am3*, 200:1 for *am86*, and 1,000:1 for *am18*. The *am3* codon is at nucleotide positions 586–588, *am18* is at 23–25, and *am86* is at 4,116–4,118 on the ϕX174 map (16).

Treatment of Depurinated DNA with Apurinic Endonuclease. The 50-μl reaction mixture contained 0.1 μg of ϕX174 DNA (either untreated or depurinated), 5 mM $MgCl_2$, 25 mM Tris·HCl (pH 7.50), 0.005% Triton X-100, 0.1 mM EDTA, and 1.1 units of apurinic endonuclease from HeLa cells. The apurinic endonuclease was a highly purified preparation kindly provided by C. M. Kane and S. Linn (17). Incubation was for 30 min at 37°C, and the reaction was terminated by addition of EDTA to 7 mM. For transfection experiments, 1.0 μg of DNA was treated in 0.5 ml with all reagents having the same concentration.

***In Vitro* DNA Synthesis.** ϕX174 *am3* viral DNA, containing 0–17 sites per single-stranded circle, was primed at a 5:1 molar ratio with *Hae* III fragment Z5 or Z8 (12). *In vitro* DNA synthesis with polymerase I was performed at 37°C for 60 min in a 25-μl reaction mixture containing 20 mM Tris·HCl (pH 8.0), 2 mM dithiothreitol, 25 μM dATP, 25 μM dGTP, 25 μM dCTP, 25 μM [α-^{32}P]dTTP (220 cpm/pmol), 10 mM $MgCl_2$, 0.20 μg of primed ϕX174 [^{3}H]DNA (18,800 cpm/μg), and *E. coli* DNA polymerase I at a 10:1 molar ratio of enzyme to template. For incorporation with AMV DNA polymerase, each reaction mixture contained 10 units of enzyme (18) and the concentration of each of the four dNTP substrates was increased to 500 μM in order to obtain synthesis of a long minus strand. Reactions were terminated by addition of EDTA to 15 mM, and the extent of synthesis was determined by measuring the acid-insoluble radioactivity in 2-μl aliquots. The reversion frequency of the products of the reactions was determined by transfection as described above.

Product Analysis. The product of the reaction obtained using Z8-primed ϕX174 *am3* viral DNA template with increasing numbers of apurinic sites was phenol extracted, dialyzed, ethanol precipitated, and then digested with restriction endonuclease *Hae* III (12) (1 unit of enzyme per 0.2 μg of product DNA). The resulting fragments were separated on a 5% polyacrylamide slab gel (12) and the dried gel was used to expose Kodak XAR-5 film for 48 hr.

RESULTS

We reported that depurination of ϕX174 *am3* DNA at pH 5.0 and 70°C does not increase the reversion frequency of the amber mutation to wild type when transfected in normal spheroplasts. However, mutagenicity is observed if the treated DNA is transfected into spheroplasts derived from bacteria that previously had been exposed to UV light (10). This mutagenesis is an SOS-dependent phenomenon because it is dependent on functional *recA*, *recF*, and *umuC* genes (19).

An important question is whether this mutagenesis is indeed caused by the apurinic sites. Because mutagenesis is a relatively rare event, the involvement of other (minor) simultaneously induced lesions in DNA cannot easily be excluded. A specific approach is afforded by the use of a highly purified apurinic endonuclease from HeLa cells. This enzyme, as reported by Kane and Linn (17), cleaves apurinic sites on single-stranded DNA. The purified apurinic endonuclease displayed a single band on a denaturing polyacrylamide gel and was devoid of detectable amounts of *N*-glycosylase, ATPase, or nuclease activity towards UV-, methyl methanesulfonate-, or OsO_4-damaged DNA substrates (17). The purity of this enzyme was evidenced in our experiment by the absence of any nonspecific endonuclease activity that might reduce the biological activity of the ϕX174 DNA. The amount of enzyme used was that required to nick >95% of the apurinic sites in depurinated RF I DNA as determined by the filter binding assay developed by Kuhnlein *et al.* (20). Depurination was not mutagenic on normal spheroplasts but was highly mutagenic on SOS-induced spheroplasts (Fig. 1). This mutagenesis was nearly completely (>90%) abolished by pretreatment of the depurinated DNA with the apurinic endonuclease. Therefore, the conclusion that the observed mutagenesis is due to the apurinic sites in the template seems to be justified.

Copying Past Apurinic Sites by DNA Polymerases *in Vitro*. We have analyzed the relationship between synthesis past apurinic sites and mutagenicity by copying ϕX174 *am3* DNA containing apurinic sites with purified *E. coli* DNA polymerase I and AMV DNA polymerase, enzymes with highly different intrinsic accuracies (21, 22). With both polymerases, the extent of DNA synthesis on primed templates was inhibited by depurination, although to different degrees (Fig. 2). At about eight sites per circle, polymerase I synthesized 12% of the amount obtained on a control nondepurinated template whereas AMV polymerase synthesized 45%. Assuming that apurinic sites completely block synthesis, one can calculate the amount of DNA synthesis (S) allowed from a single starting point for a random (Poisson) distribution of apurinic sites as follows:

$$S = \sum_{n=0} \frac{e^{-r} \cdot r^n}{n!} \cdot \frac{1}{n+1} = \frac{1}{r}(1 - e^{-r}),$$

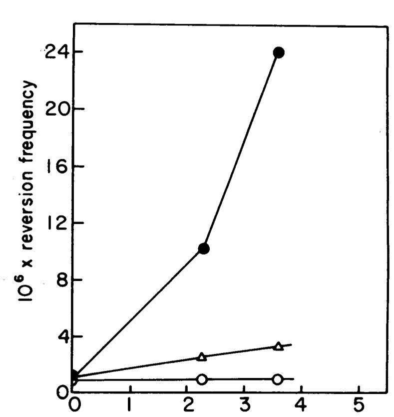

FIG. 1. Reversion frequencies of depurinated *am3* transfected on normal spheroplasts (○), SOS-induced spheroplasts (●), and SOS-induced spheroplasts first treated with apurinic endonuclease (△). The strain used was W6. The individual points represent duplicate values which differed by <10%.

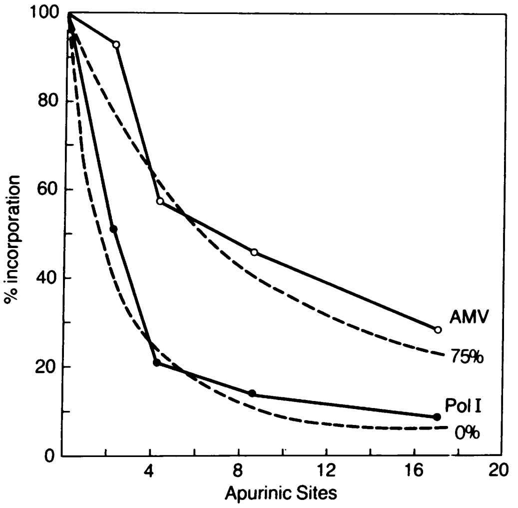

FIG. 2. Effect of depurination on extent of DNA synthesis by DNA polymerase I (Pol I) and AMV DNA polymerase on Z-8-primed ϕX174 DNA. One hundred percent synthesis by polymerase I and AMV DNA polymerase corresponds to 93 and 55 pmol/1.0 μg of ϕX174 DNA, respectively. The incorporation data were corrected for synthesis occurring on the same template in the absence of primer. The dashed lines represent theoretical curves for 0% and 75% bypass (calculation described in text).

in which r represents the average number of sites per circle and n is the actual number. For sufficiently large r, this reduces to $S = 1/r$. Fig. 2 includes this theoretical line of no bypass, as well as a calculated line for 75% bypass (obtained by substituting $0.25r$ for r). This latter line gives the best fit for the measured AMV-inhibition curve. From several similar experiments, we estimate that less than 5–10% of the apurinic sites are copied by polymerase I and approximately 75%, by AMV DNA polymerase. This differential bypass by the two enzymes is specific; it is not observed with ϕX174 templates exposed to other agents that produce blocking lesions such as UV light, *N*-acetoxyacetylaminofluorene, or *anti*-benzo[*a*]pyrene diol epoxide (results not shown).

The *Hae* III restriction endonuclease analysis of the product DNAs is displayed in Fig. 3. Incubation with both polymerases was for 1 hr to allow the maximal extent of synthesis [incorporation reaches a plateau (data not shown)]. With polymerase I (lane a) and AMV polymerase (lane f) on nondepurinated DNA, the restriction patterns were similar and as expected with a Z-8 primer. With polymerase I, an extra band was observed, presumably reflecting enzymatic action on the excess nonhybridized primer molecules. With DNA containing increasing numbers of apurinic sites, the production of the restriction fragments after synthesis by polymerase I was severely inhibited (lanes b–e); much less inhibition was observed for the same fragments with AMV polymerase (lanes g–j).

If apurinic sites are noncoding lesions, this difference in extent of synthesis with depurinated DNA should result in proportionate changes in mutagenesis. To examine this, DNA synthesized *in vitro* by both polymerase I and AMV polymerase on normal and depurinated templates was transfected in normal spheroplasts and the reversion frequency was determined (Table 1). Much more mutagenesis was observed with AMV DNA polymerase (910×10^{-6}) than with polymerase I (6.64×10^{-6}) on DNA containing two apurinic sites per circle. This *in vitro* mutagenesis was abolished by pretreatment of the DNA with apurinic endonuclease (polymerase I) or alkali (polymerase I and AMV DNA polymerase) (results not shown). The theoretical relationship (10) between the probability of an apurinic

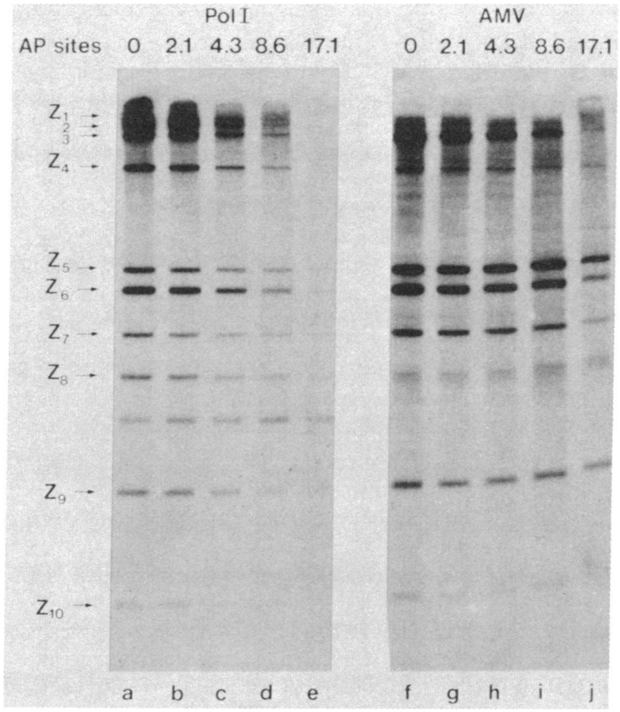

FIG. 3. *Hae* III restriction pattern of product DNA synthesized by polymerase I (Pol I) and AMV DNA polymerase.

site being copied (P) and the expected increase in reversion frequency (ΔR) is $\Delta R = (P \cdot r)/2{,}500$, based on the assumption of random depurination such that only 1 in 2,500 depurinations will take place at the *am3* position 587. Through use of this equation the measured reversion frequencies for polymerase I and AMV translate into polymerization past 4.3% and >100% of the apurinic sites at position 587, respectively. For polymerase I this compares well with the bypass of ≤10% calculated from the incorporation data (Fig. 2). The incorporation and mutagenicity data suggest more frequent bypass with the error-prone AMV DNA polymerase than with polymerase I. Mutagenicity greater than that calculated on the basis of 100% bypass could suggest a contribution of "untargeted mutagenesis" (see below).

DNA Sequence Analysis of Mutations Induced by Depurination. The specificity of the depurination-dependent *in vivo*

Table 1. Reversion frequencies of normal and depurinated ϕX174 *am3* DNA copied by DNA polymerase I and AMV DNA polymerase

	E. coli DNA polymerase I		AMV DNA polymerase	
Apurinic sites	Reversion frequency $\times 10^6$	Bypass[*]	Reversion frequency $\times 10^6$	Bypass[*]
0	<1.50	—	62.0	—
2	6.64	4.3 (<10)	910.0	>100 (75)

The background reversion frequency of uncopied DNA, which has been subtracted from the values shown, was 2.67×10^{-6}. The reversion frequency of nondepurinated DNA synthesized by different preparations of AMV DNA polymerase has varied by severalfold (22) and the mutagenicity associated with bypass of apurinic sites by different preparations has not been determined.

[*] Bypass was calculated from the reversion frequency by using the equation given in the text and after correcting the observed reversion frequency for the fact that 50% of the ϕX174 DNA molecules are copied and the expression of the minus strand is 39% (13). The numbers in parentheses are the bypass values estimated from Fig. 2.

mutagenesis as it occurs in SOS-induced spheroplasts was investigated. Knowledge of the kind of base changes that occur and the positions at which they take place are necessary to determine if mutagenesis results from misincorporation opposite potential sites of depurination, and it also allows comparison with other examples of indirect mutagenesis. Fig. 4 shows the mutagenic responses with a set of ϕX174 amber mutants. *am18* and *am86* were approximately 2-fold more mutable than *am3*, whereas *to8* was only half as mutable. In all cases, mutagenesis was dependent on SOS-induction (results not shown). Revertant plaques of *am3*, *am18*, and *am86* were chosen from separate transfections to ensure their independence and were picked at random to avoid any discrimination with regard to plaque size or character. No obvious or consistent differences in revertant plaque morphology were observed, and plating at both 30°C and 37°C showed no differences in reversion frequencies or plaque type. The average reversion frequency from which the revertants were selected was 20, 10, and 5–10 times the spontaneous reversion frequency for *am3*, *am18*, and *am86*, respectively.

The results of sequence analysis of the purified DNA of these revertants are shown in Table 2. *am3* revertants showed predominantly (12/13) the TAG-to-TTG change (A→T transversion). *am18* revertants were more diverse; however, as with *am3*, 10 of 12 changes occurred at the middle position and 80% of these changes are again TAG-to-TTG. For *am86* only a limited number of sequences were obtained. One double-base change was detected (TAG-to-CAT). Of the remaining sequences, five of six were TAG-to-TAT (G→T transversion). In conclusion it appears that mutagenesis with depurinated DNA *in vivo* is predominantly of the transversion type and occurs predominantly, although not exclusively, at positions of purines, potential sites for depurination. Furthermore, there seems to be some preference for insertion of adenine residues opposite these purine positions. TAG-to-TCG transitions have not been reported for *am18* and *am86*. Although unlikely, considering the multitude of changes already observed, it cannot be excluded that these ambers are not capable of reverting by a transition at a purine position. In that case, the evidence that depurination induces transversion in preference over transitions relies heavily on *am3* data in which 12 or 13 revertants involved an A→T transversion.

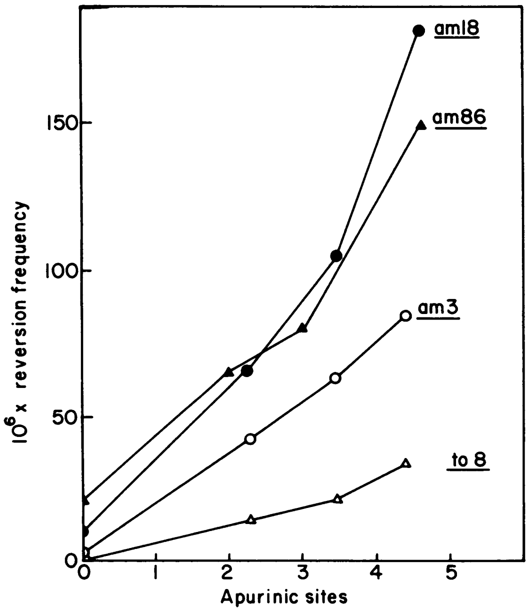

FIG. 4. Reversion frequencies obtained upon transfection of depurinated *to8*, *am3*, *am86*, and *am18* DNA on SOS-induced KT-1 spheroplasts. No increase in reversion frequency was observed on normal spheroplasts (not shown). The starting reversion frequencies were 4×10^{-7} (*to8*), 2×10^{-6} (*am3*), 10×10^{-6} (*am18*), and 20×10^{-6} (*am86*). The values given are corrected for differential burst sizes of amber and wild-type phage.

Table 2. Sequence determination of ϕX174 revertants obtained through *in vivo* mutagenesis of depurinated amber DNA

		Base substitutions in TAG codon								
		1st position			Middle position			3rd position†		
Amber*	Total	C	A	G	G	T	C	C	T	A‡
am3	13	—	—	—	1	12	0	—	—	—
am18	13	1	2§	0	—	8	2	0	—	—
am86	7	1	—	—	—	0	0	0	5	—

* The wild-type sequences are TGG for *am3* and CAG for *am18* and *am86*. 0, Changes not observed in this study but known to be viable (refs. 16 and 18; unpublished data); —, changes which are known to be nonviable or which have never been observed. Spontaneous mutants: of eight for *am3*, six were TGG and two were TTG; of six for *am18*, six were CAG.

† One double mutant (CAT) was detected.

‡ Creates nonsense codon (ochre).

§ One double base change was observed (G-T-A-G→A-A-A-G).

DISCUSSION

The studies described in this paper reveal some properties of apurinic sites which may be useful in the study of the mechanisms of mutagenesis. Significant progress has been made toward the identification of the lesions produced in DNA by mutagenic or carcinogenic compounds. A central question is how the cellular DNA replicating apparatus interacts with these lesions. In this respect it has proved useful to distinguish two types of lesions (23): miscoding lesions, which can be copied with insertion of incorrect nucleotides because of modified base-pairing properties; and noncoding lesions, which cannot be copied under normal conditions and therefore terminate DNA synthesis. Several miscoding lesions have been identified and their base-pairing properties have been studied *in vitro* (24, 25). Noncoding lesions include alterations by several environmentally important agents, such as UV light, benzo[*a*]pyrene, and aflatoxin B_1. *In vitro* studies of templates modified by noncoding lesions so far have failed to demonstrate mutagenesis. *In vivo* mutagenesis seems to require the induction of error-prone systems, of which the *E. coli* SOS-system is the best characterized (11, 26). It is hypothesized that, under induced conditions, the blocking lesion is bypassed with concomitant mutagenesis. Although the genetic evidence is substantial, there is a clear need for an *in vitro* biochemical approach for characterization and identification of the responsible factors.

One important aspect of the experiments described in this paper is that they suggest that it is possible to bypass *in vitro* an alteration of DNA which *in vivo* presumably constitutes a blocking lesion. The designations "miscoding," "noncoding," and "blocking" therefore are not absolute. The data presented here suggest that the ability to polymerize past an apurinic site relates to the intrinsic accuracy of the polymerase. Polymerase III holoenzyme (27, 28), polymerase I (13), and AMV DNA polymerase (22) copy intact DNA with decreasing accuracies, the estimated error frequencies being approximately 10^{-7}, 10^{-6}, and 10^{-4}, respectively. In comparison, the respective bypass frequencies are estimated to be <0.01% (10), 1–10%, and >75% (this paper). It should be noted that previous experiments have shown that polymerase I is capable of copying past apurinic sites (9). This was demonstrated by the isolation of double-stranded ϕX174 product DNA in the form of restriction fragments containing apurinic sites in the template strand. In that study, no attempts were made to quantitate the frequency of copying past apurinic sites. In the light of the findings in this paper, the statement that polymerase I copies apurinic sites has to be qualified as to the frequency of the event.

The relationship between the frequency by which a polymerase copies past an apurinic site and mutagenesis deserves careful consideration. Because, by definition, an apurinic site represents a noncoding lesion, polymerization opposite these lesions is expected to be highly mutagenic. For polymerase I, the relationship between bypass and mutagenicity as given in *Results* yields a reasonably good agreement between predicted and observed values. Synthesis past 5% of the apurinic sites yields a calculated reversion frequency of 8×10^{-6} for two sites per molecule (see equation in *Results* and legend to Table 1), compared to the observed value of 6.6×10^{-6} (Table 1). With AMV DNA polymerase, both the incorporation data (Figs. 2 and 3) and the mutagenicity data (Table 1) suggest more frequent bypass. Typical experiments with AMV polymerase yielded reversion frequencies greater than those calculated on the basis of 100% bypass. Furthermore, 15% of the observed base changes in mutants by using induced spheroplasts are not opposite the template purines. Finally, modest increases in mutagenesis with depurinated templates can be observed as a result of increasing the relative concentration of noncomplementary nucleotides (ref. 9; unpublished data), further suggesting that not all substitutions are directly opposite apurinic sites. Therefore, it cannot be excluded that, in addition to errors opposite AP sites, errors are also made with some high frequency in the vicinity of the lesion (untargeted mutagenesis) as also has been proposed for mutagenesis at UV dimers (29). Our system might offer the possibility of studying this interesting phenomenon *in vitro*.

At present, one can only speculate about the biochemical events in SOS mutagenesis. It might be premature to assume that one single mechanism exists for the bypass of different blocking lesions. The ease of copying past apurinic sites, compared to more bulky lesions such as UV dimers or benzo[*a*]pyrene adducts, might not be simply quantitative but may represent a more fundamental difference. Nevertheless, from the data presented here, apurinic sites offer distinct advantages in an analysis of SOS mutagenesis. *In vivo*, their bypass is rare; however, upon SOS-induction it is quite frequent. *In vitro* tests for SOS-related phenomena will soon be needed. Apurinic sites are truly noncoding and presumably produce little if any distortion of the DNA structure, in contrast to the blocking lesions of the bulky type, like pyrimidine dimers. UV-mutational spectra are complex (30, 31) whereas apurine-induced mutational spectra, although comprising a limited number of sites analyzed, seem to be relatively simple. It remains to be determined whether the specific transversion pattern observed (replacement by deoxyadenosine) is a typical feature of mutagenesis through apurinic sites or a more general SOS-related phenomenon (32, 33). Finally, it should be noted that apurinic sites are common intermediates during repair of DNA damage caused by various environmental agents (32) and, as such, may be important intermediates for mutagenesis by these agents.

We thank Dr. R. Zakour for developing the *am18* sequence analysis procedure. This work was supported by Grants CA-24845 and CA-24988, from the National Institutes of Health, PCM-7680439 from the National Science Foundation, and R-809623 from the Environmental Protection Agency.

1. Lindahl, T. & Nyberg, B. (1972) *Biochemistry* **11**, 3610–3618.
2. Lindahl, T. (1979) *Prog. Nucleic Acid Res. Mol. Biol.* **22**, 135–192.
3. Deutsch, W. A. & Linn, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 141–144.
4. Lawley, P. D. & Brookes, P. (1963) *Biochem. J.* **89**, 127–138.
5. Singer, B. (1976) *Nature (London)* **264**, 333–339.
6. Strauss, B., Scudiero, D. & Henderson, E. (1975) in *Molecular Mechanisms for Repair of DNA*, eds. Hanawalt, P. C. & Setlow, R. B. (Plenum, New York), Part A, pp. 13–24.
7. Margison, G. P. & O'Connor, P. J. (1973) *Biochim. Biophys. Acta* **331**, 349–356.
8. Shearman, C. W. & Loeb, L. A. (1979) *J. Mol. Biol.* **128**, 197–218.
9. Kunkel, T. A., Shearman, C. W. & Loeb, L. A. (1981) *Nature (London)* **291**, 349–351.
10. Schaaper, R. M. & Loeb, L. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1773–1777.
11. Witkin, E. M. (1976) *Bacteriol. Rev.* **40**, 869–907.
12. Kunkel, T. A. & Loeb, L. A. (1979) *J. Biol. Chem.* **254**, 5718–5725.
13. Kunkel, T. A. & Loeb, L. A. (1980) *J. Biol. Chem.* **255**, 9961–9966.
14. Henner, W. D., Kleber, I. & Benzinger, R. (1973) *J. Virol.* **12**, 741–747.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
16. Sanger, F., Coulson, A. R., Friedmann, T., Air, G. M., Barrell, B. G., Brown, N. L., Fiddes, J. C., Hutchinson, C. A., III, Slocombe, P. M. & Smith, M. (1978) *J. Mol. Biol.* **125**, 225–246.
17. Kane, C. M. & Linn, S. (1981) *J. Biol. Chem.* **256**, 3405–3414.
18. Houts, G. E., Miyagi, K., Ellis, C., Beard, D. & Beard, J. W. (1979) *J. Virol.* **29**, 517–522.
19. Schaaper, R. M., Glickman, B. W. & Loeb, L. A. (1982) *Mutat. Res.* **106**, 1–9.
20. Kuhnlein, U., Tsang, T. T. & Edwards, J. (1979) *Mutat. Res.* **64**, 167–182.
21. Kunkel, T. A., Schaaper, R. M., Beckman, R. A. & Loeb, L. A. (1981) *J. Biol. Chem.* **256**, 9883–9889.
22. Gopinathan, K. P., Weymouth, L. A., Kunkel, T. A. & Loeb, L. A. (1979) *Nature (London)* **278**, 857–859.
23. Radman, M., Villani, G., Boiteux, S., Kinsella, A. R., Glickman, B. W. & Spadari, S. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 937–946.
24. Gerchman, L. L. & Ludlum, D. B. (1973) *Biochem. Biophys. Res. Commun.* **308**, 310–326.
25. Singer, B., Fraenkel-Conrat, H. & Kusmierek, J. T. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1722–1726.
26. Hall, J. D. & Mount, D. W. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **25**, 53–126.
27. Loeb, L. A., Kunkel, T. A. & Schaaper, R. M. (1980) in *Mechanistic Studies of DNA Replication and Genetic Recombination*, ICN-UCLA Symposia on Molecular and Cellular Biology (Academic, New York), Vol. 19, pp. 735–751.
28. Fersht, A. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4946–4950.
29. Schaaper, R. M. & Glickman, B. W. (1982) *Mol. Gen. Genet.* **185**, 404–407.
30. Glickman, B. W. (1982) in *Induced Mutagenesis: Molecular Mechanisms and Their Implication for Environmental Protection*, eds. Lawrence, C. W., Prakash, L. & Sherman, F. (Plenum, New York), in press.
31. Coulondre, C. & Miller, J. M. (1977) *J. Mol. Biol.* **117**, 577–606.
32. Schaaper, R. M., Glickman, B. W. & Loeb, L. A. (1982) *Cancer Res.* **42**, 3480–3485.
33. Eisenstadt, E., Warren, J. A., Porter, J., Atkins, D. & Miller, J. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1945–1949.