Translesional Synthesis on DNA Templates Containing a Single Abasic Site

A MECHANISTIC STUDY OF THE “A RULE”*

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Site-specifically modified oligodeoxyribonucleotides containing a single natural abasic site or a chemically synthesized (tetrahydrofuran or deoxyribitol) model abasic site were used as templates for primer extension reactions catalyzed by the Klenow fragment of *Escherichia coli* DNA polymerase I or by calf thymus DNA polymerase α. Analysis of the fully extended products of these reactions indicated that both polymerases preferentially incorporate dAMP opposite the natural abasic site and tetrahydrofuran, while DNA templates containing the ring-opened deoxyribitol moiety block translesional synthesis, promoting sequence context-dependent deletions. The frequency of nucleotide insertion opposite the three types of abasic sites follows the order dAMP > dGMP > dCMP > dTMP. The frequency of chain extension was highest when dAMP was positioned opposite a natural abasic site. The frequency of translesional synthesis past abasic sites follows the order tetrahydrofuran > deoxyribitol > deoxyribose. The Klenow fragment promotes blunt end addition of dAMP; this reaction was much less efficient than insertion of dAMP opposite an abasic site. We conclude that the miscoding potential of a natural abasic site in vitro closely resembles that of its tetrahydrofuran analog. Ring-opened abasic sites favor deletions. Studies with polymerase α in vitro predict preferential incorporation of dAMP at abasic sites in mammalian cells.

Abasic sites in DNA arise spontaneously by hydrolysis, a process that can be accelerated by modification of purine bases (1) and by the catalytic action of N-glycosylases that remove damaged bases from DNA (2–4). The natural abasic site (Ab)1 exists as an equilibrium mixture of the cyclic hemiacetal and open chain aldehyde forms of 2’-deoxyribose (see Fig. 1) and is subject to β-elimination. This chemical reaction leads to strand scission (5); for that reason, structural analogs of deoxyribose have often been used to explore biological properties of abasic sites in DNA (6–10). Abasic site analogs include deoxyribose, a model for the open chain form of the sugar (6, 7), and tetrahydrofuran, an isoster and isoelectronic analog of deoxyribose (8, 9) that is cleaved by type II AP endonucleases, but is not subject to β-elimination (9).

The miscoding properties of natural and synthetic abasic sites have been investigated under a variety of experimental conditions using randomly or site-specifically modified DNA (6, 7, 11–15). The relative frequency of base incorporation opposite abasic sites and of chain extension from the 3’-primer terminus has also been reported (8, 9). While natural and synthetic abasic sites are structurally similar, their mutagenic potential has not been compared under the same experimental conditions. In *Escherichia coli*, synthesis past abasic sites in vitro (6–9, 11–13) and in vivo (14, 15) is accompanied by preferential incorporation of dAMP opposite the lesion, a phenomenon known as the “A rule” (16). In eukaryotes, the presence of abasic sites in DNA is associated with different mutational spectra. For example, dAMP, dCMP, and dTMP were inserted at similar frequencies opposite a natural abasic site when a plasmid vector containing this lesion was allowed to replicate in simian kidney (COS) cells (17–20). In another study in COS cells, preferential incorporation of dAMP was observed opposite the tetrahydrofuran moiety, accompanied by a small number of deletions (21). In human lymphoblastoid cells, dGMP was incorporated preferentially opposite natural abasic sites (22). In AP endonuclease-deficient strains of yeast, the frequency of A:T → C:G events increased (23); in another yeast system, dCMP was predominantly incorporated opposite the lesion (24). In this report, we used a prokaryotic and a eukaryotic DNA polymerase and DNA templates containing a single abasic site to explore the mechanistic basis underlying mutagenesis at abasic sites in DNA.

Recently, one of us (S. S.) developed a method by which site-specifically modified oligodeoxyribonucleotides could be used to quantify all base substitutions and deletions occurring during DNA synthesis in vitro (25). Combined with steady-state kinetic analysis (26, 27), this experimental system is used to compare the miscoding properties of a natural abasic site and its analogs in reactions catalyzed by the Klenow fragment of *E. coli* DNA polymerase I or by calf thymus DNA polymerase α. Our results indicate that the natural abasic site resembles closely the tetrahydrofuran moiety with respect to its miscoding properties. These lesions, which exist primarily or exclusively in a ring-closed conformation, preferentially incorporate dAMP opposite the lesion. In contrast, deoxyribose, which serves as a model for the open chain (minor) form of the natural abasic site, blocks DNA synthesis and promotes the sequence-dependent formation of 1- and 2-base deletions. In vitro, both pol α and the Klenow fragment operate according to the tenets of the A rule.

EXPERIMENTAL PROCEDURES

Materials—Organic chemicals used for the synthesis of oligodeoxyribonucleotides were supplied by Aldrich. [γ-32P]ATP (specific activity > 6000 Ci/mmol) was obtained from Amersham Corp, Cloned exo− (17,400 units/mg) and exo+ (21,200 units/mg) Klenow fragments of *E. coli* DNA polymerase I were defrayed in part by the payment of page charges. This article was defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: Ab, natural apurinic/apyrimidinic (abasic) site; F, tetrahydrofuran; Re, reduced form of the natural abasic site (deoxyribose); pol, DNA polymerase; HPLC, high performance liquid chromatography.

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polymerase I (1 unit of enzyme) catalyzes the incorporation of 1 nmol of total nucleotide into acid-insoluble form in 60 min at 37 °C and uracil-DNA glycosylase (1 unit/μl) were purchased from U. S. Biochemical Corp. intact DNA pol I and dNTPs were from Pharmacia; calf thymus DNA pol α (30,000 units/mg) (1 unit of enzyme catalyzes the incorporation of 10 nmol of nucleotide acid-insoluble material in 30 min at 37 °C using poly(dA-dT) as template primer) was from Molecular Biology Resources, Inc.; T4 polynucleotide kinase was from Stratagene; and venom phosphodiesterase I was from Worthington. Acetonitrile, triethylamine, and distilled water, all HPLC-grade, were purchased from Fisher. A Waters 990 HPLC instrument, equipped with a photodiode array detector, was used to separate and analyze modified and unmodified oligodeoxynucleotides.

Synthesis and Purification of Oligodeoxynucleotides—Oligodeoxynucleotides were synthesized by solid-state methods using an automated DNA synthesizer. Templates containing a single unmodified dG or tetrahydrofuran (F) residue at position 13 (Sequences 1, 4, 11, and 18 in Table I) were synthesized as described above. An Ab-modified 18-mer template (Template DNA (5'-CTTCCXCTTCTTTCTCTTCTTCTTCTCTCTT) or a 24-mer (5'-CTTCCXCTTCTTTCTCTTCTTCTTCTTCT) template (1.0 pmol) containing dG or a single Ab, Re, or F at the position designated X was annealed to 0.5 pmol of a 32P-labeled 12-mer primer (Sequence 5 or 19) or 0.05 units for 5 min for other pairs and 0.001 units of exonuclease pol I (exo 3' → 5') exonuclease activity was incubated at 25 or 30 °C for 1 h in 50 mM Tris-HCl (pH 8.0) containing 8 mM MgCl2 and 5 mM 2-mercaptoethanol. Primer extension reactions with pol α were conducted at 30 °C in 10 μl of 50 mM Tris-HCl (pH 8.0) containing 10 mM MgCl2, 20 mM ammonium sulfate, 2 mM dithiothreitol, and 0.5 μg/ml bovine serum albumin using a modified template (0.2 pmol) annealed to a 32P-labeled 12-mer primer (0.1 pmol). Reaction mixtures were subjected to analysis by two-phase 20% polyacrylamide gel electrophoresis (35 × 42 × 0.04 cm or 15 × 72 × 0.04 cm) with 7 μm urea present in the upper phase and no urea in the lower phase (25). Following gel electrophoresis, positions of the oligomers were established by autoradiography. Radioactivity was measured in a Packard scintillation counter. The detection limit for reaction products was 0.03% of the starting primer.

Kinetics of Nucleotide Insertion and Chain Extension—Kinetic parameters related to nucleotide insertion and chain extension were determined under conditions similar to those described for the primer extension assay (32, 33). Reaction mixtures containing varying amounts of 1.001–0.05 units of exonuclease pol I (exo 3' → 5') Klenow fragment for 60 s (C:G), 0.05 units for 90 s (A:Ab, A:Re, and A:F), 0.05 units for 3 min (G:Ab, Ab, Re, or F) primed with 0.5 pmol of 32P-labeled 12-mer (5'-AGAGGAAGAGG) to measure nucleotide insertion kinetics or with a 32P-labeled 13-mer (5'-AGAGGAAAGGAG, N = C, A, G, or T) to measure chain extension kinetics as described by Mendelman et al. (26, 27). Base insertion was measured in reactions using 0.001 units of exonuclease pol I (exo 3' → 5') Klenow fragment for 60 s (C:G), 0.01 units for 90 s (A:Ab, A:Re, and A:F), and 0.05 units for 90 s (A:G, G:Ab, G:Re, and G:F). Kinetics of extension were measured in reactions using 0.005 units of exonuclease pol I (exo 3' → 5') Klenow fragment for 60 s (C:G) and 0.05 units for 5 min for other pairs and 0.001 units of exonuclease pol I (exo 3' → 5') Klenow fragment for 60 s (C:G), 0.05 units for 3 min (G:Ab, Ab, Re, or F) primed with 0.5 pmol of 32P-labeled 12-mer (5'-AGAGGAAAGGAG) to determine relative dNTP incorporation at the blunt end of the duplex was measured in reactions using 0.005 units of exonuclease pol I (exo 3' → 5') Klenow fragment for 5 min. Samples were heated for 3 min at 95 °C in the presence of formamide and then applied to a 20% polyacrylamide gel (35 × 42 × 0.04 cm) in the presence of 7 μm urea. Following gel electrophoresis, bands were located by autoradiography and quantified as described above. Values for the Michaelis-Menten constant (Km) and the maximum velocity of the reaction (Vmax) were obtained from Hanes-Woolf plots. The ratio of primer-template to enzyme (0.05 units) is at least 20:1; similar values were reported by Boosalis et al. (34). Less than 20% of the primer is extended under the steady-state conditions used in our studies (35). All data reported represent an average of two to four independent experiments. Frequencies of nucleotide insertion (Finsert) and chain extension (Fextend) were determined relative to dC:dG according to equations derived by Mendelman et al. (26, 27), where F = (Vmax/Km)×[wrong pair]/(Vmax/Km)×[light pair]+dC:dG with "wrong pair" defined as a mismatch or any pair involving Ab, Re, or F.

RESULTS

Klenow Extension Reactions Catalyzed by the exo· and exo＋Klenow Fragments—The structures of the natural and syn-
thetic abasic sites used in these experiments are shown in Fig. 1. Primer extension reactions, catalyzed by the exo' Klenow fragment of DNA polymerase I, were conducted in the presence of four dNTPs. The expected reaction products, represented by a mixture of $^{32}$P-labeled oligodeoxynucleotides containing dC, dA, dG, dT, and 1- or 2-base deletions (25), were completely resolved by two-phase 20% polyacrylamide gel electrophoresis (72 cm), as shown in Fig. 2 (lanes 1 and 5).

DNA synthesis on an unmodified template (Fig. 2, lane 2) led to the expected incorporation of dCMP (85.2% of the starting primer) opposite dG at position 13 with no deletions. When an Ab-modified oligodeoxynucleotide was used as template (lane 3), dAMP (35.5%) was preferentially incorporated opposite the lesion; 2-base deletions (7.1%) were also formed. Similar results were obtained using an F-modified template (lane 4). In contrast, following 15 min of primer extension on an 18-mer template containing Re, the amount of fully extended product containing a 2-base deletion (32.5%) exceeded dAMP incorporation opposite the lesion (15.6%) and 1-base deletions (0.61%) (Table II). The amount of these products increased over time (Table II) and was dependent on the amount of enzyme used in the reaction (data not shown). Incorporation of dGMP and dTMP opposite abasic sites was not detected. In the presence of excess enzyme, blunt end extension was observed (slowly migrating bands in lanes 3 and 4).

The identity of the base incorporated and the position of deletions produced during translesional synthesis on Ab-modified templates were confirmed by Maxam-Gilbert sequence analysis (36). The fully extended reaction products migrating at positions 18 and 16 (Fig. 2, lane 3) represent dAMP incorporation opposite Ab and a 2-base deletion opposite the lesion, respectively (data not shown). Incorporation of dGMP and dTMP opposite abasic sites was not detected. In the presence of excess enzyme, blunt end extension was observed (slowly migrating bands in lanes 3 and 4).

Frequency of Nucleotide Insertion and Chain Extension—Kinetic parameters for nucleotide insertion opposite Ab, Re, or F and for chain extension from the 3'-primer terminus were determined under steady-state conditions as described previously (37, 38). The exo' Klenow fragment lacks 5'→3' exonuclease activity, and the exo' Klenow fragment lacks both 5'→3' and 3'→5' exonuclease activities. As shown in Table III, the relative insertion frequency ($F_{ins}$) opposite the abasic site for the exo' Klenow fragment followed the order dAMP > dGMP > dCMP > dTMP. $F_{ins}$ for dAMP incorporation opposite abasic sites was 23–48 times higher than for dAMP opposite dG and 3–8 times higher than for dGMP opposite Ab, Re, or F. $F_{ins}$ for dAMP opposite F was 2 times higher than for dAMP opposite Ab or Re. $F_{ext}$ could only be detected for the dA:Ab and dA:F pairs. $F_{ins} \times F_{ext}$, a parameter used to estimate the overall frequency of translesional synthesis (39), was 11 times higher for dF than for dA:Ab. When $F_{ins}$ for blunt end extension was measured (40), dAMP was inserted exclusively at a rate 63–122 times lower than that for dAMP incorporated opposite Ab, Re, or F.

Using the exo' Klenow fragment (Table IV), $F_{ins}$ for dAMP opposite Ab, Re, or F was 7–15 times higher than for dGMP. $F_{ins}$ for dAMP opposite Ab was 1.7 times higher than for dAMP opposite Re, but 24% less than for dAMP opposite F. $F_{ext}$ from 3'-primer termini containing dA:Ab was 3.6 times higher than from termini containing dA:Re and 31% less than from dA:F. Translesional synthesis ($F_{ins} \times F_{ext}$) past dA:Ab was estimated to be 6-fold higher than synthesis past dA:Re and 2-fold lower than past dA:F.

Effect of the 5'-Flanking Base on Base Substitutions and Deletions—Primer extension reactions were conducted using a modified oligodeoxynucleotide (Template B) in which dT is positioned 5' to Ab (Fig. 3). Fully extended products on this template contained dAMP or a 1-base deletion opposite the lesion; 2-base deletions were not detected (Fig. 3 and Table II). Upper and lower bands at position 13 represent incompletely extended products in which dGMP and dAMP have been incorporated opposite the lesion, respectively. The 14-mer and 16-mer bands also represent incompletely extended products.

Densities of bands in Fig. 3 were quantified and compared in Table II with values obtained in experiments with oligodeoxynucleotides in which dC is positioned 5' to Ab (Template A). Using this template, fully extended products containing 2-base deletions were formed (21%). The position of the inserted dAMP and 1- and 2-base deletions were confirmed by Maxam-Gilbert sequence analysis (data not shown).

**Primer Extension Reactions Catalyzed by DNA pol α**—When pol α was used to catalyze primer extension, translesional synthesis past Ab and F produced 2.1 and 5.2%, respectively, of fully extended products containing dAMP opposite the lesion and 0.4 and 1.2%, respectively, of 2-base deletions (Fig. 4A,
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Klenow fragment on templates containing abasic sites

Using an 18-mer template (A, 5′-CCTTXXCTCCT; B, 5′-CCTTXXCTCCT; X = dG or abasic site) primed with a 32P-labeled 10-mer, primer extension reactions were carried out at 25°C using 0.01 units of exo− Klenow fragment for the template containing dG and 0.4 units for that containing abasic sites as described under "Experimental Procedures." dC, dA, Δ1, and Δ2 represent the amount of the fully extended product containing dC, dA, and 1- and 2-base deletions opposite the lesion produced from the starting primer, respectively. The values in parentheses represent the relative ratio of product observed at 15 and 60 min to those at 3 min.

| Lesion | Time | dC | % | dA | % | Δ1 | % | Δ2 | % |
|--------|------|----|---|----|---|----|---|----|---|
| Template A | dG | 3 | 83.4 ± 1.2 | ND | ND | ND | ND | ND | ND |
| Ab | 3 | ND | 21.7 ± 3.1 (1.0) | 0.29 ± 0.04 (1.0) | 2.4 ± 0.6 (1.0) |
| 15 | ND | 37.8 ± 1.4 (1.7) | 0.89 ± 0.04 (3.1) | 10.4 ± 0.6 (4.4) |
| 60 | ND | 48.1 ± 1.4 (2.2) | 1.53 ± 0.15 (5.3) | 21.1 ± 1.0 (9.0) |
| Re | 3 | ND | 6.5 ± 0.4 (1.0) | 0.31 ± 0.06 (1.0) | 6.1 ± 0.4 (1.0) |
| 15 | ND | 15.6 ± 0.1 (2.3) | 0.61 ± 0.01 (2.0) | 32.5 ± 0.9 (3.3) |
| 60 | ND | 17.8 ± 0.1 (2.6) | 1.03 ± 0.19 (3.3) | 49.1 ± 1.1 (8.0) |
| F | 3 | ND | 19.4 ± 1.5 (1.0) | 0.46 ± 0.06 (1.0) | 3.3 ± 0.1 (1.0) |
| 15 | ND | 39.2 ± 0.7 (2.0) | 0.78 ± 0.11 (1.7) | 15.0 ± 3.1 (4.5) |
| 60 | ND | 42.7 ± 1.6 (2.6) | 1.43 ± 0.02 (3.1) | 24.8 ± 0.3 (7.5) |

*ND, not detectable.

In aqueous solution, Ab exists as an equilibrium mixture of 2-deoxyribose (tetrahydrofuran) and an isosteric synthetic analog of deoxyribose have been directly compared using an experimental system (25) that permits us to distinguish and quantify all possible base substitutions and 1- and 2-base deletions in vitro. When the exo− Klenow fragment of DNA pol I was used to catalyze primer extension in the presence of four dNTPs, all three types of abasic sites promoted dAMP incorporation, 2-base deletions, and a small number of 1-base deletions. Templates containing Ab or F predominantly incorporated dAMP opposite the lesion, whereas templates containing Re promoted formation of 2-base deletions.

In aqueous solution, Ab exists as an equilibrium mixture of tautomers consisting primarily of the α- and β-anomers of 2′-deoxyribofuranose, accompanied by a small amount of the

**DISCUSSION**

**Miscoding Properties of Natural and Synthetic Abasic Sites**—In this study, the mutagenic potential of Ab, a reduced form of this lesion (deoxyribitol), and an isosteric synthetic analog of deoxyribose (tetrahydrofuran) have been directly compared using an experimental system (25) that permits us to distinguish and quantify all possible base substitutions and 1- and 2-base deletions in vitro. When the exo− Klenow fragment of DNA pol I was used to catalyze primer extension in the presence of four dNTPs, all three types of abasic sites promoted dAMP incorporation, 2-base deletions, and a small number of 1-base deletions. Templates containing Ab or F predominantly incorporated dAMP opposite the lesion, whereas templates containing Re promoted formation of 2-base deletions.

In aqueous solution, Ab exists as an equilibrium mixture of tautomers consisting primarily of the α- and β-anomers of 2′-deoxyribofuranose, accompanied by a small amount of the
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**Table IV**

Kinetic parameters for nucleotide insertion and chain extension reaction catalyzed by the exo⁻ Klenow fragment of DNA polymerase I. The frequency of base insertion and chain extension was determined as described under "Experimental Procedures." Also, see the legend to Table II.

| N·X | $K_m$ (µM) | $V_{max}$ (nM/min) | $F_{ins}$ | $K_m$ (µM) | $V_{max}$ (nM/min) | $F_{ext}$ | $F_{ins} \times F_{ext}$ |
|-----|------------|-------------------|----------|------------|-------------------|----------|------------------------|
| C·G | 2.25 ± 0.94 | 25.8 ± 0.8        | 1.0      | 5.17 ± 0.26 | 96.1 ± 1.27       | 1.0      | 1.0                    |
| A·G | 67.4 ± 10.7 | 0.62 ± 0.01       | 8.02 × 10⁻⁴ | 19.9 ± 2.0 | 7.67 ± 0.49 × 10⁻² | 2.07 × 10⁻⁴ | 1.66 × 10⁻⁷ |
| A·Ab | 39.6 ± 7.1 | 9.12 ± 0.73       | 2.01 × 10⁻² | 26.4 ± 6.6 | 3.77 ± 0.21 × 10⁻² | 7.68 × 10⁻⁵ | 1.54 × 10⁻⁶ |
| A·Re | 33.3 ± 0.1 | 4.52 ± 0.21       | 1.18 × 10⁻² | 29.4 ± 7.7 | 1.18 ± 0.05 × 10⁻² | 2.15 × 10⁻⁵ | 2.55 × 10⁻⁷ |
| A·F | 35.6 ± 4.9 | 11.1 ± 0.18       | 2.72 × 10⁻² | 21.8 ± 4.9 | 4.72 ± 0.30 × 10⁻² | 1.16 × 10⁻⁴ | 3.16 × 10⁻⁵ |
| G·G | 32.2 ± 2.8 | 1.09 ± 0.02       | 2.95 × 10⁻³ | 20.6 ± 0.8 | 4.98 ± 0.04 × 10⁻¹ | 1.30 × 10⁻³ | 3.84 × 10⁻⁶ |
| G·Ab | 45.7 ± 2.0 | 1.47 ± 0.07       | 2.81 × 10⁻³ | 23.9 ± 1.3 | 5.98 ± 1.05 × 10⁻³ | 1.35 × 10⁻⁵ | 3.79 × 10⁻⁸ |
| G·Re | 38.0 ± 2.8 | 0.35 ± 0.01       | 8.03 × 10⁻⁴ | 27.6 ± 0.1 | 2.53 ± 0.08 × 10⁻² | 4.94 × 10⁻⁵ | 3.97 × 10⁻⁸ |
| G·F | 65.3 ± 2.9 | 1.47 ± 0.04       | 1.96 × 10⁻³ | 25.1 ± 5.0 | 8.62 ± 1.36 × 10⁻³ | 1.85 × 10⁻⁵ | 3.63 × 10⁻⁸ |

Fig. 3. Effect of 5'‐neighboring base on the misincoding frequencies. Using an Ab‐modified template containing dT 5′ to the lesion (5′-CTTTCCTCTCTCTCTC X = Ab), primer extension reactions using 0.4 units of exo⁻ Klenow fragment were conducted at 25 °C as described under “Experimental Procedures.” One-third of the reaction mixture was subjected to 20% polyacrylamide gel electrophoresis (35 × 42 × 0.04 cm). Lane 5 represents standards (Stn.) containing dA opposite the lesion and 1-base or 2-base deletions (Sequences 12–14).

![Fig. 3](https://example.com/fig3.png)

Fig. 4. Nucleotide incorporation opposite lesions in reactions catalyzed by pol α. Using the modified 18-mer templates (Sequences 2–4) primed with a 32P-labeled 12-mer (Sequence 6), primer extension reactions were conducted at 30 °C for 1 h using 1.2 units of pol α (A) and at 30 °C using 2.4 units of pol α (B) as described under “Experimental Procedures.” Lane 4 in A and lane 1 in B represent a mixture of oligodeoxynucleotide standards (Stn.) containing dA and dC opposite the lesion and 1- and 2-base deletions (Sequences 8–10).

![Fig. 4](https://example.com/fig4.png)

The values for these parameters are similar to those found with exo⁺, but the efficiency of translesional synthesis was 2–3 orders of magnitude lower for exo⁺ than for exo⁻. Thus, the 3′ → 5′ exonuclease function of DNA pol I acts to reduce the extent of translational synthesis at abasic sites. Similarly, when polymerase III*, the replicative polymerase of E. coli, was used in a similar primer extension assay, chain extension was blocked 1 base before the abasic site, suggesting that the 3′ → 5′ exonuclease function of this enzyme can efficiently remove a dNMP inserted opposite the lesion.²

The values reported for $F_{ins}$ and $F_{ext}$ with exo⁻ could be potentially affected by the 3′ → 5′ proofreading activity of this enzyme (40). However, differences in the relative efficiency ($V_{max}/K_m$) of exo⁻ for proofreading excision of dAMP and other nucleosides positioned opposite F at the 3′-primer terminus are at most 1.7-fold (41). Thus, although absolute values for $F_{ext}$ may be underestimated, relative values and conclusions drawn from the experiment with exo⁻ remain unchanged.

Under certain conditions, DNA polymerases perform nucleotide addition at the blunt end of duplex DNA (42). The frequency of base addition at this non-templated position was estimated; only dAMP addition was detected. This observation argues that preferential incorporation of dAMP is an intrinsic property of DNA polymerase (16). $F_{ins}$ for the addition reaction was ~2 orders of magnitude lower than comparable values for

² S. Shibutani, M. Takeshita, and A. P. Grollman, unpublished data.
dAMP insertion opposite abasic sites.

Mechanism for Deletions Induced by Abasic Sites—1- and 2-base deletions opposite abasic sites were detected when 5'-CCTCXXC- was used in the template for primer extension. Based on studies with dG-C8-acetylaminofluorene (37) and dG-N2-tetrahydrobenzo[a]pyrene (33), we have proposed a model involving template misalignment to account for such deletions. This model predicts that if extension of a newly inserted dNMP is significantly delayed, the newly inserted dNMP at the 3'-primer terminus will preferentially form a Watson-Crick pair with a template base positioned 5' to the lesion (37).

In experiments with the exo" Klenow fragment, primer extension through the 5'-CCTCXXC sequence was partially blocked opposite the abasic site (Fig. 3). \( F_{ins} \) for nucleotides opposite the lesion followed the order dAMP > dGMP > dCMP and dTMP. Thus, when dGMP is inserted opposite the abasic site, the inserted nucleotide can pair with dC 5' to the lesion to form a 1-base deletion, as shown in Fig. 6A. Alternatively, dAMP and the 5'-flanking dG in the primer could pair with TC 5' to the lesion to form a 2-base deletion. Experimentally, both products were observed. Our kinetic data indicate that Re is a more effective blocking lesion than Ab or F, and as predicted for the deletion-prone sequence used in this study, Re was more effective in promoting 2-base deletions than were ring-closed forms of the abasic site.

When the base 5' to Ab in the template was changed from dC to dT, the frequency of 1-base deletions increased (Table II). As was shown, dAMP is inserted more frequently than dGMP opposite the lesion, and 1-base deletions are generated by preferential pairing between dA and dT 5' to the lesion (Fig. 6B). This result is consistent with the general mechanism for frame-shift deletions proposed to occur in the presence of other blocking lesions (37).

dAMP incorporation increased slightly over time; however, the number of 1- and 2-base deletions increased sharply after 15 min (Table II). This delay may reflect the time required for the template to undergo a conformational change. Thus, as proposed in our model (37), kinetics of misalignment influence the relative number of base substitutions and deletions formed during translesional synthesis.

Miscoding Properties of Abasic Sites in Reactions Catalyzed by pol a—The frequency of nucleotide insertion opposite synthetic abasic sites and of chain extension from the 3'-primer terminus has been established for pol a (26, 27). A nearest neighbor effect was detected on insertion fidelity (26). The present study is the first report using a mammalian DNA polymerase and a natural abasic site in which misincorporation of dNMPs and other events have been determined site-specifically in vitro. pol a promoted incorporation of dAMP opposite Ab and F and generated 1- and 2-base deletions; lesser amounts of fully extended products were produced on templates containing Ab than on those containing F. In contrast, primer extension on an Re-modified template was blocked opposite and 1 base before the lesion. The efficiency of translesional synthesis past Re was lower than that on templates containing F. Thus, the miscoding properties of pol a and exo in vitro with respect to Ab and F are very similar. When pol δ, another mammalian replicative enzyme, was used in an analogous primer extension assay, preferential incorporation of dAMP opposite F also was detected (43).

We have conducted site-specific mutagenesis studies on a number of DNA adducts and lesions in which nucleotide misincorporation, determined by primer extension analysis and steady-state kinetics, was compared in the same sequence context with mutational specificity, determined in plasminoid replicating in bacteria and mammalian cells (44, 45). In each case, the dNMP preferentially incorporated by replicative or endogenous DNA polymerases in vitro was reflected in the mutational spectrum of the lesion as observed in cells. The miscoding properties of the natural abasic site, established in vitro with pol a and pol δ, predict dAMP incorporation at abasic sites in mammalian cells, an event that promotes G → T transversions, predominated in a site-specific mutagenesis study of the tetrahydrofuran moiety in simian kidney (COS) cells (21), but not in a similar study of natural abasic sites (20). It is conceivable that the intrinsic structural difference between Ab and F manifests itself in simian kidney cells, but not in E. coli; however, this interpretation is not supported by the in vitro studies reported here. The apparent discrepancy should be tested by side-by-side comparison of the miscoding potential of the two lesions in mammalian cells.

Conclusions—We conclude from these experiments that the miscoding properties of natural abasic sites are similar, if not identical, to those of the tetrahydrofuran analog. Analogs of the ring-opened form of the natural abasic site (deoxyribitol) tend to block translesional synthesis and to promote deletions, whereas predominantly cyclic structures (Ab and F) promote synthesis past the lesion. The preferred order of nucleotide insertion (dAMP > dGMP > dCTP > dTMP) is similar for both DNA polymerases studied. dAMP, positioned opposite Ab or F at the 3'-primer terminus, is more readily extended than other nucleotides. These kinetic studies are consistent with our observation that dAMP is preferentially incorporated into DNA
by the Klenow fragment of pol I and by pol α. Blunt end addition of dAMP, observed in primer extension assays catalyzed by the exo⁺ Klenow fragment, supports the idea that the A rule reflects an intrinsic property of this and possibly other DNA polymerases.

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