Mechanism of Frameshift (Deletion) Generated by Acetylaminofluorene-Derived DNA Adducts in Vitro†

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ABSTRACT: We have investigated the mechanism of frameshift (deletion) mutagenesis induced by acetylaminofluorene- (AAF-) derived DNA adducts. dG-AAF-modified oligodeoxynucleotides, with different bases positioned 5′ to the lesion, were annealed to ^{32}P-labeled 13-mer primers and then used in primer extension reactions catalyzed by the 3′→5′ exonuclease-free Klenow fragment of *Escherichia coli* DNA polymerase I. When the dNMP positioned opposite dG-AAF could pair with its complementary base at the 5′ flanking position, single-base deletions were produced at high frequency. Similarly, when the complementary base was two positions 5′ to the dG-AAF, two-base deletions occurred. The relative frequency of base insertions opposite dG-AAF followed the order dCMP > dAMP > dGMP > dTMP; the frequency of dNTP insertion opposite the lesion paralleled the formation of frameshift deletions. When a template designed to induce three-base deletions was used for translesion synthesis catalyzed by the exo$^-$ Klenow fragment, the expected three-base deletion was formed. When dG-AAF-modified templates containing iterated bases 5′ to the lesion were annealed to primers with the complementary dNMP positioned opposite the lesion, the dNMP inserted opposite the dG-AAF tended to pair with the complementary base 5′ to the lesion, thereby forming shorter deletions. Taken together, these results support the molecular mechanism for frameshift deletion proposed earlier by Shibutani and Grollman in which direct base insertion precedes misalignment [(1993) *J. Biol. Chem. 268*, 11703].

So-called spontaneous frameshift deletions are formed during DNA replication, presumably induced by endogenous or exogenous DNA damage (reviewed in ref *1*). The frequency of such deletions may be influenced by several factors, including DNA sequence context (*2*−*8*), the editing function of DNA polymerase (*9*, *10*), and imbalances in the dNTP[1] pool (*11*).

Fresco and Alberts (*12*) first suggested that loops forming in duplex DNA might lead to the formation of deletions. Later, Streisinger et al. (*7*, *8*) introduced the mechanism of slipped mispairing to account for the increased frequency of frameshifts detected in iterated nucleotide sequences. Fowler et al. (*13*) proposed a mechanism for base substitutions involving transient misalignment of the template-primer complex. Kunkel and co-workers described a "dislocation mutagenesis" model, invoking transient misalignment followed by realignment to explain the prevalence of base substitutions in certain sequence contexts (*14*, *15*). This group showed that frameshift errors induced by base mismatches could be initiated by nucleotide misinsertion (*16*). In earlier studies with the 3′→5′ exonuclease-free (exo$^-$) Klenow fragment of *Escherichia coli* DNA polymerase I (pol I) (*17*), we also demonstrated the influence of DNA sequence context on frameshift mutagenesis by modifying systematically the bases flanking *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF). The ability to generate one-base and two-base deletions was shown to depend on three factors: (a) the nature of the base inserted opposite the adduct, (b) the sequence context of the adduct, and (c) the overall rate of translesion DNA synthesis past the adduct. A similar mechanism was also proposed from 4-aminobiphenyl- and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole-induced mutation spectra in *Salmonella* (*18*, *19*).

To further test the foregoing model for frameshift mutagenesis, the frequency of deletions formed by the exo$^-$ Klenow fragment was determined using templates containing differing 5′-neighboring sequence contexts in which the lesion was positioned opposite dNMP at the 3′-primer terminus. A high frequency of deletions was only obtained under conditions where the dNMP opposite the lesion could pair with a complementary base 5′ to the lesion. When iterated complementary bases were at the 5′-flanking position, one-base deletions predominated. These results support our concept of the molecular events driving frameshift deletions (*17*).

EXPERIMENTAL PROCEDURES

Materials. Organic chemicals used for the synthesis of oligodeoxynucleotides were supplied by Aldrich Chemical

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[1] Abbreviations: dNTP, 2′-deoxynucleoside triphosphate; dG-AAF, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene; *N*-acetoxy-AAF, *N*-acetoxy-2-acetylaminofluorene; dG, 2′-deoxyguanosine; pol I, DNA polymerase I; exo, 3′→5′ exonuclease; F_{ins}, frequency of nucleotide insertion; PAGE, polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; RT, retention time.

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Table 1: Sequence of Oligodeoxynucleotides[a]

no.	sequence
1	5′CCTTNG^{AAF}CTACTTTCCTCT3′
2	5′CCTNC̄G^{AAF}CTACTTTCCTCT3′
3	5′GCTĀCTTTCCTCT3′
4	5′G^{AAF}CTACTTTCCTCT3′
5	5′AGAGGAAAGTAGN3′
6	5′AGAGGAAAGTAG3′
7	5′AGAGGAAAGTAGNAAGG3′
8	5′AGAGGAAAGTAGN̄AGG3′
9	5′CCGCAG^{AAF}CACCTTTCCTCT3′
10	5′AGAGGAAAGG3′
11	5′AGAGGAAAGGTGN3′
12	5′AGAGGAAAGGTGC̄GG3′

[a] Sequence of templates, primers, and standard markers. N = C, A, G, or T.

Co. [γ-^{32}P]ATP (specific activity >6000 Ci/mmol) was obtained from Amersham Corp. dNTPs were from Pharmacia. Cloned exo$^+$ (17400 units/mg) and exo$^-$ (21200 units/mg) Klenow fragments of *E. coli* DNA pol I were purchased from U.S. Biochemical Corp.; venom phosphodiesterase I and T4 polynucleotide kinase were obtained from Sigma Chemicals and Stratagene, respectively.

Synthesis and Purification of Oligodeoxynucleotides. Unmodified oligodeoxynucleotides were synthesized by solid-state methods using an automated DNA synthesizer (*20*). Oligomer templates (sequences 1 and 2 in Table 1) containing a single dG-AAF were prepared postsynthetically by reacting an unmodified oligomer with *N*-acetoxy-AAF as described previously (*17*). Modified and unmodified oligomers were purified by HPLC followed by gel electrophoresis on a 20% polyacrylamide gel in the presence of 7 M urea (*21*). A Waters 990 HPLC instrument, equipped with a photodiode array detector, was used to separate and analyze modified and unmodified oligodeoxynucleotides.

Primer Extension Reactions. An 18-mer template (1.0 pmol) containing a single dG-AAF adduct was incubated with 0.5 pmol of ^{32}P-labeled 13N-mer (5′-AGAGGAAAGTAGN, N = C, A, G, or T) at 50 °C for 3 min. To anneal the primer to the template, the reaction mixture was kept at room temperature for 30 min and then at 4 °C overnight. Using the dG-AAF-modified template primed with a ^{32}P-labeled 13N-mer, primer extension reactions were conducted in a buffered solution (10 μL) containing DNA polymerase and all four dNTPs (100 μM each). The Klenow fragment of *E. coli* DNA pol I with or without 3′→5′ exonuclease activity was incubated at 30 °C for 1 h in a solution containing 50 mM Tris-HCl, pH 8.0, 8 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. Reaction mixtures were heated at 95 °C for 3 min in the presence of formamide and subjected to 20% PAGE (35 × 42 × 0.04 cm or 15 × 72 × 0.04 cm) in the presence of 7 M urea. Following gel electrophoresis, positions of the oligomers were established by autoradiography using Kodak X-Omat XAR film. Radioactivity was measured in a Packard scintillation counter using Liquiscint (National Diagnostics). The detection limit for reaction products was 0.03% of the starting primer.

Nucleotide Insertion opposite DNA Adducts. Kinetic parameters associated with nucleotide insertion opposite dG-AAF were as described previously (*17*). Reaction mixtures containing exo$^-$ Klenow fragment (0.0005−0.05 unit) and dNTPs (0−500 μM) were incubated at 30 °C for 1.5−5 min in 10 μL of Tris-HCl buffer (pH 8.0) using 13-mer template (150 fmol; 5′G^{AAF}CTACTTTCCTCT; sequence 3 or 4 in Table 1) primed with a ^{32}P-labeled 12-mer (100 fmol; 5′AGAGGAAAGTAG; sequence 6). Samples were heated at 95 °C for 3 min in the presence of formamide and then subjected to 20% denaturating PAGE (35 × 42 × 0.04 cm). The Michaelis constants (K_m) and maximum rates of reaction (V_{max}) were obtained from Hanes−Woolf plots. Frequencies of dNTP insertion (F_{ins}) were determined relative to the dC·dG base pair according to the equation $F = (V_{max}/K_m)_{[wrong\text{-}pair]}/(V_{max}/K_m)_{[correct\ pair=dC\cdot dG]}$ (*22*, *23*).

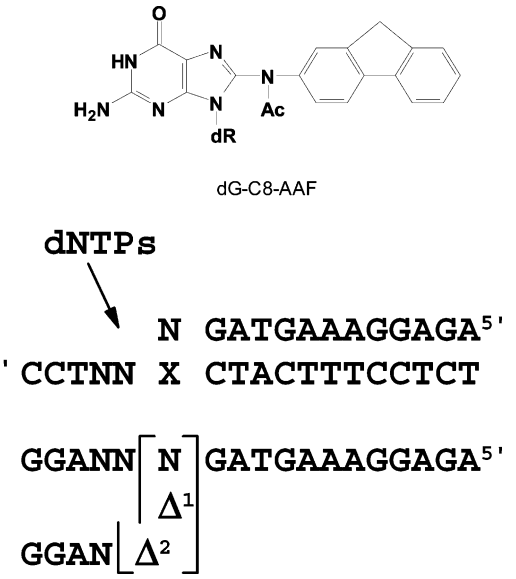

FIGURE 1: Schematic diagram of the primer extension method. N represents dC, dA, dG, or dT. X represents dG or dG-AAF.

RESULTS

Effect of the 5′-Neighboring Base on the Frequency of Deletions. Primer extension reactions catalyzed by the exo$^-$ Klenow fragment (Figure 1), using ^{32}P-labeled 13N-mers with dC, dA, dG, or dT positioned opposite dG-AAF and reaction mixtures containing all four dNTPs, were conducted on templates with different bases 5′ to the adducted base (Figure 2A). Standards, representing fully extended product with a one-base deletion, appear in lanes 5, 10, 15, and 20, respectively. When a 13-mer primer with dC (13C-mer) was used, primer extension on all dG-AAF-modified templates was retarded. When dG was the 5′-flanking base (lane 3 in Figure 2A), 57.2% one-base deletions (Δ^1) were observed. Using a 13A-mer primer, 69.7% Δ^1 were formed when dT (lane 9) was 5′ to the lesion. Using a 13G-mer or 13T-mer primer, 62.5% and 87.8% Δ^1 were detected when dC (lane 11) and dA (lane 17), respectively, constituted the 5′-flanking base. Thus, under conditions where the dNMP positioned opposite the dG-AAF lesion can pair with its complementary base 5′ to the lesion, single-base deletions are produced at high frequency (Figure 2B).

The base located two positions 5′ to the dG-AAF was varied systematically to establish the frequency of two-base deletions (Figure 3A). Fully extended products containing two base deletions are shown in lanes 5, 10, 15, and 20. Using a 13C-mer primer, 85.5% two-base deletions (Δ^2) were detected only when dG (lane 3) was two bases 5′ to the lesion. When 13A- and 13T-mer primers were used, 82.0%

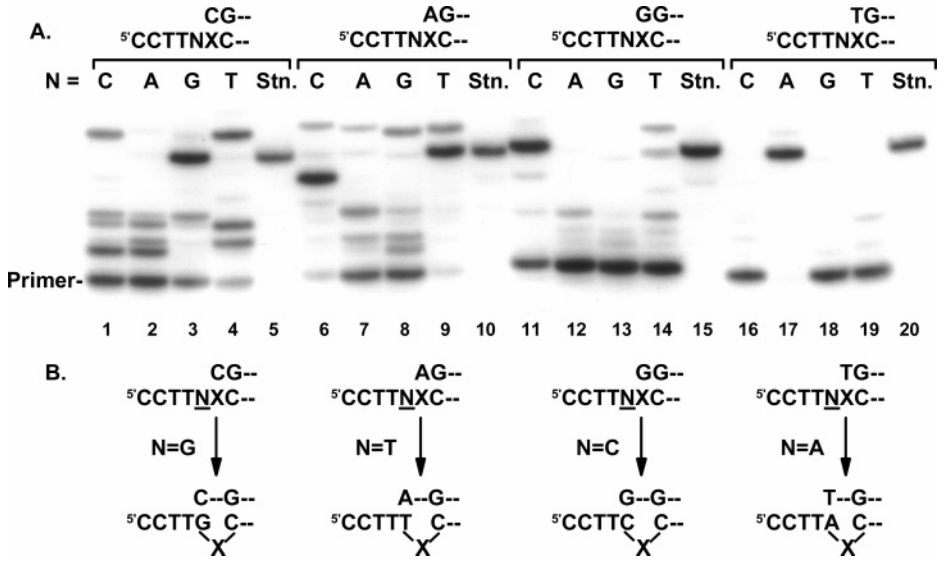

FIGURE 2: Effect of the 5′-flanking base on the frequency of one-base deletions opposite dG-AAF. (A) The 18-mer templates containing a single dG-AAF (5′CCTTNXCTACTTTCCTCT, X = dG-AAF, N = C, A, G, or T) were prepared as described by Shibutani et al. (*17*). Using dG-AAF-modified 18-mer primed with ^{32}P-labeled 13-mer with dC (lanes 1−4), dA (lanes 6−9), dG (lanes 11−14), or dT (lanes 16−19), primer extension reactions were carried out at 30 °C for 1 h in a solution containing 1.0 unit of exo$^-$ Klenow fragment and four dNTPs (100 μM each) as described under Experimental Procedures. One-third of the reaction mixture was subjected to 20% polyacrylamide gel electrophoresis. Mobilities of reaction products were compared with a synthetic 17-mer containing a single deletion (lanes 5, 10, 15, and 20, sequence 7 in Table 1). (B) Proposed mechanism for single-base deletions.

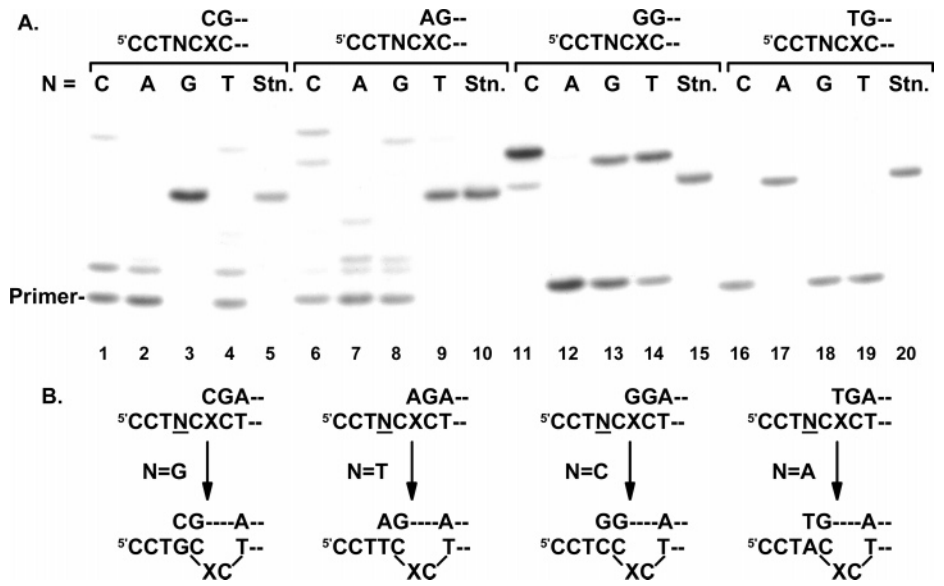

FIGURE 3: Effect of 5′-neighboring bases on the frequency of two-base deletions. (A) Using the dG-AAF-modified 18-mer template (5′CCTNCXCTACTTTCCTCT, X = dG-AAF, N = C, A, G, or T) primed with ^{32}P-labeled 13-mer with dC (lanes 1−4), dA (lanes 6−9), dG (lanes 11−14), or dT (lanes 16−19), primer extension reactions were conducted as described in the legend to Figure 2A. Mobilities of the reaction products were compared with a synthetic 16-mer containing a two-base deletion (lanes 5, 10, 15, and 20, sequence 8 in Table 1). (B) Proposed mechanism for two-base deletions.

and 90.5% Δ^2, respectively, were observed only when dT (lane 9) and dA (lane 17) were similarly positioned. Thus, two-base deletions also are formed at high frequency when the 5′-flanking base can pair with the complementary base positioned opposite dG-AAF (Figure 3B).

Using a 13G-mer primer, 15% Δ^2 were formed; however, large amounts of Δ^1 (80%) were observed when dC was two bases 5′ to the lesion (lane 11). Thus, it appears that a fraction of dGMP inserted opposite dG-AAF can pair with dC 5′ to the lesion, resulting in single-base deletions (Figure 5IA). The remaining fraction of dGMP inserted opposite the lesion can pair, together with its 5′-flanking dG, with dC 5′ to the lesion, forming two-base deletions (Figure 5IB). In this case, the amount of Δ^2 was 5-fold lower than that of Δ^1; shorter deletions predominated.

Single-base deletions also were formed when dA (lane 12), dG (lane 13), and dT (lane 14) were two bases 5′ to the lesion. With this construct, the flanking base, dC, paired with the dGMP positioned opposite the lesion to form single-base deletions.

Frequencies of dNTP Insertion opposite dG-AAF. Primers containing dNMP positioned opposite dG-AAF were used to explore the mechanism of one- and two-base deletions (Figures 2 and 3). Steady-state kinetic studies were performed to establish the frequency of dNTP insertion (F_{ins}) under conditions where dG-AAF-modified 13-mer templates were

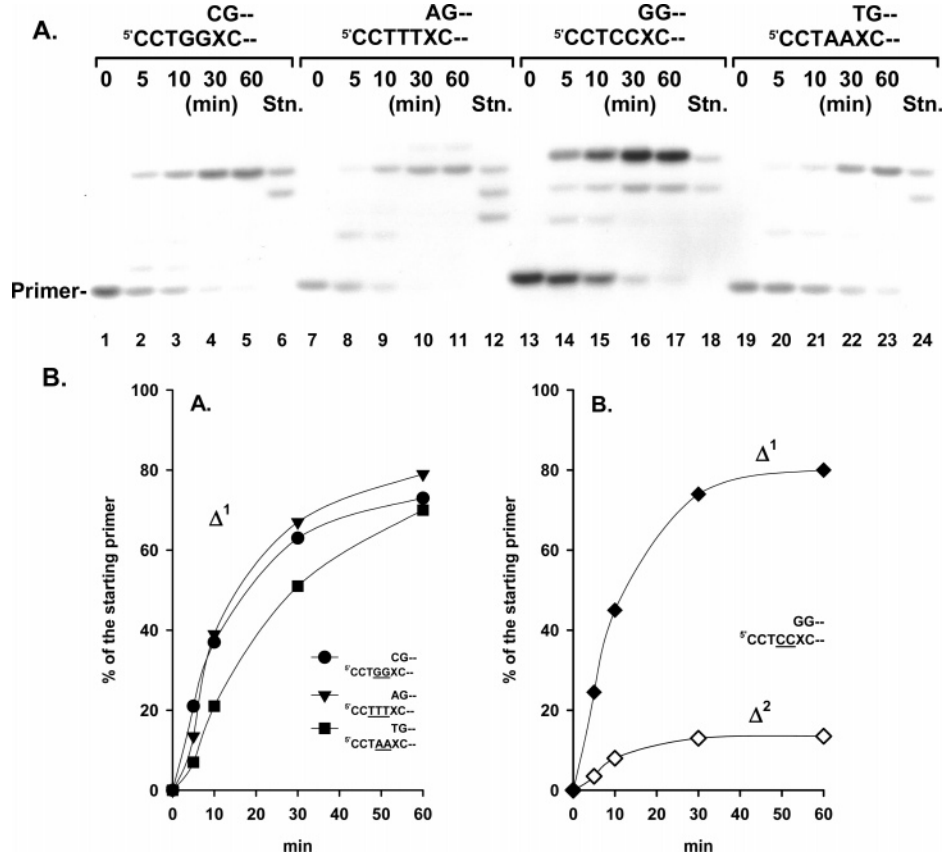

FIGURE 4: Time course of deletions on the template containing an iterated sequence 5′ to dG-AAF. (A) Using dG-AAF-modified 18-mer templates containing an iterated sequence 5′ to the lesion [5′CCTGGXCTACTTTCCTCT primed with a 13C-mer (lanes 1−5), 5′CCTTTXCTACTTTCCTCT primed with a 13A-mer (lanes 7−11), 5′CCTCCXCTACTTTCCTCT primed with a 13G-mer (lanes 13−17), or 5′CCTAAXCTACTTTCCTCT primed with a 13T-mer (lanes 19−23), X = dG-AAF], primer extension reactions were carried out as described in the legend to Figure 2A. Mobilities of the reaction products were compared with a synthetic 17-mer containing a one-base deletion, a 16-mer containing a two-base deletion, and/or a 15-mer containing a three-base deletion (lanes 6, 12, 18, and 24). (B) Formation of one- and two-base deletions on the template containing an iterated sequence 5′ to dG-AAF.

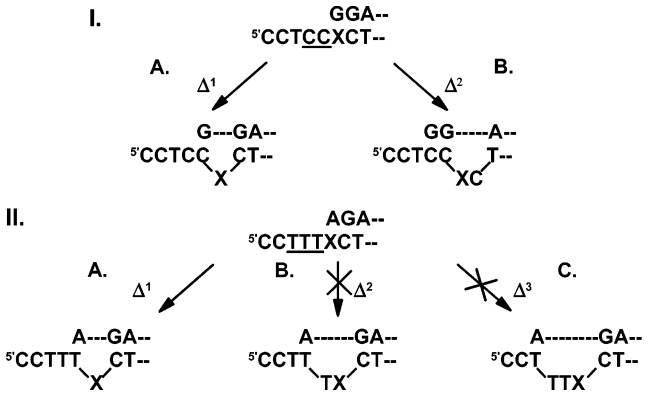

FIGURE 5: Possible mechanism of deletions on the template containing an iterated sequence 5′ to the dG-AAF.

primed with a ^{32}P-labeled 12-mer. To avoid the influence of the 5′-neighboring sequence context on frequencies of dNTP insertion, a template adducted at the 5′-terminus was used for these experiments (Table 2). The value for F_{ins} (7.9×10^{-3}) of dCTP, the correct base, opposite dG-AAF was 14, 340, or 5300 times lower than that for dATP, dGTP, or dTTP, respectively. These results are consistent with kinetic data reported previously (*17*). The frequencies of one-base and two-base deletions observed during translesion synthesis catalyzed by the exo$^-$ Klenow fragment (*17*) parallel the frequency of dNTP incorporation opposite dG-AAF. Thus, the frequency of dNTP insertion opposite the lesion appears to be a limiting factor in generating frameshift deletions in vitro.

Table 2: Frequency of Nucleotide Insertion Catalyzed by Exo$^-$ Klenow Fragment[a]

dNTP
↓GATGAAAGGAGA^{32P}
5′XCTACTTTCCTCTCCCTTT

	K_m (μM)	V_{max} (% min^{-1})	F_{ins}
N·X, X = dG			
C·G	2.8 ± 0.12[b]	43 ± 3.4[b]	1.0
N·X, X = dG-AAF			
C·X	122 ± 6.9	14.8 ± 1.35	7.9×10^{-3}
A·X	69 ± 8.1	0.61 ± 0.07	5.7×10^{-4}
G·X	24 ± 0.27	$(8.3 \pm 0.03) \times 10^{-3}$	2.3×10^{-5}
T·X	66 ± 2.9	$(1.5 \pm 0.37) \times 10^{-3}$	1.5×10^{-6}

[a] Kinetics of nucleotide insertion were determined as described in Experimental Procedures. Frequencies of nucleotide insertion (F_{ins}) were estimated by the equation $F = (V_{max}/K_m)_{[wrong\ pair]}/(V_{max}/K_m)_{[correct\ pair=dC\cdot dG]}$. X = dG or dG-AAF lesion. [b] Data expressed as mean ± SD were obtained from three independent experiments.

Frequency of Deletions on Templates Containing Iterated Sequences 5′ to the Lesion. The frequency of frameshift deletions was measured using templates containing an iterated sequence 5′ to dG-AAF. When the sequence 5′ to the adduct was dGG, dTTT, or dAA, only single-base deletions were detected (Figure 4A). The number of one-base deletions increased during the course of the reaction

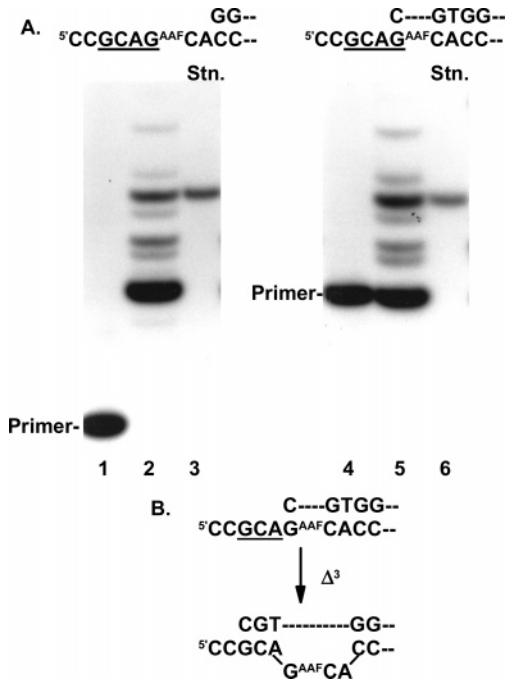

FIGURE 6: Three-base deletions induced by dG-AAF. (A) The dG-AAF-modified 18-mer template (5′CCGCAXCACCTTTCCTCT, X = dG-AAF) was primed with either ^{32}P-labeled 10-mer (sequence 10 in Table 1) or 13-mer with dC opposite the lesion (sequence 11). Primer extension reactions were carried out at 25 °C for 1 h using exo$^-$ Klenow fragment as described in the legend to Figure 2A. The mobility of the reaction product was compared with the corresponding standard 15-mer containing a three-base deletion (sequence 12 in lanes 3 and 6). (B) Proposed mechanism for three-base deletion.

with 70−75% one-base deletions observed at 60 min (Figure 4B). Using a template containing dCC 5′ to the lesion, 81% single-base and 14% two-base deletions were formed (Figure 4A); again, the fraction of single-base and two-base deletions increased over time.

Formation of Three-Base Deletions. The 18-mer templates, 5′CCGCAG^{AAF}CA-, were designed to induce three-base deletions (Δ^3), as shown in Figure 6B. Using a 5′CCGCA-G^{AAF}CA- template primed with a ^{32}P-labeled 13-mer containing dC opposite the lesion, 19% three-base deletions (Δ^3) were produced (lane 5 in Figure 6A). Using a ^{32}P-labeled 10-mer primer, 11% Δ^3 were formed (lane 2).

DISCUSSION

In vitro experimental systems have been useful in exploring molecular mechanisms by which frameshift deletions and base substitutions are formed during DNA synthesis (*17*, *24*). In the present study, primer extension reactions catalyzed by exo$^-$ and exo$^+$ Klenow fragments of DNA pol I were conducted on oligodeoxynucleotide templates modified site-specifically with dG-AAF, and we determined the influence of DNA sequence context on the formation of frameshift deletions by modifying systematically the base 5′ flanking to the lesion. Single-base deletions were obtained only under conditions where the dNMP inserted opposite dG-AAF could pair with the complementary base positioned 5′ to the lesion (Figure 2). The number of single-base deletions increased linearly during the enzymatic reaction, with the rate being similar for all base pairs tested in this system (data not shown). Two-base deletions formed in a similar manner (Figure 3). The base sequence 5′ to the dNMP opposite dG-AAF also contributed to the propensity to form two-base deletions when the 5′-flanking base was complementary to the base 5′ to the adduct in the template strand. These experiments reveal that deletions form readily when the base at the 3′-primer terminus, positioned opposite a lesion, can pair with base(s) 5′ to the lesion in the template strand. Thus, formation of frameshift deletions is influenced by the sequence context of the lesion as related to the dNTP inserted opposite the lesion.

The frequency of dNTP insertion opposite dG-AAF followed the order dCMP > dAMP > dGMP > dTMP (Table 2). This result is consistent with data obtained from earlier kinetic studies (*17*), Therefore, as dCMP inserted opposite dG-AAF is expected to form deletions when dG, its complementary base, is 5′ to the lesion, the greatest number of one-base deletions was observed when dG was 5′ to dG-AAF, as observed previously (*17*). As predicted by the kinetic study, dAMP incorporation opposite the lesion promoted the second largest amount of single-base deletions when dT was the 5′-flanking base (*17*). A similar mechanism appears to be involved in forming two-base deletions, as the frequencies of one- and two-base deletions parallel the frequency of dNTP incorporation opposite dG-AAF (*17*). Thus, the insertion frequency of dNTP opposite the lesion may be a limiting factor in generating frameshift deletions in vitro. This frequency, in turn, depends on the DNA polymerase(s) involved in translesion synthesis (*17*, *25*).

Kunkel and co-workers (*14*, *26*) showed that frameshift deletions were induced by base mismatches and proposed two alternative pathways: namely, misinsertion preceding misalignment and misalignment preceding misinsertion. The results of our studies with a single DNA adduct strongly support the former mechanism.

Slipped mispairing has been proposed to account for frameshifts observed in iterated nucleotide sequences (*7*, *8*). We designed templates containing an iterated nucleotide sequence 5′ to the dG-AAF lesion to explore preferential formation of frameshift deletions. When the TTT sequence was 5′ to dG-AAF, deletions could form in several ways; namely, dAMP located at the 3′-primer terminus could pair with the 5′-flanking dT to form a one-base deletion (Figure 5IIA), with dT positioned two positions 5′ to the lesion to form a two-base deletion (5IIB), or with dT positioned three positions 5′ to the lesion to form a three-base deletion (5IIC). As only one-base deletions were detected (Figure 4A), we conclude that the first pathway operated most efficiently under these conditions. When a shorter primer (10-mer) was previously used to initiate primer extension two bases prior to the lesion, only a one-base deletion was formed (*17*). This result is consistent with data obtained in this work using a 13-mer with dA. Similar results were observed when GG or AA was 5′ to dG-AAF (Figure 4A). Thus, when multiple opportunities for complementary base pairing are presented during a primer extension reaction, formation of the shorter deletion predominates.

When a template containing CC 5′ to the lesion was used in the reaction, both one- and two-base deletions were observed (Figure 4A). When dGMP at the 3′-primer terminus paired with dC 5′ to dG-AAF, a one-base deletion was produced (Figure 5IA). Alternatively, when GG at the 3′-primer terminus paired with CC 5′ to the lesion, two-base

deletions were formed (Figure 5IB). The amount of one-base deletions obtained was 5.8 times higher than that of two-base deletions. Thus, shorter deletions predominate when an iterated nucleotide sequence is present 5′ to the lesion in the template strand.

When the exo$^+$ Klenow fragment was used to catalyze primer extension past dG-AAF, a small number of deletions are formed (data not shown). Since the dNMP positioned opposite the lesion cannot pair tightly with the bulky dG-AAF lesion, the 3′→5′ exonuclease function of the enzyme can excise NMP from the 3′-primer terminus, limiting the number of deletions formed.

DNA pol α, a replicative enzyme in mammalian cells, promotes deletions when catalyzing DNA synthesis on site-specifically modified templates containing an apurinic/apyridiminic site (*27*) or 3,N^4-etheno-dC (*24*). DNA pol *κ* also produces one- and two-base deletions during DNA synthesis past dG-AAF (*25*). The formation of deletions induced by pol α and pol *κ* is consistent with the mechanism of deletions proposed on the basis of results with the *E. coli* enzyme (*17*). Thus, similar mechanisms for frameshift deletions may apply to mammalian and bacterial cells (*18*, *19*).

Deletions of three or more bases in the *p53* gene have been observed in human cancers (*28*). To test our model in the prediction of long-patch deletions, we used a template designed to produce three-base deletions for translesion synthesis catalyzed by exo$^-$ Klenow fragment. As predicted, three-base deletions were formed with the use of a 13-mer primer containing dCMP positioned opposite dG-AAF and a 10-mer primer. When a dG-N^2-tamoxifen-modified template (5′CATGCTGATGAATT*CCTTC*XCTTCTTTCCTCT-CCCTTT, where X is dG-N^2-tamoxifen lesion) primed with a 10-mer (5′AGAGGAAAGA) was used in a similar reaction, large amounts of five-base deletions were formed during translesion synthesis catalyzed by mammalian pol *β* (*29*). dGMP was preferentially inserted opposite the lesion and, together with the 5′-flanking sequence GAAG, paired with CCTTC 5′ to the lesion, generating a five-base deletion. A similar phenomenon was observed with pol α; however, the frequency of five-base deletions was considerably lower than that observed with pol *β* (*29*). Thus, it appears that formation of long- as well as short-patch deletions in vitro is predicted by our model.

In the mechanism proposed here, preferential base pairing 5′ to a lesion generates a bulged intermediate in the polymerase active site. Such bulges can accommodate damaged base inside or outside the helix. Crystallographic studies of ternary complexes of DNA polymerases bound to site-specifically modified duplex oligodeoxynucleotides may reveal structural details of this interaction and are central to our understanding of frameshift mutagenesis.

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