Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9583–9587, November 1991
Biochemistry

Acetylaminofluorene and aminofluorene adducts inhibit *in vitro* transcription of a *Xenopus* 5S RNA gene only when located on the coding strand

(RNA polymerase III/DNA adduct/carcinogen)

YEN-HUI CHEN, YOSHIHIRO MATSUMOTO, SHINYA SHIBUTANI, AND DANIEL F. BOGENHAGEN

Department of Pharmacological Sciences, State University of New York, Stony Brook, NY 11794

Communicated by Philip C. Hanawalt, August 5, 1991 (received for review March 26, 1991)

ABSTRACT **Unique *N*-acetyl-2-aminofluorene (AAF) or 2-aminofluorene (AF) adducts were introduced into the *Xenopus borealis* somatic 5S RNA gene between the intragenic control region and the transcription termination site. The effects of these bulky adducts on transcription were studied in a cell-free extract derived from *Xenopus laevis* oocytes. AAF and AF adducts inhibit transcription only when they are on the template strand, whereas transcription passes through these adducts when they are placed on the nontemplate strand. In the presence of the AAF or AF adduct on the template strand, transcription usually terminates one nucleotide before the altered guanine residue. Premature termination at these bulky adducts does not block reinitiation of transcription, since several transcripts are produced per gene per hour on these damaged templates.**

N-Acetyl-2-aminofluorene (AAF) and 2-aminofluorene (AF) are potent carcinogens differing only by an acetyl group. The activated forms of AAF and AF bind covalently to guanine residues to create bulky adducts (1). The predominant adducts are produced through modification at the C-8 position of guanine residues (2). AAF and AF adducts are capable of inducing base substitution and frameshift mutations (3–5). Mutations most likely occur through the action of a replicative or repair DNA polymerase. When a DNA polymerase encounters an AF or AAF adduct in the DNA template, it may either pause for a prolonged period or bypass the lesion. It is likely that this bypass or translesion synthesis frequently leads to mutation. The overall mutation rate is influenced by the efficiency of repair and by the local sequence context (6, 7). In addition, Armier *et al.* (8) have shown that AAF adducts can inhibit DNA replication by blocking the progression of the replication fork. *In vitro* DNA synthesis by *Escherichia coli* and phage DNA polymerases can be blocked by AAF lesions (9–11). AF adducts, which are thought to produce less distortion in the conformation of DNA than AAF adducts (12), are less effective in blocking DNA synthesis (13).

In recent years, studies of the repair of AAF adducts have become increasingly sophisticated. Tang *et al.* (14) have shown that the *E. coli* uvrABC excinuclease is able to recognize and incise AAF adducts *in vitro*. AAF adducts are repaired following microinjection into *Xenopus* eggs (15) and in human cell extracts (16).

In contrast to these studies of aminofluorene mutagenesis and repair, little attention has been paid to the effects of AAF and AF adducts on transcription. AAF adducts have been shown to inhibit the synthesis of the 45S rRNA precursor by eukaryotic RNA polymerase I (17) and to interfere with transcription of T7 DNA (18). However, none of these studies has examined the effects of specifically localized adducts.

We have used a 5S RNA gene as a model transcription system in which to study the effects of precisely positioned adducts. We constructed a series of recombinant 5S RNA genes that contained AAF or AF adducts located on either strand of the 5S RNA gene between the promoter and termination sequences required for transcription by RNA polymerase III. AF and AAF blocked transcription only when located on the coding strand.

MATERIALS AND METHODS

Materials. *N*-Acetoxy-2-acetylaminofluorene (*N*-acetoxy-AAF) was synthesized as described (19) and supplied by R. Gentles (SUNY, Stony Brook, NY). Oligonucleotides were synthesized as reported (20). The pBS+ and pBS− phagemids and helper phage VCS-M13 were purchased from Stratagene.

Preparation of AAF- or AF-Modified Oligonucleotides. A 15-mer containing a single G residue, 5′-CCTTCTAGAATTCCC-3′, was allowed to react with *N*-acetoxy-AAF (21). The resulting oligomer modified by AAF at the C-8 position of the G residue was purified by HPLC using a reverse-phase μBondapak C_{18} column, with elution by a linear gradient of 10–14% acetonitrile in 0.05 M triethylamine for 60 min (22). To prepare the AF-modified oligonucleotide, the oligomer containing the AAF adduct was further treated with a 1.0 M NaOH solution containing 0.25 M 2-mercaptoethanol and repurified by HPLC as described above.

Construction of 5S RNA Genes Containing a Single AAF or AF Adduct. A 5S RNA maxigene was modified and recloned to allow insertion of oligonucleotides bearing adducts. This maxigene was derived from the *Xenopus borealis* somatic-type 5S RNA gene by joining Δ3′+115 and Δ5′+105 (23) through the *Bam*HI sites at the deletion end points. This maxigene was inserted between the *Hin*dIII and *Eco*RI sites of either pBS+ or pBS− (Stratagene) along with a wild-type *X. borealis* somatic 5S RNA gene (Xbs Δ5′-48; ref. 24) as an internal control for subsequent transcription experiments. A 26-base-pair (bp) DNA fragment containing *Stu* I and *Xho* I sites separated by 15 bases was inserted into the *Bam*HI site in the maxigene (see Fig. 1). Two versions with different orientations of the insert were selected in pBS+ and in pBS− (see Fig. 1). The phagemid vectors pBS+ and pBS− were used to produce large quantities of single-stranded DNA following superinfection with helper phage (25) to allow production of specific single-stranded DNAs containing either the nontemplate strand (in pBS+) or the template strand (in pBS−) of the 5S RNA gene. The second stage of template

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AAF, *N*-acetyl-2-aminofluorene; AF, 2-aminofluorene; *N*-acetoxy-AAF, *N*-acetoxy-2-acetylaminofluorene; cccDNA, covalently closed circular DNA; ICR, internal control region.

construction was performed *in vitro*, since DNA containing adducts obviously could not be propagated in bacteria. A covalently closed circular DNA (cccDNA) containing a unique AAF or AF adduct was prepared *in vitro* essentially as described by Matsumoto and Bogenhagen (26). One hundred micrograms of duplex linear DNA resulting from digestion with *Xho* I and *Stu* I was denatured and annealed with 100 μg of the specific single-stranded DNA to form a gapped duplex. The AAF- or AF-modified 15-mer complementary to the *Stu* I/*Xho* I sequence of the gapped duplex was ligated into the gap. The resulting cccDNA was purified by equilibrium centrifugation in CsCl and ethidium bromide. Approximately 10 μg of the modified cccDNA was recovered in an average preparation.

DNA Repair Assay. The purity of the adduct-bearing plasmid DNA and its susceptibility to repair during incubation in the *Xenopus* oocyte extract were examined by digestion with *Eco*RI; this was followed by gel electrophoresis and blot hybridization. The G residue bearing the adducts is part of an *Eco*RI restriction site and its presence blocks digestion by *Eco*RI. To determine whether DNA repair occurred, adduct-bearing DNAs were incubated in the extract under the same conditions used for transcription assays. After incubation with or without the oocyte extract, the plasmid DNA was digested with *Hin*dIII and *Eco*RI. The restriction fragments were electrophoresed and detected by Southern hybridization using ^{32}P-labeled RNA probes as follows. A truncated 5S RNA gene, Xbs Δ3′+97, was inserted in the polylinker region of vector pT7/T3-19 (BRL). A ^{32}P-labeled strand-specific 5S RNA probe ("T", see Fig. 2*C*) was prepared using T7 RNA polymerase to hybridize to the template strand of restriction fragments; the RNA probe "NT" was prepared using T3 RNA polymerase to detect the nontemplate strand of restriction fragments.

***In Vitro* Transcription.** *In vitro* transcription was performed in *Xenopus laevis* oocyte S150 extracts (27, 28) as described by McConkey and Bogenhagen (29). Ten nanograms of template-containing plasmid DNA and 50 ng of nonspecific plasmid DNA were incubated with the S150 extract in 20-μl reaction mixtures including 15 mM Tris (pH 7.9), 15 mM Hepes (pH 7.4), 60 mM KCl, 7 mM $MgCl_2$, 2 mM dithiothreitol, 5 mM β-glycerol phosphate, 2 mM ATP, 200 μM UTP and CTP, 50 μM GTP, and 5 μCi of [α-^{32}P]GTP (1 Ci = 37 GBq). Under these conditions, approximately three transcripts were synthesized per gene per hour.

The labeled transcripts ([^{32}P]GMP) were sized by electrophoresis in 6% polyacrylamide gels containing 8 M urea and detected by autoradiography without intensification. Densitometric scanning was employed to quantify the transcripts. In some experiments, the transcription efficiency was determined by assaying the radioactivity in the gel slices directly.

RNA Sequencing. The template used for RNA sequencing was adapted from a clone prepared by M. Sands in which transcription by T7 RNA polymerase initiates at the exact 5′ guanylyl residue of Xbs 5S RNA (30). This T7 5S promoter was joined at the *Eco*RV site of the Xbs 5S RNA gene (residue 31) to the maxigene diagramed in Fig. 1*A* (pBS+). The DNA was cleaved at the *Hin*dIII site, two nucleotides downstream of the gene in order to perform runoff transcription by T7 RNA polymerase in the presence of 3′-deoxyribonucleotide chain terminators. RNA sequencing reactions were modified from those of Axelrod and Kramer (31). The same template was used for DNA sequencing in the presence of 2′,3′-dideoxyribonucleotide chain terminators. DNA sequencing was performed using the Sequenase kit (United States Biochemical).

RESULTS

Construction of 5S RNA Maxigenes Containing Unique AAF or AF Adducts. Efficient transcription of 5S RNA genes is initiated *in vitro* under the control of the internal control region (ICR; from +45 to +95) and terminates at a cluster of T residues surrounding residue +120. Substitutions or insertions of a nonspecific DNA sequence between residues +95 and +118 do not appear to have serious effects on transcription *in vitro* (23, 24). We first constructed two modified 5S RNA genes cloned in pBS− and pBS+ phagemid vectors that would permit oligonucleotides to be inserted between the ICR and the termination site required for RNA polymerase III transcription as described in *Materials and Methods* (Fig. 1*A*). The final construct cloned in pBS+ allows production of single-stranded DNA containing the nontemplate strand of the 5S RNA gene, whereas the construct in pBS− allows production of single-stranded DNA containing the template strand of the 5S RNA gene. These single-stranded circular DNAs were annealed to the appropriate *Stu* I/*Xho* I-cut duplex DNA to generate gapped heteroduplexes. AAF- or AF-modified synthetic oligonucleotides were ligated into the gap of the heteroduplex to place the adduct on either the template or nontemplate strand of the 5S RNA maxigene (Fig. 1*B*). A wild-type 5S RNA gene (120 bp) was included in these plasmid constructs as a *Hin*dIII insert upstream of the adduct-bearing maxigene to provide an internal control for transcription.

The structures of the ligated cccDNAs were confirmed by blot hybridization with strand-specific RNA probes prepared

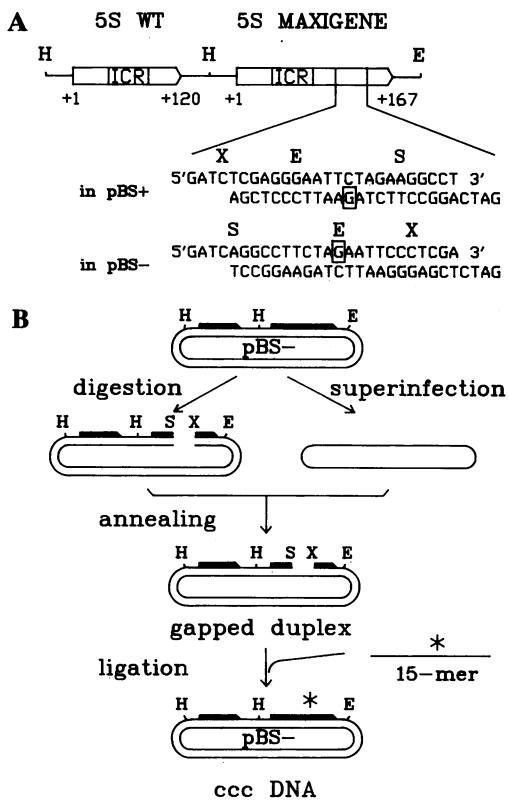

FIG. 1. Construction of a 5S RNA gene containing a unique AAF- or AF-modified guanine residue. (*A*) Map of a 5S RNA gene constructed as described in the text. Genes with the desired orientation of the 26-bp fragment were selected in pBS+ and pBS− vectors to allow placement of an isolated G residue (boxed) on the template strand (pBS+) or nontemplate strand (pBS−) of the gene. The sequence of the 26-bp fragment containing *Xho* I (X), *Eco*RI (E), and *Stu* I (S) restriction sites is shown. WT, wild type. (*B*) Preparation of AAF- or AF-modified cccDNA by *in vitro* ligation. This example shows the preparation of DNA modified on the nontemplate strand. The AAF- or AF-modified 15-mer was ligated to fill the gap between the *Stu* I and *Xho* I sites on the nontemplate strand of the gapped heteroduplex.

as described in *Materials and Methods*. Both probes contain sequences derived from the 5′ portion of the 5S RNA gene. The two RNA probes synthesized by the T7 or T3 RNA polymerase, respectively, hybridize to the template or the nontemplate strand of the 5S RNA genes (Fig. 2). Blot hybridization with these probes confirmed the presence of adducts in the DNA, since the adduct located on a G residue of the *Eco*RI site prevents cleavage by the enzyme. Preparations of ligated DNA were routinely checked to confirm that the DNA was *Eco*RI resistant. If a fraction of the *Stu* I/*Xho* I-treated DNA used to prepare gapped heteroduplexes was not completely cut by both enzymes, the unmodified DNA would be self ligated so that we would detect cleavage at the *Eco*RI site. As shown in Fig. 2, neither AAF- nor AF-modified DNA was susceptible to *Eco*RI digestion at the adduct site. The results shown in Fig. 2 also indicate that neither adduct is repaired efficiently *in vitro* under the conditions we have used (Fig. 2 *A* and *B*, lanes 3 and 5). Scans of the blots shown in Fig. 2 and other experiments suggest that >95% of DNA contains adducts after incubation in the extract.

Transcription of 5S RNA Genes Containing AF and AAF Adducts. We sought to minimize the amount of DNA used in *in vitro* transcription experiments since only small amounts of DNA were produced by our ligation methods. It has been shown that the efficiency of *in vitro* transcription can be increased by using small amounts of specific template DNA mixed with nonspecific carrier DNA (32).

The results of *in vitro* transcription of 5S RNA genes containing adducts are shown in Fig. 3. Similar results were

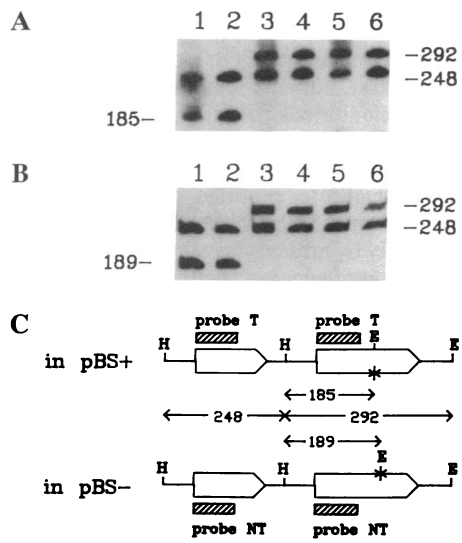

FIG. 2. The adduct-containing DNA is homogeneous and is not repaired *in vitro*. (*A*) Control DNA without adducts (lanes 1 and 2), DNA containing an AF adduct (lanes 3 and 4), or DNA containing an AAF adduct (lanes 5 and 6) on the template strand was incubated in the presence (lanes 1, 3, and 5) or absence (lanes 2, 4, and 6) of the *X. laevis* oocyte S150 extract for 2 hr. The recovered DNAs were digested with *Eco*RI and *Hin*dIII and detected by blot hybridization with RNA probe T (*C*). A 185-nucleotide band results from *Eco*RI digestion (lanes 1 and 2). A 292-nucleotide band reflects resistance to *Eco*RI digestion (lanes 3–6). The 248-nucleotide band is an internal control resulting from the *Hin*dIII fragment containing the control wild-type 5S RNA gene. (*B*) An experiment identical to that in *A* was performed with templates containing lesions on the nontemplate strand. In this case, RNA probe NT (*C*) was used for blot hybridization. (*C*) Diagram showing the hybridization of RNA probes to restriction fragments of modified DNA (*). RNA probe T contains part of the sequence of the nontemplate strand of the 5S RNA gene to detect the template strand of DNA. RNA probe NT contains the sequence of the template strand of 5S RNA gene to detect the nontemplate strand of DNA.

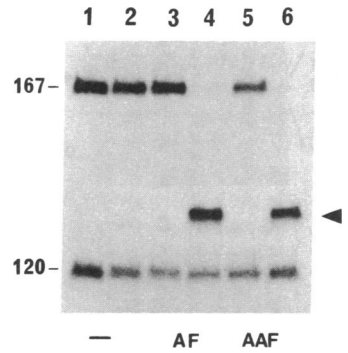

FIG. 3. Transcription of adduct-containing 5S RNA genes. An autoradiogram of a denaturing gel of transcripts of six independent plasmid constructs is shown. Lanes 1 and 2, transcripts of control templates in pBS− and pBS+, respectively, without adducts (see Fig. 2*C*). Transcripts of the wild-type gene and maxigene are 120 and 167 nucleotides in length. Lanes 3–6, transcripts of the tandem constructs in which the maxigene contains either an AF adduct or AAF adduct on the nontemplate (lanes 3 and 5) or template strand (lanes 4 and 6), respectively. The arrowhead indicates truncated transcripts.

obtained with AF- and AAF-modified templates. In each case, truncated transcripts (arrowhead in Fig. 3, lanes 4 and 6) were synthesized when the adducts resided on the template strand, whereas lesions on the nontemplate strand had no effect. We reproducibly observed a small amount of apparently full-length (167 nucleotides) maxigene transcripts for templates bearing AF or AAF residues on the template strand (Fig. 3, lanes 4 and 6). The proportion of full-length transcripts of genes containing AF or AAF adducts on the template strand averaged ≈5% in several experiments. This low rate of full-length transcription is more appreciable in experiments presented below. These full-length transcripts might result from a low frequency of transcription through AF or AAF adducts. However, at present we cannot rigorously rule out the possibility that they derive from efficient transcription of the low fraction of DNA templates that might lack adducts due to either incomplete modification of the initial DNAs or a low rate of DNA repair.

We performed the experiment shown in Fig. 4 to determine the precise site for AAF- or AF-induced termination of transcription. In this experiment, the truncated transcripts were subjected to electrophoresis along with an RNA sequence ladder representing the sequence of the full-length maxigene transcript used in our studies. This RNA sequence ladder was produced by chain-terminator sequencing using T7 RNA polymerase to transcribe a plasmid in which the T7 promoter is juxtaposed to the 5S RNA maxigene. In this construct, T7 RNA polymerase initiates with the correct 5′ sequence of 5S RNA and continues through the 26-bp sequence inserted into the 5S RNA gene. As shown in Fig. 4*A*, the majority of transcripts (arrowhead) of the AAF- and AF-modified templates appear to terminate one nucleotide before the modified G residue in the template strand. A minor fraction of transcripts appears to terminate two nucleotides before the lesion. This interpretation is limited in part by difficulty we have routinely observed in obtaining a clear RNA sequence through this portion of the template sequence. Such ambiguities are not uncommon in RNA sequencing. Fig. 4*B* shows that the DNA sequence ladder produced from the same template is free of artifacts.

Transcription Reinitiation by RNA Polymerase III. The truncated transcripts observed in Figs. 3 and 4 result from stalling of RNA polymerase III at lesions on the template strand. If the stalled RNA polymerase were unable to dissociate from the template and release the nascent transcript, this might be expected to block further rounds of transcrip-

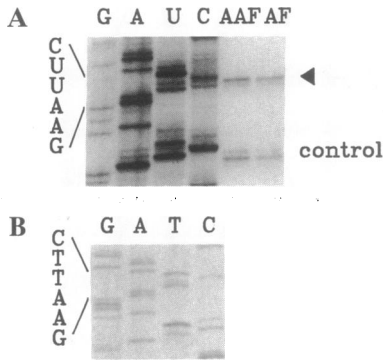

FIG. 4. Termination of transcription at the adduct-bearing site. (*A*) An autoradiogram of a denaturing polyacrylamide gel is shown in which transcripts similar to those in Fig. 3, lanes 4 (AF) and 6 (AAF), were run alongside an RNA sequence ladder. The RNAs labeled control are transcripts of the wild-type 5S RNA gene included as a control in the transcription template. The RNA sequence was obtained from a plasmid without adducts constructed so that a T7 RNA polymerase promoter overlaps the sequence at the 5′ end of the 5S RNA gene (see text). This alteration of the consensus T7 promoter sequence reduces the efficiency of the promoter and may contribute to occasional aberrant bands in the RNA sequence. In particular, we note that shadow bands appear in lane C below the authentic C residue in the sequence GAAUUC. See text for discussion of arrowhead. (*B*) An autoradiogram of a DNA sequencing gel was obtained from the same template as that in *A*.

tion. Therefore, we monitored the accumulation of transcripts in reactions continuing for periods as long as 4 hr. Under these conditions, the truncated transcripts accumulated with time in proportion to the transcripts of the control gene (Fig. 5). However, with increasing periods of incubation, some apparent degradation of the truncated transcripts was observed. Xing and Worcel (33) have shown that 5S RNA is stabilized by a base-paired 3′ terminus that is absent in these truncated transcripts. These results indicate that RNA polymerase III transcription complexes do not stall for a protracted period of time at the site of the DNA adduct. We conclude that the transcription complex can be released from the template to allow reinitiation of multiple rounds of transcription.

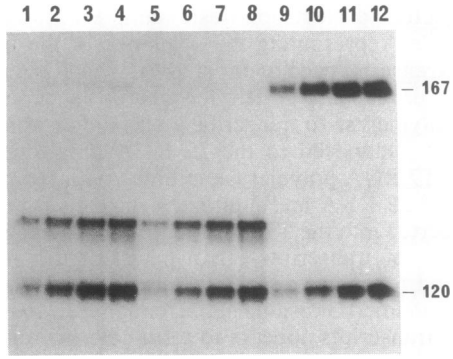

FIG. 5. Bulky lesions on the template strand do not block reinitiation of transcription. Transcription was performed using 10 ng of template DNA along with 50 ng of carrier DNA in reactions containing 60 μg of *X. laevis* oocyte S150 extract protein. The DNA templates for the reaction mixtures in lanes 1–4 and lanes 5–8 were modified with AAF and AF, respectively, on the template strand of the 5S RNA maxigene. The DNA template for the reaction mixtures in lanes 9–12 was a control plasmid without modification of the 5S RNA maxigene. The transcription reactions were allowed to proceed for 1 hr (lanes 1, 5, and 9), 2 hr (lanes 2, 6, and 10), 3 hr (lanes 3, 7, and 11), or 4 hr (lanes 4, 8, and 12). Sizes of transcripts are indicated in nucleotides.

DISCUSSION

It has only recently become possible to examine the effects of precisely localized DNA damage. The development of methods to study site-specific repair and mutagenesis has been reviewed by Naser *et al.* (34). To our knowledge, experiments that examine the effects of precisely localized adducts on transcription by a eukaryotic RNA polymerase have not been reported previously. We have incorporated HPLC-purified oligonucleotides into specific sites downstream of the sequences required for transcription by RNA polymerase III. Control experiments showed that the templates were essentially homogeneous at the start of the reaction and that the lesions are not repaired under the conditions of our *in vitro* transcription reactions (Fig. 2). Other authors have found that AF and AAF adducts can be repaired under some conditions. Orfanoudakis *et al.* (15) reported that the microinjected AAF-modified DNA can be repaired in *Xenopus* eggs. Hansson *et al.* (16) have recently documented DNA repair synthesis at the site of an AAF lesion in mammalian cell extracts. In these reactions, the extent of repair synthesis was enhanced by supplementation with the *E. coli* uvrABC excinuclease. It would appear that some limiting DNA repair activities are absent from the whole cell *Xenopus* oocyte extract used in our experiments. It may be that additional manipulation of reaction conditions or supplementation with repair activities absent from the oocyte extract would allow detection of repair of these adducts. We have taken advantage of the stability of these adducts *in vitro* to examine their effects on transcription. Lesions located on the nontemplate strand had no deleterious effects on transcription, despite the likelihood that they would result in a distortion of the local structure of the DNA (35–38). In contrast, adducts located on the template strand provided an efficient block to transcription. The observation that AAF and AF adducts have similar effects on transcription was unexpected for two reasons. (*i*) AAF and AF adducts are thought to produce different effects on the conformation of modified DNA, although these two compounds differ only by an acetyl group. AAF covalently bound to the C-8 position of guanine is considered to cause rotation of the guanine residue around the N-glycosidic bond to displace the guanine residue out of the DNA helix of B-form DNA (35, 36). This conformational shift is described as the base displacement or insertion–denaturation model. In contrast, AF is thought to produce a different sort of distortion in the DNA helix. Potential energy calculations suggest that AF adducts on the C-8 position of guanine would be directed away from the DNA helix (37, 38). Recently, this minor groove binding model for an AF-guanylyl adduct has been supported by NMR data (12). (*ii*) AAF and AF adducts have been shown to have different effects on DNA synthesis by *E. coli* and phage DNA polymerase (9–11, 13). In those experiments, DNA synthesis was inhibited more effectively by AAF adducts than by AF adducts. Our data indicate that RNA polymerase III does not discriminate between these two adducts.

Transcription of the majority of the truncated transcripts terminates one nucleotide before the guanylyl residue containing an AAF or AF adduct (Fig. 4). Apparently the RNA polymerase does not efficiently incorporate a nucleotide opposite the altered guanine. The polymerase may simply have a high probability of dissociating from the template when it is forced to pause at a lesion. However, the observation of a minor fraction of full-length transcripts suggests that RNA polymerase III may polymerize through AF or AAF lesions part of the time, as noted above. If translesion synthesis occurs, it is a relatively rare event. If future experiments can conclusively show readthrough of these

lesions, it should be possible to determine the nucleotide incorporated opposite the adduct.

The result that AAF or AF adducts on the template strand present a barrier to elongation by RNA polymerase is not surprising. Indeed, we had expected that RNA polymerase III might stall for a prolonged period of time. Instead, the results in Fig. 5 show that the adduct-bearing templates clearly support multiple rounds of transcription. This requires that RNA polymerase III must dissociate from the elongation complex and reinitiate. It will be interesting to determine whether RNA polymerases I and II have the same ability.

In intact cells that are competent to repair this sort of DNA damage, it is possible that the differential action of the RNA polymerase on two strands is part of a mechanism to identify lesions in the template strand of transcribed sequences. Hanawalt and his colleagues (39–41) have found that UV-induced pyrimidine dimers are more efficiently repaired within actively transcribed genes than in the genome as a whole. Mellon *et al.* (42) have clearly shown that UV-induced DNA lesions are removed more efficiently from the template strand than from the nontemplate strand of mammalian genes. This apparent targeting of repair activity may be coupled with transcription (43). In an elegant set of experiments, Selby and Sancar (44) attempted to observe enhanced repair dependent on the (A)BC excinuclease at sites where a bacterial RNA polymerase had stalled at a pyrimidine dimer on the transcribed strand. Their inability to observe such an enhancement of repair activity may indicate that their *in vitro* transcription and repair reactions did not exhibit the same sort of control observed *in vivo*. How strand-specific repair occurs and whether it will be observed in genes transcribed by RNA polymerase III are still unknown. It may be possible to extend the experiments reported in this paper to help resolve some of these questions.

We thank Robert Rieger for the synthesis of oligonucleotides and Arthur P. Grollman for comments on the manuscript. This work was supported by a grant from National Institute of Environmental Health Sciences (ES04068).

1. Miller, J. (1970) *Cancer Res.* **30,** 559–573.
2. Heflich, R. H. (1988) *Environ. Mol. Mutagen.* **11,** 167–181.
3. Fuchs, R. P. P., Schwartz, N. & Daune, M. P. (1981) *Nature (London)* **294,** 657–659.
4. Moriya, M., Takeshita, M., Johnson, F., Peden, K., Will, S. & Grollman, A. P. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1586–1589.
5. Gupta, P. K., Johnson, D. L., Reid, T. M., Lee, M.-S., Romano, L. J. & King, C. M. (1989) *J. Biol. Chem.* **264,** 20120–20130.
6. Seeberg, E. & Fuchs, R. P. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 191–194.
7. Burnouf, D., Koehl, P. & Fuchs, R. P. P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4147–4151.
8. Armier, J., Mezzina, M., Leng, M., Fuchs, R. P. P. & Sarasin, A. (1988) *Carcinogenesis* **9,** 789–795.
9. Moore, P. D., Rabkin, S. D. & Strauss, B. S. (1980) *Nucleic Acids Res.* **8,** 4473–4484.
10. Moore, P. D., Rabkin, S. D., Osborn, A. L., King, C. M. & Strauss, B. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7166–7170.
11. Strauss, B. S. & Wang, J. (1990) *Carcinogenesis* **11,** 2103–2109.
12. Norman, D., Abuaf, P., Hingerty, B. E., Live, D., Grunberger, D., Broyde, S. & Patel, D. J. (1989) *Biochemistry* **8,** 7462–7476.
13. Michaels, M. L., Johnson, D. L., Reid, T. M., King, C. M. & Romano, L. J. (1987) *J. Biol. Chem.* **262,** 14648–14654.
14. Tang, M.-S., Bohr, V. A., Zhang, X.-S., Pierce, J. & Hanawalt, P. C. (1989) *J. Biol. Chem.* **264,** 14455–14462.
15. Orfanoudakis, G., Gilson, G., Wolff, C. M., Ebel, J. P., Befort, N. & Remy, P. (1990) *Biochimie* **72,** 271–278.
16. Hansson, J., Munn, M., Rupp, W. D., Kahn, R. & Wood, R. D. (1989) *J. Biol. Chem.* **264,** 21788–21792.
17. Kaplan, L. A. & Weinstein, I. B. (1976) *Chem. Biol. Interact.* **12,** 99–108.
18. Millette, R. L. & Fink, L. M. (1975) *Biochemistry* **14,** 1426–1432.
19. Lotlikar, P. D., Scribner, J. D., Miller, J. A. & Miller, E. C. (1966) *Life Sci.* **5,** 1263–1269.
20. Takeshita, M., Chang, C. N., Johnson, F., Will, S. & Grollman, A. P. (1987) *J. Biol. Chem.* **262,** 10171–10179.
21. Shibutani, S., Gentles, R. G., Iden, C. R. & Johnson, F. (1990) *J. Am. Chem. Soc.* **112,** 5667–5668.
22. Shibutani, S., Gentles, R., Johnson, F. & Grollman, A. P. (1991) *Carcinogenesis* **12,** 813–818.
23. Bogenhagen, D. F. & Brown, D. D. (1981) *Cell* **24,** 261–270.
24. Bogenhagen, D. F., Sakonju, S. & Brown, D. D. (1980) *Cell* **19,** 27–35.
25. Vieira, J. & Messing, J. (1987) *Methods Enzymol.* **153,** 3–11.
26. Matsumoto, Y. & Bogenhagen, D. F. (1991) *Mol. Cell. Biol.* **11,** 4441–4447.
27. Matsumoto, Y. & Bogenhagen, D. F. (1989) *Mol. Cell. Biol.* **9,** 3750–3757.
28. Glikin, G. C., Ruberti, I. & Worcel, A. (1984) *Cell* **37,** 33–41.
29. McConkey, G. A. & Bogenhagen, D. F. (1988) *Genes Dev.* **2,** 205–214.
30. Sands, M. S. & Bogenhagen, D. F. (1991) *Nucleic Acids Res.* **19,** 1791–1796.
31. Axelrod, V. D. & Kramer, F. R. (1985) *Biochemistry* **24,** 5716–5723.
32. Wolffe, A. P., Jordan, E. & Brown, D. D. (1986) *Cell* **44,** 381–389.
33. Xing, Y. Y. & Worcel, A. (1989) *Genes Dev.* **3,** 1008–1018.
34. Naser, L. J., Pinto, A. L., Lippard, S. J. & Essigmann, J. M. (1988) in *DNA Repair: A Laboratory Manual of Research Procedures*, eds. Friedberg, E. C. & Hanawalt, P. C. (Dekker, New York), Vol. 3, pp. 205–217.
35. Nelson, J. H., Grunberger, D., Cantor, C. R. & Weinstein, I. B. (1971) *J. Mol. Biol.* **62,** 331–346.
36. Grunberger, D. & Santella, R. M. (1982) in *Mechanisms of Chemical Carcinogenesis*, eds. Harris, C. C. & Cerutti, P. A. (Liss, New York), p. 155.
37. Broyde, S. & Hingerty, B. E. (1983) *Biopolymers* **22,** 2423–2441.
38. Evans, F. E., Miller, D. W. & Beland, F. A. (1980) *Carcinogenesis* **1,** 955–959.
39. Bohr, V. A., Smith, C. A., Okumoto, D. S. & Hanawalt, P. C. (1985) *Cell* **40,** 359–369.
40. Madhani, H. D., Bohr, V. A. & Hanawalt, P. C. (1986) *Cell* **45,** 417–423.
41. Mellon, I., Bohr, V. A., Smith, C. A. & Hanawalt, P. C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8878–8882.
42. Mellon, I., Spivak, G. & Hanawalt, P. C. (1987) *Cell* **51,** 241–249.
43. Mellon, I. & Hanawalt, P. C. (1989) *Nature (London)* **342,** 95–98.
44. Selby, C. P. & Sancar, A. (1990) *J. Biol. Chem.* **265,** 21330–21336.