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Mutagenic replication in human cell extracts of DNA containing site-specific *N*-2-acetylaminofluorene adducts

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ABSTRACT **We have analyzed the effects of site-specific *N*-2-acetylaminofluorene (AAF) adducts on the efficiency and frameshift fidelity of bidirectional replication of double-stranded DNA in a human cell extract. Plasmid vectors were constructed containing the simian virus 40 origin of replication and single AAF adducts at one of three guanines in the *Nar* I sequence GGCGCC in a *lacZ* reporter gene. The presence of an AAF adduct diminishes replication efficiency in HeLa cell extracts by 70–80%. Replication product analyses reveal unique termination sites with each damaged vector, suggesting that when the replication fork encounters an AAF adduct, it often stops before incorporation opposite the adduct. We also observed a higher proportion of products representing replication of the undamaged strand compared to the damaged strand. This suggests that the undamaged strand is replicated more readily, either by uncoupling the first fork to encounter the lesion or by replication using the fork arriving from the other direction. Also included among replication products are covalently closed monomer-length molecules resistant to cleavage at the AAF-modified *Nar* I site. This resistance is characteristic of substrates containing the AAF adduct, suggesting that translesion bypass had occurred. Transformation of *Escherichia coli* cells with the replicated damaged DNA yielded *lacZα* revertant frequencies significantly above values obtained with undamaged DNA or with damaged DNA not replicated *in vitro*. This increase was only seen with the substrate modified at the third guanine position. Analysis of mutant DNA demonstrated the loss of a GC dinucleotide at the *Nar* I sequence. Generation of this position-dependent AAF-induced frameshift error in a human replication system is consistent with previous observations in *E. coli* suggesting that, after incorporation of dCMP opposite modified guanine in the third position, realignment of the template-primer occurs to form an intermediate with two unpaired nucleotides in the template strand.**

N-2-Acetylaminofluorene (AAF) is a potent carcinogen that binds primarily at the C-8 position of guanine to form *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene adducts, hereafter termed AAF adducts (1). Studies in *Escherichia coli* have demonstrated that AAF is a strong mutagen for 1- and 2-base deletions (2, 3) and that its mutagenic potential depends on location. Thus the adduct is most mutagenic when at those guanine positions within repetitive sequences that permit slippage to form misaligned intermediates during replication (4, 5). Mutagenesis is also higher for adducts on the lagging strand during *E. coli* DNA replication than for adducts on the leading strand (6).

In attempting to understand the processes in human cells that convert DNA adducts into mutations, we have been studying the accuracy of replication of damaged DNA *in vitro* by using HeLa cell extracts. Initial studies using UV-irradiated simian virus 40 (SV40) origin-containing substrates have demonstrated mutagenic translesion bypass of pyrimidine dimers by the human replication apparatus (7, 8). Detailed analysis of mutational specificity with substrates containing randomly distributed photoproducts revealed both site- and strand-specific differences in replication error rates for base substitutions (9).

Understanding site- and strand-specific differences in replication fidelity with damaged DNA is important for full appreciation of the risk posed by human carcinogens. Much of our understanding of how the major AAF adduct is mutagenic in model prokaryotic systems comes from observations made possible by the construction of DNA substrates containing a single AAF adduct at only one position in all the molecules (10). We take advantage of this strategy in the present study of a eukaryotic system by placing AAF adducts at specific nucleotide positions within a *lacZα* reporter gene in a vector containing the SV40 origin. Three modified substrates were prepared, each with the adduct at a different guanine in the *Nar* I recognition sequence 5′-GGCGCC-3′. These substrates were replicated in a HeLa cell extract and the products were characterized extensively. We wanted to determine whether replication was inhibited, whether complete replication of the leading and lagging strands occurred with equal probability, whether lesion bypass occurred, and, if so, whether translesion replication led to position-specific two-base deletions consistent with the model inferred from studies in *E. coli*.

MATERIALS AND METHODS

Materials. *E. coli* strains and plasmid vectors (pNar) have been described (5). Tumor (T) antigen was from Molecular Biology Resources (Milwaukee). All other enzymes were purchased from New England Biolabs or United States Biochemical. Radionuclides were from Amersham. HeLa cell extract was prepared by the method of Li and Kelly (11). Plasmids containing single AAF adducts at unique guanines were prepared as described (6). Unmodified substrate was prepared by the same procedure as for modified DNAs, starting with mock treatment of the oligonucleotide used to introduce the site-specific adduct.

Replication Reactions. The replication reaction method has been described in detail (12). Reaction mixtures (25 μl) containing [α-^{32}P]dCTP, 20 ng of DNA, and other components as indicated were incubated for 4 hr at 37°C and then processed as described (12). One-tenth of the reaction products was analyzed to determine total incorporation.

Product Analysis. An aliquot of each replication reaction mixture, with and without T antigen, was resolved by electrophoresis (55 V) on a 1.1% agarose gel containing ethidium

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Abbreviations: AAF, *N*-2-acetylaminofluorene; SV40, simian virus 40; T antigen, SV40 large tumor antigen; RF, replicative form.

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bromide (0.2 μg/ml). The dried gel was subjected to autoradiography or exposed to a phosphor screen for scanning and quantitation of products using a Molecular Dynamics PhosphoImager.

Enzymatic Purification of Replicative Form I (RFI) Products. Some replicated samples were processed to remove all products except RFI (covalently closed circular double-stranded DNA). Samples were treated first with exonuclease III for 1 hr at 37°C in a reaction mixture (30 μl) containing 50 mM Tris·HCl (pH 8.0), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, bovine serum albumin at 100 μg/ml, 20 units of exonuclease III, and ≈5 ng of replicated DNA. The enzyme was inactivated by incubation at 70°C for 20 min. One unit of native T7 DNA polymerase was added, and the samples were incubated for 1 hr at 37°C. The polymerase was then inactivated for 10 min at 70°C.

Analysis of Translesion Bypass. After treatment to obtain RFI products, samples were digested with 1 unit of restriction enzyme *Bsa*HI for 1 hr at 60°C. This enzyme recognizes sequences similar to those recognized by *Nar* I but is more efficient in cleaving the site of interest. However, when the *Nar* I site is modified with AAF, neither enzyme incises the DNA (see below). The samples were subjected to agarose gel electrophoresis and quantitation as described above. Translesion bypass was calculated by dividing the intensity of the band resistant to cleavage by the sum of the intensity of the two cleavage products plus the resistant band, after subtracting background values.

Determination of Reversion Frequencies. *lacZα*-complementation revertant frequencies were determined by electroporation of *E. coli* JM103 to score colonies on LB indicator plates containing ampicillin, as described (4). The assay scores errors that restore the correct reading frame of the gene as blue colonies. White colonies represent the remaining total of replicated molecules. Revertants were confirmed by picking, regrowing, and streaking on new plates. Some revertants were screened for loss of the *Nar* I site by digesting purified RFI DNA with *Bsa*HI. To confirm that the revertants contained 2-bp deletions, several mutant DNAs were sequenced by the double-stranded sequencing technique of United States Biochemical.

Analysis of Replication of the Two Strands. Replicated samples were treated to obtain RFI products as described above, desalted, and digested with restriction endonucleases *Pvu* II and *Eco*RI in a 30-μl reaction mixture, using the conditions specified by the manufacturer for *Pvu* II. The samples were desalted, lyophilized, dissolved in 10 μl of formamide loading dye, and resolved on an 8% polyacrylamide sequencing gel. Material in the dried gel was quantitated as above.

RESULTS

Description of Substrates. DNA substrates were prepared containing site-specific AAF adducts at one of three guanine residues in the *Nar* I sequence (Fig. 1). These substrates are referred to as G1, G2, and G3; G0 is the unmodified control DNA. They contain the SV40 replication origin and thus are suitable substrates for replication *in vitro* using human cell extracts. The modified *Nar* I site is located in the polylinker region of the *lacZα* reporter gene of pUC8. The adducts are located on the leading strand of replication if it is assumed the sequence is replicated by the closest fork emanating from the SV40 origin. The reading frame in the reporter gene is +2 (−1), which allows for −2 frameshift errors to be detected as blue colonies on indicator plates. Errors other than $3n + 1$ and $3n - 2$ will not be scored in this assay.

Replication of Damaged Plasmids in a HeLa Cell Extract. Reactions were performed in a HeLa cell extract in the presence and absence of T antigen. As measured by incorporation of [α-^{32}P]dCTP into acid-insoluble DNA in a 4-hr reaction, T-antigen-dependent replication was inhibited 60–70% by a lesion at any of the three guanines (total incorporation, 50–70 pmol), compared to an unmodified control (170 pmol).

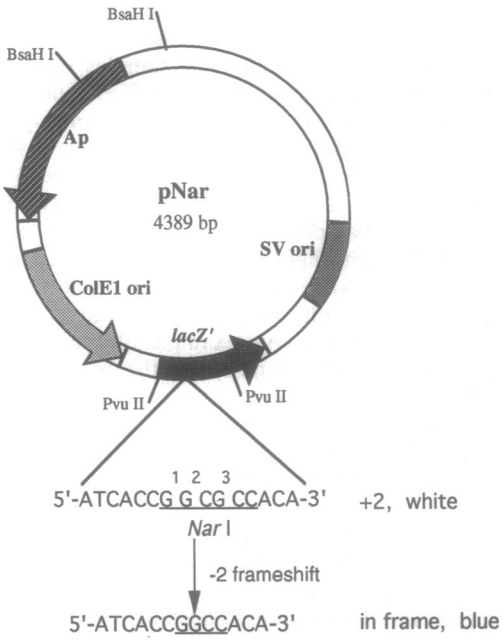

FIG. 1. Map of plasmid pNar. Vectors contained either no modification or unique AAF adducts in the *lacZα* gene as shown. For reference, the G3 vector contains the adduct at nt 2136. The closest fork emanating from the SV40 origin must replicate 966 nt to copy (as the leading strand) the adduct in G3 (i.e., 22% of the total size of the plasmid). Also denoted are the locations of restriction sites used in bypass (*Bsa*HI) and uncoupling (*Pvu* II) measurements. The location of the *Eco*RI site used in uncoupling measurements is shown more precisely in Fig. 4*A*. Ap is the resistance gene for ampicillin, β-lactamase.

Aliquots of the deproteinized radiolabeled samples were resolved on an agarose gel (Fig. 2). Qualitatively, the products with the modified substrates in the presence of T antigen are similar to those with undamaged DNA and include the production of covalently closed monomer-length RFI products despite the presence of an AAF adduct in all molecules of the modified DNAs. With the damaged plasmids, all product species are reduced in amount—e.g., for each modified plasmid, RFI products were about 20% of the control value.

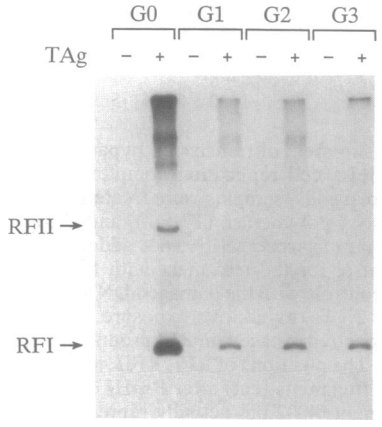

FIG. 2. Agarose gel electrophoretic analysis of replication products (for description, see text). TAg, T antigen. +, T antigen added; −, T antigen not added.

Enzymatic Purification of RFI Products. To characterize further the products of complete replication, we developed a method to purify RFI products. The samples were treated sequentially with exonuclease III, a 3′ → 5′ exonuclease that can attack nicks, blunt-ends, and 3′ recessed ends of duplex DNA, and native T7 polymerase, which, in the absence of dNTPs, also has a highly active and processive 3′ → 5′ exonuclease activity. Treatment with either enzyme alone removes a substantial portion of the replication products other than RFI, with slightly more RFII (nicked circular double-stranded DNA) remaining (data not shown). However, treatment with both exonucleases effectively digested all but the RFI products. An example of the enrichment achieved is shown in Fig. 3, lane 2, with the untreated sample shown in lane 1 for comparison. Consistently, >80% of the RFI band remained after treatment, and >90% of the radioactive material found in other bands was eliminated. Similar results were obtained whether the samples contained damage or not.

Measurement of Translesion Bypass. To establish whether replicative bypass of AAF adducts had occurred, we wanted to determine whether adducts remained in replicated DNA. We attempted three approaches, digestion with ABC excinuclease, alkaline hydrolysis, and restriction endonuclease digestion. The first two approaches were unsuccessful. However, we found that covalent linkage of AAF to the *Nar* I site rendered it completely resistant to cleavage by an isoschizomer of *Nar* I, *Bsa*HI, which was also found to cleave efficiently the unmodified site (data not shown). Thus, after exonuclease treatment of replicated DNA to obtain RFI products, samples were digested with *Bsa*HI and resolved on an agarose gel (Fig. 3). With undamaged (G0) DNA, incision at the recognition site converted the RFI DNA into two bands running just ahead of RFI DNA (compare lanes 2 and 3). Digestion of the replicated G1, G2, and G3 DNA samples yielded similar bands. However, an additional band at the position expected for the uncleaved larger fragment was also detected readily with all three damaged substrates. The portion of resistant DNA in each modified sample is 5% of the total of all three bands combined. Similar results were obtained using samples not pretreated with exonucleases (data not shown).

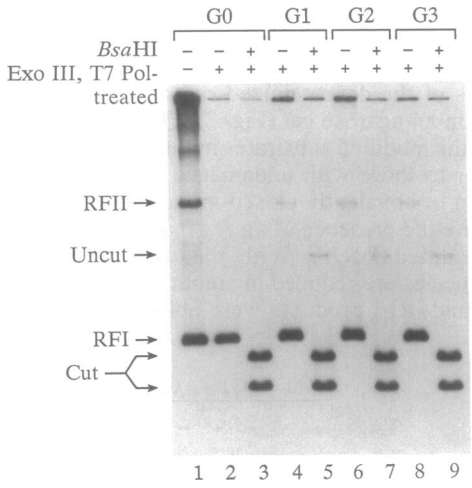

FIG. 3. Measurement of translesion bypass of site-specific AAF adducts by a HeLa cell replication complex. After replication of AAF-modified plasmids, samples were treated with exonuclease III (Exo III), T7 DNA polymerase (T7 Pol), and *Bsa*HI (as indicated) and resolved on an agarose gel (−, not added; +, added). Lane 1 serves as a control for the treatment with the exonucleases. The corresponding controls with the damaged DNA were similar to those shown in Fig. 2. To avoid overexposure, approximately equal amounts of radioactivity, based on RFI products, were loaded on the gel in each lane. The positions of RFI, RFII, the uncut fragment, and the two smaller fragments (cut) after *Bsa*HI cleavage are indicated. The uncut fragment (4007 bp) actually represents the larger of two fragments generated by *Bsa*HI when the *Nar* I site is modified by AAF (or no longer exists). The smaller fragment (382 bp) is not seen in this figure. Cleavage of the unmodified *Nar* I site yields the cut products observed (2204 and 1803 bp).

Termination Bands and Unequal Replication of the Two Strands. Semiconservative replication of double-stranded DNA is expected to yield two product molecules, each containing one parental strand and one daughter strand (which in these experiments will be radiolabeled). To investigate whether the presence of damage in one strand but not its complement affected the probability of replication of the two strands, replication products were digested with *Eco*RI and *Pvu* II to generate two fragments whose complementary strands differed in length by 4 bases (Fig. 4*A*). Samples were treated with these enzymes either with or without exonuclease pretreatment to obtain RFI products, and the products were analyzed by electrophoresis in an 8% sequencing gel (Fig. 4*B*). With undamaged G0 DNA, the intensities of the two complementary strands of both fragments are equal, after correcting for the amount of cytosine (the radiolabeled nucleotide) present in each strand. However, for the three AAF-modified replicated substrates, the intensities of the

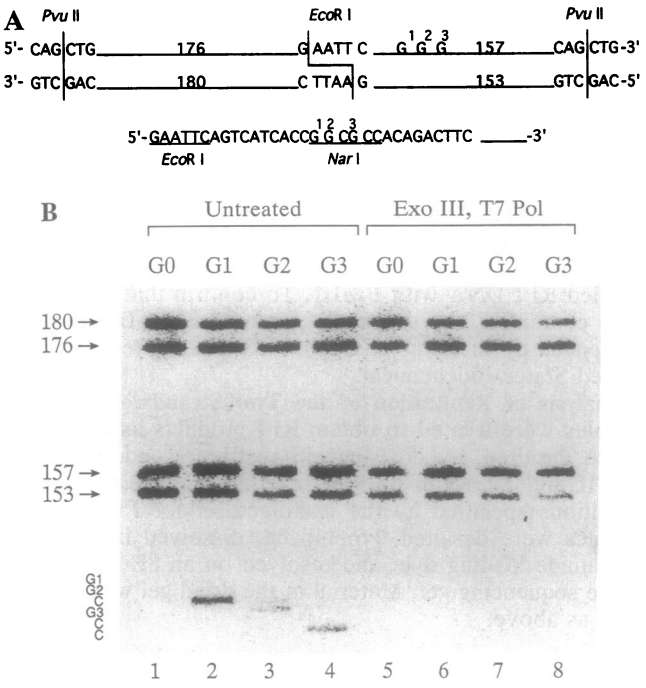

FIG. 4. Determination of strand asymmetry and termination bands among replication products. (*A*) Description of restriction sites and the strands they generate. Replication products, either untreated or treated with exonucleases to obtain RFI products, were digested with *Pvu* II and *Eco*RI to generate two fragments whose individual strands differ in length by 4 nt. The exact length in nucleotides is noted above each strand, and the location of the AAF-modified guanines in the 157-nt strand is indicated. Below the fragments is the sequence of the 157-nt strand in the region of the *Nar* I site, with the guanine residues indicated. From this, the exact length of bands representing sites of termination for each substrate can be determined by comparison to a sequence ladder (data not shown). (*B*) PhosphoImager print of denaturing polyacrylamide gel containing resolved strands of *Pvu* II–*Eco*RI fragments depicted in *A* (lanes 1–4). The length of each strand is indicated to the left. The second series of four samples (lanes 5–8) were exonuclease-treated (Exo III, T7 Pol). The sites of presumed termination for each untreated damaged sample are indicated according to their position opposite the bases within the *Nar* I sequence shown at the lower left.

two strands of each fragment are not equal. In all cases, the less-intense band is for the strand complementary to the strand containing the adduct. Similar inequalities were obtained with samples not pretreated with exonuclease (Fig. 4*B*, lanes 1–4) or that were subjected to exonuclease digestion to obtain RFI products prior to restriction endonuclease digestion (Fig. 4*B*, lanes 5–8). The data are consistent with diminished replication of the adduct-containing strand.

Analysis of the samples not pretreated with exonuclease (Fig. 4*B*, lanes 1–4) also reveals single bands of higher mobility for each of the three AAF-containing substrates but not for undamaged DNA. Their lengths are consistent with the relative initial placement of the AAF adduct in the three substrates. Thus, the band in the G1 lane is 1 nt longer than that in the G2 lane and 3 nt longer than that in the G3 lane. Each band is equivalent to the length predicted for a strand cut at one end by *Pvu* II and terminating 1 or 2 bases short of the adduct in each substrate. The lane representing the G2 DNA also has a second band of lesser intensity (seen more readily in a longer exposure) that is 1 nt longer than the main termination band.

Mutation Frequencies of Replicated AAF-Modified DNAs. To examine whether replication of AAF-modified substrates was mutagenic, replicated samples were digested with restriction endonuclease *Dpn* I to remove unreplicated or incompletely replicated (e.g., θ structures) molecules, and the resulting samples were used to transform *E. coli* host cells to score colony colors. Blue colonies represent mutants having the correct reading frame restored, and white colonies represent the remaining total of replicated molecules.

First, transfections without prior replication *in vitro* were performed to establish background revertant frequencies for each of the vectors. These values ranged from 0.4 to 17 $\times$ 10^{-4} (Table 1). Untreated G1 and G2 DNA yield frequencies slightly above the value for undamaged DNA. Sequence analysis of blue revertants from G1 and G2 substrate constructions demonstrated that they were not −2 deletions at the *Nar* I site (X.V. and R.P.P.F., unpublished results) and are perhaps construction artifacts. The frequency for G3 DNA is much higher (Table 1) and sequence analysis revealed that these were indeed −2 deletions at the *Nar* I site (ref. 4 and X.V. and R.P.P.F., unpublished results).

Table 1. Revertant frequencies with and without replication *in vitro*

Substrate	Total colonies, no.	Blue colonies, no.	Rev. freq. ($\times 10^{-4}$)
Unreplicated DNA, not incubated in extract, not treated with *Dpn* I			
G0	23,893	1	0.4
G1	9,153	2	2.2
G2	9,032	4	4.4
G3	48,589	81	17
Unreplicated DNA, incubated in extract without TAg, not treated with *Dpn* I			
G0	6,230	0	≤1.6
	12,590	0	≤0.8
G1	6,405	0	≤1.6
	11,550	0	≤0.9
G2	5,740	0	≤1.7
	9,760	0	≤1.0
G3	22,075	25	11
	18,060	6	3.3
Replicated DNA, incubated in extract with TAg, then treated with *Dpn* I			
G0	18,798	2	1.1
	6,029	0	≤1.7
G1	5,131	3	5.8
	9,024	3	3.3
G2	6,464	0	≤1.5
	9,527	1	1.0
G3	14,425	114	79
	14,060	75	53

For the two experiments shown, the first value for the control without T antigen (TAg) matches the first value listed for the equivalent substrates replicated with T antigen. Rev. freq., revertant frequency.

The revertant frequency values for all substrates incubated in the extract in the absence of T antigen (Table 1, two experiments shown for each vector) were no higher than those for the untreated samples, demonstrating that extract-dependent mutagenesis is not seen in the absence of T-antigen-dependent replication. Note that revertant frequencies were actually lower with the G3 substrate after incubation without T antigen (11 and 3.3 $\times$ 10^{-4}) compared to the untreated G3 control (17 $\times$ 10^{-4}). This may reflect removal of some AAF adducts by the extract.

After T-antigen-dependent replication *in vitro* and *Dpn* I treatment to digest unreplicated molecules (12), the revertant frequencies for replicated G0, G1, and G2 DNA (Table 1) remained similar to the control values. Thus, replication was not detectably mutagenic with these substrates. However, the frequencies for replicated G3 DNA were 7- to 16-fold higher than for the same substrate incubated in the extract without T antigen (Table 1, compare 11 $\times$ 10^{-4} to 79 $\times$ 10^{-4} or 3.3 $\times$ 10^{-4} to 53 $\times$ 10^{-4}). This suggests that *in vitro* replication past the G3 adduct is mutagenic. The difference provides probably a minimum estimate of replication infidelity *in vitro*, since repair of the adduct or additional rounds of replication of the undamaged strand (see above) will reduce the apparent differences in revertant frequencies between the unreplicated and replicated G3 substrate.

Several revertant colonies from the replicated G3 substrate were picked, grown as overnight cultures, and streaked on selective indicator plates to confirm their blue-colony phenotypes. DNA from these blue revertants were sequenced and all were found to have the sequence GGCC in place of the *Nar* I sequence, demonstrating that the predicted −2 frameshift event had occurred.

DISCUSSION

This study was initiated to determine the effects of single AAF adducts placed at specific locations in a DNA sequence on replication catalyzed by the human multiprotein replication apparatus. The first effect noted was inhibition of replication, as determined by diminished total incorporation and by examination of replication products (Fig. 2). The position of specific product bands observed with the AAF-modified substrates but not undamaged DNA (Fig. 4*B*, lanes 1–4) is consistent with some degree of termination of replication without insertion of a nucleotide opposite the adduct. This is similar to observations with purified DNA polymerases, demonstrating that they terminate synthesis without inserting a nucleotide opposite an AAF adduct (13, 14).

With these site-specifically modified substrates, little or no RFI product would be observed if AAF adducts were absolute blocks to progression of both strands of the replication fork. However, much of the T-antigen-dependent incorporation with the modified DNA was into covalently closed monomer-length circular DNA (Fig. 2). These completely replicated molecules could be generated in several ways. Excision repair of the adduct before replication would restore the modified vectors to the G0 state. This may also explain the lower revertant frequency for G3 DNA after incubation in the extract without T antigen compared to the unincubated control (Table 1).

Alternatively, when the replication fork encounters the lesion, fully replicated DNA would be produced by complete

replication of the undamaged strand even if synthesis of the damage-containing strand was inhibited. This is supported by the observation of unique position-specific bands among replication products and the unequal distribution of product strands biased in favor of replication of the undamaged strand (Fig. 4). One possibility for the strand bias in replication products is that when the closest fork emanating from the SV40 origin encounters the lesion, replication of the two strands becomes uncoupled. The identical amounts of complementary strand products observed with the G0 substrate DNA suggest that replication of the two strands of undamaged DNA is coupled. Evidence for uncoupled SV40 DNA replication has been obtained *in vivo*, when infected cells are treated with an inhibitor of protein synthesis (15). The accumulation of large quantities of single-stranded DNA in cells (16, 17) is also consistent with the possibility that under some circumstances replication might be uncoupled. Also AAF-induced uncoupling of *E. coli* replication was suggested by the observation that when two or three AAF adducts were present on only one strand of double-stranded plasmid molecules, only progeny from the undamaged strand were recovered (ref. 18 and X.V. and R.P.P.F., unpublished results). A second possibility is that replication of both strands at the first fork is inhibited but that replication by the fork arriving from the other direction completes the undamaged strand, but not the damaged strand. Further experiments will be required to distinguish between these two possibilities.

The detection of RFI DNA products resistant to restriction endonuclease cleavage at the originally modified *Nar* I site (Fig. 3) is consistent with the interpretation that some of the covalently closed monomer-length circular DNA observed after replication of AAF-modified DNA (Fig. 2) results from replicative bypass of AAF adducts. Hypothetically, if all adducts were bypassed, 50% of the products would be resistant to cleavage, yet only 5% of the RFI DNA was resistant (Fig. 3). This difference and the observed inhibition of replication by the adduct demonstrate clearly that not all adducts are bypassed. The amount of cleavage-resistant product may also be influenced by subsequent rounds of replication of the undamaged strand, repair prior to replication, and the presence of mutations at the cleavage site.

In *E. coli*, AAF adducts yield primarily 1-base deletions in homopolymeric runs of G·C base pairs and 2-base deletions in repeated GpC dinucleotide sequences (2, 3). Two models have been proposed to explain the latter deletions. One suggests that they result from processing of AAF-modified GpC sequences independently of replication (ref. 19 and references therein), while the second (3, 20) suggests that they result from a replication slippage mechanism similar to that proposed for 1-base frameshifts (5). In this model, the replication apparatus first incorporates cytosine opposite a modified guanine. Then template-primer slippage occurs and a misaligned intermediate forms wherein the two terminal bases in the primer (3′-CpG-5′) hydrogen bond with a repeated downstream complementary 5′-GpC-3′ dinucleotide in the template. Continued synthesis from this intermediate, now containing an unpaired modified G*pC dinucleotide, leads to a complementary strand 2 bases shorter than the template strand (i.e., a 2-base deletion error). This model predicts that mutagenesis should result from adducts in the G3 but not the G1 or G2 positions, because, given the sequence at the *Nar* I site, only the former would allow formation of a misaligned intermediate stabilized by two correct terminal base pairs. This prediction was realized in *E. coli* when site-specifically modified substrates were introduced into cells and a high revertant frequency was observed only with the substrate modified at the G3 position (4, 6).

Two major objectives of this study were to determine whether replication of AAF-modified DNA in a human system was mutagenic and, if so, whether mutagenesis depended on adduct location. The data in Table 1 reveal mutagenesis that depends on the presence of an AAF adduct and on SV40-origin-dependent replication in a HeLa cell extract. Moreover, an increase in −2 frameshift errors was only seen with the substrate modified at the G3 position. These data strongly support the idea that 2-base deletions result from strand slippage during replication to generate a misaligned intermediate. Given indications of some adduct removal in the extract and possible additional rounds of replication of the undamaged strand, the reversion frequencies shown in Table 1 probably underestimate the full mutagenic potential of the G3 adduct during replication.

The present study represents our initial attempt using site-specifically modified substrates to examine the effects of a major adduct of a known carcinogen on the efficiency and fidelity of replication in a human system. The strategy used here provides an opportunity to examine other fundamentally important issues. For example, do AAF adducts induce other types of mutations during human DNA replication? Also, the AAF adducts were located on the leading strand relative to the nearest replication fork emanating from the origin. Are substrates containing AAF adducts on either the leading or lagging strands replicated with equal efficiency and mutagenicity?

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