Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6943–6947, November 1984
Biochemistry

Nonrandom binding of the carcinogen *N*-hydroxy-2-acetylaminofluorene to repetitive sequences of rat liver DNA *in vivo*

(DNA damage/excision repair/^{32}P assay/repetitive sequence/DNA isolation)

RAMESH C. GUPTA

Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030

Communicated by Devendra Lal, July 23, 1984

ABSTRACT **We have examined the distribution of individual adducts in repetitive DNA sequences of rat liver *in vivo* after a single dose of the carcinogen *N*-hydroxy-2-acetylaminofluorene. Repetitive fragments [82, 125, 179, 225, and 370 base pairs (bp)] were isolated by digestion of hepatic DNA with *Hin*dIII restriction endonuclease (EC 3.1.23.21) and gel electrophoresis. As assayed by ^{32}P postlabeling, no qualitative differences were observed between the DNA-bound metabolites in repetitive sequences and total DNA, but preferential binding to these sequences occurred. After 1 day of treatment, the amounts of *N*-hydroxy-2-acetylaminofluorene-induced adducts were found to be 13.8, 2.0, and 3.0 times higher in 179-, 225-, and 370-bp repeats, respectively, than in total DNA, while 82- and 125-bp repeats showed no differences. The relative distribution of individual adducts varied among the various sequences. After 9 days, all five sequences showed 1.3–1.7 times higher binding as compared to total DNA. In contrast, a random binding was observed when DNA reacted *in vitro* with an active metabolite, *N*-acetoxy-2-acetylaminofluorene. Taken together, these results suggest that the enrichment and differential excision of adducts in the repetitive DNA sequences may be a function of the nuclear organization of DNA. This application of the ^{32}P assay constitutes a means to study the DNA damage and excision repair *in vivo* in chromatin structural components, including transcribed and nontranscribed multiple-copy genes, in a much more sensitive and precise way than has been hitherto possible.**

It is axiomatic that covalent binding of ultimate carcinogens to cellular macromolecules, particularly DNA, may be concerned in the initiation of malignancy. Extensive work has been done on the characterization of the nature of carcinogen binding to DNA (1). However, the intragenomic distribution of specific DNA lesions induced *in vivo* by chemical carcinogens, such as aromatic amines, is not well known. This aspect of carcinogen DNA interaction may be important because the structure of chromatin can affect both the initial distribution of DNA damage and its accessibility to repair enzymes (2). Using ^{3}H- or ^{14}C-labeled 2-acetylaminofluorene (AcNHFln), benzo[*a*]pyrene, dimethylnitrosamine, and aflatoxin B1, it has been shown that these carcinogens bind preferentially to presumed template active chromatin (3–6), nucleosomal linker DNA (7–10), matrix-bound DNA (11–13), and/or ribosomal genes (14).

The rat genome contains 8%–10% of its sequences in highly repetitive forms, as determined by renaturation kinetics (e.g., see ref. 15). The analysis of this DNA with restriction endonucleases and gel electrophoresis has revealed a series of discrete bands, indicating the presence of repetitive DNA elements (16–19). Digestion with *Hin*dIII nuclease shows at least five fragments [82, 125, 179, 225, and 370 base pairs (bp)] (19). Only 179-bp (19) and 370-bp (20) elements have been sequenced thus far and shown to be at least 85% homogeneous with respect to their sequences (19, 20). These and the other *Hin*dIII repeats, which give discrete bands on polyacrylamide gels (19), can therefore be used as probes for damage and repair studies in specific cellular DNA sequences.

Adducts in specific DNA sequences have not been analyzed in the past because of methodological limitations. Such studies are, however, rendered possible with the aid of a recently developed ^{32}P assay (21), which requires only 1–2 μg of DNA for replicate analyses and enables the detection of a single adduct in 10^{7}–10^{8} total nucleotides. In the present study, we have examined the intragenomic distribution of individual adducts in the *Hin*dIII repetitive DNA sequences in rats after the administration of a potent hepatocarcinogen, *N*-hydroxy-2-acetylaminofluorene (OH-AcNHFln). We found that this chemical bound preferentially to the repetitive DNA sequences as compared to total DNA. The 179- and 370-bp repeats showed high initial binding but adduct removal was also rapid in these DNA sequences.

MATERIALS AND METHODS

Chemicals. OH-AcNHFln and (*N*-acetoxy-2-acetylaminofluorene (AcO-AcNHFln) were obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository. *Hin*dIII restriction endonuclease (EC 3.1.23.21) and proteinase K (EC 3.4.21.14) were from Boehringer Mannheim. RNase T_1 (EC 3.1.27.3) and RNase A (EC 3.4.27.5; Type III-A) were from Calbiochem–Behring and Sigma, respectively. Materials required for the ^{32}P assay were the same as described (21). [γ-^{32}P]ATP was synthesized as described (21).

***In Vivo* Modification of DNA.** Male Sprague–Dawley rats (180–200 g; 3 rats per group) were given a single i.p. dose (40 mg/kg) of OH-AcNHFln in 0.3 ml of dimethyl sulfoxide; control rats received dimethyl sulfoxide alone. Standard laboratory diet and water were supplied ad lib. Animals were sacrificed 1 day and 9 days after treatment, livers were excised, minced, pooled from each group, and frozen immediately for storage at −70°C until isolation of DNA.

***In Vitro* Modification of DNA.** Rat liver DNA (3 mg) was incubated with AcO-AcNHFln (0.09 μmol) at 23°C for 10 min in 3 ml of 5 mM Bis-Tris, pH 7.0/10% ethanol. Noncovalently bound material was removed by extracting 3 times

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Abbreviations: AcNHFln, 2-acetylaminofluorene; OH-AcNHFln, *N*-hydroxy-2-acetylaminofluorene; AcO-AcNHFln, *N*-acetoxy-2-acetylaminofluorene; dG-C8-AcNHFln, *N*-acetyl-*N*-(deoxyguanosin-8-yl)-2-aminofluorene; dG-N^{2}-AcNHFln, 3-(deoxyguanosin-N^{2}-yl)-2-acetylaminofluorene; dG-C8-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; bp, base pair(s).

with ether and the DNA was recovered by precipitation with ethanol.

Isolation of DNA and Repetitive DNA Fragments. DNA was isolated by a procedure adapted and modified from methods reported previously (22–24). Frozen tissue (1 g) was thawed in 10 ml of 1% $NaDodSO_4$/1 mM EDTA, homogenized with Polytron homogenizer (speed control position 5) for 10 sec, and the homogenate was incubated at 38°C for 30 min with proteinase K (500 μg/ml). After the addition of 0.5 ml of 1 M Tris·HCl (pH 7.4), the homogenate was extracted successively with 1 vol each of phenol (5 min), 1:1 mixture of phenol/Sevag (chloroform/isoamyl alcohol, 24:1) (3 min), and Sevag (3 min). The extractions were done in 50-ml polypropylene tubes. The phases were separated by centrifugation (29,000 × *g* at 4°C, 10 min for the first extraction and 5 min for subsequent extractions). After the addition of 0.1 vol of 5 M NaCl, DNA was precipitated by the gradual addition of 1 vol of absolute ethanol precooled to −20°C. After inverting the tube several times, the DNA lump was removed with forceps and rinsed briefly in 70% ethanol to remove salt. Traces of ethanol were removed with a Pasteur pipette and DNA was dissolved, without letting the precipitate dry, in 2 ml of 0.01× NaCl/Cit/1 mM EDTA (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate). Residual RNA was destroyed by incubation at 38°C for 30 min with a mixture of RNase T_1 (50 units/ml) and RNase A (100 μg/ml) in 50 mM Tris·HCl (pH 7.4). After the extraction of this solution with Sevag, DNA was recovered from the aqueous phase as described above, dissolved in 2 ml of 0.01× NaCl/Cit/0.1 mM EDTA, and its concentration was estimated spectrophotometrically considering 20 A_{260} units/mg. The solution was stored at −70°C after quick freezing in dry ice/acetone and was rapidly thawed before using. This method enabled the isolation of large (10–30 mg) and small (100–200 μg) amounts of high molecular weight DNA (M_r, $\geq 3 \times 10^7$) in high yields (1.9–2.3 mg per g of rat liver), and in a relatively short time (3–4 hr). The A_{230}/A_{260} and A_{260}/A_{280} ratios of 0.40 ± 0.03 and 1.82 ± 0.05, respectively, are in agreement with published values (23, 24).

To prepare the repetitive DNA fragments, carcinogen-modified DNA (3 mg) was digested with *Hin*dIII restriction endonuclease (3000 units) in 6 ml of 20 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/50 mM NaCl/1 mM dithiothreitol/bovine serum albumin (10 μg/ml). After incubation at 38°C for 3 hr, the reaction mixture was extracted with 1 vol of Sevag and DNA was recovered by ethanol precipitation. The DNA was dissolved in 700 μl of 0.01× NaCl/Cit/0.1 mM EDTA, and mixed with reference 5′-^{32}P repetitive fragments (82, 125, 179, 225, and 370 bp; 150,000 cpm of each); see legend of Fig. 1 for their preparation. After adding sucrose (5%) and bromophenol blue (0.1 μg/μl), the digest was electrophoresed on two 5% polyacrylamide slab gels (30 × 20 × 0.2 cm) (25) until the dye marker had run down the gel. The radioactive fragments were detected by screen-enhanced autoradiography (4°C, 14–16 hr) and eluted from the gel by homogenization in 7–8 ml of 1× NaCl/Cit, followed by incubation at 38°C for 45 min. The supernatant was collected by centrifugation, the sediment was reextracted with 2–3 ml of 1× NaCl/Cit, and the combined supernatant was extracted with an equal volume of Sevag to remove gel contaminants. DNA was recovered by ethanol precipitation and dissolved in 50 μl of water. Average yields of 82-, 125-, 179-, 225-, and 370-bp fragments were 2.8, 3.4, 5.6, 4.3, and 12 μg, respectively, as estimated spectrophotometrically.

^{32}P Postlabeling Assay. DNA and DNA fragments were digested with a mixture of micrococcal nuclease and spleen phosphodiesterase to deoxynucleoside 3′-monophosphates, which were then converted to 5′-^{32}P-labeled deoxynucleoside 3′,5′-bisphosphates by T4 polynucleotide kinase-catalyzed transfer of [^{32}P]phosphate from [γ-^{32}P]ATP. The ^{32}P adducts were analyzed by a four-directional TLC procedure (21) with modifications (26). Specific conditions are described in the legend of Fig. 2. To calculate adduct levels, total nucleotides were analyzed by one-dimensional PEI-cellulose TLC after appropriate dilution of the labeled digest (26). Adduct levels were determined as described (26). Calculations were done according to relative adduct labeling (RAL)

$$= \frac{\text{cpm in adduct nucleotide(s)}}{\text{cpm in total nucleotides}} \times \frac{1}{\text{dilution factor}}.$$

The RAL values were then translated into fmol adducts per μg of DNA by multiplying RAL × 10^7 × 0.3, assuming 1 μg of DNA = 0.3 × 10^7 fmol of nucleotides (26).

RESULTS

OH-AcNHFln Damage *in Vivo*. We measured the distribution of adducts in five *Hin*dIII repetitive DNA sequences and total hepatic DNA of OH-AcNHFln-treated rats by anion-exchange PEI-cellulose TLC analysis of ^{32}P-labeled adduct nucleotides. As reported elsewhere (26), the ^{32}P fingerprinting analysis of DNA modified as described above showed two acetylated [*N*-acetyl-*N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AcNHFln) and 3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene (dG-N^2-AcNHFln)] and one deacetylated [*N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF)] known adducts as well as a previously unobserved acetylated adenine derivative and four chromatographically related unknown acetylated derivatives. The latter adducts have recently been characterized as the undigested dinucleotides of the structure dpXpNp, where X is dG-C8-AcNHFln and N is A, T, C, or G (unpublished observations).

We have previously reported that digestion of rat DNA with *Hin*dIII restriction endonuclease and polyacrylamide gel electrophoresis shows discrete bands, as visualized by a sensitive silver-staining procedure (19), indicating the presence of repetitive DNA sequences. The DNA repeats contain only small (<10%–15%) amounts of other DNA sequences as background, as shown by sequence analysis for at least 179- and 370-bp repeats (19, 20). In the present study, we used ^{32}P-labeled authentic reference DNA fragments (Fig. 1) to isolate the DNA repeats from carcinogen-treated and untreated DNAs. Fig. 2 shows the fingerprints obtained from total DNA (Fig. 2*a*) and DNA repeats (Fig. 2 *b–f*) in rats killed 1 day after treatment.

Qualitatively, no differences were observed between the DNA-bound metabolites in the DNA repeats and total DNA. However, striking differences were noticeable in the relative intensities of several adduct spots in the total DNA fingerprint and in fingerprints obtained from DNA repeats. The distribution of lesions were obtained by Čerenkov counting of adduct and normal nucleotides and is displayed in Fig. 3*a*. An examination of the data revealed that, while 82- and 125-bp repeats contained the same concentration of adducts as total DNA, the total binding levels were 13.8, 2.0, and 3.0 times higher in 179-, 225-, and 370-bp repeats, respectively. In 82- and 125-bp repeats, practically no differences were observed in the concentration of any of the individual adducts as compared to their respective values in total DNA. The concentration of each of dG-C8-AcNHFln and dG-C8-AF in 225- and 370-bp repeats were found to be ≈2 and 3–3.5 times higher, respectively, than in total DNA. The respective increase in the concentration of dG-N^2-AcNHFln in these two repeats was 1.5 and 2 times. More striking differences in the adduct distribution were found in the 179-bp repeat. The concentration of various acetylated adducts—namely, no. 6 (an adenine derivative), dG-C8-AcNHFln, and dG-N^2-AcNHFln—were found to be ≈19, 36, and 10 times higher, respectively, than in total DNA, but no difference

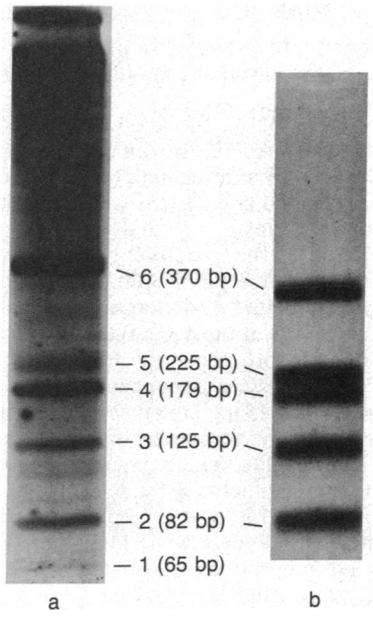

FIG. 1. Fractionation of reference 5′ ^{32}P-labeled *Hin*dIII repeats of rat hepatic DNA. (*a*) Total DNA digest; (*b*) purified DNA fragments. DNA (200 μg) was digested with *Hin*dIII restriction endonuclease (see text) and subjected to 5′ ^{32}P-labeling by T4 polynucleotide kinase under conditions described (25), except that carrier-free [γ-^{32}P]ATP (500 μCi; 1 Ci = 37 GBq) was used and the reaction was carried out in a 100-μl volume. The labeled fragments were resolved on a 5% polyacrylamide gel and detected by screen-enhanced autoradiographic exposure (1–2 min).

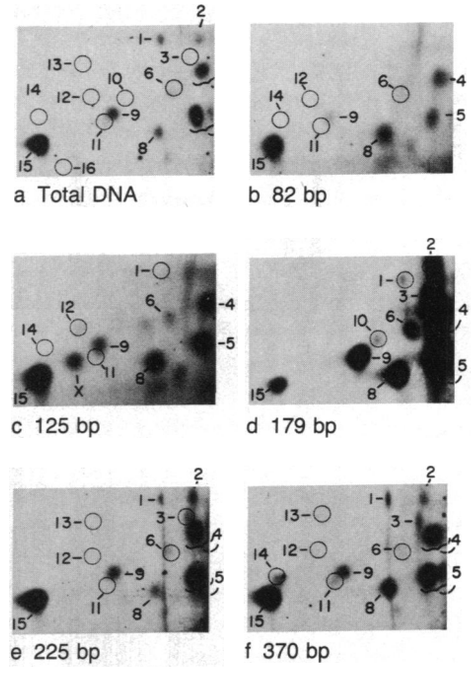

FIG. 2. ^{32}P fingerprints of the repetitive DNA fragments and total DNA from rat liver 1 day after a single dose of OH-AcNHFln (40 mg/kg). The fragments were generated by digestion of the hepatic DNA with *Hin*dIII endonuclease. Total DNA and DNA fragments were digested and subjected to ^{32}P-labeling and mapping by four-directional (D) PEI-cellulose TLC. About 300 μCi of labeled digest was chromatographed. Development was in 1 M LiCl (D1) and 2.5 M ammonium formate (pH 3.5) (D2), resulting in the removal of normal nucleotides and $^{32}P_i$, while the adducts retained at or close to the origin were then resolved by development in 3 M lithium formate/7 M urea, pH 3.5 (D3), followed by 0.6 M LiCl/0.5 M Tris·HCl/7 M urea, pH 8.0 (D4). The chromatogram was finally developed in the direction of D4 in 0.35 M $MgCl_2$ (26). The D3 was from bottom to top, and D4 was from left to right; D1 and D2 were opposite D3 and D4, respectively (21). Screen-enhanced autoradiography was at −80°C for 8 hr. Spots requiring extended exposure for detection have been circled. Unmarked spots denote background contaminants that were also present in DNA from untreated rats. Spots 8, 9, and 15 are 3′,5′-bisphosphates of dG-C8-AcNHFln, dG-N^2-AcNHFln, and dG-C8-AF, respectively, and spots 11 and 12 are the ring-opened forms of dG-C8-AF (26). Spots 4 and 5 represent four acetylated dinucleotides of the structure dpXpNp, where X is dG-C8-AcNHFln and N is A, T, C or G (see *Results*). Spot 6, a tentatively identified acetylated adenine derivative (26). Other spots represent unknowns. Spot marked X in *c*, representing <5% of the total adducts, was observed repeatedly from this DNA fragment and was absent in control DNA.

was found in the concentration of dG-C8-AF. We also analyzed ^{32}P base analysis (21) of these repetitive fragments to find out whether the preferential binding observed here was due to higher guanine content. The data presented in Fig. 3*a* showed that this was not the case, as DNA repeats (19.5%–20.8%) in fact contained slightly lower guanine content than total DNA (22.3%).

To determine the extent of repair in the specific sequences, adduct distribution was examined after a 9-day treatment. Three main adducts (dG-C8-AcNHFln, dG-N^2-AcNHFln, and dG-C8-AF) were detected in the fingerprints obtained from total DNA as well as DNA repeats (not shown). Evaluation of the adduct levels indicated a somewhat preferential binding to all five repeats (Fig. 3*b*). The 225-bp repeat showed 1.7 times as much adducts as total DNA, while the other four repeats showed 1.3–1.4 times as much adducts. A comparison of the total adduct concentrations measured after 1 day and 9 days indicated that adduct removal occurred in the order 179 bp > 370 bp > 225 bp > total DNA > 82 bp > 125 bp. The ratios of C8-AcNHFln/N^2-AcNHFln lesions in the various repeats 82 bp (6.2), 125 bp (6.8), 179 bp (16.5), 225 bp (5.4), 370 bp (7.7), and total DNA (4.4), dropped to ≈0.5 in each case, indicating that the relative excision rates of the two lesions were comparable in total DNA and various repeats, except the 179-bp repeat in which C8-AcNHFln lesions were removed much more rapidly than N^2-AcNHFln lesions. The ratios of C8-AF/N^2-AcNHFln lesions in total DNA, 82-bp repeat, and 370-bp repeat dropped from the respective values of ≈7.5, 6, and 10 to a value of 2 in each case and from a value of 7 to 4 in the case of the 125-bp repeat and from 11 to 2.5 in the case of the 225-bp repeat. These results indicated that C8-AF lesions were excised from total DNA as well as repetitive sequences more efficiently than N^2-AcNHFln lesions, but the extent of excisions varied. In the 179-bp repeat, however, C8-AF lesions appeared to be removed less efficiently than N^2-AcNHFln lesions, as suggested by a significant increase (from 0.7 to 2.8) in the ratio of C8-AF/N^2-AcNHFln.

AcO-AcNHFln Damage *in Vitro*. The adduct distribution in rat hepatic DNA was also examined after reaction *in vitro* with this ultimate carcinogen to determine whether the preferential binding *in vivo* to the repetitive DNA sequences occurred because of some chromatin structural effects. This metabolite binds mainly to the C8 and N^2 positions of guanine and in trace amounts to adenine residues in DNA (26, 27). DNA samples in which 0.5%–2% of the nucleotides were modified with AcNHFln were found unsuitable for the present study because of the poor yields of the *Hin*dIII repetitive fragments. This was possibly due to the formation of localized denatured regions (28), which were not cleaved by the double-strand-specific restriction enzyme. A typical DNA preparation used here contained 0.14% AcNHFln-modified nucleotides. No qualitative differences were observed in the ^{32}P fingerprints obtained from total DNA and

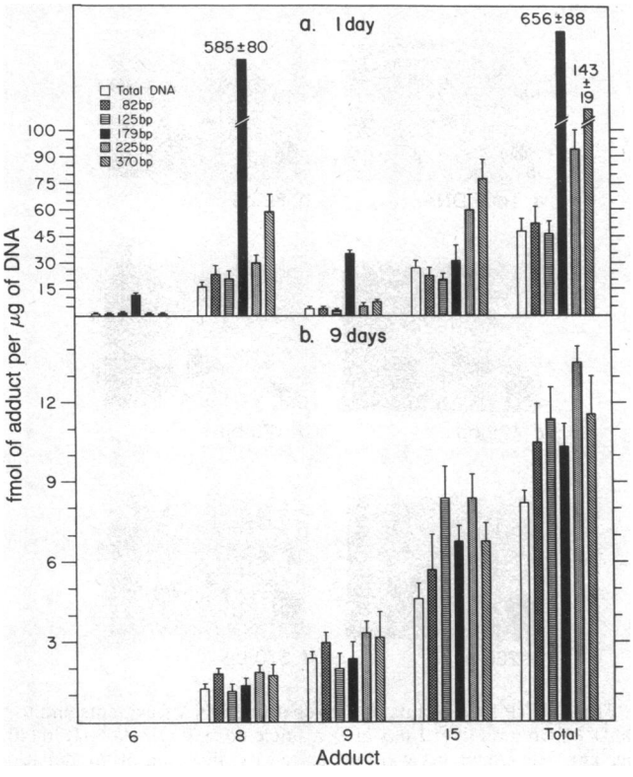

FIG. 3. Distribution of adducts in *Hin*dIII repetitive DNA sequences and total DNA of rats dosed with OH-AcNHFln for the indicated lengths of time. Adduct concentrations were evaluated as described in text. The data represent an average of 3–4 analyses from one batch of treated animals. Total concentration of dG-C8-AcNHFln (spot 8) was obtained by adding radioactivity in spots 4 and 5 to spot 8 radioactivity. Radioactivity in spots 11 and 12 was added to spot 15 (dG-C8-AF) radioactivity, as they were ring-opened forms of the latter. Spot 9, dG-N^2-AcNHFln. Minor spots (1–3, 7, 10, 13, 14, and 16; Fig. 2) comprise ≈10% of the total adduct radioactivity and are not included here. The contents of the principal reacting base guanine in the repetitive sequences and total DNA were 19.7% (82 bp), 20.0% (125 bp), 20.8% (179 bp), 19.7% (225 bp), 19.5% (370 bp), and 22.3% (total DNA).

179-, 225-, and 370-bp repeats (not shown). Quantitatively, the total adduct concentrations in fmol per μg of DNA were calculated to be 4110 (total DNA), 3420 (179 bp), 3270 (225 bp), and 2910 (370 bp), indicating a more or less random distribution. The specific adduct concentrations in the repetitive sequences and total DNA were also comparable. Similar conclusions were drawn when another DNA preparation with 0.026% AcNHFln-modified nucleotides was used. These results indicate that the preferential binding to the repetitive DNA sequences observed *in vivo* may be related to their organization in heterochromatin and not to any sequence specificity.

DISCUSSION

The results described in this communication have shown a nonrandom distribution of the OH-AcNHFln adducts in *Hin*dIII rat repetitive DNA sequences *in vivo*. Initially, some sequences (179, 225, and 370 bp) contained 2–14 times as much OH-AcNHFln adducts as total DNA, while others (82 and 125 bp) contained similar levels. Nine days after treatment, however, the adduct enrichment was less pronounced; all five repeats showed 1.3–1.7 times higher adduct concentrations than in total DNA. We have demonstrated that this reflects a differential excision of adducts. In contrast, DNA reacting *in vitro* with an active metabolite (AcO-AcNHFln) showed a random binding. Taken together, these results suggest that the nonrandom binding to the *Hin*dIII repeats may be a function of the chromatin structure in which they are organized.

In male rat liver DNA, the acetylated adducts (dG-C8-AcNHFln and dG-N^2-AcNHFln) are thought to be generated from a reactive sulfate intermediate (e.g., see ref. 29), while the major deacetylated adduct (dG-C8-AF) probably results from an *N,O*-acetyltransferase-catalyzed product (30–32) or deacylation to give the reactive species, *N*-hydroxy-2-aminofluorene (33). A striking initial enhancement of the various acetylated adducts (adducts 6, 8, and 9; Fig. 3*a*), but a lack of enhancement of the deacetylated derivative (adduct 15; Fig. 3*a*) in a 179-bp repeat and to a lesser extent in 82- and 125-bp repeats suggest dependence on the proximity of the respective metabolizing enzymes. It is possible that the enzyme(s) inducing the formation of the acetylated derivatives are enriched, while the enzyme inducing the formation of the deacetylated adduct is deficient in the chromatin fraction(s) in which 82-, 125-, and 179-bp repeats are organized. The latter enzyme is known to be highly species and tissue specific (32, 34), but nothing is known about its distribution within a cell type. The deacetylated adduct is considered quite stable (31) unless it is subjected to long (15–20 hr) exposure to pH 9 (27) or pH <6 as experienced by us. Since all repetitive fragments were isolated and assayed in parallel, any nonspecific loss of this adduct from these three repeats during the experimental manipulation appears highly unlikely.

A random distribution of lesions has been shown in DNAs of different degrees of repetitiveness in human cells exposed to UV_{254} or AcO-AcNHFln (35) and mouse skin exposed to 9,10-dimethyl-1,2-benzanthracene (36). As to the distribution of lesions in specific cellular DNA sequences, it has been reported that, whereas damage in cultured African green monkey cells by UV_{254} resulted in the same frequency of pyrimidine dimers in α and non-α sequences, the repair of chemical adducts formed with the DNA damaging agents AcO-AcNHFln, furocoumarins, and aflatoxin B1 was lower in α-DNA than in bulk DNA (37, 38). These studies suggested that the repair of different kinds of DNA damage could be affected to different extents by some property of this tandemly repeated heterochromatic DNA (37). The nonrandom distribution of both acetylated and nonacetylated adducts in the various rat *Hin*dIII repeats shown in the present study also suggest dependence on the nuclear organization of these specific sequences. Like monkey α-DNA, which showed the same initial binding as total DNA but deficient repair, the 82- and 125-bp rat repeats showed similar initial binding but 30%–40% deficient repair. On the other hand, the 179- and 370-bp rat repeats showed significantly higher initial binding, and so was the rapid removal; the 225-bp rat repeat showed higher initial binding but the excision repair paralleled that in total DNA. It may be pointed out that, although 179-bp rat α-type repeat and 172-bp monkey α-DNA exhibit a significant (37%) sequence homology (19), they are very different with respect to DNA damage and excision repair. It is reasonable to assume that this difference may be due to their different organization in chromatin. For instance, monkey α-DNA is arranged in long tandem arrays and comprises 15%–20% of the total nuclear DNA (e.g., see ref. 39), while 179-bp rat sequence appears to be organized in small tandem arrays and corresponds to <1% of the genomic weight (19).

The repetitive sequences are located primarily in the relatively genetically inert heterochromatin (e.g., see ref. 40). The functions of the large collection of repeated DNA families have not been elucidated thus far. However, several functions have been suggested, including involvement in chromosome pairing, control of gene expression, participation in DNA replication, and processing of messenger RNA

precursors (40–42). The abundance of carcinogen binding to DNA repeats can be expected to influence one or more of the above putative functions. It will be interesting to determine whether repetitive sequences, in general, are preferential targets for other classes of carcinogens also.

The author thanks Drs. Frederick A. Beland and Harold C. Smith for critical reading of the manuscript and Kurt Randerath for providing laboratory facilities. The excellent technical assistance of Nutan R. Dighe is acknowledged with gratitude. I also thank Linda M. Whiteman for typing the manuscript. OH-AcNHFln and AcO-AcNHFln were obtained through the courtesy of the National Cancer Institute Chemical Carcinogen Reference Standard Repository. This work was supported by National Cancer Institute Grant CA-30606.

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