Distribution of Deoxyribonucleic Acid Repair Synthesis among Repetitive and Unique Sequences in the Human Diploid Genome†

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ABSTRACT: DNA repair synthesis was studied in contact-inhibited (non-S-phase) human diploid fibroblasts (WI-38) after damage primarily to pyrimidine bases (ultraviolet radiation, 254 nm) or purine bases (*N*-acetoxy-2-acetylaminofluorene or 7-bromomethylbenz[*a*]anthracene). The distribution of repair synthesis among sequences of different degrees of repetitiveness was examined by following the reassociation kinetics of sheared (450 nucleotide length), denatured DNAs labeled with [^{3}H]thymidine during repair synthesis. DNAs labeled during replicative synthesis served as standards. The kinetics of reassociation for these DNAs were identical between C_0t 0.01 and 10,000 (mol sec)/l. and suggested that families of sequences of all degrees of repetitiveness are repairable or partially repairable after either type of damage. Reassociated DNAs were subjected to thermal elution chromatography and found to have similar elution profiles. These data suggested that the similarity of the reassociation kinetics was not the result of artifacts such as the failure of hydroxylapatite to distinguish between poorly matched and well-matched sequences. Experiments in which cells were damaged over a 100-fold dose range with *N*-acetoxy-2-acetylaminofluorene (1–100 μM) and reassociated to C_0t 100 revealed at most only a small shift in the distribution of repair synthesis from repeated to unique sequences. Calculations of the amount of damage at the doses used and its distribution among the DNA fragments used for these studies demonstrated that no interference with strand reassociation would be expected in these experiments. The results of the present study suggest that DNA damage in human cells, involving either purines or pyrimidines, is repairable to about the same extent in both unique and repetitive sequences.

It is now well established that mammalian cells of many origins can repair DNA damage induced by a wide variety of agents (for references, see Cleaver, 1973, 1974; Legator and Flamm, 1973; Lieberman, 1974); little is known, however, about the functional significance of repair, especially as it relates to gene expression, mutagenesis, or carcinogenesis. One approach which might help clarify the significance of repair synthesis is an analysis of its intragenomal distribution (Lieberman and Poirier, 1974). Although the data are still incomplete and largely inferential, eukaryotic DNAs appear to consist of families of sequences which have different cellular functions (*e.g.*, Britten and Kohne, 1968; Britten and Davidson, 1969; Brown *et al.*, 1972; Flamm, 1972; Davidson *et al.*, 1973). It would be of interest to know whether all such sequences are repairable or if repair is selective. A previous investigation of repair synthesis in cultured mouse cells demonstrated approximately as much repair synthesis on a specific activity basis in satellite DNA as in the remainder of the genome (Lieberman and Poirier, 1974). A report from another laboratory indicated that there is uniform distribution of repair synthesis among repetitive and unique sequences in HeLa cells damaged with ultraviolet radiation (Meltz and Painter, 1973).

We here report an analysis of the distribution of repair synthesis among families of repetitive and unique sequences from the human diploid genome. To simplify the analysis, a uniform cell population, consisting of confluent (contact-inhibited), diploid fibroblasts (WI-38), was used. With this system rapid changes in nuclear physiology which might be associated with changes in gene expression or the cell cycle (Rovera *et al.*, 1971) were avoided. DNA repair synthesis was studied in response to agents (ultraviolet radiation, NA-AAF, and 7BrMeBA)[1] which produce relatively well-characterized damage to purine and pyrimidine bases (Miller *et al.*, 1966; Setlow and Carrier, 1966; Kriek *et al.*, 1967; Dipple *et al.*, 1971).

Materials and Methods

Preparation of DNAs Labeled during Repair Synthesis. The details of methods have been published elsewhere (Lieberman and Poirier, 1973). In brief, confluent WI-38 human, diploid fibroblasts (passage 22–26) are almost devoid of S-phase cells, and remaining replicative synthesis may be suppressed by the addition of hydroxyurea. If such cells are treated with uv radiation or electrophilic chemicals (Miller, 1970; Dipple *et al.*, 1971) and[^{3}H]dT is added, 95–98% of the incorporation (*e.g.*, Figure 1) occurs as the result of repair synthesis, except under conditions of minimal damage in which about 90% of the incorporation is attributable to repair synthesis (*e.g.*, Figure 4). In the present study [*methyl*-^{3}H]dT (5–20 μCi/ml; 50 Ci/mmol) was added 30–40 min after hydroxyurea (final concentration 10 mM) and 20 min after the addition of the damaging agent. The following agents were used: ultraviolet radiation (λ 254 nm, at a flux of 11 erg mm^{-2} sec^{-1} and a fluence of 200 erg/mm^2; 20 J/m^2); NA-AAF at a final concentration of 1–100 μM (provided by the Standard Reference Compound Bank of the Carcinogenesis Program; National Cancer Institute); 7BrMeBA at a final concentration of 1 μM (a gift of Dr. A. Dipple). Two or five hours later cells were harvested, and DNA was prepared from crude nuclei by CsCl centrifugation as pre-

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[1] Abbreviations used are: 7BrMeBA, 7-bromomethylbenz[*a*]anthracene; NA-AAF, *N*-acetoxy-2-acetylaminofluorene; $T_{E,50\%}$, midpoint of a thermal elution profile on hydroxylapatite.

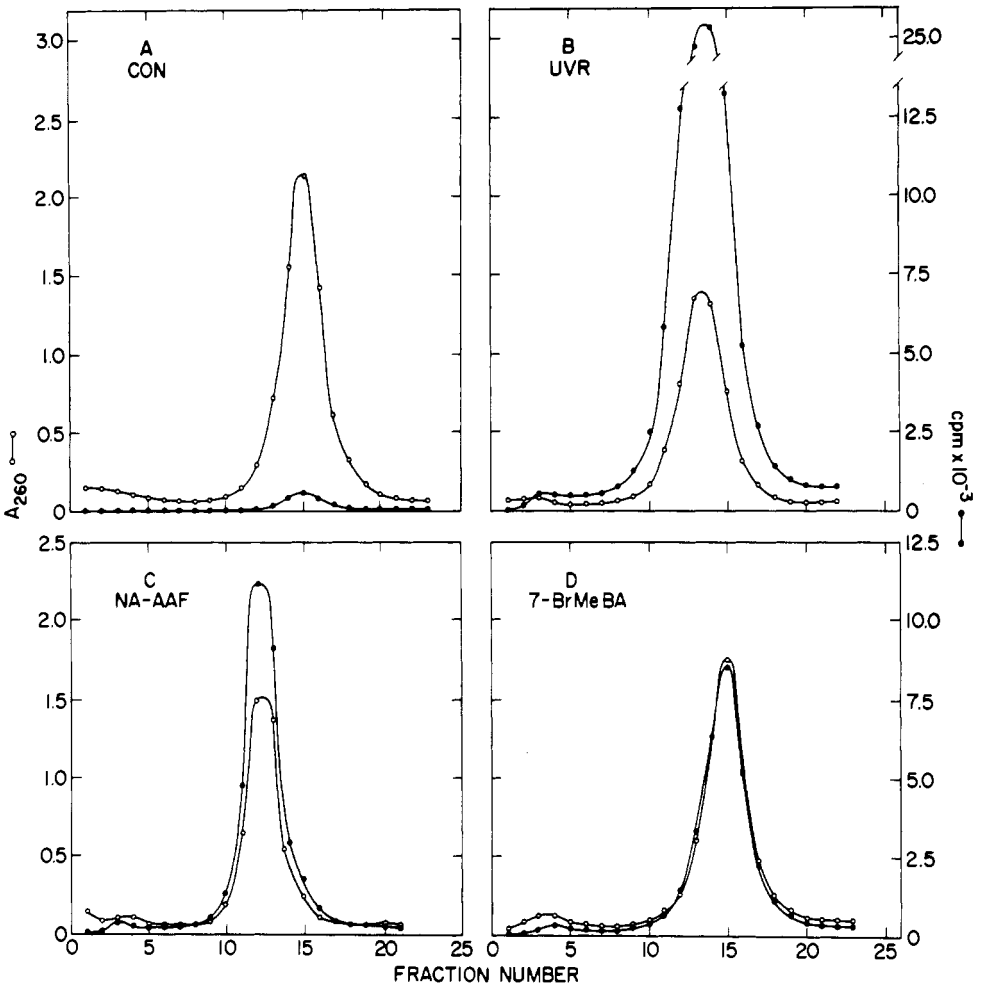

FIGURE 1: Repair synthesis in confluent human diploid fibroblasts. Cells were treated with hydroxyurea (10 mM final concentration) followed 20 min later by ultraviolet radiation (200 erg/mm^2) (B); NA-AAF (10 μM final concentration) (C); or 7BrMeBA (1 μM final concentration) (D). Control cells (A) received only hydroxyurea; 40 min after hydroxyurea, cells were treated with [*methyl*-^{3}H]thymidine (8.7 μCi/ml; 50 Ci/mol) for 2 hr. Preparative CsCl gradients were used to extract DNA from detergent-prepared nuclei (Lieberman and Poirier, 1973). The initial density of CsCl was adjusted to 1.710 in 0.01 M Tris buffer (pH 7.5); fraction 1 represents the bottom of the gradient. Samples of 0.36 ml were diluted with 0.20 ml of H_2O and read at 260 nm. Aliquots of 0.1 ml were counted in Instagel at 30% efficiency. Specific activities of the samples were (A) control, 88.9 dpm/μg; (B) repair after ultraviolet radiation, 6624 dpm/μg; (C) repair after NA-AAF, 2710 dpm/μg; (D) repair after 7BrMeBA, 1625 dpm/μg.

viously described (Flamm *et al.*, 1972; Lieberman and Poirier, 1973) or occasionally by a phenol-*m*-cresol method (see below). Both types of preparation gave similar results in the experiments described below. These DNAs had specific activities of 1500–8000 dpm/μg.

Preparation of DNA Labeled during Replicative Synthesis. Replicated DNAs which served as a standard for these experiments were prepared from asynchronous cells labeled with [^{3}H]dT (0.1 μCi/ml; 6.7 Ci/mmol) for 6–12 hr and had specific activities in the range of 3000–12,000 dpm/μg.

Preparation of Human Liver DNA. Since large amounts of DNA were needed to "drive" reactions to high C_0t, DNA was prepared from human liver by a modified phenol-*m*-cresol method (Kirby and Cook, 1967). Initial homogenization was in a Waring Blender for 2 min, at 4° and low speed setting. Deproteination after RNase treatment was carried out by chloroform–isoamyl alcohol (20:1; v/v). Glycogen was removed by centrifugation at 40,000 rpm in a 60 Ti Beckman rotor for 1 hr (Dingman and Sporn, 1962).

When cultured cells were used, DNA was prepared from detergent-prepared nuclei either by a single extraction or by the full method with the RNase and chloroform–isoamyl alcohol steps. These two types of preparation resulted in DNA which behaved similarly to that prepared by the CsCl method. The removal of glycogen by centrifugation was omitted in preparing DNA from cultured cells.

Reassociation of DNAs Labeled during Repair and Replication. In one series of experiments C_0t curves were run as follows: DNAs from cell culture, labeled during repair or replicative synthesis, were dialyzed into 0.14 M sodium phosphate buffer (pH 6.8) and mixed with human liver DNA in the same buffer to give 12 mg of DNA in 30 ml. These were sonicated (Hoyer *et al.*, 1973) to yield a single strand length of 450 nucleotides, dialyzed against distilled water, concentrated to 10 mg/ml, and adjusted to 0.14 M sodium phosphate. Samples were denatured by heating to 100° for 6 min. They were reassociated in 0.05–5 ml volumes at 60° for varying periods of time to obtain C_0t values from 0.01 to 10,000 (mol sec)/l. calculated from A_{260} native DNA/2 hours (Kohne and Britten, 1972). DNA concentrations in reassociation vessels ranged from 0.1 to 10 mg/ml. Samples were diluted to 8 ml, frozen in an alcohol–Dry Ice bath, and stored at −70° for analysis (Rosen *et al.*, 1973); 500-μg samples (75,000–300,000 dpm) were fractionated into single- and double-stranded DNA by passage over a 3-ml column of freshly boiled hydroxylapatite (Bio-Gel HTP, Bio-Rad, Richmond, Calif.) at 60°. The column was washed three times with 8 ml of 0.14 M sodium phosphate (containing 0.02% sodium dodecyl sulfate), followed by

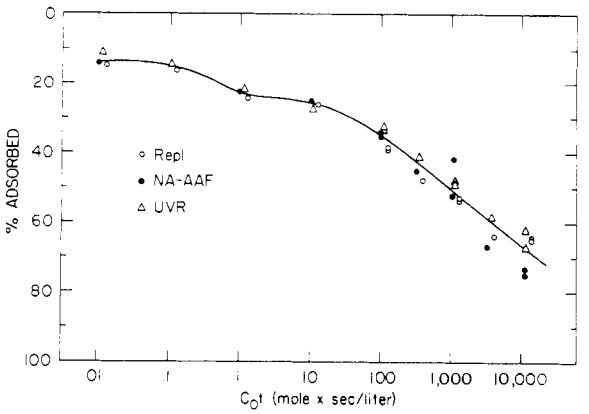

FIGURE 2: Reassociation kinetics of DNAs labeled during repair or replicative synthesis. DNA samples from cells undergoing repair synthesis after damage with NA-AAF (●) or ultraviolet radiation (Δ) were reannealed to different C_0t values as described in the text. A DNA with label introduced during replication (○) served as a control. Ordinate is % adsorbed to hydroxylapatite at 60° in 0.14 M sodium phosphate buffer (pH 6.8).

four washes with 8 ml of 0.4 M sodium phosphate (containing 0.02% sodium dodecyl sulfate) to elute double-stranded material. The hydroxylapatite was then dissolved in 1 N HCl. Aliquots (7.14 ml) of the 0.14 M washes and 2 ml of the 0.4 M washes plus 5.14 ml of H_2O were counted in 10 ml of Instagel (Packard, Downers Grove, Ill.) at an efficiency of 28%. From these data the percentage of single- and double-stranded DNA in each sample was calculated. Recovery of DNA from the column was greater than 99.5% as judged by counting the acid digest of the hydroxylapatite (22% eff.).

In another series of experiments, repaired and replicated DNAs were separated into repetitive and unique sequences by reassociating to C_0t 100 without the addition of exogenous liver DNA. The procedure was similar to that described above. Sheared, heat-denatured samples were incubated at 0.1 mg/ml in 2-ml aliquots for 100 hr. Storage and fractionation were as above.

Thermal Elution Chromatography of Reassociated DNAs. Three columns were run in parallel, each monitored by a calibrated thermometer with an expanded scale which was read to the nearest 0.1°. Portions of the first and second 0.4 M sodium phosphate wash were pooled, diluted to 0.10 M buffer, and passed over hydroxylapatite at room temperature; 8 ml of 0.10 M buffer containing 0.02% sodium dodecyl sulfate was added, and the column temperature was raised to 60°. After 1–2-min equilibration the wash was collected. The effluent from sample application and the wash were counted (8 ml vol. + 10 ml of Instagel). In most cases over 99% of the material bound to hydroxylapatite at 60°. This procedure was repeated through 97.5°; the hydroxylapatite was then dissolved in 1 N HCl, and an aliquot was counted. Cumulative percentage eluted was plotted as a function of temperature and the midpoint of the elution profile ($T_{E,50\%}$) was estimated graphically; 2.5° increments were used close to the $T_{E,50\%}$ and 5° increments well below and above the $T_{E,50\%}$. Values were compared to the $T_{E,50\%}$ of sonicated, native DNA with a [^{3}H]dT label introduced during replication (M. W. Lieberman and M. C. Poirier, manuscript in preparation), and the difference between these values ($\Delta T_{E,50\%}$) was used to calculate the percentage of mismatch, based on the observation that a 1° lowering of the $T_{E,50\%}$ represents about a 1.5% mismatch (Laird *et al.*, 1969).

Results

Repair Synthesis in Confluent WI-38 Cells. Repair synthesis was measured by following the incorporation of [^{3}H]dT into the DNA of non-S-phase cells. Hydroxyurea was added to suppress replicative synthesis in cells escaping contact inhibition (Painter and Cleaver, 1967; Roberts *et al.*, 1971; Lieberman and Poirier, 1973, 1974). It has been suggested that this system may have advantages over a bromodeoxyuridine density labeling system when the repaired DNAs are to be further analyzed since no foreign nucleotides (*e.g.*, bromouracil deoxyribonucleotide) are present (Lieberman, 1974). Figure 1 presents CsCl gradients of DNA from cells undergoing repair synthesis. From the specific activities of the DNAs (legend to Figure 1) it is apparent that 95–98% of the label introduced in this manner is the result of DNA repair synthesis; 2–5% represents the upper limit of contamination since the small amount of remaining S-phase synthesis in hydroxyurea-treated cells (Figure 1A) is probably further suppressed by these agents (*e.g.*, Lieberman and Poirier, 1973, 1974). Parenthetically, it may be noted that one may measure the repair of purine damage with [^{3}H]thymidine because both purines and pyrimidines are inserted into repair patches after damage to purine bases (Cleaver, 1973; Lieberman and Poirier, 1973).

Reassociation Kinetics of DNAs Labeled during Repair or Replicative Synthesis. DNAs labeled during repair synthesis from cells treated with ultraviolet radiation (200 erg/mm^2) or NA-AAF (10 μM) were obtained from the CsCl gradients in Figure 1 and similar gradients. These and a DNA labeled during replicative synthesis were reassociated to different C_0t values in a reaction driven by unlabeled human liver DNA (Figure 2). As originally pointed out by Britten and Kohne (1968), C_0t curves of eukaryotic DNAs are a composite of reassociation rates of many families of unique and repeated sequences. The data indicate that under our annealing conditions (60°; 0.14 M sodium phosphate) about 10–14% of the human genome consists of rapidly reassociating (highly repetitious) sequences (reassociated by C_0t 0.01), about 20% of the genome consists of families of moderate repetition ($0.01 < C_0t \simeq 100$), and the remainder of the genome (approximately 65%) is made up of "unique" sequences and sequences of low repetition, operationally defined as those reassociated at $C_0t \geq 100$. These findings are similar to those reported by Saunders *et al.* (1972).

Inspection of Figure 2 reveals that within the limits of the technique DNAs labeled during repair synthesis have the same kinetics of reassociation as DNA labeled during replication. It appears that repair patches (*e.g.*, Cleaver, 1968; Regan *et al.*, 1971; Setlow and Regan, 1972) are distributed among DNA sequences of all degrees of repetitiveness. Damage to both pyrimidines (primarily thymine-containing dimers, Setlow and Carrier, 1966) and purines (primarily guanine adducts; Krieck *et al.*, 1967) is repairable in all such sequences.

Thermal Elution Chromatography of Reassociated DNA Sequences. Because it was not clear precisely how much variation in secondary structure hydroxylapatite discriminated against, it was important to demonstrate that the reassociated DNAs (fraction bound at 60°) from cells in repair synthesis and those in replicative synthesis were equivalent. Consequently, DNA samples reassociated to $C_0t \simeq 100$, 1000, and 10,000 were subjected to thermal elution chromatography. As illustrated in Figure 3, reassociated-repaired and reassociated-replicated DNAs had similar elution profiles at each C_0t value and eluted at a lower temperature than a native, sheared DNA marker. Table I presents the $T_{E,50\%}$ data derived from a series of hydroxylapatite columns and calculations of the per cent mismatch at different C_0t values. Note that for any C_0t value the $T_{E,50\%}$ data are similar. The per cent mismatch is least for

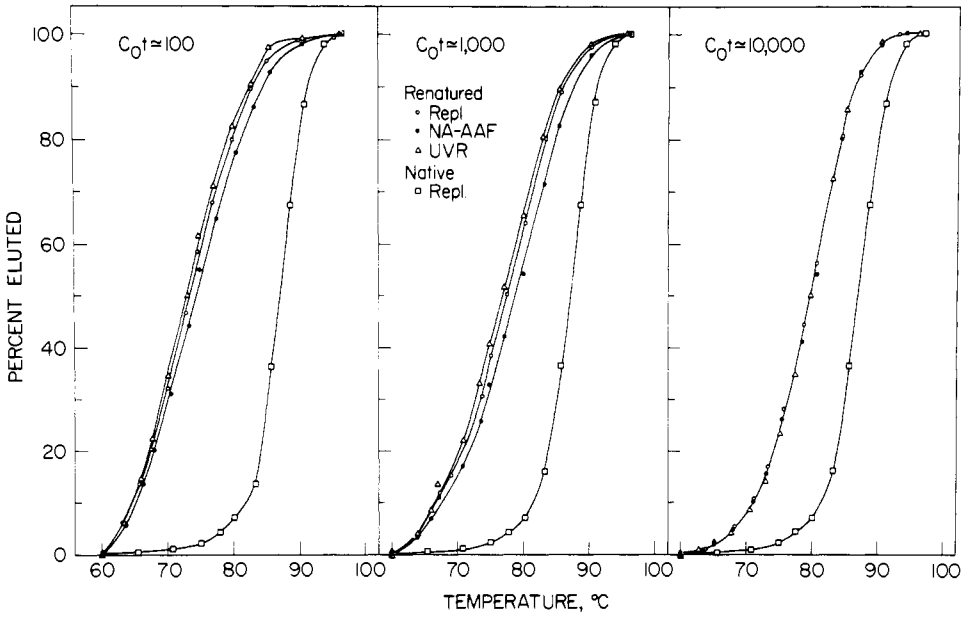

FIGURE 3: Thermal elution chromatography of reassociated DNAs. Reassociated DNA samples from Figure 2 were reloaded on hydroxylapatite columns after dilution to 0.10 M sodium phosphate and eluted as described in the text. Reassociated replicated DNA (○); reassociated DNA with label introduced during repair synthesis after NA-AAF (●), or ultraviolet radiation (△). Sheared, native, replicated DNA (□) is included as a standard. Left panel: DNAs reassociated to $C_0t \simeq 100$; center panel, DNAs reassociated to $C_0t \simeq 1000$; right panel, DNAs reassociated to $C_0t \simeq 10{,}000$. Ordinate is cumulative percentage eluted from hydroxylapatite.

C_0t 10,000 and greatest for C_0t 100. This finding may reflect the extent to which each fraction is made up of unique sequences and families of similar, but not identical sequences (*e.g.*, Britten and Davidson, 1969; Hough and Davidson, 1972). The $T_{E,50\%}$ data and the per cent mismatch calculations suggest that there is no fundamental difference in the degree or type of binding to hydroxylapatite of DNA labeled during repair or replicative synthesis and support the conclusions based on C_0t data presented in Figure 2.

Reassociation Kinetics of "Repaired DNAs" in the Absence of Exogenous (Liver) DNA. The distribution of repair synthesis between repetitive and unique sequences was examined in DNA from cells damaged with ultraviolet radiation (200 erg/mm^2), 7BrMeBA (1 μM), or NA-AAF (1–100 μM). DNA labeled during repair synthesis was prepared from these cultures, sheared and denatured as before, and reassociated to C_0t 100 in the absence of exogenous liver DNA. This C_0T value was chosen to separate DNA into "repetitious" and "unique" sequences on the basis of previous findings (Figure 2; Saunders *et al.*, 1972). Table II demonstrates that about the same proportion of DNA labeled by repair synthesis after treatment with ultraviolet radiation, 7BrMeBA, or NA-AAF or by replicative synthesis binds to hydroxylapatite after reassociating to C_0t 100. A slightly lower percentage of DNA reassociated to C_0t 100 is bound to hydroxylapatite in this set of experiments than in those presented in Figure 2; however, both sets of results are similar. These data support the conclusion that repair patches are present in both repetitive and unique DNA sequences. Examination of the $T_{E,50\%}$ and per cent mismatch (Table II) suggests slightly less mismatching at C_0t 100 in these experiments than in those presented in Figure 2 and Table I. Whether slight increases in the $T_{E,50\%}$ such as those seen with ultraviolet radiation or 7BrMeBA will prove real requires further study.

Reassociation Kinetics of DNAs from Cells Treated with Different Doses of NA-AAF and Showing Different Amounts of Repair Synthesis. A previous analysis of different amounts of repair synthesis induced in cultured mouse cells by uv radiation showed no difference in the distribution of repair synthesis between satellite and mainband DNA at different dose levels

TABLE I: Thermal Stability of Reassociated DNAs on Hydroxylapatite.

Description	$\sim C_0t$	$T_{E,50\%}$	$\Delta T_{E,50\%}$[c]	% Mismatch[d]
Replicated[a]	100	73.5	−13.9	21
Repair after NA-AAF[a]	100	74.2	−13.2	20
Repair after uv radiation[a]	100	73.0	−14.4	22
Replicated[a]	1,000	77.5	−9.9	15
Repair after NA-AAF[a]	1,000	78.7	−8.7	13
Repair after uv radiation[a]	1,000	76.9	−10.5	16
Replicated	10,000	79.9 ± 0.2[b]	−7.5	11
Repair after NA-AAF	10,000	80.5 ± 0.8[b]	−6.9	10
Repair after uv radiation[a]	10,000	80.6 ± 0.6[b]	−6.8	10

[a] Duplicates pooled. [b] ± range of duplicate determinations. [c] Compared to native, sheared DNA with a $T_{E,50\%} = 87.4 \pm 0.5$ (S.D.) (M. W. Lieberman and M. C. Poirier, manuscript in preparation). [d] Calculated from 1.5% mismatch/1° (Laird *et al.*, 1969).

TABLE II: Extent of Binding to Hydroxylapatite and Thermal Stabilities of DNAs Reassociated to C_0t 100.

Description[a]	Sample No.	% Bound to HAP	$T_{E,50\%}$	$\bar{X}T_{E,50\%}$	$\Delta T_{E,50\%}$[c]	% Mismatch[c]
Replicated	1	29.7	75.8	76.1	−11.3	17
	2	29.2	76.4 ± 0.2[b]			
Repair after 200 erg/mm² uv radiation	1	25.1	79.0	79.0	−8.4	13
	2	25.1	79.1			
Repair after 1 μM 7BrMeBA	1	27.2	78.2 ± 0.1	78.2	−9.2	14
Repair after 1 μM NA-AAF	1	31.3	77.8	77.8	−9.6	14
	2	24.7	77.9			
Repair after 3 μM NA-AAF	1	22.2	77.7	77.7	−9.7	15
Repair after 10 μM NA-AAF	1	24.8	76.6 ± 0.1	76.8	−10.6	16
	2	22.2	77.1 ± 0.1			
Repair after 100 μM NA-AAF	1	20.8	77.2 ± 0.1	77.0	−10.4	16
	2	20.4	76.9			

[a] All samples were reassociated to C_0t = 100. [b] ± range of duplicate determinations. [c] Calculated as in Table I.

(Lieberman and Poirier, 1974). An experiment similar in concept was designed utilizing WI-38 cells and NA-AAF as the damaging agent. Cells were exposed to 0, 1, 3, 10, or 100 μM NA-AAF, and repair synthesis was measured by following the incorporation of [³H]dT into DNA for 5 hr in the presence of hydroxyurea (Lieberman and Poirier, 1973). The distribution of repair synthesis between repetitive and unique DNA (defined as extent of reassociation at C_0t 100) was measured for DNA from the NA-AAF treated groups and compared to replicated DNA. The results of this experiment are seen in Figure 4. The top panel illustrates that a 10–30-fold increase in the amount of incorporation of [³H]dT into DNA in the presence of hydroxyurea is seen following treatment with NA-AAF. After these DNAs, labeled during repair synthesis, were sheared, denatured, and reassociated to C_0t 100, binding to hydroxylapatite was studied (Figure 4, lower panel; Table II). A similarly treated, replicated DNA served as a control. Over the dose range used, there is no substantial shift in the distribution of repair synthesis; whether, the small shift from repetitive to unique sequences seen at high doses is significant cannot be determined from the present data. However, it is unlikely to be the result of the inability of damaged DNA to reassociate properly since the $T_{E,50\%}$ values and per cent mismatching of these DNAs and controls are similar. In addition, it may be demonstrated (see Discussion) that there is probably too little damage present to interfere significantly with reassociation.

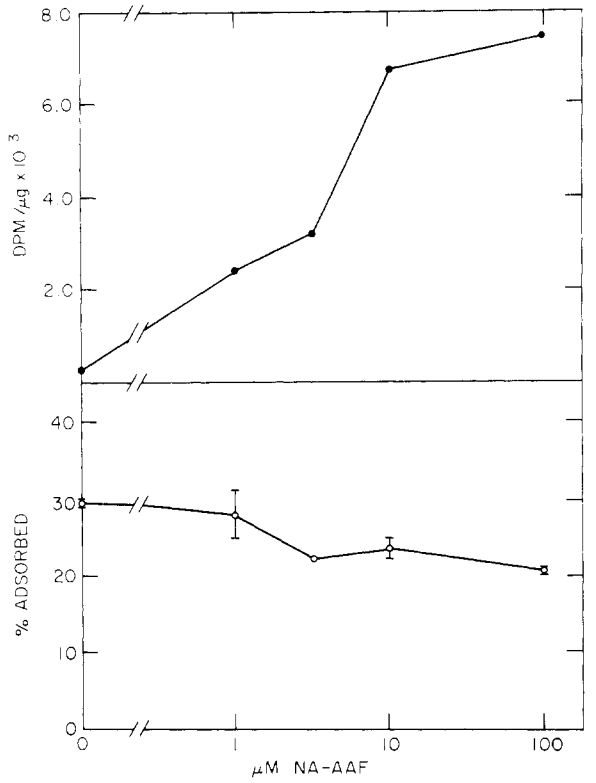

FIGURE 4: Amount and distribution of repair synthesis as a function of dose of NA-AAF. The upper panel presents data on the extent of repair synthesis after various doses of NA-AAF (●). Treatment of cells was similar to that described in Figure 1, except that 16 μCi/ml of [³H]dT was added for 5 hr. DNA was prepared by a phenol–*m*-cresol method (see text). The value at a zero dose of NA-AAF is the hydroxyurea control. The lower panel presents data on the binding of these DNAs to hydroxylapatite after shearing, denaturation, and reassociation to C_0t 100 (○). Bars represent the range of duplicate samples. The value at a zero dose of NA-AAF is from a replicated DNA similarly treated.

Discussion

DNA repair synthesis occurs in sequences of all degrees of repetitiveness in the genome of contact-inhibited human diploid fibroblasts. A recent study of repair synthesis in asynchronously growing HeLa cells damaged with ultraviolet radiation (400 erg/mm²; 254 nm) reached similar conclusions (Meltz and Painter, 1973). While there may well be substantial heterogeneity of response within any class of repeated sequences and large numbers of unrepaired sequences, it appears that the overall extent of repair synthesis for DNA sequences of different degrees of repetitiveness is about the same. Thermal elution chromatography data suggest that these results are probably not an artifact due to failure to discriminate between well-matched and poorly matched duplexes. Of interest is the observation that the distribution of repair synthesis is similar regardless of whether damage is primarily to purine moieties (guanine adducts) or to pyrimidine moieties (thymine-containing cyclobutyl dimers). Apparently a wide variety of DNA sequences of presumably different function and bearing different lesions are repairable in cells whose genome is highly restricted in terms of both transcription and replication (*e.g.*, Rovera *et al.*, 1971).

It is known that extensive base damage may affect the reassociation kinetics of DNA (Bonner *et al.*, 1973). A few calculations (illustrated here for ultraviolet radiation damage) indicate that under the conditions of the present study too little

damage is present to affect our results. One may first consider the likelihood of a dimer and a repair patch occurring on the same 450 nucleotide fragment. Assuming random damage and about 1 lesion/3000 nucleotides (calculated from Cleaver and Trosko, 1970), one may calculate that about 2% $(450/3000)^2$ of the sheared single-stranded pieces will contain two dimers. If one assumes that 20% of the dimers are removed at random in 5 hr (*i.e.*, are replaced by repair patches) and that the patch size is about 100 nucleotides (*e.g.*, Cleaver, 1968; Regan *et al.*, 1971), then the probability of an unrepaired dimer occurring on the same piece as a dimer undergoing repair is about 0.004 (0.02×0.2). Since the chances are about 3 in 4 that a 100 nucleotide patch will *not* include both dimers, then the probability of a repaired patch and an unexcised dimer occurring on a fragment to be reassociated is about 0.003. Thus, the situation in which a repair patch and a dimer occur on the same fragment can be ignored for practical purposes.

One may now consider the situation in which a dimer and a repair patch occur on different fragments. If exogenous carrier is added in excess (10–50-fold) to drive the reaction, the effect of dimer-containing strands on reassociation will be small: *i.e.*, while 15% (450/3000) of the pieces contain dimers, the addition of a tenfold excess of liver DNA reduces the amount to 1.5%. Since there is only 1 dimer/450 nucleotides (2 damaged bases/450 nucleotides or about 0.5% damage), even in the experiments run without the addition of liver DNA there would probably be little effect on reassociation (*i.e.*, only 15% of the fragments contain 0.5% damaged bases). Although, this approximation has not taken into account such factors as local distortion in the region of pyrimidine dimers, etc., it is clear that the presence of unexcised dimers will have at most a small effect on only a small percentage of the DNA. In agreement with this calculation are the above data which show no differences in the extent of reassociation between ultraviolet radiation damaged and control DNA.

Acknowledgments

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