Efficiency of Formation of Pyrimidine Dimers in SV40 Chromatin in Vitro†

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ABSTRACT: The efficiency of formation of pyrimidine dimers by 254-nm light was studied in mixtures of SV40 chromatin and DNA extracted from that chromatin. At high doses (beyond 380 J/m^2), fewer dimers are formed in chromatin than in DNA for a given dose of radiation. This difference is about 10% as saturation with pyrimidine dimers is approached at 6840 J/m^2. Conversely, at biologically repairable doses (up to 40 J/m^2, less than 2 dimers/genome), significantly more dimers are produced in the chromatin than in the DNA. A maximum increase of about 50% occurs at doses producing 0.5–20 dimers/genome. With isolated nucleosomes from this chromatin, a maximum increase in dimer formation of 77% was observed. Therefore, the increased dimer formation in the whole chromatin can be wholly accounted for in the nucleosome portion.

In order to understand DNA repair in eukaryotic cells, the effect of chromatin structure on DNA damage and repair enzymes must be investigated thoroughly. Repair DNA synthesis is reported to occur preferentially in nuclease-sensitive (noncore) DNA shortly after damage by alkylating agents (Bodell & Banarjee, 1979; Tlsty & Lieberman, 1978; Bodell, 1977; Oleson et al., 1979) or UV radiation (Cleaver, 1977; Williams & Friedberg, 1979; Smerdon & Lieberman, 1978; Smerdon et al., 1979). However, the extent to which repaired nucleotides shift into nuclease-resistant DNA is still unclear. There are at least three obvious explanations for the early repair label being in nuclease-sensitive DNA: (i) the nucleosome shields the DNA within it from damaging agents so that most of the damage is in the linker DNA; (ii) the DNA in nucleosomes is inaccessible to repair enzymes, and the nucleosomes migrate slowly if at all; (iii) the DNA repair process or the DNA damage removes or disrupts nucleosomes, making the DNA contained in them nuclease sensitive. Although many DNA damaging agents are reported to act preferentially on linker DNA (Jahn & Litman, 1977, 1979; Metzger et al., 1977; Ramanathan et al., 1976), the few studies done with agents either known to react preferentially with nucleosome core DNA or believed to damage core and linker DNA approximately equally have indicated that early repair label is still found in nuclease-sensitive DNA. These findings apparently rule out (i) above. Likewise, in studying the action of the carcinogen 7-(bromomethyl)benz[*a*]anthracene, Oleson and co-workers (1979) showed that most of the damage from this agent is in nucleosome core DNA, yet the damage is removed at the same rate from core and linker regions, and essentially all of the early repair label is in nuclease-sensitive DNA. This supports model iii and is especially interesting in view of a preliminary study which proposes that factors exist which specifically mediate the actions of repair enzymes in chromatin (Mortelmans et al., 1976).

Many of the studies of distribution of repair label in chromatin have employed UV radiation as a damaging agent. However, no careful study of the effect of chromatin on pyrimidine dimer formation at various UV doses has been published. Therefore, we have chosen to undertake such a study.

Materials and Methods

Preparation of SV40 Minichromosomes. Monkey kidney cell line CV1 was grown to confluence in half-gallon roller bottles in Dulbecco's modified Eagles medium plus 5% fetal calf serum and infected with SV40 strain 776 at a multiplicity of infection of 40–50. At 24-h postinfection, [*methyl*-^{3}H]-thymidine (15 Ci/mmol) was added to a concentration of 0.02 mCi/mL of medium. At 40-h postinfection, old medium and label were removed, and the cells were rinsed twice with phosphate-buffered saline (per liter: 8.0 g of NaCl, 0.2 g of KCl, 1.15 g of $Na_2HPO_4 \cdot 2H_2O$, and 0.2 g of KH_2PO_4) and twice with Tris–EDTA (10 mM Tris-HCl, pH 7.9, and 5 mM EDTA). Each bottle of cells was then scraped into 12.4 mL of Tris–EDTA, allowed to stand 30 min at 4 °C, and then lysed with five strokes in a Dounce homogenizer. Nuclei were then pelleted by centrifugation at 2100*g* for 5 min, resuspended in 30 mL of 10 mM Tris-HCl, pH 6.8, 10 mM EDTA, 0.13 M NaCl, and 0.25% Triton X-100, and centrifuged at 4750*g* for 5 min. The pellets were then resuspended in 3.1 mL of the above buffer titrated to pH 8.0, and suspensions were placed on a rotary table at 4 °C. The nuclear suspensions were made 0.1 M in phenylmethanesulfonyl fluoride (PMSF) after 30 min, then shaken for an additional 2.5 h, and collected by centrifugation at 2600*g* for 5 min. The nuclear extract was then passed over a Bio-Gel A-5m column (2 × 41 cm bed dimensions) equilibrated in buffer C [10 mM Tris-HCl, pH 7.5, 0.13 M NaCl, 0.2 mM EDTA, and 1.0 mM dithiothreitol (DTT)]. The minichromosomes eluted as a sharp peak coincident with the void volume (Figure 1) while free PMSF, Triton X-100, and proteins less than 5 × 10^6 daltons were included in the gel. All columns and tubes were glass that had been siliconized by treatment with dichlorodimethylsilane (5% in CCl_4). The chromatin was stored in ice and was stable for at least 4 weeks as judged by the sedimentation coefficient and percent form I DNA.

Preparation of SV40 DNA. DNA for each series of experiments was extracted from the purified chromatin to which it was being compared. To an aliquot of chromatin solution were added 0.2 volume of 5 M NaCl, 0.1 volume of 6% Sodium dodecyl sulfate ($NaDodSO_4$) in 0.1 M Tris-HCl, pH 7.5, 1.3 volumes of water-saturated phenol (adjusted to pH 8.0 by addition of Tris base), and 0.65 volume of chloroform–isoamyl alcohol (24:1). The sample was mixed by shaking 3 times and then centrifuged at 5000*g* for 5 min. The aqueous phase was reextracted with 1 volume of chloroform–isoamyl alcohol, centrifuged again, then concentrated to 0.5–0.6 mL by vacuum

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dialysis, and loaded onto a Sepharose CL-2B column (1.0 × 26 cm bed dimensions) equilibrated in buffer C. The column was run with a 25-cm head pressure at room temperature, and 1.0-mL fractions were collected. DNA eluted in a sharp peak in the void volume, whereas $NaDodSO_4$ was retarded by the column.

Micrococcal Nuclease Digestion of Chromatin. SV40 chromatin in buffer C was adjusted to 3.4 mM $CaCl_2$ with 1.0 M $CaCl_2$, and micrococcal nuclease was added to a concentration of 50 μg/mL. Digestion was carried out at 37 °C until 20% of the DNA was rendered acid soluble (9 min). The reaction was stopped by addition of 0.2 volume of 0.4 M EDTA. Mononucleosomes were then separated from polynucleosomes and small acid-soluble digestion products by chromatography on Bio-Gel A-5m as described by Shaw et al. (1976).

Pyrimidine Dimer Assays by Thin-Layer Chromatography. Aliquots of DNA or chromatin were placed in hydrolysis tubes and brought to 1 mL with H_2O. To each tube were added 25 μg of calf thymus DNA in 25 μL and then 1 volume of 20% trichloroacetic acid (Cl_3CCOOH). After 15 min at 0 °C, DNA was pelleted at 27 500*g* for 15 min. Each tube was drained briefly, and then 0.15 mL of 98% formic acid was added. The tubes were sealed and incubated at 175–180 °C for 1 h. After the tubes were cooled to room temperature, they were immersed into liquid nitrogen, their seals were broken, and formic acid was removed in an Evapomix vacuum evaporator. Each sample was taken up in 50 μL of H_2O and spotted onto a Polygram Sil G thin-layer plate (prewashed with H_2O). Chromatography was carried out as described by Goldman & Friedberg (1973) in ethyl acetate–1-propanol–H_2O (4:1:2). After the chromatogram was taped with Scotch tape, each lane was cut into 0.5-cm segments and each segment placed in a scintillation vial and eluted with 0.5 mL of H_2O for 1 h. An aliquot of 5 mL of aqueous fluor was then added, and the samples were counted. The data of Unrau et al. (1973) were used to calculate total pyrimidine dimers per SV40 genome from percent of thymine label in dimers.

Superhelical Nicking Assay with T4 UV Endonuclease. SV40 DNA containing pyrimidine dimers was treated with a several-fold excess of T4 UV endonuclease for 1 h at 37 °C in 0.025 M KPO_4, pH 7.4, 0.2 M NaCl, 0.088 mg/mL acetylated bovine serum albumin, and 1.0 mM EDTA. Reactions were stopped by addition of 1 volume of 0.1% $NaDodSO_4$. The superhelical nicking assay was then done as described by Kuhnlein et al. (1976) to obtain the number of nicks introduced per SV40 genome. It was assumed that the number of nicks introduced under these conditions was equal to the number of pyrimidine dimers present.

Irradiation of Chromatin–DNA or Nucleosome–DNA Mixtures. Minichromosomes and DNA extracted from them (both in buffer C) were mixed and irradiated in a 1-cm quartz cuvette at an exposure rate of 4.1 J/(m^2 s). The UV source was a Westinghouse Sterilamp G15T8, whose output was calibrated by potassium ferroxalate actinometry (Jagger, 1967) after an initial operation for 100 h to allow aging of the lamp. The lamp was "warmed up" for 20 min and its output checked with a Laterjet meter before each experiment. Optical densities at 254 nm were negligible so that exposure equaled dose; nevertheless, samples were mixed and irradiated together rather than separately to ensure that any small differences in dimer content were not due to protein absorption (internal filter effect) or light scattering. After irradiation, minichromosomes and DNA were separated by sedimentation through a 5–25% sucrose gradient for 3.5 h at 36 000 rpm in an SW41 rotor and collected from the tube bottom. Minichromosome peaks (60–65 S) and DNA peaks (21 S) were well separated. Aliquots were taken from the peak fractions to assay for pyrimidine dimer content. The T4 UV endonuclease assay was used for very low UV doses which produce around 0.5–2 dimers per SV40 genome. In this case, the irradiated SV40 chromatin was first phenol extracted.

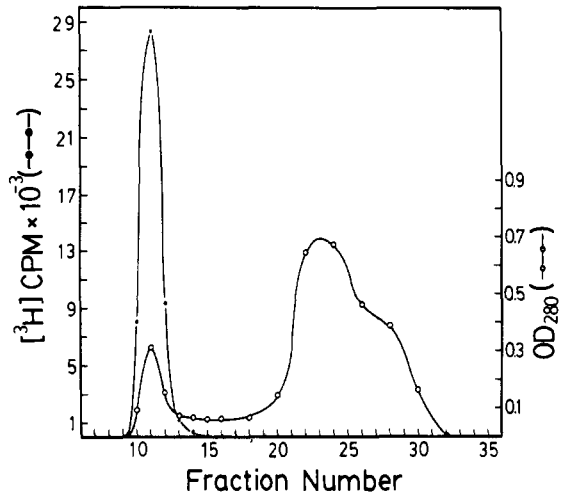

FIGURE 1: Elution profile of agarose A-5M column used to separate SV40 minichromosomes from free proteins, Triton, and PMSF: ^3H cpm (●); OD_{280} (○).

Where nucleosome cores and DNA were irradiated together, separation was carried out on an agarose A5M column identical with that used in the preparation of nucleosome cores. DNA was in the void volume while nucleosome cores were included in the gel.

Results

Purification of SV40 Chromatin and DNA. Attempts to purify substrate amounts of chromatin by published procedures involving sucrose gradient ultracentrifugation resulted in unacceptable losses of material due to binding to vessel walls. In one typical preparation, 15% of the chromatin was lost due to binding to the walls of the polyallomer centrifuge tubes, 51% of the remaining chromatin was lost by binding to the dialysis membrane, and another 23% was lost during overnight storage in a polypropylene tube. With additional losses onto pipets and tubes used to collect sucrose gradients, the total yield was around 1–2%.

The alternative purification procedure described here employs an agarose A-5M sieving column to remove contaminating proteases and nucleases as well as Triton and PMSF. The chromatin is excluded from the gel and elutes as a sharp peak in the void volume (Figure 1) within minutes of loading. The column and all fraction and storage tubes were of siliconized glass. The overall recovery was 94%, and the chromatin obtained was intact as judged by sedimentation coefficient (~61 S), percent form I DNA (90%), and electron microscopy. Specific activity was around 2.5 × 10^5 cpm/μg of DNA, with 360–500 μg of DNA from a preparation of 4 half-gallon roller bottles. The chromatin was stable for at least 4 weeks.

Extraction of DNA was also carried out in such a way as to minimize nicking of DNA and losses due to binding. The removal of $NaDodSO_4$, traces of solvent, and extraction buffer was by chromatography on Sepharose CL-2B. The DNA eluted as a sharp peak in the void volume within minutes of loading with a recovery greater than 98%. All extraction tubes, columns, and fraction tubes were siliconized glass.

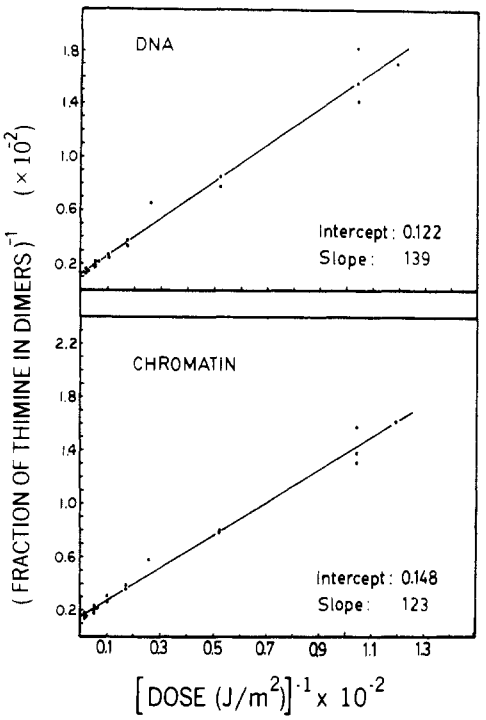

FIGURE 2: Dose response for pyrimidine dimer formation in SV40 chromatin and DNA.

Pyrimidine Dimer Formation in Minichromosomes vs. DNA. After irradiation, mixtures of DNA and minichromosomes were separated by sucrose gradient centrifugation and analyzed separately for pyrimidine dimer content. For moderate to very high doses of UV irradiation, dimers were measured by thin-layer chromatography. The data are obtained directly from thin-layer chromatograms as the fraction of thymine label in the dimer peak. If chromatin structure has no influence on pyrimidine dimer formation, the dose response for dimer formation should be the same for chromatin and DNA. In fact, they are not (Figure 2). Double-reciprocal plots for dose at 254 nm vs. fraction of thymine in dimers yield straight lines with good correlation coefficients, but the plots for SV40 chromatin and DNA have distinctly different intercepts and slopes. At high doses, fewer pyrimidine dimers are formed in chromatin than in DNA. In addition, extrapolation of these plots suggests that SV40 DNA and SV40 chromatin will saturate at 8.22% and 6.76% thymine in dimers, respectively, at infinite dose.

More interesting from the point of view of DNA repair is that at doses lower than about 380 J/m^2 more pyrimidine dimers are formed in the chromatin than in the DNA. This can be seen either by viewing the dose–response plots or by directly comparing the DNA and chromatin values (Figure 3). The dashed line shows the result expected if there were no effect of chromatin structure on pyrimidine dimer formation. The data points clearly do not fit this line. The linear least-squares fit to the data is shown by the solid line.

To study the kinetics of dimer formation at very low doses (less than 40 J/m^2), we monitored dimers by the susceptibility of these structures to nicking by T4 UV endonuclease and subsequent quantitation of these nicks. At these low doses, pyrimidine dimers form in SV40 chromatin at about 150% of their rate of formation in DNA (Figure 4).

When the data from thin-layer chromatography (Figures 2 and 3) are converted from fraction of thymine in dimers to dimers per SV40 genome [by using the data of Unrau et al. (1973)], they can be combined with the data from the nicking assay on a single graph (Figure 5). The plot shows the data

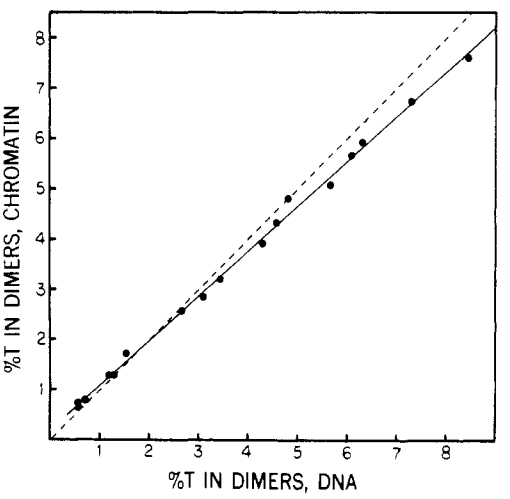

FIGURE 3: Comparison of percent of thymine label in dimers for SV40 chromatin and DNA. Result expected if chromatin structure had no effect (---); least-squares fit to the data (—), with correlation coefficient = 0.998.

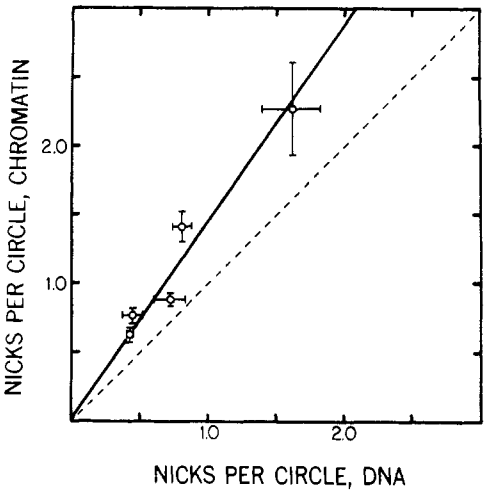

FIGURE 4: T4 UV endonuclease sites formed in SV40 chromatin and DNA at doses below 40 J/m^2. Result expected if chromatin structure had no effect (---); least-squares fit to the data (—), with correlation coefficient = 0.981.

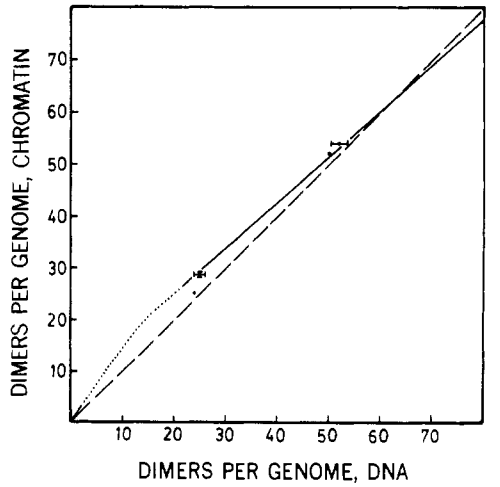

FIGURE 5: Data from Figures 3 and 4 converted to dimers per genome and combined on a single graph to show relative dimer formation in chromatin and DNA for doses between 0 and 400 J/m^2. Region not accessible to either assay (···); result expected if chromatin structure had no effect (---); least-squares fit to the data (—).

for doses less than 400 J/m^2 and includes the cross-over point at about 380 J/m^2 where equal numbers of dimers are formed

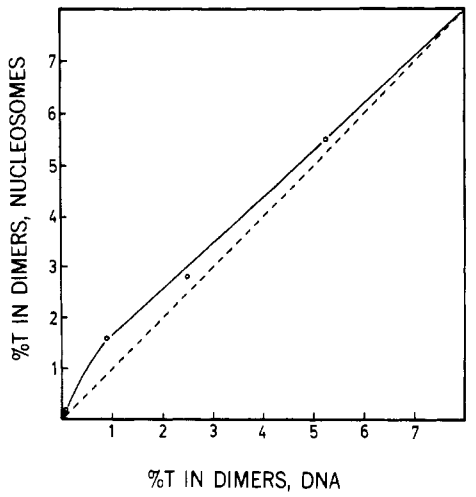

FIGURE 6: Direct comparison of percent thymine label in dimers for SV40 nucleosomes and DNA. Result expected if nucleosome structure has no effect (---); best fit to data (—).

in both DNA and chromatin. The dotted line connects the data from the two assays, and the dashed line again is that expected if chromatin structure had no effect on dimer formation.

Pyrimidine Dimer Formation in Nucleosomes. Since the internucleosome linker DNA is thought to be relaxed B-form DNA, it seemed likely that the difference seen between chromatin and DNA might be due to special constraints on the DNA specifically contained in the nucleosomes. Thus, it was of interest to compare pyrimidine dimer formation in isolated nucleosome cores to that in intact DNA. The comparison (Figure 6) indicates that at low doses pyrimidine dimers form in nucleosomal DNA at about 177% of their rate of formation in free DNA (from the best straight line through the first three points which represent doses up to about 141 J/m^2). This is in good agreement with the data from intact minichromosomes since approximately 85% of the DNA in the minichromosome is contained in nucleosomes. At higher doses, the difference in dimer content between nucleosomes and free DNA decreases, and both seem to approach the same saturation value.

Discussion

These studies show that at doses up to at least 40 J/m^2 pyrimidine dimers form in SV40 chromatin at about 150% of their rate of formation in SV40 DNA. At higher doses, the difference becomes smaller, until the values become equal at about 380 J/m^2. Beyond 380 J/m^2, fewer dimers are formed in the chromatin than in DNA for a given dose.

The low-dose results are of special interest for studies of DNA repair since these doses produce biologically repairable levels of damage. If the difference seen between chromatin and DNA are due to constraints on the DNA contained in nucleosomes, then one can predict the rate of dimer formation in nucleosomes from their rate of formation in intact chromatin. With the assumption that there are about 190–200 base pairs associated with each SV40 nucleosome (Bellard et al., 1976; Griffith & Christiansen, 1978; Germond et al., 1975; Shelton et al., 1980), about 22 nucleosomes per SV40 genome (Griffith & Christiansen, 1978; Shelton et al., 1980), and 5224 base pairs per genome (Fiers et al., 1978), then about 80–85% of the DNA in a minichromosome is in nucleosomes. Dividing the relative rate of dimer formation in whole chromatin by the fraction of DNA contained in nucleosomes (0.8–0.85), we predict that the relative rate of dimer formation in nucleosome cores would be 1.76–1.87; i.e., dimers would be expected to form in nucleosomes or nucleosome cores at about 180% the rate of formation in free DNA. In fact, the experiment with isolated nucleosomes gave a relative rate of 177% in this same dose range, in good agreement with the predicted value.

At high doses, dimer formation in nucleosome cores differs from dimer formation in intact chromatin. Whereas in intact chromatin there is a crossover so that fewer dimers were formed in chromatin than in DNA at high doses, in free nucleosome cores the values slowly approach the free DNA values as saturation in dimers is approached. The absence of covalently closed DNA circles in the case of free nucleosome cores may account for this difference, since high levels of pyrimidine dimers can significantly alter the superhelicity of covalently closed circular DNA (Woodworth-Gutai et al., 1977; Denhardt & Kato, 1973), and thus might also alter nucleosome structure in the intact SV40 chromatin.

All of the studies of distribution of repair nucleotide label following UV irradiation of eukaryotic cells have been done at doses less than 84 J/m^2. Since SV40 nucleosomes are composed of host cell histones and organized into nucleosomes as in cell chromatin (Lake et al., 1973; Shelton et al., 1980), it is likely that similar kinetics of dimer formation occur in the cellular nucleosomes. Thus, in the cell studies, dimers were probably preferentially formed in DNA contained in the nucleosomes. Yet in these same studies, repair label was first found in nuclease-sensitive DNA. Our data argue against the possibility that dimers were selectively formed in linker DNA. Instead, when taken together with the appearance of repair label in nuclease-sensitive DNA in vivo, they tend to support the suggestion of Oleson et al. (1979) that repair processes in nucleosomes in some way make the nucleosomal DNA nuclease sensitive.

Wu and co-workers (1980) found that ethidium bromide preferentially binds DNA in nucleosomes at low concentrations whereas the opposite is true at high concentrations. This is analogous to the formation of pyrimidine dimers in minichromosomes where the enhanced formation at low doses is reversed at high doses. Pyrimidine dimers, like bound ethidium bromide molecules, unwind the DNA helix (Camerman & Camerman, 1968; Denhardt & Kato, 1973), although the unwinding due to each dimer is smaller than that produced by each bound ethidium bromide molecule. Wu et al. (1980) observed distortion of the nucleosomes when ethidium bromide was bound and attributed the enhanced binding at low concentrations to relief of superhelical stress in the nucleosomes. Thus, it is especially important to note that patterns of damage in DNA in chromatin at low doses (or concentrations) of damaging agent may be the opposite of the pattern found at higher doses (or concentrations). Therefore, studies of the effect of chromatin structure on DNA damage should not be widely extrapolated.

There are several possible explanations for enhanced dimer formation in nucleosomes at low UV doses. In addition to unwinding the DNA helix by about 8°, each dimer kinks the DNA (Camerman & Camerman, 1968), producing a flex point by disrupting the stacking interaction and breaking hydrogen bonds between bases. It has been shown that pyrimidine dimer formation reduces the chain stiffness parameter of superhelical DNA (Triebel et al., 1979). Other workers have also reported increased flexibility in DNA after UV irradiation (Moroson & Alexander, 1961). Increased flexibility in nucleosomal DNA might allow tighter binding of the DNA to the protein core, thus stabilizing the kink (dimer) and decreasing the reverse photoreaction (monomerization of dimers) which also occurs at 254 nm. Conversely, preexisting

kinks or unstacked bases might act as sites of preferential dimer formation for analogous reasons.

Another possibility is that the conformation of the DNA in the nucleosome differs from that of free DNA in such a way that the 5–6 double bonds of adjacent pyrimidines are brought closer together. A "C" conformation would do this, and there is evidence from circular dichroism studies that DNA in nucleosomes is in a C-like conformation (Baase & Johnson, 1979; Bina-Stein, 1978; Zhurkin et al., 1979).

A final possibility is photosensitization by the histones. However, in view of the low aromatic content of histones, we consider this the least likely of the several possible explanations for enhanced dimer formation in nucleosomes at low doses.

References

Baase, W. A., & Johnson, W. C., Jr. (1979) *Nucleic Acids Res. 6*, 797–814.

Bellard, M., Oudet, P., Germond, J.-E., & Chambon, P. (1976) *Eur. J. Biochem. 70*, 543–553.

Bina-Stein, M. (1978) *J. Biol. Chem. 253*, 5213–5219.

Bodell, W. J. (1977) *Nucleic Acids Res. 4*, 2619–2628.

Bodell, W. J., & Banarjee, M. R. (1979) *Nucleic Acids Res. 6*, 359–370.

Camerman, N., & Camerman, A. (1968) *Science (Washington, D.C.) 160*, 1451–1452.

Cleaver, J. E. (1977) *Nature (London) 270*, 451–453.

Denhardt, D. T., & Kato, A. C. (1973) *J. Mol. Biol. 77*, 479–494.

Fiers, W., Contreras, R., Haegeman, G., Rogiers, R., Van de Voorde, A., Van Heuverswyn, H., Van Iterreweghe, J., Volckaert, G., & Ysebaert, M. (1978) *Nature (London) 273*, 113–120.

Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M., & Chambon, P. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1843–1847.

Goldmann, K., & Friedberg, E. C. (1973) *Anal. Biochem. 53*, 124–131.

Griffith, J. D., & Christiansen, G. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 215–226.

Jagger, J. (1967) in *Introduction to Research in Ultraviolet Photobiology*, Prentice-Hall, Englewood Cliffs, NJ.

Jahn, C. L., & Litman, G. W. (1977) *Biochem. Biophys. Res. Commun. 76*, 534–540.

Jahn, C. L., & Litman, G. W. (1979) *Biochemistry 18*, 1442–1449.

Kuhnlein, V., Penhoet, E. E., & Linn, S. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1169–1193.

Lake, R. S., Barban, S., & Salzman, N. P. (1973) *Biochem. Biophys. Res. Commun. 54*, 640–649.

Metzger, G., Wilhelm, F. X., & Wilhelm, M. L. (1977) *Biochem. Biophys. Res. Commun. 75*, 703–710.

Moroson, H., & Alexander, P. (1961) *Radiat. Res. 14*, 29–49.

Mortelmans, K., Friedberg, E. C., Slor, H., Thomas, G., & Cleaver, J. E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2757–2761.

Oleson, F. B., Mitchell, B. L., Dipple, A., & Lieberman, M. W. (1979) *Nucleic Acids Res. 7*, 1343–1361.

Ramanathan, R., Rajalakshmi, S., Sarma, D. S. R., & Farber, E. (1976) *Cancer Res. 36*, 2073–2079.

Shaw, B. R., Herman, T. M., Kovacic, R. T., Beadreau, G. S., & Van Holde, K. E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 505–509.

Shelton, E. R., Wassarman, P. M., & De Pamphilis, M. L. (1980) *J. Biol. Chem. 255*, 771–782.

Smerdon, M. J., & Lieberman, M. W. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4238–4281.

Smerdon, M. J., Kastan, M. B., & Lieberman, M. W. (1979) *Biochemistry 18*, 3732–3738.

Tlsty, T. D., & Lieberman, M. W. (1978) *Nucleic Acids Res. 5*, 3261–3273.

Triebel, H., Reinert, K.-E., Bar, H., & Lang, H. (1979) *Biochim. Biophys. Acta 561*, 59–68.

Unrau, P., Wheatcroft, R., & Olive, T. (1973) *Biochim. Biophys. Acta 312*, 626–632.

Williams, J. I., & Friedberg, E. C. (1979) *Biochemistry 18*, 3965–3971.

Woodworth-Gutai, M., Lebowitz, J., Kato, A. C., & Denhardt, D. T. (1977) *Nucleic Acids Res. 4*, 1243–1256.

Wu, H.-M., Dattagupta, N., Hogan, M., & Crothers, D. M. (1980) *Biochemistry 19*, 626–634.

Zhurkin, V. B., Lysov, Y. P., & Ivanov, V. (1979) *Nucleic Acids Res. 6*, 1081–1096.