Effect of Histone H1 Removal on the Distribution of Ultraviolet-Induced Deoxyribonucleic Acid Repair Synthesis within Chromatin†

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ABSTRACT: Because histone H1 plays an important role in the organization of chromatin, we have examined its possible involvement in determining the distribution within chromatin of nucleotides incorporated by UV-induced DNA repair synthesis. It has been shown previously that immediately after repair synthesis almost all of the newly incorporated nucleotides are rapidly digested to acid-soluble form by staphylococcal nuclease [Smerdon, M. J., & Lieberman, M. W. (1980) *Biochemistry 19*, 2992–3000]. The selective removal of histone H1 from intact nuclei by low pH does not alter this enhanced nuclease sensitivity and, therefore, does not induce any rearrangements of chromatin structure (as detected by staphylococcal nuclease) in newly repaired regions of DNA. However, H1 removal results in 5–12% of the bulk DNA in nuclei becoming more nuclease sensitive. We conclude that, prior to nucleosome rearrangement, either newly repaired regions of DNA lack histone H1 or, if present, this histone confers less resistance to the digestion of these regions by staphylococcal nuclease than to the digestion of the bulk of the DNA in chromatin. Furthermore, following nucleosome rearrangement in newly repaired regions of DNA, removal of histone H1 does not restore the enhanced nuclease sensitivity of these regions. Thus, the increased resistance to nuclease digestion acquired by these regions during nucleosome rearrangement is not conferred in any simple way by the presence of histone H1. Finally, examination of the electrophoretic profiles of the submonomer fragments generated by staphylococcal nuclease from newly repaired DNA and bulk DNA indicates that following nucleosome rearrangement (at least within 30 min) histone H1 is present in most (or all) of the newly repaired regions of DNA.

It is now clear that most of the genome in eukaryotes is organized as a series of repeating units known as nucleosomes [for recent reviews, see Chambon (1977), Isenberg (1979), McGhee & Felsenfeld (1980) and Kornberg & Klug (1981)]. One important consequence of this subunit structure of chromatin is the apparent need for nucleosome rearrangement during DNA processing events.

Our laboratory and others have demonstrated that nucleosome rearrangement occurs following the excision repair of damage induced by ultraviolet (UV)[1] radiation and UV-mimetic agents in cultured human cells (Smerdon & Lieberman, 1978, 1980; Smerdon et al., 1979; Williams & Friedberg, 1979; Tlsty & Lieberman, 1978; Oleson et al., 1979; Bodell & Cleaver, 1981).[2] However, to date, little is known about the mechanism of this rearrangement. The following two possibilities have been proposed (Smerdon & Lieberman, 1978): (1) nucleosome rearrangement in newly repaired regions of DNA is due to a constitutive process (i.e., a normal cellular process that occurs in the presence or absence of DNA damage and repair); (2) nucleosome rearrangement is induced by the process of damage and/or repair (e.g., an obligatory, final step in the excision repair process). Although thus far a definitive demonstration of either possibility has not been possible, we have proposed a model which explains all of our results to date (Lieberman et al., 1979). In essence, this model depicts the rearrangement that we observe as a "refolding" of the DNA into the original nucleosome structure following an "unfolding" event caused by the excision repair process. Thus, we have begun to focus on ways of testing this model. In the present paper, we report our studies on the possible role of histone H1 in this rearrangement process.

Although the exact role(s) of histone H1 in the packaging of DNA in chromatin is (are) not known, a number of studies suggest that it may be involved in higher order packaging (or condensation) of nucleosomes (Littau et al., 1965; Mirsky et al., 1968; Bradbury et al., 1973; Oudet et al., 1975; Lewis et al., 1976; Noll & Kornberg, 1977; Renz et al., 1977; Thoma & Koller, 1977, 1981; Griffith & Christiansen, 1977; Keller et al., 1977; Gaubatz et al., 1977; Thoma et al., 1979). Also, H1 appears to be much less prevalent in regions of chromatin undergoing transcription (Simpson & Reeck, 1973; Shirley & Anderson, 1976; Lishanskaya & Mosevitsky, 1976; Lau & Ruddon, 1977; Chiu et al., 1977; Letnansky, 1978; Djondjurov et al., 1979; Levy et al., 1979), and during replication, H1 appears to associate with the daughter strand after (and separate from) the core histones (Worcel et al., 1978). These observations suggest that histone H1 may be responsible for the packaging of nucleosomes in regions of chromatin where DNA is not being "actively processed" and that H1 can bind and, perhaps, refold the nucleosome unit following a processing event. Thus, it is possible that H1 may play a role in the rearrangement of nucleosome structure following DNA repair. For instance, H1 may be partially (or totally) displaced from the nucleosome during DNA repair, and rearrangement may involve the refolding of nucleosomal DNA by the reassociation of histone H1.

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[1] Abbreviations: UV, ultraviolet; dThd, thymidine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; cpm, counts per minute.

[2] We note that Williams & Friedberg (1979) reported that the redistribution of repair-incorporated nucleotides in chromatin was more limited than what we observe. As discussed in Smerdon & Lieberman (1980), the difference between our calculations and those of Williams & Friedberg (1979) is that these authors calculated only the amount of redistribution occurring *after* a 1-h pulse-labeling period, whereas our calculations were for the *total* amount of redistribution occurring after repair synthesis.

0006-2960/82/0421-3879$01.25/0

In the present paper, we have examined the effect of selective removal of histone H1 from nuclei on the chromatin distribution characteristics of repair-incorporated nucleotides following different extents of nucleosome rearrangement. The necessary criteria for such a study are the following: (1) the procedure for removing H1 must not *itself* induce nucleosome rearrangement; (2) this procedure must be selective for histone H1 (i.e., not remove other histones or nonhistone proteins). Both of these criteria appear to be met by the H1 extraction method of Lawson & Cole (1979). This method does not induce changes in the average nucleosome repeat length and appears to be quite selective for removal of histone H1 (Lawson & Cole, 1979; Weischet et al., 1979; Weischet, 1979; Smerdon & Lieberman, 1981).

Materials and Methods

Cell Culture, Damage, and Labeling. Human diploid fibroblasts [lines IMR-90 (Nichols et al., 1977) and AG1518] were labeled during the growth phase with 0.02–0.025 μCi/mL [^{14}C]dThd (~50 mCi/mmol; Amersham or New England Nuclear), grown to confluence, treated with either 5 or 10 mM hydroxyurea, and irradiated (predominantly 254 nm; 2 W/m^2) with 12 J/m^2 UV irradiation as described previously (Smerdon et al., 1978, 1979). Under these conditions, the amount of replicative synthesis is reduced to very low levels, while there is little (or no) effect on either the amount of repair synthesis or the rate and extent of nucleosome rearrangement in newly repaired regions (Smerdon et al., 1978, 1979). Cells were pulse labeled for 30–45 min with 5–10 μCi/mL [^{3}H]dThd (40–60 Ci/mmol; Amersham or New England Nuclear) immediately after irradiation. Cells were then either harvested immediately or subjected to a chase period prior to harvest as described previously (Smerdon et al., 1979). [For the approximate number of cells used per experiment, as well as the specific activities of the DNA under these conditions, see Smerdon & Lieberman (1980).]

Nuclei Preparation and H1 Removal. Nuclei were prepared, and histone H1 was extracted, using the method of Lawson & Cole (1979) [see also Smerdon & Lieberman (1981)]. This method involves extraction of purified nuclei with low-pH buffers and resuspension in digestion buffer (50 mM Tris, pH 7.5, 25 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.25 M sucrose). All buffers contained 0.1 mM phenylmethanesulfonyl fluoride. Displaced and retained nuclear proteins were prepared and analyzed by electrophoresis as previously described (Smerdon & Lieberman, 1981).

Nuclease Digestions and Electrophoresis. Staphylococcal nuclease digestions of nuclei [(3.5–7) × 10^6/mL] suspended in digestion buffer, agarose gel electrophoresis, and data analysis were all carried out as described elsewhere [e.g., see Smerdon & Lieberman (1980)]. Electrophoresis of DNA samples on 6% polyacrylamide gels was carried out according to Axel et al. (1974). Gels were assayed for radioactivity as described previously (Smerdon et al., 1978; Smerdon & Lieberman, 1980). The sizes of the staphylococcal nuclease digestion products were determined from calibration of gels with *Hin*fI restriction fragments of ϕX174 RF DNA (prepared by Dr. Steven Dresler).

Results

Effect of H1 Removal on Staphylococcal Nuclease Digestion Kinetics. We have examined the effect of histone H1 removal on the distribution of UV-induced DNA repair sythesis in nuclei from two different human diploid fibroblast cell lines. In each case, nuclei were prepared from confluent cells, and histone H1 was selectively removed by extraction

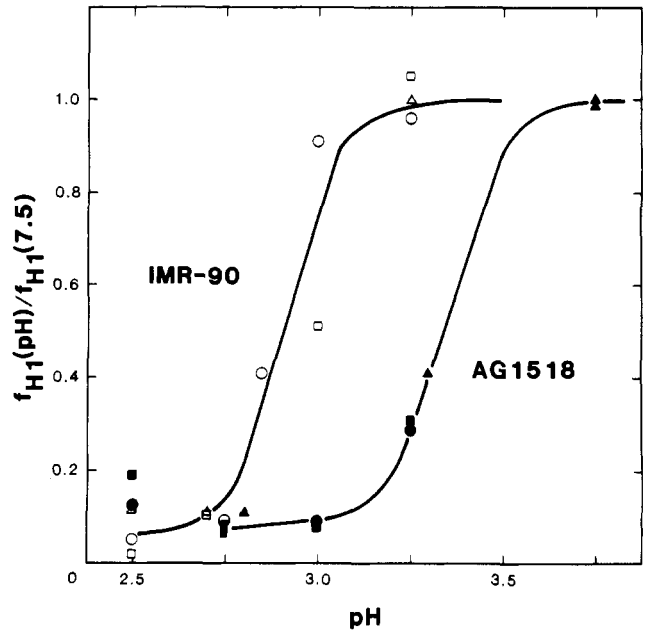

FIGURE 1: Fraction of histone H1 retained by IMR-90 nuclei (open symbols) and AG1518 nuclei (closed symbols), relative to the fraction at pH 7.5, as a function of the pH of the extraction buffer. In each case, nuclei were prepared from confluent cells and extracted in acidic buffer by using the method of Lawson & Cole (1979). The fraction of H1 retained was determined from sodium dodecyl sulfate gel profiles of total acid-soluble nuclear proteins (Smerdon & Lieberman, 1981). Different symbols represent different experiments.

in acidic buffers (Lawson & Cole, 1979). As recently reported (Smerdon & Lieberman, 1981), nuclei from IMR-90 cells retain ≥95% of their total H1 down to a pH of 3.25, while ≤10% of the H1 is retained at pH 2.7 (e.g., see Figure 1). When nuclei from AG1518 cells are used, however, the curve for H1 displacement is shifted (~0.4 pH unit) to higher pH values (Figure 1). Therefore, nuclei from AG1518 cells yield an H1 displacement curve that is similar to that reported by Lawson & Cole (1979) for HeLa cell nuclei. Interestingly, these authors also stated that nuclei from "two other cell lines" yielded an H1 displacement curve that was shifted to more acidic values (as we have found for IMR-90 cells). Thus, the pH required for displacement of 50% of the total H1 from nuclei is, at least somewhat, cell type specific.

For examination of the effect of selective removal of histone H1 on the relative nuclease sensitivity of repair-incorporated nucleotides, cells were prelabeled with [^{14}C]dThd during replication, grown to confluence, irradiated with UV light, and labeled with [^{3}H]dThd during repair synthesis. As reported previously, when nuclei are prepared from cells which are harvested immediately after the pulse-labeling period (i.e., 0-h chase) and incubated with staphylococcal nuclease or DNase I, many of the repair-incorporated nucleotides are rapidly digested to acid-soluble form [e.g., see Smerdon & Lieberman (1980)]. As shown in Figure 2 (left panel, solid circles), this is also the case when IMR-90 nuclei are first extracted at pH 3.25 (no H1 release). The data in Figure 2 represent the ratio of the fraction of ^{3}H cpm [$f(^3H)$; repaired DNA] to the fraction of ^{14}C cpm [$f(^{14}C)$; bulk DNA] rendered acid soluble by staphylococcal nuclease as a function of the amount of bulk DNA rendered acid soluble [$f(^{14}C)$]. For low extents of digestion, the amount of repair-incorporated nucleotides released relative to the amount of bulk DNA released is much greater than that for larger extents of digestion. This finding indicates that the repair-incorporated nucleotides are more nuclease sensitive than the DNA as a whole in chromatin. These data

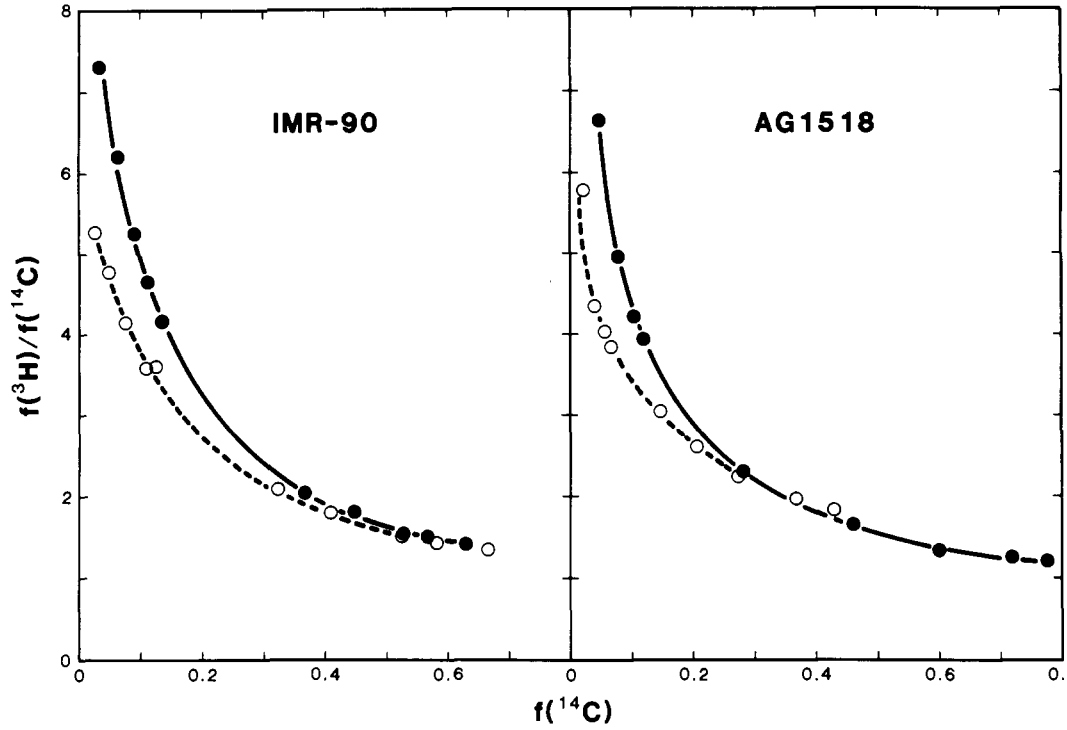

FIGURE 2: Ratio of the fraction of ^3H cpm [$f(^3H)$; repaired DNA] and the fraction of ^{14}C cpm [$f(^{14}C)$; bulk DNA] rendered acid soluble by staphylococcal nuclease as a function of the fraction of ^{14}C cpm rendered acid soluble. Cells were prelabeled with [^{14}C]dThd, irradiated with 12 J/m^2 UV light, pulse labeled with [^3H]dThd, and harvested immediately after the pulse-labeling period (i.e., no chase). The data shown are for IMR-90 nuclei (left panel) extracted at pH 3.25 (●) and 2.70 (○) and AG1518 nuclei (right panel) extracted at pH 7.50 (●) and 3.00 (○). Staphylococcal nuclease digestions were performed as follows: At time zero, nuclease was added, and aliquots were taken at 3, 6, 9, 12, and 15 min for the determination of acid-soluble cpm (Smerdon et al., 1978). Additional nuclease was added at 20 min, and aliquots were taken at 30, 40, 60, 80, and 100 min for IMR-90 nuclei and 24, 30, 40, 50, and 60 min for AG1518 nuclei. For the digestion of AG1518 nuclei, the final concentration of nuclease added at time zero was 0.15 (pH 7.50) and 0.075 (pH 3.00) unit/10^6 nuclei, and at 20 min, the final concentration of nuclease added was 1.66 (pH 7.50) and 0.83 (pH 3.00) unit/10^6 nuclei. Nuclease concentrations for the digestion of IMR-90 nuclei are given in legend to Figure 4.

were identical with those obtained for IMR-90 nuclei that were extracted at pH 7.5 (data not shown) and similar to our previous results (Smerdon et al., 1978, 1979; Smerdon & Lieberman, 1978, 1980). This enhanced sensitivity to staphylococcal nuclease digestion is also seen when IMR-90 nuclei are extracted at pH 2.7 (≥90% H1 removed; Figure 2, open circles); however, there is a small, yet reproducible, decrease in the overall sensitivity of the repair-incorporated nucleotides in going from pH 3.25 to pH 2.7. A similar decrease was observed for AG1518 nuclei; however, in this case, the decrease in overall sensitivity occurs between pH 3.75 and pH 3.0 (Figure 2, right panel). (In this case, there was no change in the data from pH 7.5 to pH 3.75.) Thus, for each cell type, the small decrease in nuclease sensitivity of repair-incorporated nucleotides appears to correspond to the removal of histone H1 from these nuclei rather than some nonspecific effect on the chromatin structure induced by exposure of nuclei to low pH. One explanation for this decrease is that displacement of histone H1 by low pH causes some nucleosome rearrangement which in turn decreases the enhanced sensitivity of the repaired DNA via partial randomization. Alternatively, this decrease may result from an *increase* in the amount of *bulk DNA* that is nuclease sensitive, relative to the amount of repaired DNA that is nuclease sensitive, following H1 removal.

One cannot distinguish between these two possibilities from the data as presented in Figure 2; therefore, the data were analyzed by a method previously described by us (Smerdon et al., 1978, 1979; Smerdon & Lieberman, 1980). In using this method, we plot the *difference* between the fractions of the two isotopes rendered acid soluble by staphylococcal nuclease [$f(^3H) - f(^{14}C)$] vs. the fraction of ^{14}C cpm rendered acid soluble.[3] From these normalized difference curves, one can determine the fraction of repair-incorporated nucleotides that is nuclease sensitive (f_s) and the fraction of the total DNA that is nuclease sensitive (ξ). The ratio f_s/ξ is determined from the initial slope of these curves (Smerdon et al., 1979), while ξ is determined from the intercept of the lines defined by the initial and final slopes. The normalized difference curves for the data from IMR-90 nuclei (Figure 2) are shown in Figure 3. As can be seen, there is both a small decrease in the initial slope of this curve and a small increase in the value of ξ following extraction of nuclei at pH 2.7.

This analysis was performed on nuclease digestion data from IMR-90 nuclei extracted at different pH values over the range of 2.5–3.25. The values for f_s and ξ, as well as the relative amount of histone H1 retained by the nuclei, are shown in Figure 4. The data demonstrate that the change in ξ correlates well with the removal of histone H1, and, more importantly, the value of f_s remains essentially constant following different extents of H1 removal. [Note that a nearly constant value of f_s vs. pH is predicted for the changes observed here for ξ when it is assumed that nucleosome rearrangement in repaired regions does not occur during H1 removal (Figure

[3] In past reports, we have used the difference (ΔH) between the ^3H cpm released from nuclei at various values of $f(^{14}C)$ and the corresponding ^3H cpm released from the purified DNA from those nuclei. These values were normalized by dividing by the total ^3H cpm incorporated into the DNA ($H_{100\%}$). In equating $f(^3H) - f(^{14}C)$ with $\Delta H/H_{100\%}$, we assume that the ratio of the total ^3H cpm and ^{14}C cpm ($H_{100\%}/C_{100\%}$) is equal to ^3H cpm/^{14}C cpm for the purified DNA (Smerdon et al., 1979).

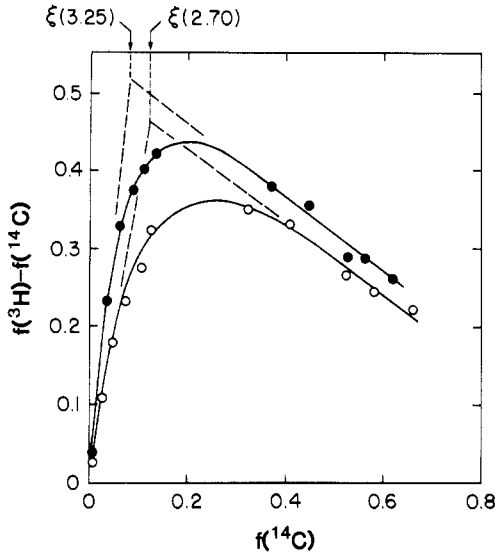

FIGURE 3: Normalized difference curves for the data in Figure 2 (IMR-90 nuclei).[3] Definitions of $f(^3H)$ and $f(^{14}C)$ are given in Figure 2. Dashed lines indicate the initial and final slopes, as well as the values of ξ (i.e., fraction of bulk DNA that is nuclease sensitive) for each set of data. Open and closed symbols are the same as those in Figure 2.

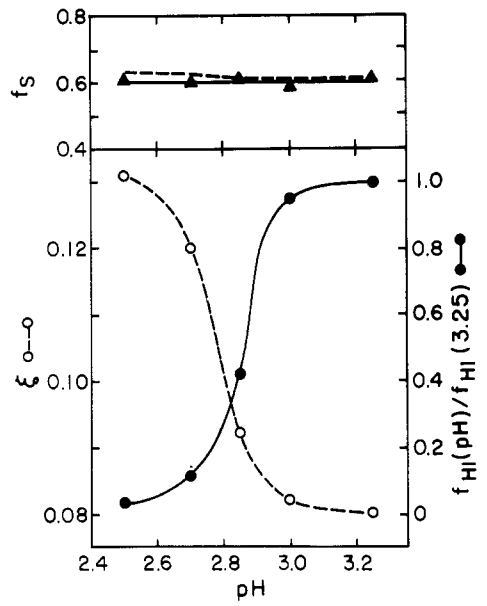

FIGURE 4: Fraction of repair-incorporated nucleotides that is nuclease sensitive (f_s), fraction of bulk DNA that is nuclease sensitive (ξ), and fraction of histone H1 retained by nuclei (relative to pH 3.25) as a function of the pH of the extraction buffer. IMR-90 nuclei were prepared and incubated with staphylococcal nuclease as described in the legend to Figure 2. Final concentration of nuclease added at time zero was 0.15 (pH 3.25), 0.113 (pH 3.00), 0.09 (pH 2.85), 0.075 (pH 2.70), and 0.06 (pH 2.50) unit/10^6 nuclei. Final concentration of nuclease added at 20 min was 1.4 (pH 3.25), 1.05 (pH 3.00), 0.84 (pH 2.85), 0.70 (pH 2.70), and 0.56 (pH 2.50) unit/10^6 nuclei. In each case, normalized difference curves were generated (e.g., Figure 3) and the values of f_s and ξ determined (see text). The dashed line in the upper panel indicates values of f_s calculated (eq A4 of the Appendix) for the changes in ξ from the value at pH 3.25.

4; top panel, dashed line).] Similar results were obtained for AG1518 nuclei in the pH range 2.75–3.75 (data not shown). Furthermore, we have examined the amount of repair-incorporated label in DNA from isolated nucleosome cores and find no increase in the amount of this label in the core DNA from H1-depleted nuclei as compared to that from control nuclei (J. F. Watkins and M. J. Smerdon, unpublished results). This

Table I: Effect of H1 Removal on the Nuclease Sensitivity of Repaired DNA following the Rapid Phase of Rearrangement[a]

chase time (h)	f_s/ξ pH 7.5	pH 2.7	calcd[b]
0	5.84	3.21	3.55
3	1.65	1.54	1.42

[a] IMR-90 cells were pulse labeled for 30 min immediately after irradiation and either harvested immediately or subjected to a chase period of 3 h. Nuclei were prepared, extracted in either pH 7.5 or pH 2.7 buffer, and incubated with 0.4 (pH 7.5) or 0.2 (pH 2.7) unit/10^6 nuclei staphylococcal nuclease. The data were analyzed as described in the text. f_s/ξ is the ratio of the fraction of repair-incorporated nucleotides that is nuclease sensitive (f_s) to the fraction of bulk DNA that is nuclease sensitive (ξ). [b] Calculated values of f_s/ξ were determined from the equation (see eq A4 of the Appendix) f_s/ξ (calcd) = $[f_s(\text{pH } 7.5) + \phi_r[\xi(\text{pH } 2.7) - \xi(\text{pH } 7.5)]]/\xi(\text{pH } 2.7)$ where $f_s(\text{pH } 7.5) = f_s$ determined for nuclei extracted at pH 7.5, $\xi(\text{pH } x) = \xi$ determined for nuclei extracted at pH x, and, from eq A3 of the Appendix, $\phi_r = [f_s(\text{pH } 7.5) - 1]/[\xi(\text{pH } 7.5) - 1]$. These values are expected if no nucleosome rearrangement takes place during H1 removal (Appendix).

result is predicted if H1 removal does not change the value of f_s. Thus, the change in the initial slope of the normalized difference curve (and, therefore, f_s/ξ) following H1 removal appears to result from the change in ξ only. This change in ξ, which varied between 5 and 12% (four different experiments), suggests that a small fraction of the genome becomes more nuclease sensitive relative to newly repaired DNA following removal of histone H1. Furthermore, the fact that H1 removal has little or no effect on f_s suggests that this histone confers less resistance to the digestion of newly repaired DNA (before rearrangement) than to the DNA as a whole in chromatin.

We note that the value of $\xi \simeq 0.10$ that we report here (Figures 3 and 4) is less than the value we have previously reported for these damage and labeling conditions (i.e., ξ = 0.15–0.20; Smerdon et al., 1979; Smerdon & Lieberman, 1980). This difference reflects the difference in digestion buffers used in the past and present reports. We have observed an inverse relationship between the value of ξ and the ionic strength (and/or divalent cation concentration) of the digestion buffer used. Values of ξ = 0.20–0.30 have been obtained for buffers with low ionic strengths (~10 mM) and divalent cation concentrations (0.1 mM) (Smerdon & Lieberman, 1978, and unpublished results), whereas values of ξ = 0.08–0.12 have been obtained for buffers with higher ionic strengths (40–80 mM) and divalent cation concentrations (2–4 mM) (present report and unpublished results). This apparent dependence of ξ on the ionic strength (and/or divalent cation concentration) may result from changes in the nucleosome (or higher order) structure induced by the changes in buffer conditions (Thoma & Koller, 1977, 1981; Renz et al., 1977; Gordon et al., 1978, 1979; Stratling et al., 1978; Dieterich et al., 1979; Fullmer & Fasman, 1979; Lewis, 1979; Martinson et al., 1979; Stratling, 1979; Thoma et al., 1979; Wu et al., 1979).

As reported previously (Smerdon & Lieberman, 1978), following repair synthesis nucleosome rearrangement takes place. This rearrangement process is characterized by an early, rapid phase of redistribution (i.e., during the first 1–2 h) where some 80–90% of the repair-incorporated nucleotides become uniformly distributed in chromatin. To investigate the effects of H1 removal on the distribution within chromatin of repair-incorporated nucleotides following the rapid phase of redistribution, we examined the nuclease digestion properties of repaired regions in nuclei from IMR-90 cells subjected to a 3-h chase period and extracted at either pH 7.5 or pH 2.7.

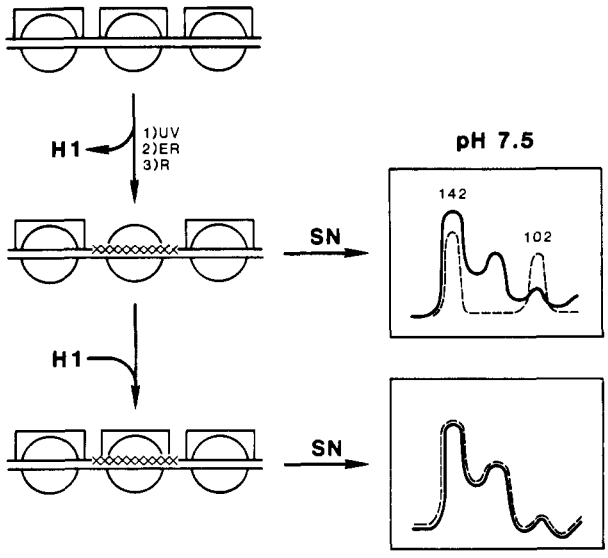

FIGURE 5: Schematic representation of "repair patches" that are uniformly distributed in nucleosomes following the rapid phase of rearrangement. The panels on the right show the predicted gel profiles for nuclei extracted at pH 7.5 both before and after H1 association (see text). The dashed line represents repaired DNA, and the solid line represents bulk DNA. Abbreviations: UV, UV irradiation; ER, excision repair; R, rearrangement; SN, staphylococcal nuclease.

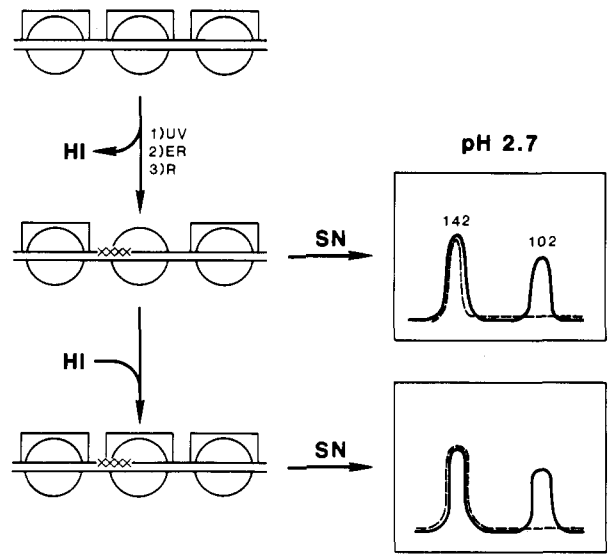

FIGURE 6: Schematic representation of "repair patches" that do not extend into the 102-bp fragment region of the nucleosome following the rapid phase of rearrangement. The panels on the right show the predicted gel profiles for nuclei (from IMR-90 cells) extracted at pH 2.7 both before and after H1 association (see text). See Figure 5 for details.

An example of the results of these experiments is shown in Table I in which we have listed the values for the relative nuclease sensitivity (f_s/ξ) that were obtained from one experiment. As can be seen, following rearrangement there was only a slight decrease in f_s/ξ when H1 was removed, and this decrease is similar to the expected decrease if the only change induced by H1 removal is the change in ξ (Table I, calculated f_s/ξ). Thus, specific removal of H1 from nuclei does not restore the enhanced nuclease sensitivity of repaired DNA that is seen prior to its rearrangement.

Effect of H1 Removal on the Electrophoretic Profiles of Staphylococcal Nuclease Digestion Products. Since one explanation for the small decrease in f_s/ξ upon removal of H1 is that H1 may be displaced from the newly repaired regions of DNA, we examined these regions *following rearrangement* for the presence of H1. This was done by taking advantage of our recently reported observation that the removal of histone H1 from nuclei alters the digestion of nucleosome core DNA by staphylococcal nuclease (Smerdon & Lieberman, 1981). This alteration is characterized by a marked increase in the relative intensity of the 102 ± 4 base pair(s) (bp) submonomer fragment on gels when some 20–50% of the DNA is rendered acid soluble (e.g., Figure 7). Before examining the data, however, it is helpful to consider the predictions made by specific models.

In Figures 5 and 6, we have schematically outlined two models which involve the displacement of H1 during excision repair and the reattachment of this histone *after* the rapid phase of rearrangement. The corresponding gel profiles predicted for the submonomer fragments are also shown in each case. In both models, following the rapid phase of rearrangement a nucleosome "core particle" is assumed to be present in the newly repaired region. We have made this assumption on the basis of previous results (Smerdon & Lieberman, 1978, 1980) and the fact that removal of H1 does not restore the enhanced nuclease sensitivity of newly repaired DNA following rearrangement (Table I). (Thus, rearrangement must involve more than simply a restoration of the protection of repaired DNA solely by H1.) The difference between the two models is that in one case (Figure 5) the predicted gel profiles are based upon a random (or nearly random) distribution of repair-incorporated nucleotides in the core region, while in the other (Figure 6) the 102-bp fragment region of the core particle is devoid of repaired DNA. It is clear that these two possibilities would yield differences in the submonomer patterns for newly repaired DNA and bulk DNA either before reassociation of H1 (Figure 5) or at all times after rearrangement (Figure 6).

To test these possibilities, we obtained gel profiles for different extents of digestion of nuclei from cells that were labeled for 30 min after irradiation and either harvested immediately or subjected to a chase period of 4 h. An example for cells that were harvested immediately (no chase) is shown in Figure 7. It is important to note that most (or all) of the nucleosome cores containing repair-incorporated label are in nucleosomes that have undergone rearrangement prior to the time of harvest (Smerdon & Lieberman, 1978, 1980; Tlsty & Lieberman, 1978). Therefore, the data represent regions of newly repaired DNA that have undergone rearrangement during the 30-min pulse period. As can be seen, when ~40% of the bulk DNA is rendered acid soluble, the profiles of the bulk-labeled DNA and repair-labeled DNA are similar both before (Figure 7a) and after (Figure 7b) H1 removal. Furthermore, the ratio of the two labels is similar for the 102- and 142-bp peaks at different extents of digestion (Figure 8). (We note that for early chase times, the 102-bp peak is actually slightly enriched in repair-labeled DNA.) Also shown in Figure 8 are the ratios for cells which were subjected to a 4-h chase period. As can be seen, the ratio of the two labels has increased in each peak and is essentially the same. Thus, we can exclude the possibility outlined in Figure 6. Furthermore, these data indicate that histone H1 is present in most (or all) of the newly repaired nucleosomes that have undergone rearrangement either during the 30-min pulse period or during the subsequent chase period.

Discussion

We have shown that the removal of histone H1 from two different lines of human fibroblast nuclei by low pH results in little or no change in the overall distribution within chromatin of repair-incorporated nucleotides prior to nucleosome rearrangement (Figures 2 and 3). However, for each of these

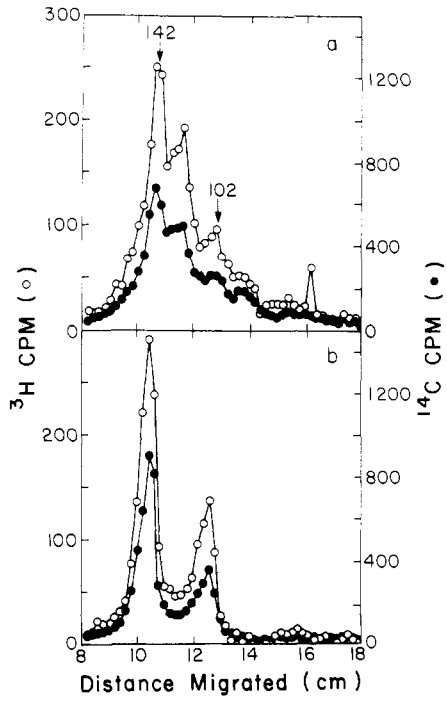

FIGURE 7: Polyacrylamide gel profiles of staphylococcal nuclease digestion products from IMR-90 nuclei extracted at pH 7.5 (a) or pH 2.7 (b). Nuclei were from cells harvested immediately after a 30-min pulse-labeling period. Panels show profiles for both the bulk DNA (^{14}C) and repaired DNA (^{3}H). Nuclei were incubated with 0.66 (pH 7.5) or 0.20 (pH 2.7) unit/10^6 nuclei staphylococcal nuclease, and digestions were terminated when the fraction of ^{14}C cpm rendered acid soluble was 0.40 (a) and 0.39 (b). The arrows in panel a indicate the positions of the 142- and 102-bp peaks.

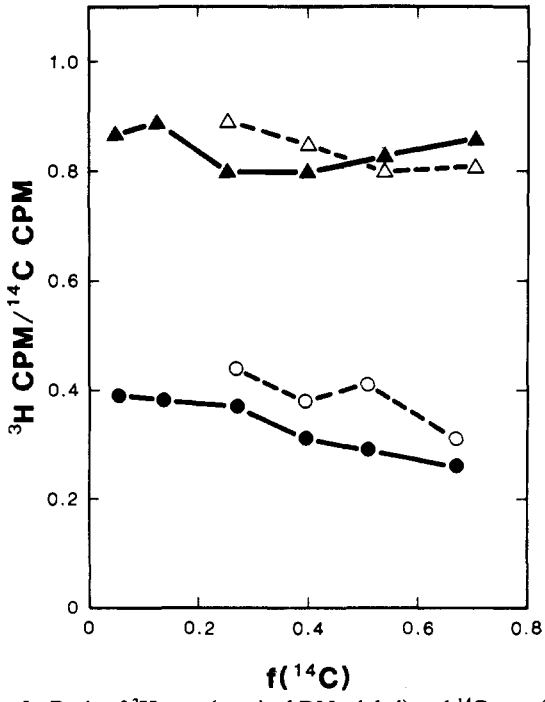

FIGURE 8: Ratio of ^{3}H cpm (repaired DNA label) and ^{14}C cpm (bulk DNA label) in the 142-bp peak (closed symbols) and 102-bp peak (open symbols) as a function of the fraction of ^{14}C cpm rendered acid soluble by staphylococcal nuclease. IMR-90 nuclei were extracted at pH 2.7, digested with staphylococcal nuclease, and electrophoresed as described in the legend of Figure 7. Data are for cells exposed to a chase period of 0 (●, ○) or 4 h (▲, △).

cell lines, we do observe a small increase in the fraction of bulk DNA that is nuclease sensitive (ξ) following H1 removal (Figures 2 and 3). Therefore, if histone H1 is present in the newly repaired regions of DNA (prior to their rearrangement), then this histone confers less resistance to the digestion of these regions by staphylococcal nuclease than to the bulk of the DNA in chromatin. Alternatively, histone H1 may be absent from newly repaired DNA (see the introduction). Furthermore, if rearrangement results from the reassociation of H1 with the nucleosomes in newly repaired regions, the data in Table I suggest that this process cannot be reversed by simply removing H1 from nuclei since f_s/ξ did not increase following H1 removal. However, the results in Table I do not necessarily indicate that H1 is not involved in the rearrangement process. Clearly, the onset of nucleosome rearrangement may be induced by the reassociation of H1, which then initiates the restoration of the strong interactions between nucleosome core DNA and core histones. Once these strong interactions are restored, one would expect that they would not be broken by the removal of H1.

The possibility that histone H1 may be absent from newly repaired DNA for extended times after the rapid phase of rearrangement was evaluated by examining the electrophoretic profiles of the submonomer fragments from newly repaired DNA and bulk DNA. The electrophoresis results (Figure 7) demonstrated that, following the rapid phase of nucleosome rearrangement, the repair-labeled DNA yields a submonomer fragment pattern that changes following H1 removal. This change is identical with that observed for the bulk DNA label and is the same as that reported previously for ethidium bromide stained bulk DNA (Smerdon & Lieberman, 1981). Furthermore, the ratios of the two labels for the 142-bp peak (total core DNA) and 102-bp peak (markedly enhanced in relative mass in the absence of H1) were similar both immediately after a 30-min pulse period and after a 30-min pulse followed by a 4-h chase (Figure 8). Since the shortest pulse time used was 30 min, we conclude that within 30 min after repair synthesis histone H1 is present in most (or all) of the newly repaired regions of DNA that have undergone nucleosome rearrangement (Figures 5 and 6). Also, we conclude that the 102-bp fragment region of core DNA is not markedly enhanced or deficient in repair-incorporated label under these conditions (Figure 6).

Finally, we note that our results both verify and extend the observations of Lawson & Cole (1979) and Weischet (1979), who showed that the low pH extraction method does not induce the formation of compact oligomers. In the present study, we have used a different, and perhaps more sensitive, criterion for nucleosome rearrangement and also do not observe any measurable rearrangement induced by removal of H1 by low pH.

Acknowledgments

We thank Dr. Steven Dresler for his help in the analysis of the agarose gel electrophoresis data and for the generous gift of *Hin*fI ϕX174 RFI restriction fragments. We also thank Juanita Belanger and Suey Lan for their excellent technical assistance, Holly Schumacher for her assistance in preparation of the manuscript, and Dr. Raymond Reeves for his critical review of the manuscript.

Appendix

If we assume that the nuclease digestion properties of a given "repair patch" reflect only one of two possible states (i.e., unrearranged and rearranged), then

$$f_s = \phi_u f_s^u + \phi_r f_s^r \quad \text{(A1)}$$

where ϕ_i = fraction of repair patches in state i, f_s^i = fraction

of repair-incorporated nucleotides in state i that is nuclease sensitive, and u and r denote unrearranged and rearranged, respectively.

If we also assume that the repaired DNA in the unrearranged state is totally nuclease sensitive (i.e., $f_s^u = 1$; Smerdon & Lieberman, 1978, 1980) and in the rearranged state is totally randomized (i.e., $f_s^r = \xi$), then

$$f_s = \phi_u + \phi_r\xi \quad \text{(A2)}$$

and since $\phi_u + \phi_r = 1$

$$f_s = \phi_r(\xi - 1) + 1 \quad \text{(A3)}$$

Therefore, if the removal of histone H1 changes only the value of ξ and does not induce any rearrangement, then ϕ_r remains constant and the new value of f_s (i.e., f_s') will be

$$f_s' = f_s + \phi_r\Delta\xi \quad \text{(A4)}$$

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