Nucleosome Rearrangement in Vitro. 1. Two Phases of Salt-Induced Nucleosome Migration in Nuclei†

John F. Watkins and Michael J. Smerdon*
Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164-4660
Received March 19, 1985

ABSTRACT: We have investigated the salt- and temperature-induced rearrangement of nucleosomes in both intact and H1-depleted nuclei from human cells. In agreement with previous reports on the rearrangement of nucleosomes in isolated chromatin or chromain fragments, we observed a decrease in the average nucleosome repeat length following incubation of nuclei at 37 °C in elevated salt concentrations. However, this decrease occurred in two distinct phases. First, incubation of H1-depleted nuclei at 37 °C for as little as 10 min in low-salt, isotonic buffer (containing 0.025 M KCl) resulted in a shift in the limiting repeat value from ~190 to 168 base pairs (bp). A similar shift was observed for intact nuclei incubated at 37 °C for 1 h in buffer containing near-physiological salt concentrations (i.e., 0.175 M KCl). This limiting repeat value was maintained in both intact and H1-depleted nuclei up to a salt concentration of 0.45 M KCl in the incubation buffer. Second, at salt concentrations of 0.625 M KCl, a limiting repeat of ~146 bp was obtained, and the nuclei had clearly lysed. During the first shift in repeat length, little additional exchange of nuclear proteins occurred compared to nuclei kept on ice in a low-salt buffer. This was the case even though the conditions used to monitor exchange were optimized by using a high DNA to chromatin ratio. On the other hand, a significant increase in the exchange of nuclear proteins, and formation of nucleosomes on the naked DNA, was observed during the shift in repeat length to 146 bp. Furthermore, in the following report [Watkins, J. F., & Smerdon, M. J. (1985) *Biochemistry* (following paper in this issue)], we demonstrate that nucleosomes do not form in unfolded, newly repaired DNA during the first shift in repeat length while ~50% of this DNA acquires a nucleosome structure during the second shift in repeat length. These results suggest that cooperative, lateral migration of nucleosomes ("sliding") is responsible for the shift from ~190 to 168 bp but this process may be accompanied by histone exchange in the formation of the "compact oligomers".

An important consequence of the subunit structure of chromatin is the apparent need for nucleosome rearrangement during DNA processing events. Indeed, there is now evidence for rearrangements of chromatin structure during DNA replication and repair, as well as gene transcription [see recent reviews by Annunziato & Seale (1983), Lieberman (1982), and Reeves (1984)]. Whether the features of nucleosome rearrangement are similar for each of these cases is unknown. However, the fact that such rearrangements exist in vivo provides the impetus to understand which factors are important in allowing nucleosome rearrangements to take place in vitro and whether these factors may play a role under physiological conditions. Furthermore, it is important to establish what rearrangements of chromatin structure can occur under the conditions used to assess rearrangements in vivo.

Perhaps the easiest approach to follow at least one form of nucleosome rearrangement in vitro has been the measurement of nucleosome migration. Beard (1978) used a hybrid of linearized SV40 chromosomes and naked DNA to demonstrate nucleosome migration at 37 °C in 0.15 M NaCl. Subsequently, several laboratories reported on the importance of salt concentration and temperature, as well as the histone H1 content of chromatin, in determining the degree of nucleosome migration in isolated chromatin or chromatin fragments (Steinmetz et al., 1978; Spadafora et al., 1979; Weischet, 1979). In these studies, nucleosome migration was detected by the formation of closely spaced nucleosomes [≤145 base pair (bp)[1] repeat], or "compact oligomers", in H1-depleted chromatin initially having a native nucleosome spacing (i.e., >180 bp). Shortly after these studies, it was reported that staphylococcal nuclease itself can induce nucleosome migration in H1-depleted chromatin when digestion is carried out at elevated salt concentrations (i.e., >0.3 M; Weischet & Van Holde, 1980). Recently, Stein and co-workers have established conditions to promote the "reversal" of this compaction of nucleosome spacing by the addition of histone H5 to H1- and H5-depleted chromatin (Stein & Kunzler, 1983; Stein & Bina, 1984).

Concerning the rearrangement of nucleosomes in newly replicated regions of chromatin, several authors have reported that the nucleosome repeat of nascent (or immature) chromatin is shorter than that of mature chromatin (Murphy et al., 1978, 1980; Levy & Jakob, 1978). Indeed, when nuclei from HeLa cells (Annunziato & Seale, 1982) or Chinese hamster cells, blocked with hydroxyurea (D'Anna & Prentice, 1983), are digested with staphylococcal nuclease, the nascent chromatin yields a limiting nucleosome repeat of ~165 bp. The conditions used by these authors to carry out the nuclease digestions (i.e., low salt and, in one case, low temperature) clearly met the criteria established in the previous studies on isolated chromatin (discussed above) for the prevention of nucleosome migration in vitro. However, Jackson et al. (1981) presented evidence that in *nuclei* these criteria may be different and proposed that the formation of shorter repeat lengths in nascent chromatin is caused by the selective nucleosome rearrangement in these regions during the nuclease digestion. In the present report, we show that a stable 168 bp nucleosome repeat can be formed, at least in short stretches of oligo-

† This study was supported by National Institutes of Health Grant ES02614. M.J.S. is the recipient of an NIH Research Career Development Award.

[1] Abbreviations: bp, base pair(s); dThd, thymidine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; PCA, perchloric acid; HMG, high-mobility group.

0006-2960/85/0424-7279$01.50/0

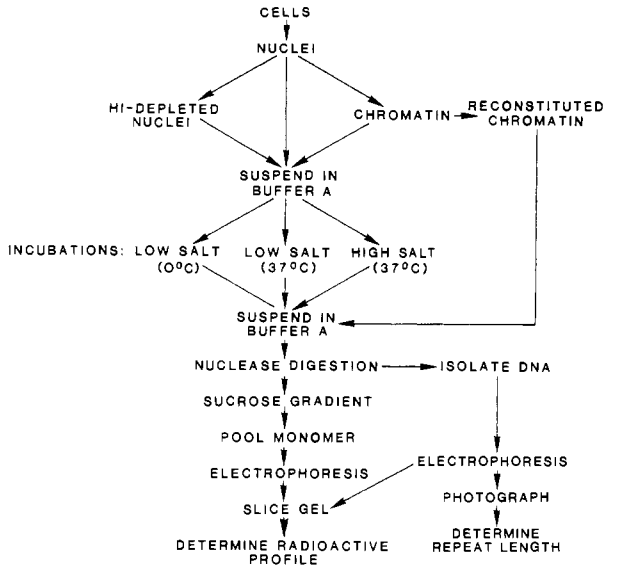

FIGURE 1: Flow chart of experimental procedures. For details, see Materials and Methods.

nucleosomes, in H1-depleted nuclei under low ionic strength conditions at 37 °C.

Materials and Methods

For clarification of the experimental procedures used in this paper, a flow chart is presented in Figure 1.

Cell Culture and Labeling. Normal human diploid fibroblasts (strains AG1518 and IMR-90) were grown in culture as described by Smerdon et al. (1982a). Cells between passage 10 and passage 18 were split 1:3 and labeled with 25–50 nCi/mL [^{14}C]dThd (50 mCi/mmol; New England Nuclear) or 10 nCi/mL [^{3}H]dThd (50–80 Ci/mmol; New England Nuclear) for 3–6 days. The medium was then replaced with fresh medium (not containing labeled nucleotides) and grown to confluence. For the DNA competition studies, cells were cultured and labeled with [^{3}H]dThd or [^{14}C]dThd as described previously, except in one experiment where 10 nCi/mL [^{14}C]lysine (317 mCi/mmol; New England Nuclear) was used in place of [^{14}C]dThd.

Preparation of Intact and H1-Depleted Nuclei and Chromatin. Both intact and H1-depleted nuclei were prepared by the method of Lawson & Cole (1979). As described previously (Smerdon et al., 1982b), >90% of the histone H1 is selectively extracted from AG1518 nuclei at pH 3.00 and from IMR-90 nuclei at pH 2.70. Alternatively, histone H1 was extracted by dissociation with 0.625 M KCl and subsequent centrifugation. In this latter method, purified nuclei were suspended in buffer A (50 mM Tris, pH 7.5, 25 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.25 M sucrose) containing 0.6 M KCl and stirred slowly for 30 min at 4 °C. The suspension was then centrifuged at 300*g* for 2 min, the supernatant was removed, and the salt extraction of histone H1 was repeated once more on the nuclear pellet. The H1-depleted nuclei were then resuspended in buffer A. The number of nuclei in each sample was determined prior to the salt incubation step (Figure 1) by counting an aliquot of the nuclei on a "bright-line" hemacytometer (American Optical Corp.) viewed through a phase-contrast microscope. Chromatin (hypotonically lysed nuclei) was prepared from the nuclear suspension by dialysis against 1 mM Tris, pH 7.4, at 4 °C for 10–12 h. All buffers contained 0.1 mM phenylmethanesulfonyl fluoride, and all procedures were performed on ice unless otherwise indicated.

Salt Incubations. The salt concentration of nuclei or chromatin, suspended in buffer A, was raised to 0.625 M KCl and 2 mM EDTA by dropwise addition of a solution consiting of buffer A, 3 M KCl, and 10 mM EDTA while stirring rapidly. When intermediate salt concentrations were used (see text), buffer A containing 10 mM EDTA and 5 times the final concentration of KCl was added. The resulting mixtures were incubated at 37 °C for 1 h with stirring every 10 min. The EDTA and excess salt were then removed either by dialysis against buffer A or by two low-speed centrifugation washings (300*g*) for short times (2 min) with buffer A. Serving as controls, some nuclei were suspended in low-salt buffer (buffer A and 2 mM EDTA) and either kept on ice during the incubation step or incubated at 37 °C exactly like the high-salt samples.

Nuclease Digestions. Nuclei (or an equivalent amount of chromatin), suspended in buffer A at a concentration of (3.5–7.0) × 10^6/mL, were incubated with staphylococcal nuclease (Worthington) at 37 °C as described elsewhere [e.g., see Smerdon & Lieberman (1980)]. With the exception of the H1-depleted nuclei prepared by salt dissociation at 4 °C and the H1-depleted nuclei incubated in low-salt buffer at 0 °C (Figure 1), all samples were preincubated for 10 min at 37 °C prior to the addition of staphylococcal nuclease. In the case of the two exceptions, the samples were left on ice during the preincubation period and incubated at 37 °C only *after* the addition of nuclease. The concentration of enzyme used is indicated in the figure legends. At various times during the digestion, the reaction was terminated by adding an aliquot of the reaction mixture to a tube on ice containing 0.1 volume of 0.1 M EDTA (pH 7) and vortexing. Ice-cold 70% perchloric acid (PCA) was then added to a portion of the terminated reaction mixture (final concentration 7% PCA), and the resulting mixture was vortexed and left on ice for 20 min. The PCA-precipitated sample was then centrifuged for 10 min in an Eppendorf micro centrifuge (Model 5412), and the resulting supernatant was assayed for radioactivity. For those samples to be used for the determination of the nucleosome repeat length, the remainder of the terminated reaction mixture was then incubated with proteinase K (EM Biochemicals) and the DNA ethanol-precipitated as previously described (Smerdon & Lieberman, 1980).

For the determination of the 100% digestion value, an aliquot of the nuclei was first incubated with 20 μg/mL proteinase K for 3 h at 37 °C followed by a 1-h incubation at 37 °C with 250 units/mL staphylococcal nuclease. The digestion was stopped with EDTA as above, ice-cold 70% PCA was added to a final concentration of 7%, the samples were vortexed, and the radioactivity of duplicate aliquots was determined. (Originally, the PCA-precipitated samples were left on ice and centrifuged, and the supernatant was assayed for radioactivity as with the partially digested samples. However, we have observed no difference in the level of radioactivity between the acid-soluble fraction following centrifugation and the suspension prior to centrifugation and have, therefore, omitted the centrifugation step in the determination of the 100% digestion value.)

DNA Competition Experiments. Naked DNA, labeled with [^{3}H]dThd, and hypotonically lysed nuclei (chromatin), labeled with [^{14}C]dThd or [^{14}C]lysine, were mixed in buffer A at a 2:1 (w/w) ratio, respectively, prior to salt treatment. The naked DNA was prepared by isopycnic centrifugation in neutral CsCl gradients centrifuged in a Beckman VTi 65 rotor as described by Smerdon et al. (1978). The concentration of naked DNA was determined from the absorbance at 260 nm (1 ODU = 50 μg/mL), and the amount of DNA in the chromatin was estimated by assuming each nucleus contained

6.4 pg of DNA (Kornberg, 1980). The final concentration of DNA in the mixture was 50–100 μg/mL. Following the salt incubation, the samples were dialyzed overnight against a 1000× volume of buffer A. The [^{3}H]dThd-labeled DNA/[^{14}C]lysine-labeled chromatin mixtures were then dialyzed against a 1000× volume of 10 mM Tris and 2 mM EDTA, pH 7.4, for 14 h with one change of buffer after 2 h. The dialyzed samples were layered on 5–20% linear sucrose gradients (40 mL) containing 2 mM EDTA (pH 7.4) and centrifuged for 2.25 h at 30 000 rpm in a Beckman VTi 50 rotor using a Beckman L8-70 ultracentrifuge. The gradients were fractionated (0.8 mL/fraction) from bottom to top by using an LKB microperpex peristaltic pump (Model 2132) in conjunction with an LKB Redirac fraction collector (Model 2112). The radioactivity of each fraction was determined by adding Ready-Solv MP (Beckman) and counting in a Beckman LS 7500 liquid scintillation counter.

Those samples containing a mixture of [^{3}H]dThd-labeled DNA and [^{14}C]dThd-labeled chromatin were partially digested by staphylococcal nuclease in buffer A, dialyzed against 10 mM Tris and 2 mM EDTA, pH 7.4, and layered on 5–20% linear sucrose gradients containing 2 mM EDTA (pH 7.4). The gradients were then centrifuged for 3.25 h at 49 000 rpm in a Beckman VTi 50 rotor and fractionated as above, and 50 μL of each fraction was assayed for radioactivity. Those fractions corresponding to the mononucleosome peak were pooled and digested with proteinase K, and the DNA was ethanol-precipitated for electrophoresis.

Electrophoresis. Electrophoresis was carried out on 2.8% or 1.3% horizontal agarose slab gels as described by Smerdon et al. (1978). The gels were stained with 2 μg/mL ethidium bromide, illuminated (Ultra-violet Products, Inc., transilluminator), and photographed with a Polaroid MP-4 camera using a red filter and Polaroid type 55 film. For those samples to be assayed for radioactivity, the gel lanes were cut into 2-mm slices, placed into glass scintillation vials, and dissolved in 2 mL of H_2O with heat. The dissolved slices were then assayed for radioactivity as described previously.

Determination of Repeat Length. Photographic negatives of the gels were scanned on either a Beckman DU spectrophotometer equipped with a linear transport device or a Beckman CDS-100F gel scanner and integrator system. Sizes of the staphylococcal nuclease digestion products were then obtained from the calibration curve generated by plotting the log of the band size (in bp) of *Hin*fI restriction fragments of *ϕX174* RF DNA, or *Hin*cII restriction fragments of λ DNA, against the migration distance.

The nucleosome repeat length of total DNA can then be calculated by the linear regression method of Thomas & Furber (1976). In this method, the size of the digestion products is plotted against the band number, and the slope of the line from a least-squares fit of the data is equal to the repeat length. However, with increasing extents of nuclease digestion, the number of oligonucleosome bands decreases with only dimer, trimer, and, perhaps, tetramer DNA bands visible on the gel. For these greater extents of digestion, the linear regression method is less accurate since only two or three points can be used to determine the line. Except for early digestion points, our data fall into this category, and therefore, an alternate method was developed. In this method, the value of the repeat length (RL) for any given oligomer is calculated from the expression

$$\mathrm{RL}(n) = \frac{L_n - nL_1}{n-1} + L_1 \qquad (1)$$

where n = the oligomer band number (e.g., dimer = 2, trimer = 3), L_1 = the size (in base pairs) of the monomer band, and L_n = the size of the n^{th} oligomer band. It is important to note that L_1 is not necessarily equal to core-sized DNA (146 bp) but is equal to whatever value is obtained from the calibration curve. The first term on the right-hand side of eq 1 is equivalent to the average "effective" size of the linker DNA for the n^{th} oligomer band [i.e., it is the size of linker DNA not accounted for by the monomer size (L_1)]. Thus, for a repeat of 200 bp, one might expect L_1 = 200 bp at very small extents of digestion, which means the first term of eq 1 would be zero. With increasing extents of digestion, L_1 decreases, and the effective linker value increases accordingly to yield a repeat length close to 200 bp. The repeat length of total DNA can be determined from the arithmetic mean of the repeat length values obtained for each oligomer. Thus, we can write [realizing that eq 1 reduces to $(L_n - L_1)/(n-1)$]

$$\mathrm{RL} = \frac{\sum_{n=2}^{m} [(L_n - L_1)/(n-1)]}{m-1} \qquad (2)$$

where m = the largest resolvable oligomer band number.

We have compared the repeat lengths of 70 samples using the linear regression method and our method (eq 2). Each sample had oligomer DNA bands resolvable to at least the tetramer level. The average difference between the repeat length values from the two methods, plus or minus 1 standard deviation, was 0.3 ± 2.0 bp with a range from −4.0 to 3.8 bp. Thus, for low to moderate extents of digestion, the two methods give comparable results.

Results

Effect of H1 Removal on Salt-Induced Rearrangement. The salt-induced rearrangement of nucleosome cores in human fibroblast nuclei was examined by monitoring the change in the nucleosomal repeat length. In each case, intact or H1-depleted nuclei were incubated in the presence of various salt concentrations at 0 or 37 °C, resuspended in isotonic buffer, and digested with staphylococcal nuclease, and the resulting DNA fragments were electrophoresed on agarose gels (see Figure 1). As reported by others for isolated chromatin or chromatin fragments (Beard, 1978; Spadafora et al., 1979; Weischet, 1979; Wasylyk & Chambon, 1980; Glotov et al., 1982), the shift in repeat length was found to be dependent upon a combination of the concentration of salt during the incubation step, the temperature, and whether or not the nuclei were depleted of histone H1.

When intact nuclei were examined, the general effect of an increase in the concentration of salt during the 37 °C incubation step was a decrease in the average nucleosome repeat length obtained following relatively large extents of digestion. This effect is seen most clearly when one determines the nucleosome repeat length as a function of the fraction of DNA rendered acid soluble by staphylococcal nuclease. As shown in Figure 2 (open circles), when intact nuclei were incubated in low-salt buffer, the average nucleosome repeat length remained close to the maximum value of 193 bp even after 20% of the labeled DNA had been digested to acid-soluble form. On the other hand, following incubation in high-salt buffer (buffer A plus 0.6 M KCl) at 37 °C, the repeat length decreased to a "limiting" value of ~146 bp when 20% of the DNA was rendered acid soluble (Figure 2, open triangles). (We note that following this latter treatment, the nuclei appeared ruptured when viewed through a phase microscope.) Thus, the overall change observed (i.e., between the maximum average repeat length and the limiting repeat length obtained

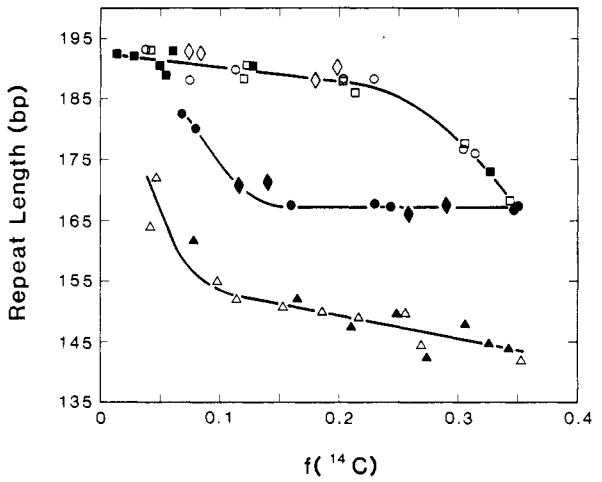

FIGURE 2: Nucleosome repeat length as a function of the fraction of DNA [$f(^{14}C)$] rendered acid soluble by staphylococcal nuclease. Intact (open symbols) and H1-depleted (closed symbols) nuclei were prepared from confluent human fibroblasts (strain AG1518) and labeled with [^{14}C]dThd during the growth phase, as described in Smerdon et al. (1982b). The data are for nuclei suspended in low-salt buffer (buffer A and 2 mM EDTA) and incubated for 1 h at 0 (■, □) or 37 °C (●, ○) and for nuclei suspended in high-salt buffer (buffer A, 0.6 M KCl, and 2 mM EDTA) and incubated for 1 h at 37 °C (▲, △). Following these incubations, the nuclei were resuspended in buffer A, preincubated at 37 °C for 10 min, and digested at 37 °C with staphylococcal nuclease [except for the H1-depleted sample incubated at 0 °C (■), which was kept on ice during the preincubation period]. The resulting DNA fragments were isolated and coelectrophoresed with *Hin*fI restriction fragments of ϕX174 RF DNA on agarose gels, and the repeat length was determined as described under Materials and Methods. We have included the results obtained for IMR-90 nuclei which were incubated on ice in buffer A, preincubated for 10 min at 37 °C, and digested with staphylococcal nuclease (♦, ◊).

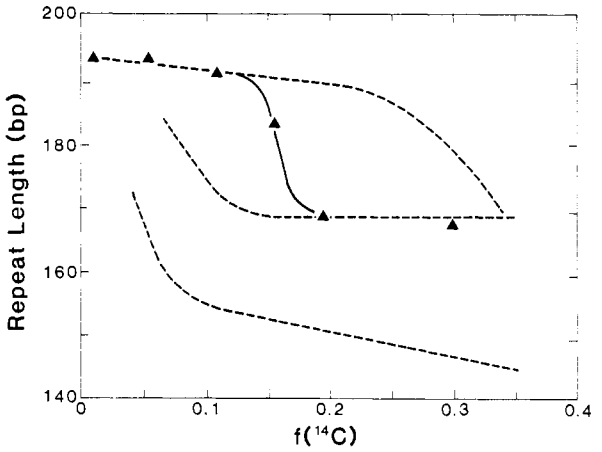

FIGURE 3: Nucleosome repeat length as a function of the fraction of DNA [$f(^{14}C)$] rendered acid soluble by staphylococcal nuclease for chromatin depleted of histone H1 by high-salt treatment. Nuclei from confluent human fibroblasts (strain AG1518), labeled during the growth phase with [^{14}C]dThd, were suspended in high-salt buffer (buffer A, 0.6 M KCl, and 2 mM EDTA) and incubated at 0 °C for 1 h, while stirring slowly, to dissociate histone H1. The resulting chromatin was then washed with buffer A, resuspended in this buffer, and digested with staphylococcal nuclease in the following manner: Nuclease was added to the suspension on ice (final concentration 4 units/10^6 nuclei), and the suspension was placed in a 37 °C water bath. Aliquots were removed at 1, 1.5, 2, 2.5, 3.25, and 4 min after addition of the nuclease, and the digestion was terminated with EDTA. The temperature of the incubation mixture at each digestion time was 23 (1 min), 28 (1.5 min), 32 (2 min), 35 (2.5 min), 36 (3.25 min), and 37 °C (4 min). The resulting DNA fragments were electrophoresed on 2.8% agarose gels and the repeat lengths determined as in Figure 2. The dashed lines indicate the fits to the repeat length data presented in Figure 2.

after high-salt incubation) was ~47 bp, or the (average) maximum length of linker DNA between adjacent core particles. Furthermore, the gel scans of staphylococcal nuclease digestion products from nuclei incubated in the presence of high salt at 37 °C showed a higher background at earlier digestion times than nuclei incubated in the presence of low salt, as well as an increased amount of nucleosome dimers (data not shown; although see Figure 5). These latter results are in complete accordance with previous studies by Spadafora et al. (1979) on isolated chromatin fragments and indicate that exposure of intact nuclei to elevated salt concentrations (0.625 M KCl) at 37 °C results in the formation of "compact" nucleosome dimers and oligomers.

When H1-depleted nuclei were incubated at 0 °C (Figure 2, closed squares), we obtained the same repeat length values as intact nuclei incubated at either 0 or 37 °C. In this case, staphylococcal nuclease was added to an ice-cold suspension of nuclei, and the digestion was carried out while the sample warmed to 37 °C (i.e., there was no preincubation at 37 °C prior to addition of nuclease). On the other hand, when the incubation in low-salt buffer was performed at 37 °C, a major distinction between the repeat lengths of intact and H1-depleted nuclei was observed. In this case, the repeat length of the H1-depleted nuclei rapidly decreased during the first 15% of digestion and leveled off at 168 bp (Figure 2, closed circles). Furthermore, when H1-depleted nuclei were first incubated at 0 °C in low-salt buffer, and then preincubated for 10 min at 37 °C prior to addition of nuclease and digestion at 37 °C, the resulting repeat length fell to ~168 bp. This result is illustrated in Figure 2 (diamonds) for intact and H1-depleted nuclei from IMR-90 human fibroblasts. Thus, exposure of H1-depleted nuclei to 37 °C for as little as 10 min resulted in a redistribution of a significant fraction of the nucleosome cores to give an average repeat of ~168 bp.

The transition to the 168 bp repeat length was also examined in nuclei which had been depleted of histone H1 (and most non-histone chromosomal proteins) in high-salt buffer at 4 °C. In this case, the repeat length remained high (~193 bp) during the early digestion times (and lower extents of digestion), indicating that the high-salt treatment *alone* at 4 °C did not affect the repeat length (Figure 3). This result is also in agreement with previous reports (Steinmetz et al., 1978; Spadafora et al., 1979; Weischet, 1979). However, as the temperature of the sample increased during the digestion, a transition to the 168 bp repeat length occurred. [It is interesting to note that when the temperature of the buffer was monitored under identical digestion conditions, the drop in repeat length occurred at the same time the temperature reached 37 °C (see legend to Figure 3).] This decrease in repeat length is strikingly similar to that obtained for nuclei depleted of H1 by extraction in low-pH buffer (Figure 2), although the acid-extracted nuclei did not show this change unless they were preincubated at 37 °C for at least 10 min. This observation may reflect the differences in selectivity by the two methods of histone H1 depletion: The low-pH method is highly specific for displacement of histone H1 (Lawson & Cole, 1979; Smerdon & Lieberman, 1981), while the high-salt method removes non-histone proteins along with H1. Therefore, certain non-histone chromosomal proteins may play a role in maintaining the native nucleosome repeat length. Alternatively, these results may reflect differences between the constraints placed upon the nucleofilament in chromatin and nuclei. (As examined by phase-contrast microscopy, the nuclei remain intact when H1 is extracted at low pH but are ruptured when incubated in high salt.) In any event, a stable 168 bp repeat was generated by incubation at 37 °C in the

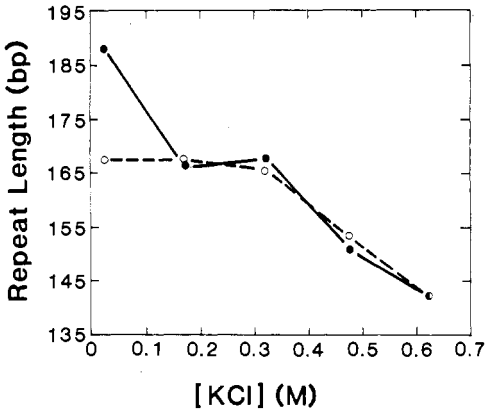

FIGURE 4: Nucleosome repeat length of intact (●) and H1-depleted (○) nuclei as a function of the salt concentration used during the incubation step. Nuclei from AG1518 cells were incubated for 1 h at 37 °C in buffer A containing 2 mM EDTA and KCl at a final concentration of 0.025, 0.175, 0.325, 0.475, or 0.625 M. Following salt treatment, the nuclei were resuspended in buffer A and incubated with staphylococcal nuclease until 23–31% of the DNA was rendered acid soluble. The nuclease digestion products were then electrophoresed on 2.8% agarose gels along with *Hin*fI restriction fragments of ϕX174 RF DNA, and the nucleosome repeat lengths were determined (Materials and Methods).

absence of histone H1 and increased salt concentrations [although the action of staphylococcal nuclease cannot be ignored (see Discussion)].

Effect of Varying Salt Concentrations on Rearrangement. Intact and H1-depleted nuclei were incubated for 1 h at 37 °C in buffer A containing 0, 0.15, 0.30, 0.45, or 0.60 M additional KCl. Following the incubation, the nuclei were resuspended in buffer A and digested with staphylococcal nuclease, and the repeat length was determined. The extent of digestion was 23–29% for the intact nuclei and 25–31% for the H1-depleted nuclei (i.e., within the range where the greatest differences in repeat lengths were expected; Figure 2). A plot of the repeat length vs. KCl concentration is presented in Figure 4. The results show that H1-depleted nuclei treated with as much as 0.30 M addition salt in the incubation buffer were still able to maintain a ~168 bp repeat, while 0.45 M KCl reduced the repeat to 154 bp and 0.60 M KCl led to formation of compact oligomers and a repeat of 143 bp. A control sample (kept on ice during the incubation step) gave a repeat of 191 bp (data not shown). Surprisingly, when intact nuclei were examined, the ~168 bp repeat was formed in nuclei that had been incubated in as little as 0.15 M additional salt. The original repeat length (~190 bp) was obtained from these nuclei incubated in buffer A alone, and the same extent of rearrangement as that seen for the H1-depleted nuclei was obtained for 0.45 and 0.60 M KCl. The shift in repeat length at 0.15 M KCl for intact nuclei can be seen directly in Figure 5B. The data represent profiles obtained for ^{3}H-labeled DNA, isolated from nuclei incubated in buffer A and 0.15 M KCl, and ^{14}C-labeled DNA, isolated from nuclei incubated in buffer A only, that was electrophoresed in the same gel lane on an agarose gel. Even though the nuclei in each case were digested to approximately the same extent (24% for ^{3}H-labeled DNA and 20% for ^{14}C-labeled DNA) by staphylococcal nuclease, the dimer and trimer bands labeled with ^{3}H were shifted to lower DNA sizes relative to the ^{14}C-labeled DNA, while the monomer bands had the same mobility in each case. A more sensitive method for detecting such a shift is shown in Figure 5A where the ratio of the two labels is plotted as a function of migration. While this ratio is almost constant over the monomer band, it is clear that the dimer and trimer bands did not coelectrophorese. We note that when the ^{3}H-labeled nuclei were incubated in buffer A only (i.e., the same as the ^{14}C-labeled nuclei) and the nu-

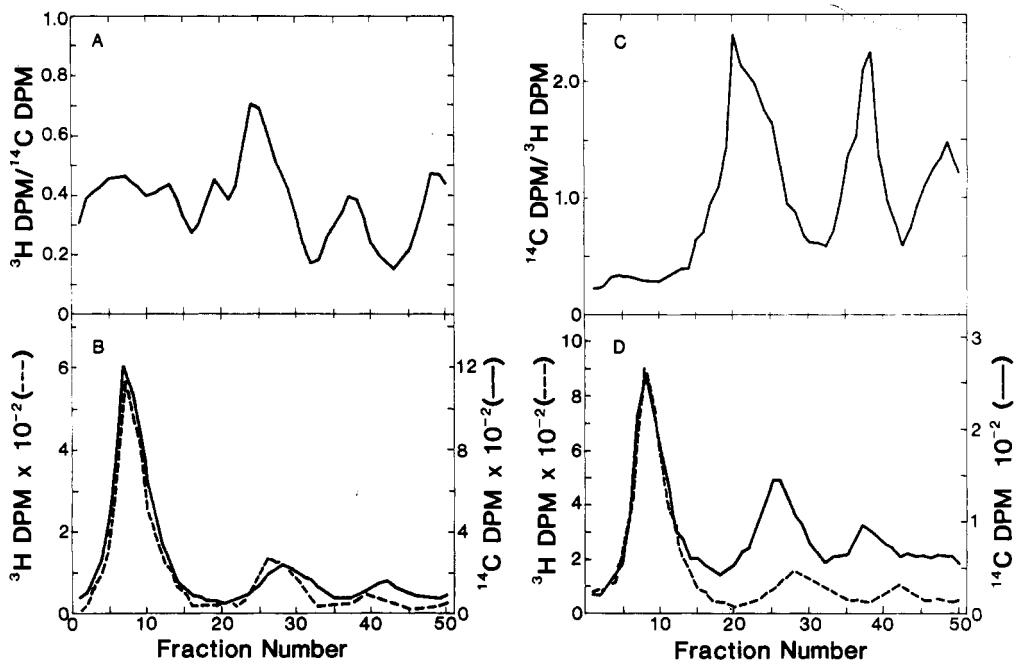

FIGURE 5: Gel electrophoresis profiles of radiolabeled DNA fragments from nuclei, following different incubation conditions, digested with staphylococcal nuclease. Nuclei were isolated from AG1518 cells labeled during the growth phase with either [^{3}H]dThd or [^{14}C]dThd. For panels A and B, ^{3}H-labeled nuclei were incubated at 37 °C for 1 h in buffer A containing 0.175 M KCl, and ^{14}C-labeled nuclei were incubated at 0 °C in buffer A only. The nuclei were then resuspended in buffer A and digested with staphylococcal nuclease until 24% (^{3}H) or 20% (^{14}C) of the DNA was rendered acid soluble. The DNA fragments from each sample were combined and coelectrophoresed on a 2.8% agarose gel. For panels C and D, DNA fragments from ^{3}H-labeled nuclei incubated in buffer A at 0 °C were coelectrophoresed with DNA fragments from ^{14}C-labeled nuclei incubated at 37 °C in buffer A containing 0.6 M KCl. In this case, 19% (^{3}H) or 20% (^{14}C) of the DNA was rendered acid soluble. The gel lanes were sliced and the radioactivity profiles determined (Materials and Methods). In each case, both the amount of each label (B and D) and the ratio of the two labels (A and C) were determined. The direction of migration is from right to left with the monomer peak being between fractions 6 and 12.

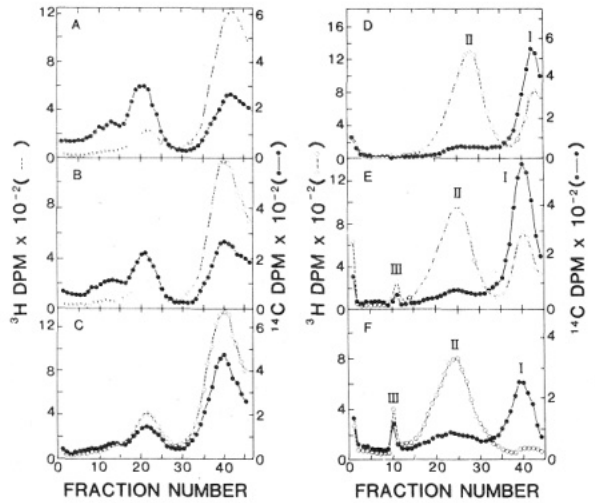

FIGURE 6: Sucrose gradient profiles from DNA competition experiments. Naked DNA (^{3}H labeled; open circles) was mixed 2:1 with chromatin (closed circles) labeled with [^{14}C]dThd (panels A–C) or [^{14}C]lysine (panels D–F). The mixture was incubated for 1 h in low-salt buffer at 0 °C (A and D), in intermediate-salt buffer (0.175 M KCl) at 37 °C (B and E), or in high-salt buffer (0.625 M KCl) at 37 °C (C and F). The samples were then subjected to sucrose density centrifugation (Materials and Methods) either immediately (D–F) or following partial digestion with staphylococcal nuclease (A–C). The direction of sedimentation is from right to left. In panels A–C, the nucleosome monomer peak is between fractions 16 and 26.

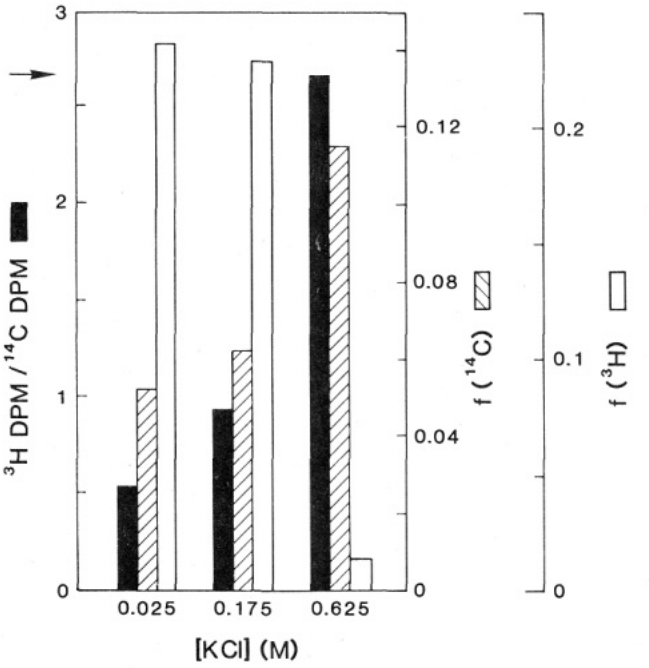

FIGURE 7: Comparison of the extent of protein and nucleosome core exchange between chromatin and naked DNA at three different salt concentrations. The ^{3}H dpm/^{14}C dpm ratio (solid bars) was obtained from integration of the electrophoretic profile of the core DNA that was prepared from core particles isolated on the sucrose gradients shown in Figure 6, panels A–C. The arrow indicates the ratio of total ^{3}H dpm to total ^{14}C dpm in the unfractionated sample. The hatched bars represent the fraction of labeled protein (^{14}C) in peak II, and the open bars represent the fraction of naked DNA (^{3}H) in peak I of Figure 6, panels D–F. (See the legend to Figure 6 for details.)

clease digestion products coelectrophoresed with the ^{14}C-labeled DNA, a constant ^{3}H/^{14}C ratio across the gel was obtained (data not shown). This same analysis was performed on control and high-salt-treated nuclei (Figure 5, panels C and D). A comparison of panels B and D of Figure 5 reveals the larger shift in the dimer and trimer bands for high-salt-treated nuclei, thereby further demonstrating the extent of salt-induced rearrangement from low to intermediate salt concentrations, and from intermediate to high salt concentrations.

DNA Competition Studies. We were interested in determining if these rearrangements of chromatin structure proceeded by a "sliding" process only (i.e., lateral movement of nucleosome cores along the DNA without complete dissociation from the DNA) or whether the exchange of histones from one DNA strand to another could also take place under these conditions. Two approaches were taken to address this question.

First of all, chromatin, containing [^{14}C]lysine-labeled proteins, was prepared from nuclei by hypotonic lysis to facilitate the transfer of any dissociated proteins to competing naked DNA strands. [As shown in the following paper (Watkins & Smerdon, 1985), this method of chromatin preparation does not induce any measurable nucleosome rearrangements.] The ^{14}C-labeled chromatin was then mixed in a 1:2 ratio with ^{3}H-labeled DNA and incubated in buffer A containing either 0, 0.15, or 0.6 M addition salt. This high DNA to chromatin ratio was chosen to optimize the conditions for exchange (Germond et al., 1976) since we were interested to know whether *any* exchange could take place under our conditions (see Discussion). The samples were then dialyzed against a low-salt buffer and subjected to sucrose gradient centrifugation (Materials and Methods). The radioactivity profiles are presented in Figure 6 (panels D–F). In control gradients containing only naked DNA or chromatin, all of the ^{3}H-labeled DNA sedimented in the same location as peak I, while >90% of the chromatin radioactivity pelleted [<10% remained at the very top of the gradient (data not shown)]. For the profiles in Figure 6, panels D–F, <9% of the DNA and ~50% of the labeled proteins pelleted. Of the labeled proteins that did not pellet, some appeared in peak I and most likely represent "free proteins" (i.e., not bound to the DNA) since unbound proteins also appeared at this position in the chromatin control gradient. (These proteins are presumably weakly bound in the chromatin and are released in low ionic strength buffer.) The remaining labeled protein appears to have bound to the naked DNA, causing an increase in the sedimentation rate of this DNA from peak I to peak II. With increasing salt concentrations, the amount of protein in peak II increased while the amount of free DNA (peak I) decreased. Furthermore, following the incubations in 0.15 and 0.6 M KCl, a third peak (peak III) was observed. At present, we do not know what nucleoprotein structure peak III represents. The fraction of protein label in peak II [$f(^{14}C)$] and the fraction of DNA label in peak I [$f(^3H)$] are plotted in Figure 7. The data show that while the amount of protein exchange in the samples incubated at the two lower salt concentrations is similar, there is a significant increase in the amount of exchange in the high-salt sample. These results indicate that at least some protein exchange can take place during the incubation step and there is a marked increase in this exchange when samples are incubated in the high-salt-containing buffers.

To determine whether the exchange of protein from chromatin to naked DNA involved formation of nucleosome core particles, the DNA and chromatin were labeled with different radioactive isotopes of dThd. After incubation in buffer A with 0, 0.15, or 0.60 M added salt, the samples were equilibrated to buffer A by dialysis and partially digested with staphylococcal nuclease. Monomer nucleosomes were then isolated on sucrose gradients (Figure 6, panels A–C). Two observations can be made from these gradient profiles: (1) In each case, a small fraction of naked DNA cosediments with the monomer peak from the chromatin sample; and (2) with

increasing salt concentrations, there is an increase in the amount of ^{3}H-labeled (naked) DNA, relative to the amount of ^{14}C-labeled (chromatin) DNA, located in this peak. To assure that the ^{3}H-labeled DNA in the monomer peak represented DNA folded into nucleosome-like structures (rather than simply large DNA fragments cosedimenting with the same *s* value as core particles), the DNA from the monomer peak was electrophoresed on agarose gels, and the gel lanes were sliced and assayed for radioactivity. The ^{3}H and ^{14}C profiles were identical, yielding a single peak centered around 146 bp (data not shown). The ratio of the total ^{3}H and ^{14}C dpm in the 146 bp band yields a quantitative measure of the amount of originally naked DNA that was protected from nuclease digestion following incubation at the different salt concentrations. As shown in Figure 7 (solid bars), there is only a small change in this ratio in going from an incubation in buffer A at 0 °C to an incubation in buffer A and 0.15 M KCl at 37 °C. However, a significant increase in the amount of protected naked DNA was obtained following incubation in high salt. In fact, the ratio of (originally) naked DNA label (^{3}H) to chromatin label (^{14}C) in the isolated monomer core particles is identical with the ratio of *total* ^{3}H to *total* ^{14}C in the mixture (Figure 7, arrow). This result indicates that the exchange of nucleosome cores reached equilibrium during the 1-h incubation in high salt (i.e., each of the labeled DNA populations had the same number of nucleosome cores per unit length of DNA).

Discussion

In this report, we have examined the salt-induced rearrangement of nucleosome cores in nuclei by monitoring the change in the nucleosomal repeat length. In agreement with previous reports on isolated chromatin or chromatin fragments (Spadafora et al., 1979; Weischet & Van Holde, 1980), our results show that incubation of nuclei at 37 °C and elevated salt concentrations gives a decrease in the average nucleosome repeat length. Unexpectedly, however, we found this change to occur in two distinct phases. First of all, a drop in the repeat length from 193 bp to a limiting value of 168 bp was obtained when intact nuclei were incubated for 1 h at 37 °C and intermediate salt concentrations (0.175–0.325 M KCl; Figure 4). Furthermore, when nuclei were depleted of histone H1, this repeat length was obtained following incubation at 37 °C for as little as 10 min in low-salt buffer (0.025 M KCl; Figure 2). Second, following incubation in high salt (0.625 M KCl) at 37 °C, a limiting repeat of ~146 bp was obtained for both initially intact and H1-depleted nuclei (Figure 2). This latter treatment had caused most of the nuclei to lyse.

In comparing the various treatments in this study, we note that at low extents of digestion [i.e., $f(^{14}C) \leq 0.05$], only a few base pairs separate the repeat length values of H1-depleted nuclei incubated in low-salt buffer at 37 °C and those incubated at 0 °C. On the other hand, distinct differences in repeat length were observed when 10–25% of the DNA had been digested to acid-soluble form (Figure 2). At digestions greater than 25%, the average repeat length of low-salt-treated (0 °C) H1-depleted nuclei and low-salt-treated (0 and 37 °C) intact nuclei gradually decreased until ~35% of the DNA was digested (the maximum digestion value at which a repeat length could be obtained). At this point, these samples had the same nucleosome repeat length as H1-depleted nuclei incubated in low salt at 37 °C. This decrease in repeat length is presumably due to preferential cleavage of long linkers and exonucleolytic trimming of linker ends (Klevan & Crothers, 1977; Lohr et al., 1977; Martin et al., 1977; Noll & Kornberg, 1977). Therefore, although it is clear from the data in Figure 2 that a significant fraction of the chromatin has undergone some form of rearrangement to yield stable repeat lengths of 168 and ~146 bp, the sharp decrease in repeat length value during the first 10–12% of digestion indicates that a heterogeneous population of linker lengths exists in these samples following salt treatment.

The extent to which the action of staphylococcal nuclease plays a role in this in vitro rearrangement process is not known. Weischet & Van Holde (1980) have shown that staphylococcal nuclease can induce the formation of compact oligomers (120*n* + 30 bp repeat) in H1-depleted chromatin when digested is carried out at 37 °C in 0.3–0.35 M salt. These authors also found evidence for the formation of compact oligomers when H1-depleted nuclei were digested under the same conditions; however, it was clear the nuclei had lysed. In our experiments, the 168 bp repeat was formed under low-salt conditions in nonlysed, H1-depleted nuclei (Figures 2 and 4). Furthermore, intact nuclei also yielded a 168 bp limiting repeat following incubation at 37 °C in 0.15 M additional salt (Figure 4). It is important to note that these latter nuclei were resuspended in low-salt buffer prior to the addition of nuclease. These results, along with those of Weischet & Van Holde (1980), suggest that the change in repeat length that we observe in nuclei is due primarily to nucleosome rearrangement during the incubation step rather than during the nuclease digestion.

The implication that histone H1 plays a role in stabilizing the native nucleosome repeat is clear from these studies. The formation of the 168 bp repeat occurred at low ionic strength in H1-depleted nuclei (at 37 °C) while the intact nuclei maintained a normal nucleosome repeat under the same conditions (Figure 2). [It is interesting to note that Stein & Kunzler (1983) have shown that this shift in nucleosome repeat can be reversed by the addition of histone H5 to H1- and H5-depleted chromatin.] Somewhat surprisingly, the 168 bp repeat was formed in *intact* nuclei following incubation at 37 °C in near-physiological ionic strength buffer. However, this change in repeat length was not completely unexpected since histone H1 has been reported to exchange between sites at physiological ionic strengths at 0 °C (Caron & Thomas, 1981). In fact, efficient exchange of H1 was reported at ionic strengths of 50–80 mM at 4 °C (Lasters et al., 1981; Louters & Chalkley, 1984). We would expect, therefore, that under our experimental conditions, efficient exchange of H1 was taking place. Continuous exchange of H1 among various sites could lead to destabilization of individual nucleosomes for the time H1 was not bound (or only partially bound). Thus, during the course of the salt incubation, many of the nucleosomes may be in a "destabilized state" and are free to undergo rearrangement to the 168 bp repeat.

We also performed DNA competition experiments to determine if the changes in nucleosome repeat are accompanied by exchange of nuclear proteins. We used hypotonically lysed nuclei for these experiments to facilitate any exchange of proteins to a free DNA substrate. Furthermore, we used a high DNA to chromatin ratio (2:1) to minimize the average distance between the free DNA and nucleoprotein and, therefore, mimic more closely the situation of a protein-free DNA strand within nuclear chromatin. The results of these experiments (Figures 6 and 7) suggest that the initial drop in repeat length to 168 bp occurs by a sliding (or lateral migration) mechanism. This statement is partially based on the fact that although some nuclear proteins were exchanged in the low-salt-treated (0 °C) mixture, no significant increase in exchange was observed when the salt concentration was increased by 0.15 M and the temperature elevated to 37 °C.

On the other hand, this shift in salt concentration and temperature had a marked effect on the repeat length. Furthermore, the exchange of histones has been demonstrated to be dependent not only on the salt concentration and the temperature but also on the time (Louters & Chalkley, 1984). Thus, the level of exchange observed in these two samples may be attributed to the length of time the DNA and chromatin were suspended together in the same solutions (30–40 h). (Alternatively, this "exchange" may represent the binding of free histones in the mixture to the naked DNA.) The concept that nucleosomes slide along the DNA at these salt concentrations is consistent with the conclusions of Beard (1978) and Glotov et al. (1982) for linearized SV40 minichromosomes.

Incubation in the high-salt buffer resulted in significant exchange of nuclear proteins to the free DNA (Figures 6 and 7). We believe that this exchange involves transfer of nucleosome core proteins, which then assemble into core structures, for several reasons. First of all, the transfer of proteins resulted in the formation of particles that protected ~146 bp of DNA from staphylococcal nuclease digestion and cosedimented with bona fide core particles on sucrose gradients (Figure 6). It is known that corelike structures ("beads") can also be formed by HMG proteins, histone H2A–H2B complexes, and histone H3–H4 tetramers, as well as the normal histone octamer complex (Sollner-Webb et al., 1976; Jorcano & Ruiz-Carrillo, 1979; Mathis et al., 1980). However, although H2A, H2B, and HMG proteins can be dissociated from chromatin even at the lowest ionic strength used here (Jensen & Chalkley, 1968; Ilyin et al., 1971; Burton et al., 1978; Louters & Chalkley, 1984), the resulting DNA–protein complexes will not protect fragments characteristic of nucleosomes from nuclease digestion (Sollner-Webb et al., 1976; Jorcano & Ruiz-Carrillo, 1979; Mathis et al., 1980). Histones H3 and H4 have been shown to readily transfer to naked DNA in 0.6 M salt, and the "half-nucleosomes" formed can protect DNA fragments as long as 146 bp; however, the lengths, rate of appearance, and stability of the DNA fragments produced by nuclease digestion are clearly different from digests of normal chromatin (Varshavsky & Ilyin, 1974; Burton et al., 1978; Jorcano & Ruiz-Carrillo, 1979). Since core-sized DNA was protected even after extensive digestion in our experiments (45–50% acid soluble), and since the gel profiles of the ^{3}H-labeled and ^{14}C-labeled core DNA were superimposable, H3–H4 tetramers are probably not the species bound to the originally naked DNA. Thus, by all the criteria examined, it appears that bona fide core particles were formed on the competing DNA.

When the chromatin and DNA were incubated in high salt, exchange of nucleosomes reached equilibrium (Figure 7), indicating that the mechanism for nucleosome rearrangement at this salt concentration and temperature could be the result of *both* exchange and nucleosome sliding. The method by which nucleosome core histones migrate from one DNA strand to another is unknown; however, under our conditions, it is likely that the histones were "titrated" off the chromatin by the naked DNA since no evidence exists for nucleosome dissociation below 0.6 M salt in the absence of added DNA or histone, except in the case of monomer nucleosomes (Cotton & Hamkalo, 1981). Thus, the drop in repeat length to ~146 bp that we observed for high-salt-treated nuclei may be the result of both an exchange of histones between different DNA strands in chromatin and migration of histones along the same DNA strand.

A model summarizing the results of the present report is shown in Figure 8. As indicated, the change in repeat length

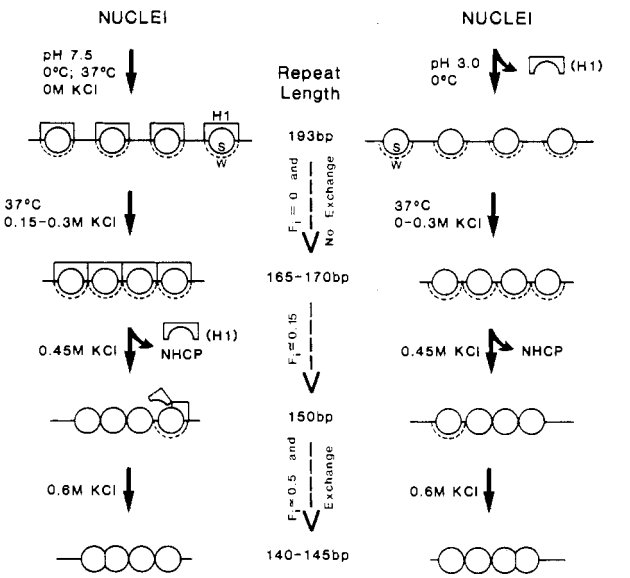

FIGURE 8: Schematic representation of the different phases of nucleosome rearrangement in intact (left) and H1-depleted (right) nuclei in vitro. The letters S and W refer to the strong and weak interactions of the core histones in the 168 bp chromatosome, respectively (Tatchell & Van Holde, 1979; Belyavsky et al., 1980; Marion et al., 1983), and NHCP refers to non-histone chromosomal proteins. Along the dashed arrows in the center is denoted the amount of nuclear protein exchange associated with each transition, as well as the fraction of nonnucleosomal repair patches that are folded into nucleosomes following the transition [F_i; from Watkins & Smerdon (1985)].

from 193 bp to a limiting value of 165–170 bp is not associated with any additional exchange of core proteins over that of control nuclei. In addition, in the following report (Watkins & Smerdon, 1985) we show that this change in repeat length is not accompanied by any nucleosome formation in newly repaired DNA (F_i = 0; Figure 8). On the other hand, as the limiting repeat length shifts to 140–145 bp, a marked increase in protein exchange takes place, and about half of the newly repaired patches of DNA become folded into nucleosome structures ($F_i \simeq 0.5$; Figure 8). Thus, these two different phases of nucleosome rearrangement are associated with quite different effects on both nuclear protein exchange and nonnucleosomal repair patches in nuclei. We note that the model in Figure 8 depicts only one of several possible modes for obtaining the different repeat lengths we observe. In the case shown, there is a *sequential* change in nucleosome spacing where the native repeat contracts to a chromatosome repeat, which is stable at 37 °C and at low to moderate salt concentrations. The chromatosome repeat then contracts to the compact oligomer repeat at the higher salt concentrations. In this scheme, the weak histone–DNA interactions at the ends of the chromatosome are considered to be sufficiently strong at salt concentrations ≤0.3 M and 37 °C to maintain a full two turns of DNA around the core histone surface and present a barrier to compact oligomer formation. This is the case, even though migration of histone octomers, through the transient formation of *both* strong and weak histone–DNA bonds (Beard, 1978), can occur at these salt concentrations and temperature. With increasing salt concentration, the weak histone–DNA interactions are sufficiently reduced to allow unfolding of the DNA at the ends of the chromatosome and, hence, the tighter compaction of the nucleofilament. An alternative possibility is one in which the shift from the native nucleosome spacing to the compact oligomer involves a different pathway than the shift to the chromatosome repeat. In this case, the changes we observe would represent two distinct modes of nucleosome rearrangement rather than two different

phases of the same migration process. For instance, the formation of the 168 bp repeat could be the result of nucleosome sliding, while the formation of the 146 bp repeat could be the result of an exchange mechanism.

Finally, several laboratories have observed a 165–170 bp nucleosome repeat in newly replicated DNA which appears to occur prior to H1 deposition (Murphy et al., 1978, 1980; Levy & Jakob, 1978; Annunziato & Seale, 1982). However, Smith et al. (1984) have recently reported that when histones are cross-linked to newly replicated DNA prior to nuclease digestion, a native nucleosome repeat is obtained. These results, along with those of the present study, raise the possibility that newly replicated regions of chromatin may selectively undergo nucleosome rearrangement during nuclear isolation or nuclease digestion due to their reduced H1 content.

ACKNOWLEDGMENTS

We thank Dr. Raymond Reeves, Dr. Brinda Ramanathan, Jim Gale, and Khalifah Sidik for helpful discussions. We also thank Suey Lan for excellent technical assistance and Margaret Kelnhofer for help in preparation of the manuscript.

REFERENCES

Annunziato, A. T., & Seale, R. L. (1982) *Biochemistry 21*, 5431–5438.

Annunziato, A. T., & Seale, R. L. (1983) *Mol. Cell. Biochem. 55*, 99–112.

Beard, P. (1978) *Cell (Cambridge, Mass.) 15*, 955–967.

Belyavsky, A. V., Bavykin, S. G., Goguadze, E. G., & Mirzabekov, A. D. (1980) *J. Mol. Biol. 139*, 519–536.

Burton, D. R., Butler, M. J., Hyde, J. E., Phillips, D., Skidmore, C. J., & Walker, I. O. (1978) *Nucleic Acids Res. 5*, 3643–3663.

Caron, F., & Thomas, J. O. (1981) *J. Mol. Biol. 146*, 513–537.

Cotton, R. W., & Hamkalo, B. A. (1981) *Nucleic Acids Res. 9*, 445–457.

D'Anna, J. A., & Prentice, D. A. (1983) *Biochemistry 22*, 5631–5640.

Germond, J., Bellard, M., Oudet, P., & Chambon, P. (1976) *Nucleic Acids Res. 3*, 3173–3192.

Glotov, B. O., Rudin, A. V., & Severin, E. S. (1982) *Biochim. Biophys. Acta 696*, 275–284.

Ilyin, Yu. V., Varshavsky, A. J., Mickelsaar, U. N., & Georgiev, G. P. (1971) *Eur. J. Biochem. 22*, 235–245.

Jackson, V., Marshall, S., & Chalkley, R. (1981) *Nucleic Acids Res. 9*, 4563–4581.

Jensen, R. H., & Chalkley, R. (1968) *Biochemistry 7*, 4388–4395.

Jorcano, J. L., & Ruiz-Carrillo, A. (1979) *Biochemistry 18*, 768–774.

Klevan, L., & Crothers, D. M. (1977) *Nucleic Acids Res. 4*, 4077–4089.

Kornberg, A. (1980) in *DNA Replication* (Bartlett, A. C., Brewer, P., & McNally, R., Eds.) pp 19–20, W. H. Freeman, San Francisco, CA.

Lasters, I., Muyldermans, S., Wyns, L., & Hamers, R. (1981) *Biochemistry 20*, 1104–1110.

Lawson, G. M., & Cole, R. D. (1979) *Biochemistry 18*, 2160–2166.

Levy, A., & Jakob, K. M. (1978) *Cell (Cambridge, Mass.) 14*, 259–267.

Lieberman, M. W. (1982) in *DNA Repair, Chromosome Alterations, and Chromatin Structure* (Natarajan, A. T., Obe, G., & Altmann, H., Eds.) pp 103–111, Elsevier Biomedical Press, Amsterdam.

Lohr, D., Corden, J., Tatchell, K., Kovacic, R. T., & Van Holde, K. E. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 79–83.

Louters, L., & Chalkley, R. (1984) *Biochemistry 23*, 547–552.

Marion, C., Roux, B., & Coulet, P. R. (1983) *FEBS Lett. 157*, 317–321.

Martin, D. Z., Todd, R. D., Lang, D., Peri, P. N., & Gerrard, W. T. (1977) *J. Biol. Chem. 252*, 8269–8277.

Mathis, D. J., Kindelis, A., & Spadafora, C. (1980) *Nucleic Acids Res. 8*, 2577–2590.

Murphy, R. F., Wallace, R. B., & Bonner, J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5903–5907.

Murphy, R. F., Wallace, R. B., & Bonner, J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3336–3340.

Noll, M., & Kornberg, R. (1977) *J. Mol. Biol. 109*, 393–404.

Reeves, R. (1984) *Biochim. Biophys. Acta 782*, 343–393.

Smerdon, M. J., & Lieberman, M. W. (1980) *Biochemistry 19*, 2992–3000.

Smerdon, M. J. & Lieberman, M. W. (1981) *J. Biol. Chem. 256*, 2480–2483.

Smerdon, M. J., Tlsty, T. D., & Lieberman, M. W. (1978) *Biochemistry 17*, 2377–2386.

Smerdon, M. J., Lan, S. Y., Calza, R. E., & Reeves, R. (1982a) *J. Biol. Chem. 257*, 13441–13447.

Smerdon, M. J., Watkins, J. F., & Lieberman, M. W. (1982b) *Biochemistry 21*, 3879–3885.

Smith, P. A., Jackson, V., & Chalkley, R. (1984) *Biochemistry 23*, 1576–1581.

Sollner-Webb, B., Camerini-Otero, R. D., & Felsenfeld, G. (1976) *Cell (Cambridge, Mass.) 9*, 179–193.

Spadafora, C., Oudet, P., & Chambon, P. (1979) *Eur. J. Biochem. 100*, 225–235.

Stein, A., & Kunzler, P. (1983) *Nature (London) 302*, 549–550.

Stein, A., & Bina, M. (1984) *J. Mol. Biol. 178*, 341–363.

Steinmetz, M., Streek, R. E., & Zachau, H. G. (1978) *Eur. J. Biochem. 83*, 615–628.

Tatchell, K., & Van Holde, K. E. (1979) *Biochemistry 18*, 2871–2880.

Thomas, J. O., & Furber, V. (1976) *FEBS Lett. 66*, 274–280.

Varshavsky, A. J., & Ilyin, Yu. V. (1974) *Biochim. Biophys. Acta 340*, 207–217.

Wasylyk, B., & Chambon, P. (1980) *Eur. J. Biochem. 103*, 219–226.

Watkins, J. F., & Smerdon, M. J. (1985) *Biochemistry* (following paper in this issue).

Weischet, W. O. (1979) *Nucleic Acids Res. 7*, 291–304.

Weischet, W. O., & Van Holde, K. E. (1980) *Nucleic Acids Res. 8*, 3743–3755.