fraction, contamination by cartilage matrix molecules was probably insignificant.

The results of the present study may be summarized as a model for proteoglycan synthesis and deposition in fetal mammalian bone. Three species of proteoglycan are synthesized: a large CS and a small DS proteoglycan, which are located in the nonmineralized matrix, and a small CS proteoglycan, which is initially present in the nonmineralized matrix but subsequently becomes incorporated into the mineralized matrix. Some of the small CS proteoglycan undergoes a strong interaction with collagen which renders it resistant to extraction with denaturing solvents and demineralizing agents.

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References

Campo, R. D. (1981) *Calcif. Tissue Int. 33*, 89–99.
Fisher, L. W., Termine, J. D., Dejter, S. W., Whitson, S. W., Yanagishita, M., Kimura, J. H., Hascall, V. C., Kleinman, H. K., Hassell, J. R., & Nilsson, B. (1983) *J. Biol. Chem. 258*, 6588–6594.
Fransson, L. A. Anseth, A., Antonopoulos, C. A., & Gardell S. (1970) *Carbohydr. Res. 15*, 73–89.
Hascall, V. C., & Sajdera, S. W. (1970) *J. Biol. Chem. 245*, 4920–4930.
Hauschka, P. V., Lian, J. B., & Gallop, P. M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3925–3929.
Herring, G. M. (1968) *Biochem. J. 107*, 41–49.
Hunter, G. K., Heersche, J. N. M., & Aubin, J. E. (1983) *Biochemistry 22*, 831–837.
Hunter, S. J., & Schraer, H. (1983) *Arch. Biochem. Biophys. 220*, 272–279.
Linde, A., Jontell, M., Lundgren, T., Nilson, B., & Svanberg, U. (1983) *J. Biol. Chem. 258*, 1698–1705.
Maier, G. D., Lechner, J. H., & Veis, A. (1983) *J. Biol. Chem. 258*, 1450–1455.
Mosher, D. F., Schad, P. E., & Kleinman, H. K. (1979) *J. Clin. Invest. 64*, 781–787.
Mulder, G. J., & Scholtens, E. (1978) *Biochem. J. 172*, 247–251.
Reddi, A. H., Hascall, V. C., & Hascall, G. K. (1978) *J. Biol. Chem. 253*, 2429–2436.
Sugahara, K., Ho, P.-L., & Dorfman, A. (1981) *Dev. Biol. 85*, 180–189.
Tanzer, M. L., & Hunt, R. D. (1963) *Science (Washington, D.C.) 141*, 1270–1272.
Termine, J. D., Kleinman, H. K., Whitson, S. W., Conn, K. M., McGarvey, M. L., & Martin, G. R. (1981a) *Cell (Cambridge, Mass.) 26*, 99–105.
Termine, J. D., Belcourt, A. B., Conn, K. M., & Kleinman, H. K. (1981b) *J. Biol. Chem. 256*, 10403–10408.
Wasteson, A. K. (1971) *J. Chromatogr. 59*, 87–97.

# Two-Stage Maturation Process for Newly Replicated Chromatin†

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ABSTRACT: HTC cells have been labeled by short exposures to [$^3$H]thymidine in order to identify newly synthesized DNA. By either isolating nuclei directly or isolating them after an extensive fixation with formaldehyde, we have been able to identify two phases in the maturation process of newly replicated chromatin. The first phase which is relatively brief (<5 min) is reflected in a diffuse, irregular organization of nucleosomes on new DNA immediately postreplicatively. The second phase which lasts from 5 to 30 min postreplication is characterized by a normal repeat length for the nucleosomes which are nonetheless more weakly bound than bulk nucleosomes. This is reflected in increased sliding during nuclease digestion as well as increased nuclease sensitivity and the presence of easily dissociated histones which has been described by other workers.

Studies investigating the structure of eucaryotic chromatin in the vicinity of the replication fork have shown that the nature of nucleosomes associated with this region of DNA differs from that seen for nucleosomes associated with nonreplicating DNA. Within 5–25 min of DNA synthesis, these immature nucleosomes undergo a rearrangement in which they attain the structural properties of bulk or mature chromatin (DePamphilis & Wassarman, 1980).

Some of the structural characteristics associated with immature chromatin include a lower buoyant density, an increased sensitivity to digestion by nucleases, and a decreased affinity between new H3 and $H_4$ and new DNA (Jackson & Chalkley, 1981; Seale, 1975, 1976, 1978; Walters & Hildebrand, 1976; Klempnauer et al., 1980; Levy & Jakob, 1978; Jackson et al., 1981). In addition, several investigators have reported that the nucleosomal repeat length for nascent chromatin is 15–50 base pairs (bp) shorter than that calculated for bulk chromatin (Murphy et al., 1978, 1980; Levy & Jakob, 1978; Annunziato et al., 1982). The difference in repeat lengths is thought to be due to a shorter linker region, but there has been no adequate explanation for the mechanism by which immature nucleosomes can rearrange to acquire the longer nucleosomal repeat length of bulk chromatin. Data obtained in this laboratory indicate that the shorter nucleosomal repeat may be generated during the micrococcal nuclease digestion process itself, perhaps as a consequence of the decreased affinity between newly replicated DNA and new H3 and H4 (Jackson et al., 1981).

The decreased affinity of new H3 and H4 for newly rep-

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licated DNA leads to a partial dissociation of these histones even under the conditions of low ionic strength that are used to isolate nuclei in studies of newly replicated chromatin (Jackson et al., 1981). As a consequence, we have sought to study the properties of intact, newly replicated chromatin by cross-linking histones to DNA in whole cells so that dissociation of histones and DNA cannot occur during subsequent isolation procedures. We conclude that immediately after replication the organization of the nucleosomes with respect to one another is irregular but that within 5 min after DNA synthesis the organization of nascent chromatin resembles that of bulk chromatin. We show that sliding of newly assembled nucleosomes occurs on newly synthesized DNA during micrococcal nuclease digestion. This phenomenon results in an apparent shorter spacing for nascent chromatin which can be inhibited by the cross-linking procedures we describe.

## Materials and Methods

*Materials.* [$^3$H]Thymidine (71 Ci/mmol) was purchased from Schwarz/Mann. Thymidine, proteinase K (protease, type XI), and RNase A were obtained from Sigma. Formaldehyde was purchased from Fischer Scientific. Micrococcal nuclease was obtained from Worthington Biochemical Corp.

*Labeling of Cells and Isolation of Nuclei.* HTC cells were pulse labeled as described by Jackson & Chalkley (1981). We have previously shown that our pulse-labeling procedures do not adversely affect the normal replication of these cells (Jackson et al., 1976). Cells were harvested at mid-log phase [(4–5) × $10^5$ cells/mL] and pulsed with [$^3$H]thymidine for 2 min at 37 °C in DMEM (Dulbecco's minimal Eagle's medium) without serum. The pulse was terminated by centrifuging cells at 6000 rpm and resuspending the cells in warm DMEM medium (~1 × $10^7$ cells/mL) containing 2 mM thymidine. At the end of the chase periods, the cells were centrifuged at 6000 rpm and were either frozen in a dry ice/ethanol bath (control cells) or resuspended in DMEM medium (4 × $10^5$ cells/mL) containing 1% formaldehyde at 4 °C. The cells were harvested by centrifugation after stirring in the cold (4 °C) for 18 h. The cells were then resuspended in fresh DMEM and stirred an additional 24 h at 4 °C in order to remove the formaldehyde.

Nuclei were isolated by homogenization in a buffer containing 0.25 M sucrose, 10 mM $MgCl_2$, 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 8.0, and 1% Triton X-100. Subsequently, the nuclei were centrifuged at 2500 rpm for 5 min. The nuclei were treated in this manner 2 more times. After an additional wash in the same buffer as above without the Triton X-100, the nuclei were resuspended in buffer containing 0.25 M sucrose, 10 mM $MgCl_2$, 10 mM Tris, pH 8, 10 mM NaCl, and 1 mM $CaCl_2$ at a DNA concentration of 1 mg/mL ($A_{260}$ = 20).

*Micrococcal Nuclease Digestion.* Nuclei from both fixed and unfixed HTC cells were isolated as described above. Micrococcal nuclease was added at a concentration of 0.5 unit/$A_{260}$. Digestions were terminated at the appropriate times by pipetting an aliquot of cells into a 5-fold excess of 20 mM ethylenediaminetetraacetic acid (EDTA)/0.5% sodium dodecyl sulfate (SDS), pH 8.0. The DNA fragments from unfixed cells were isolated by incubating the samples with proteinase K (1 μg/25 μg of DNA) at 37 °C for 2 h. The DNA was then precipitated with ethanol, and the pellet was lyophilized. The dried pellet was resuspended in 20 mM Tris, 5 mM sodium acetate, and 0.5 mM EDTA, pH 7.9. RNase A was added (2.5 μg/100 μg of DNA) and incubated at 37 °C for 1 h. Prior to isolation of the DNA fragments as described above for unfixed nuclei, the samples from fixed cells were incubated in the presence of SDS at 37 °C for 3 days to remove protein–DNA cross-links.

*Analysis of Nucleosomal Repeat Lengths.* DNA fragments isolated as described above were analyzed by electrophoresis on a 1.2% agarose gel with Tris–acetate buffer. The gels were stained with ethidium bromide, photographed, impregnated with 2,5-diphenyloxazole (PPO), and fluorographed by the procedure of Laskey & Mills (1975). The sizes of the DNA fragments were calculated from a standard curve derived from the concurrent electrophoresis of a partial *Eco*RI digest of p93-50. This molecular weight standard is a derivative of pBR322 containing the HSV tk$^+$ gene and 50 copies of a genomic 93 bp repeat sequence from rat cells (Sealy et al., 1981). The size of the nucleosome multimers (number of base pairs) was plotted vs. the multimer size, and the nucleosome repeat length was then calculated as the slope of the line which was obtained by linear regression analysis (Annunziato & Seale, 1982; Noll & Kornberg, 1977).

Nucleosome repeat lengths were calculated as described previously except for the 30-min time point for the unfixed cells. Due to variations in the repeat lengths and extents of digestions obtained from the ethidium gels from three different experiments and since one would expect the ethidium to yield very similar repeat lengths, the values for the 30-min time point for two of the experiments were set equal to the 60-min time point for the third experiment (same extent of digestion), and the remaining repeat length values were normalized accordingly. The repeat lengths from the fluorograms were multiplied by the same factors as the ethidium values.

## Results

*Nucleosomes Immediately after the Replication Fork Are Not Regularly Organized.* Chromosomal material found immediately after the replication fork was identified by a short (2-min) pulse of [$^3$H]thymidine followed by nuclease digestion of nuclei isolated from these cells. The distribution of newly synthesized DNA in oligonucleosomes seen during the time course of the nuclease digestion is shown in Figure 1. As judged by the sizes of the DNA fragments, newly synthesized DNA is digested somewhat more rapidly than bulk DNA, in agreement with the observations of previous workers (Jackson & Chalkley, 1981; Seale, 1975, 1976, 1978; Walters & Hildebrand, 1976; Klempnauer et al., 1980; Levy & Jakob, 1978; Jackson et al., 1981). At early times of digestion, the bulk nucleosomal material has a normal oligonucleosome ladder. In distinct contrast, however, newly synthesized DNA appears to have been organized in an irregular manner which leads to a diffuse distribution of DNA sizes which are partially protected against nuclease attack. Despite the apparently irregular distribution of nucleosomes on new DNA, it is clear that much of the newly synthesized DNA is in some sort of nucleosomal organization since after extended digestion some of the new DNA is found in material of ≃145–150 bp in size (Figure 1). A similar experiment performed with HeLa cells showed an identical result (data not shown).

We were concerned that newly synthesized DNA might be unusually degraded during the isolation procedures. If the newly synthesized DNA is digested with *Eco*RI, a typical 93 bp ladder of the highly repeated DNA is obtained on a fluorogram, indicating that the DNA as a whole seems to be intact (data not shown).

There would appear to be several possible explanations for this observation. (1) In the cell, newly assembled nucleosomes are in fact organized with the same regularity as seen for bulk nucleosomes, but the new nucleosomes are not tightly bound and migrate to random positions during the digestion process.

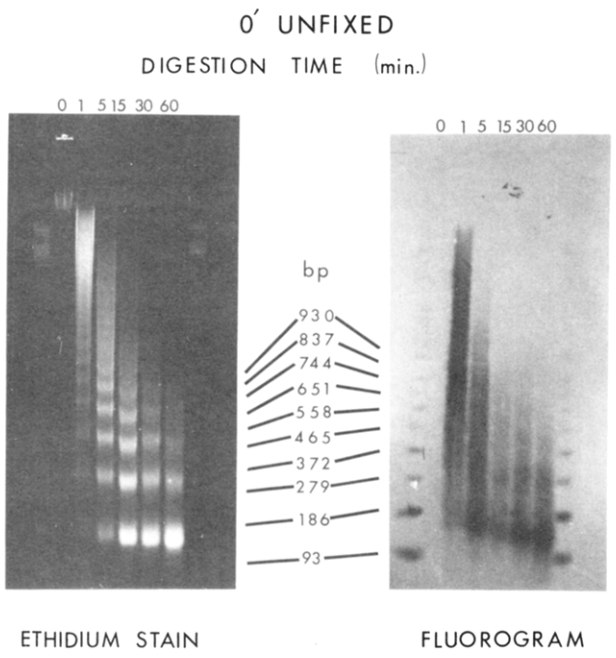


FIGURE 1: Newly replicated chromatin has a diffuse organization immediately after it is synthesized. Agarose gel electrophoresis of DNA fragments isolated from micrococcal nuclease digested nuclei. Nuclei were prepared from HTC cells which had been pulsed with [$^3$H]thymidine for 2 min. The DNA standard is a partial *Eco*RI digest of p93-50 (see Materials and Methods) which has been end labeled with [$^3$H]dATP. The ethidium-stained fragments represent the bulk chromatin, and the fluorogram represents those fragments which are newly synthesized. Conditions for nuclease digestion and gel electrophoresis are as described under Materials and Methods.

This point is addressed (and in fact excluded) in later experiments. (2) The nucleosomes are organized in an irregular manner on both new arms of the replication fork. (3) Nucleosomes are organized in an irregular manner after the replication fork on one new arm whereas the other arm contains nucleosomes with regular spacing. There is a suggestion from previous work that the last possibility may be the correct proposal (Annunziato et al., 1981). However, synthesis of a distribution consisting of 50% normal spacing from an ethidium bromide stained gel and 50% of a diffuse pattern such as that obtained by a partial digestion of deproteinized DNA does not reproduce the highly diffuse pattern seen in the fluorogram of Figure 1 (data not shown).

*Irregularly Spaced Nucleosomes on Newly Replicated DNA Rapidly Become Organized into a Regular Array.* The nonregular organization of nucleosomes on very recently replicated DNA is rapidly followed by arrangement into a detectable array of oligonucleosomes as shown in Figure 2. Five minutes after the termination of a short [$^3$H]thymidine pulse, a nucleosomal pattern is clearly detectable as indicated in the fluorogram by the ladder of partially digested DNA fragments. Nonetheless, the pattern of newly synthesized oligomeric DNA fragments displayed on the flurogram is different in one key way from that shown by the fragments representing the nuclease-digested bulk chromatin, seen in the ethidium-stained gel. The nucleosomal repeat (calculated as shown in Figure 2B) for the earliest time point in the digestion of new chromatin (5 min after synthesis) is ≅180 bp. However, as the digestion proceeds, the nucleosomal repeat of the new chromatin drops to 152 bp. The repeat size is calculated by a graphical analysis of all points in the oligonucleosome ladder (see Materials and Methods). We find that the decrease in repeat size is shown not only by smaller (more digested) fragments but also by higher oligonucleosomes up to at least a 4-mer, indicating either (a) that this reflects a lower repeat value for extended tracts of newly organized nucleosomes on new chromatin or (b) that nucleosome sliding can occur over a range of several nucleosomes in new chromatin, presumably reflecting a much decreased affinity between newly organized histone cores and new DNA. We also observed changes in the nucleosomal repeat length in mature nucleosomal material as a function of the extent of digestion as has been described by other workers (Johnson et al., 1978; Humphries et al., 1979). In our hands, we see a shift in the apparent nucleosomal repeat from ≅190 bp at earliest times of digestion to ≅170 bp at very late stages when monomer and dimer fragments constitute 80% of the digestion products (P. A. Smith and R. Chalkley, unpublished results).

Thus, we have identified two phases in the maturation of new chromatin. An initial phase appears to reflect the presence of irregularly spaced nucleosomes (Figure 1), and a second phase is reflected in a difference of nucleosome repeat size between new chromatin and bulk material (Figures 2 and 3). Although the first phase is complete in less than 5 min, the second phase appears to last until 30 min. This was ascertained by following a 2-min pulse of [$^3$H]thymidine with chase periods up to 60 min after removal of radiolabel. The nucleosomal repeat sizes of radiolabeled (new) and ethidium bromide stained (bulk) chromatin were then measured as a function of micrococcal nuclease digestion time. The difference in nucleosomal repeat size of ≅15–30 bp seen at early times in the chase (5 and 15 min) is completely abolished 60 min after the synthesis of the new chromatin as shown in Figure 3.

*The Short Nucleosomal Repeat of New Chromatin Is Not Seen If Cells Are Fixed before Nuclease Digestion.* It has been previously established that several of the core histones in new chromatin are bound unusually weakly (Jackson et al., 1981). This led us to postulate the hypothesis that the unusual, short nucleosomal repeat seen in new chromatin after micrococcal nuclease digestion might be a consequence of a digestion-induced sliding of weakly bound histone cores. In order to test this hypothesis, we have cross-linked histones to the DNA before cell disruption so that sliding is impossible. We have previously shown that even the less tightly bound histones on new chromatin are efficiently cross-linked by formaldehyde during a whole cell fixation (Jackson & Chalkley, 1981).

HTC cells were exposed to a short pulse (2 min) of [$^3$H]-thymidine in an experimental protocol analogous to that described above. The intact cells were then fixed with formaldehyde. Nuclei were isolated as described previously and digested with micrococcal nuclease. Digestion of fixed nuclei from cells collected 5 min after termination of the pulse yields a bulk nucleosome repeat pattern similar to that seen in unfixed material as shown in the ethidium stain of Figure 4. The newly replicated material shown in Figure 4, however, does differ in one important aspect from the newly replicated material seen in Figure 2, namely, that the reduction in the apparent nucleosomal repeat at later stages of digestion is much less than that seen following digestion of unfixed nuclei. As seen in Figure 3, the nucleosomal repeat size for the 5-min fixed material drops from ≅185 to ≅175 bp (compared to 180 to 152 bp for unfixed nuclei). The small decrease observed in fixed nuclei is highly reproducible and may reflect a preference for digestion of longer spacer material at early times in the digestion (Johnson et al., 1978; Humphries et al., 1979). A summary analysis of the nucleosome repeat sizes of both fixed and unfixed material obtained after various chase points is shown in Figure 3. This summary figure indicates that in fixed cells the nucleosome repeat lengths of newly synthesized

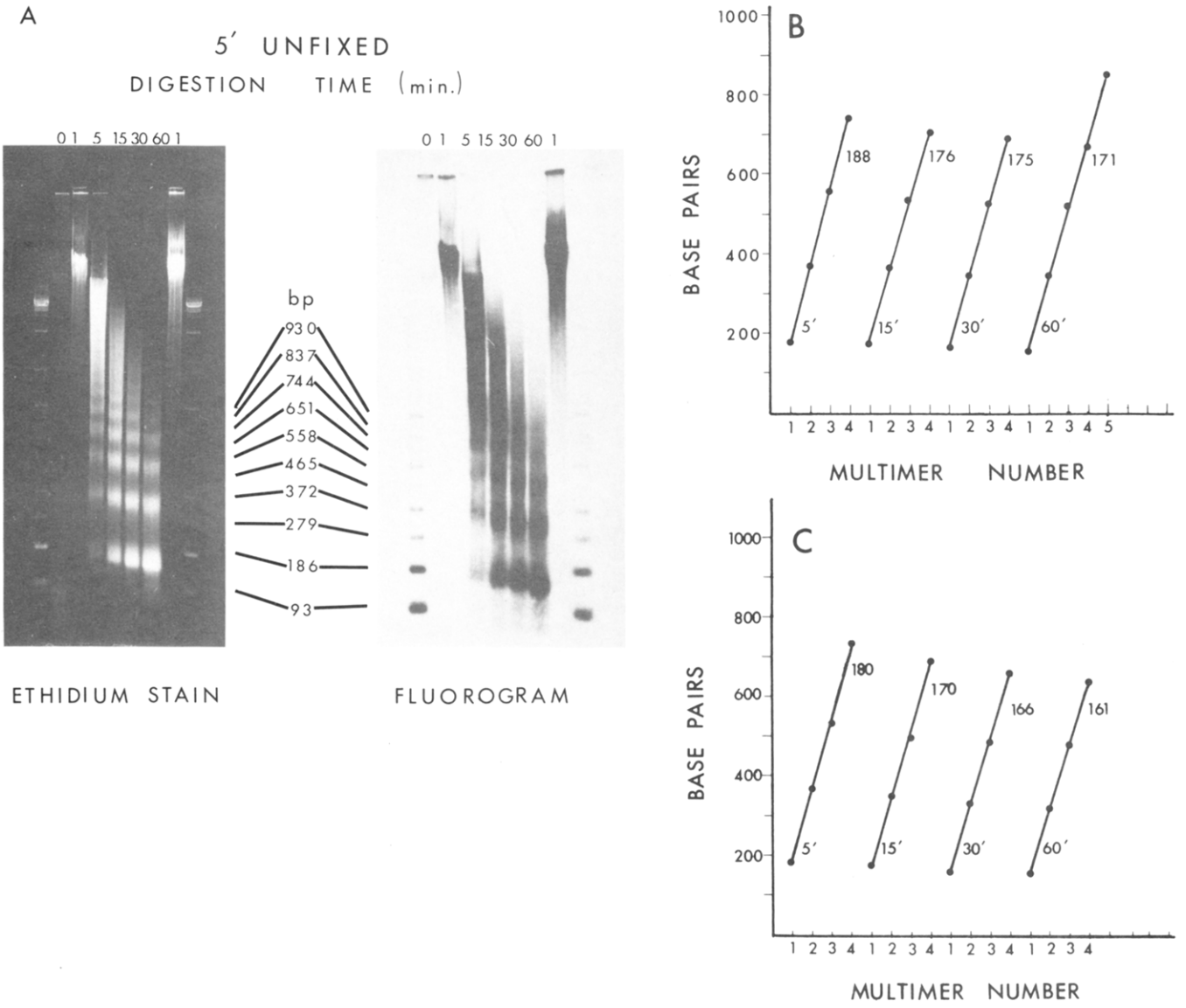


FIGURE 2: Appearance of regularly spaced nucleosomes on recently replicated chromatin. Agarose gel electrophoresis of DNA fragments isolated from HTC nuclei which have been subjected to micrococcal nuclease digestion. The nuclei were pulsed for 2 min with [$^3$H]thymidine and chased for 5 min with 2 mM cold thymidine. Electrophoresis was performed as in Figure 1. (A) Ethidium-stained fragments represent the bulk chromatin, and the fluorogram represents those fragments which are labeled with [$^3$H]thymidine. (B) Repeat lengths for the ethidium-stained fragments from a 5-min unfixed experiment were calculated as the slope of the line where the multimer size (number of base pairs) was plotted vs. the multimer number. All of the lines were obtained by linear regression analysis; correlation coefficients were as follows: 5 min, 1.0; 15 min, 1.0; 30 min, 1.0; 60 min, 0.999. (C) Repeat lengths for the [$^3$H]thymidine-labeled fragments were calculated as in (B). Correlation coefficients were as follows: 5 min, 1.0; 15 min, 0.998; 30 min, 1.0; 60 min, 1.0.

chromatin are not significantly different from those seen for bulk chromosomal material at any of the time periods after chromatin synthesis.

We have used the fixation approach to assay if the different nucleosome pattern seen immediately after DNA synthesis (2 min pulse) is due to sliding of weakly bound histones. Cells were labeled by a 2-min pulse of [$^3$H]thymidine, collected, and fixed immediately in the cold. After digestion with micrococcal nuclease, a DNA distribution on agarose electrophoresis was obtained as shown in Figure 5.

Analysis of the fluorogram of Figure 5 enables us to assay the organization of nucleosome cores on immediately post-replicative chromatin. We observe a diffuse pattern analogous to that seen for unfixed nuclei (Figure 1). This indicates that the nonregular spacing of nucleosomes found within 1–2 min after replication is not due to an increased tendency of the more loosely bound histone to slide during isolation and digestion of the chromatin.

## Discussion

We have been able to discern two stages of maturation of newly synthesized chromatin. In the first stage, nucleosomes appear to be present as indicated by the formation of 145 bp cores after nuclease digestion (Figures 1 and 5); however, they are not organized in a normal type of regular array. In addition, previous work has indicated that they are more labile toward nucleases and that the core histones do not appear to bind as tightly to DNA as seen in mature chromatin (DePamphilis & Wassarman, 1980; Jackson et al., 1981). We do not find any compelling evidence to suggest that 50% of the new chromatin is normally organized and 50% is diffuse, though this remains a formal possibility. In the second stage of maturation, which occurs in HTC cells within 5 min after synthesis, the nucleosomes appear to adopt the organization and spacing characteristic of mature chromatin. Data supporting the occurrence of a second stage of maturation have been obtained from digestions of both fixed and unfixed nuclei.

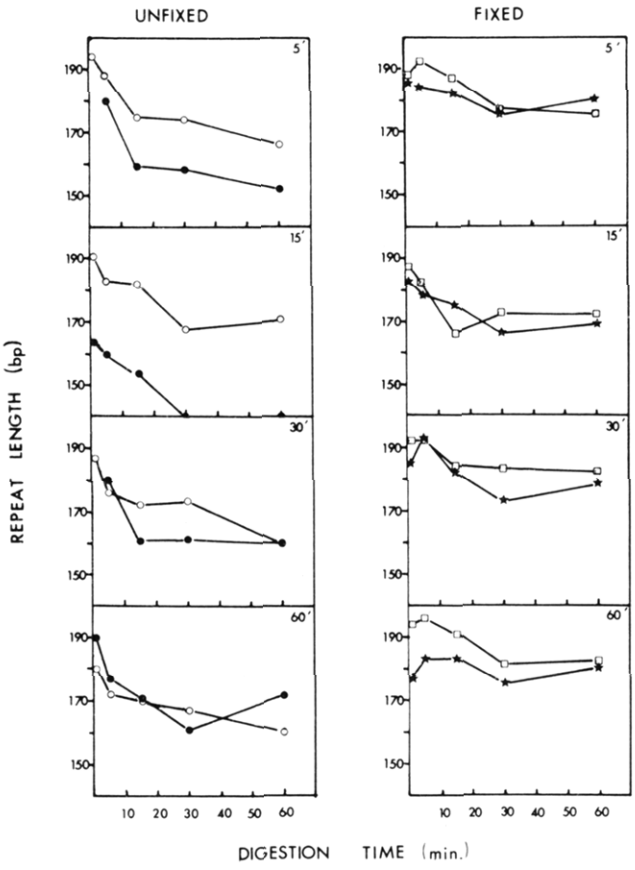


FIGURE 3: Nucleosome repeat lengths obtained from micrococcal nuclease digestion of both unfixed and fixed HTC nuclei. Nuclei were prepared from HTC cells that had been pulsed with [$^3$H]thymidine for 2 min and which were then chased in 2 mM thymidine for 5, 15, 30, and 60 min. The repeat lengths are plotted as a function of the digestion time for each of the chase periods for nuclei obtained from both unfixed and fixed HTC cells. Nucleosome repeat lengths were calculated as described under Materials and Methods. Closed circles and closed stars represent repeat lengths obtained from newly replicated material from unfixed and fixed cells, respectively, and open circles and open boxes represent repeat lengths obtained from bulk material from unfixed and fixed cells, respectively.

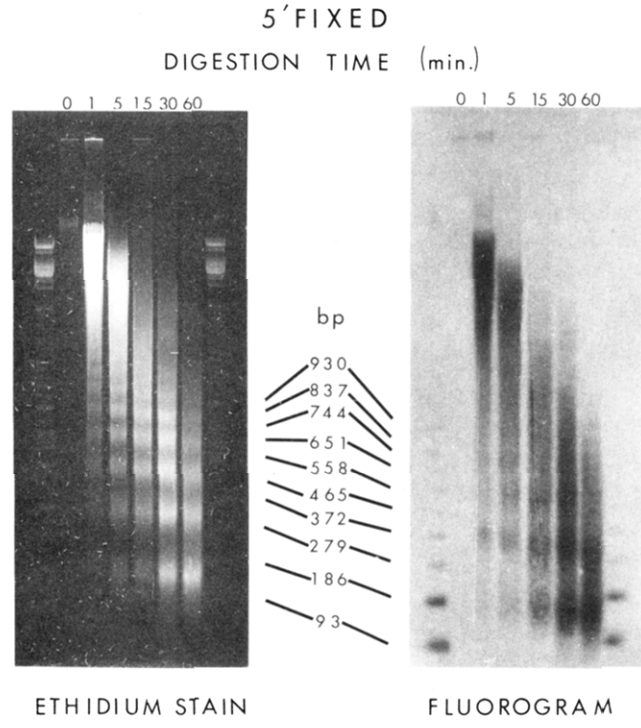


FIGURE 4: Repeat lengths of newly synthesized chromatin from formaldehyde-fixed HTC cells are very similar to those for bulk chromatin from the same cells. Agarose gel electrophoresis of DNA fragments isolated from nuclei obtained from formaldehyde-fixed HTC cells was performed as described in Figure 1. The cells were pulsed with [$^3$H]thymidine for 2 min, chased with cold thymidine (2 mM) for 5 min, and fixed with 1% formaldehyde for 18 h at 4 °C as described under Materials and Methods. After micrococcal nuclease digestion, the formaldehyde-induced cross-links were reversed as described under Materials and Methods.

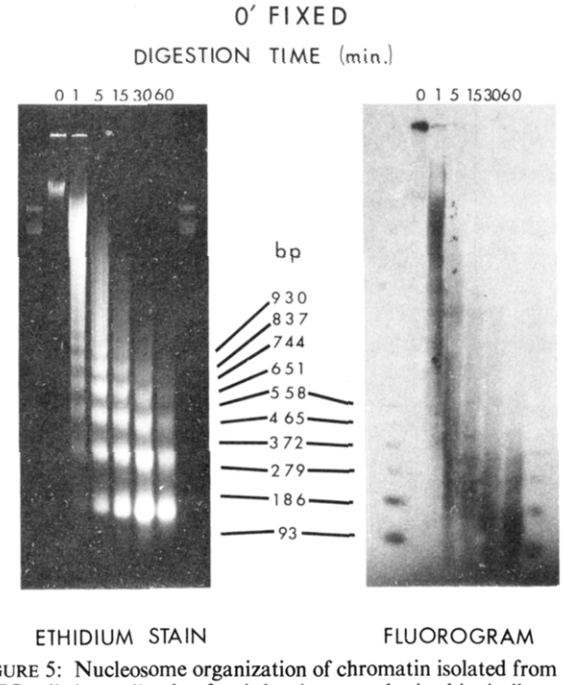


FIGURE 5: Nucleosome organization of chromatin isolated from fixed HTC cells immediately after it has been synthesized is similar to that of unfixed material. Agarose gel electrophoresis was performed as described in Figure 4. DNA fragments were isolated from micrococcal nuclease treated nuclei obtained from HTC cells pulsed with [$^3$H]-thymidine for 2 min. The cells were fixed with 1% formaldehyde for 18 h as described under Materials and Methods prior to micrococcal nuclease digestion.

For example, nucleosomes from unfixed HTC nuclei appear to be unusually inclined to slide to a lower nucleosome repeat during the digestion process. However, digestion of fixed nuclei with micrococcal nuclease results in a repeat length for newly synthesized chromatin essentially the same as that found for bulk chromatin. Finally, by 30 min, the chromatin largely loses its increased ability to slide, and by 60 min, the repeat length of newly replicated chromatin from unfixed nuclei is very similar to that of bulk chromatin from unfixed nuclei.

We have tested the reproducibility of these results in several ways. The entire experiment has been performed several times, and in addition, several electrophoretic runs of the different experiments have been made. Despite the differences of comparing mean values for repeat size from experiment to experiment because of inherent biological variations, we find that the mean standard deviation on a repeat length is 4.9 ● 3.2. This is sufficient for us to describe a difference in repeat size, for example at 5 min, of 16 bp out of 174 bp, which is significant at the 95% confidence limit (Student's $t$ test).

A similar observation of a lower nucleosomal repeat for newly replicated chromatin than that found for bulk material has been made by several laboratories, including that of Seale. The latter group argued that since the lower repeat size is obtained even following nuclease digestion at 4 °C then it is unlikely to be due to a nuclease-induced sliding phenomenon (Annunziato et al., 1982). We and others have observed that bulk chromatin exhibits some sliding during the later stages

of digestion at 37 °C which is largely prevented if digestion proceeds in the cold (P. A. Smith and R. Chalkley, unpublished results). However, bulk nucleosomal material is a poor model for nucleosomes on new DNA, inasmuch as there may be a significant decrease in the affinity of core histones for new DNA (Jackson et al., 1981).

The origin of the low affinity between some histones and DNA in new chromatin is not known. Since the affinity between DNA and purified histones reflects that existing in bulk chromatin, we must surmise that some other agent is acting to weaken DNA–histone bonds in new chromatin (Jackson et al., 1981). The cell may exploit the weak binding of H3 and H4 to develop the most appropriate nucleosome spacing after replication has been followed by a period of diffuse organization of nucleosomes. Previous results obtained from this laboratory indicate that H3 and H4 are the histones which are deposited on new DNA at the replication fork and are also the histones most sensitive to low-salt extraction from new chromatin (Jackson et al., 1981). The diffuse phase of maturation may represent the stage before nucleosomes adopt their final positions, perhaps as a consequence of the placement of regulatory proteins which bind preferentially to particular DNA sequences. Thus, nucleosome positions characteristic of the chromatin structure of the terminally differentiated state may be established at this time.

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References

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

Annunziato, A., Schindler, R. K., Thomas, C. A., & Seale, R. (1981) *J. Biol. Chem. 256*, 1180–1886.

DePamphilis, M. L., & Wassarman, P. M. (1980) *Annu. Rev. Biochem. 49*, 627–646.

Humphries, S. E., Young, P., & Carroll, D. (1979) *Biochemistry 18*, 3223–3231.

Jackson, V., & Chalkley, R. (1981) *Cell (Cambridge, Mass.) 23*, 121–134.

Jackson, V., Granner, D., & Chalkley, R. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2266–2269.

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

Johnson, E. M., Allfrey, V. G., Bradbury, E. M., & Matthews, H. R. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1116–1120.

Klempnauer, K., Fanning, E., Otto, B., & Knippers, R. (1980) *J. Mol. Biol. 136*, 359–374.

Laskey, R., & Mills, A. (1978) *Eur. J. Biochem. 56*, 335–341.

Levy, A., & Jakob, K. M. (1978) *Cell (Cambridge, Mass.) 14*, 757–767.

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. D. (1977) *J. Mol. Biol. 109*, 393–404.

Seale, R. (1975) *Nature (London) 255*, 247–249.

Seale, R. (1976) *Cell (Cambridge, Mass.) 9*, 423–429.

Seale, R. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2717–2721.

Sealy, L., Hartley, J., Donelson, J., Chalkley, R., Hutchison, N., & Hamkalo, B. (1981) *J. Mol. Biol. 145*, 291–318.

Walters, R. A., & Hildebrand, C. E. (1976) *Biochem. Biophys. Res. Commun. 73*, 157–163.