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Shortest Nucleosomal Repeat Lengths during Sea Urchin Development Are Found in Two-Cell Embryos†

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ABSTRACT: Prior to fertilization, sperm possess one of the longest nucleosome repeat lengths yet determined [~250 base pairs (bp) for the sea urchin *Strongylocentrotus purpuratus*]. We show here that the two-cell embryo has an average repeat size of 189 ± 2 bp as probed by micrococcal nuclease; this is the shortest average nucleosomal subunit reported for *S. purpuratus*. By the eight-cell stage, the average nucleosome repeat increases to 201 ± 2 bp, and it subsequently increases further during development. These results indicate that a dramatic rearrangement of chromatin occurs upon fertilization and that this chromatin remodeling continues through early development. When two-cell embryos are labeled for 30 min with [^{3}H]thymidine and digested briefly, they exhibit nuclease-hypersensitive fragments averaging 308 bp in size, which are consistent with the size of protected DNA units in replication intermediate complexes at blastula stage (as described by Levy and Jacob [Levy, A., & Jacob, K. M. (1978) *Cell (Cambridge, Mass.) 14*, 259]). Our results are consistent with two general propositions: (1) long repeat lengths are found in highly differentiated cells, and (2) short repeat lengths are characteristic of cells more active in cell division. Our data would also imply that a rapid increase in the DNA complement, e.g., in the transition from haploid to diploid state following fertilization, is accompanied by a shortening of the average size of DNA in a nucleosome after replication.

The average repeat length of DNA in the nucleosome, once assumed to be nearly constant at 200 base pairs (Kornberg, 1974), has been found to vary widely from 160 bp (base pairs) in lower eukaryotes like yeast (Lohr et al., 1977) to about 250 bp in sea urchin sperm (Arceci & Gross, 1980a; Spadafora et al., 1976). Even within one species the basic subunit repeat has been observed to differ dramatically depending on the tissue origin and state of development of the cell, indicating that two tissues from the same animal can have the genome

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packaged in two different ways (Morris, 1976; Compton et al., 1976). During development, the repeat length of chromatin also changes. Increases occur during embryonic development of the sea urchin (Arceci & Gross, 1980; Savic et al., 1981) and erythropoesis in avian cells (Weintraub, 1978). In contrast, decreases in the repeat length were reported during development of rat and rabbit neurons (Thomas & Thompson, 1977; Ermini & Kuenzle, 1978).

To understand the molecular basis of variations in the nucleosomal repeat lengths in chromatin and the possibility that these variations are involved in the regulation of gene expression, we characterized nucleosome structures in the sea urchin *Strongylocentrotus purpuratus* immediately following fertilization (Shaw et al., 1981). In the present paper, we use micrococcal nuclease to examine the size of DNA in the nucleosome core and the length of the subunit repeat in the two-cell and eight-cell stages. We show that the size of the average DNA repeat length at the two-cell stage is 12 bp shorter than at the eight-cell stage and 30 bp shorter than in the 300-cell blastula-stage embryo. Whereas it is difficult to interpret differences in repeat lengths between different organisms, the documented changes in the subunit length reported during the maturation in a single organism suggest that a rearrangement of DNA and proteins subsequent to fertilization is manifested in substantial changes in the chromatin structure.

Materials and Methods

Cells and Culture Conditions. Strongylocentrotus purpuratus were purchased from Pacific Bio Marine, Venice, CA. Eggs were obtained by dissecting gonads out of scissor-opened *S. purpuratus* or by spontaneous shedding after 0.5 M KCl coelomic injections; they were separated from large particulate material by filtration through a 200-μm nylon net. Oocyte contamination was eliminated by allowing the eggs to settle twice in artificial seawater (Instant Ocean) and then removing the supernatant. Eggs were allowed to settle a third time in seawater containing 1 mM aminotriazole and then fertilized in its presence. The extent of fertilization was measured by microscopic observation of the presence of fertilization membranes. Only cultures with better than 95% fertilization, no polyspermy, and a high degree of synchrony were utilized. After single-cell embryos were washed 3 times with cold seawater to reduce excess sperm, radiolabel was added. Cells were grown in [^{3}H]thymidine (2.5–10 mCi/L) shortly after fertilization until they were harvested at the appropriate stage. When harvested, 85–95% of the embryos were midway through the cell cycle. Fertilization membranes were removed by passing the embryos through a nylon 53-μm net (Small Parts Inc.). Embryos were then pelleted by a 5-min centrifugation at 1000 rpm in a Sorvall HB-4 rotor. This and subsequent steps were performed at 0–4 °C.

Nuclear Isolation and Digestion. Nuclei from two-cell-stage embryos are very fragile and difficult to isolate pure and intact, unlike nuclei from other eukaryotes and later stage sea urchin embryos. Therefore, the following procedure must be carried out with extreme care to avoid disruption of the higher order structure and resulting contamination of the chromatin with yolk material and endogenous nucleases.

Embryos were washed twice in IG buffer [0.1 M sodium phosphate, 0.1 M LiCl, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), pH 6.0] supplemented with 25 mM ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) following Shaw et al. (1981). The pellet was then washed twice in IG buffer without EDTA and homogenized with two strokes of a loose fitting pestle of a Dounce homogenizer. The homogenate was poured into a centrifugation tube, and an equal volume of a solution of 0.1% Triton X-100–1 mM $CaCl_2$ in IG was added and gently mixed to produce a final Triton X-100 concentration of 0.05%. The homogenate was pelleted at 5000 rpm for 5 min and washed in IG buffer. Nuclei were then washed twice and resuspended in either KPS [0.085 M KCl, 0.3 M sucrose, 5 mM 1,4-piperazinediethanesulfonic acid (Pipes), 0.5 mM PMSF, pH 7.0] or A4 buffer (60 mM KCl, 15 mM NaCl, 10 mM MgCl, 0.1 mM EDTA, 0.5 mM PMSF, pH 7.4). Providing that nuclease digestions were initiated within 30 min following homogenization, only minimimal endogenous nuclease action was observed. Such nuclei were digested at 37 °C with 1 unit/OD_{260} of micrococcal nuclease (Worthington) in KPS or A4 buffer with 1 mM $CaCl_2$. Digestions took place in DNA concentrations of 40–80 OD/mL and were terminated by the addition of 0.1 volume of 100 mM EDTA and immediate vortexing. Subsequent analysis indicated no differences in the repeat length obtained in KPS or A4 buffer.

Gel Electrophoresis. DNA was isolated and electrophoresed either on 3.8% acrylamide gels as described by Cognetti & Shaw (1982) or, for accurate sizing of DNA, on 1.2% agarose slab gels ($32 \times 18 \times 0.15$ cm^3). The running buffer was 50 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.2, 20 mM sodium acetate, and 1 mM EDTA [E buffer of Loening (1967)]. Samples of 1–20 μg of sea urchin DNA in 4–20 μL of 1/10 E buffer with 5% Ficoll and 0.005% bromophenol blue were applied per slot after preelectrophoresis of the gel for 20 min at 70 V. For sizing, each digestion time point was electrophoresed in two adjacent lanes, one containing sample and the other containing sample and either SV40 DNA (Bethesda Research Laboratories) restricted with *Sau*I or ϕX174 DNA restricted with *Hae*III (New England Nuclear). All lanes also contained pBR 322 DNA (gift of Dr. Bill Jack) restricted with *Eco*RI and *Pvu*I as internal standards (2293 and 2068 bp). Electrophoresis was carried out at 60 V for 1 h and 240 V for the remaining time. Gels were fluorographed with Enhance (New England Nuclear). Developed films were scanned by using a Zeinah soft laser densitometer on line to a Digital Electronics computer and printed on a Hewlett-Packard x–y plotter.

Calculations. DNA was sized with a program on the basis of the mobility vs. reciprocal length relationship first noted by Southern (1979). A Fortran version of this program was reported by Schaffer & Sederoff (1981); we used a version transcribed to Basic by Dr. R. Schaffer (personnal communication). This program gave agreement to within 1% in predictions for known restriction fragments. For each digestion time point, a repeat length value was obtained from a plot of fragment length (as predicted by the Schaffer and Sederoff program) as a function of band order (i.e., oligomer number) for that gel lane. Since all lanes contained two long fragments (pBR 322 DNA restricted with *Eco*RI and *Pvu*I) as internal standards, the distance from the largest pBR 322 restriction fragment (2294 bp) to the center and maximum of each band was measured. This was considerably more accurate than measuring from the top of the gel, which invariably was distorted in the drying process required for fluorography. Linear-regression analysis of DNA size vs. number of nucleosomes yields a slope for each time point; the slope gives the repeat length that represents the most probable average center-to-center distance between nucleosomes. This method of analysis produces a repeat length that is free from the effects of degradation from the ends since an n-mer nucleosome should experience exonucleolytic shortening similar to an $(n + 1)$-mer

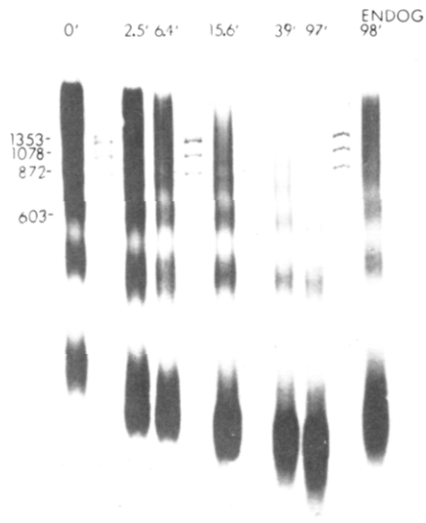

FIGURE 1: Fluorograph of a 3.8% polyacrylamide gel showing timed digest of nuclei isolated from two-cell embryos. Marker lanes contained ϕX174 replicative-form DNA restricted with *Hae*III.

nucleosome. Thus, this technique is more accurate than measurements affected by exonucleolytic shortening of the nucleosomal DNA. Reported errors indicate 95% confidence limits with respect to the mean of the slopes.

Results

To determine the average spacing between nucleosomes within the nucleus, we labeled embryos with [^{3}H]thymidine, harvested them at the two-cell stage following the first cleavage, and digested isolated nuclei with micrococcal nuclease. Purified DNA fragments from these digestions were first electrophoresed on 3.8% acrylamide gels (Figure 1). As can be seen in Figure 1, a pattern typical of a nucleosome repeat was obtained for two-cell embryos. Although endogenous nucleases are active, it is clear that most of the DNA is present as large oligomers at the zero time point. As digestion with micrococcal nuclease proceeds, the proportion of short oligomers and monomers increases while that of longer oligomers decreases. In order to accurately determine the size of the digestion fragments, this DNA was electrophoresed on 1.2% agarose slab gels (Figure 2). The average chromatin repeat for two-cell nucleosomes was determined to be 190 ± 1 bp for culture A and 187 ± 4 bp for culture B (Figure 3). There was little or no change in the repeat length determined for the first 5–40% of digestion, as shown in Figure 3. Although differences have been reported in calf thymus and erythrocyte chromatin for the repeat size measured from brief digests compared to longer term digests [see Martin et al. (1977)], we observed little or no difference in the size of two-cell repeats as a function of time (Figure 3), indicating that most of the early cleavage stage chromatin may be devoid of structurally different domains having different nuclease sensitivities that are characteristic of differentiated cells. The whole genome appears rather homogeneous in its structure and accessibility to nuclease. The slight digestion evident for the zero incubation time of two-cell nuclei reflects the large amounts of endogenous nuclease present in the early cleavage stages of development as demonstrated in Figure 1. Since this nuclease did not generate any core or smaller size DNA (i.e., ≤146 bp; cf. zero time, Figures 1 and 2) and since there were no variations in repeat size at intermediate digestion times, it is unlikely that it affected the calculated repeat length. The endogenous nuclease did, however, complicate this project until

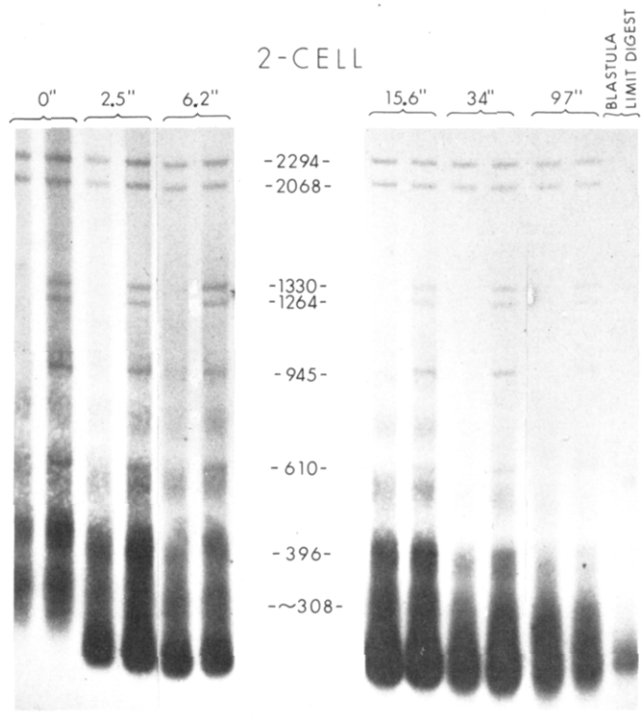

FIGURE 2: Fluorograph of agarose gel electrophoresis depicting a timed digest of nuclei isolated from two-cell embryos (culture B). Digestion times (in minutes) are indicated above lanes. Measured sizes are given in base pairs. All lanes contained pBR 322 digested with *Eco*RI and *Pvu*I. One lane of each time point also contained *Sau*I restriction fragments of SV40 DNA.

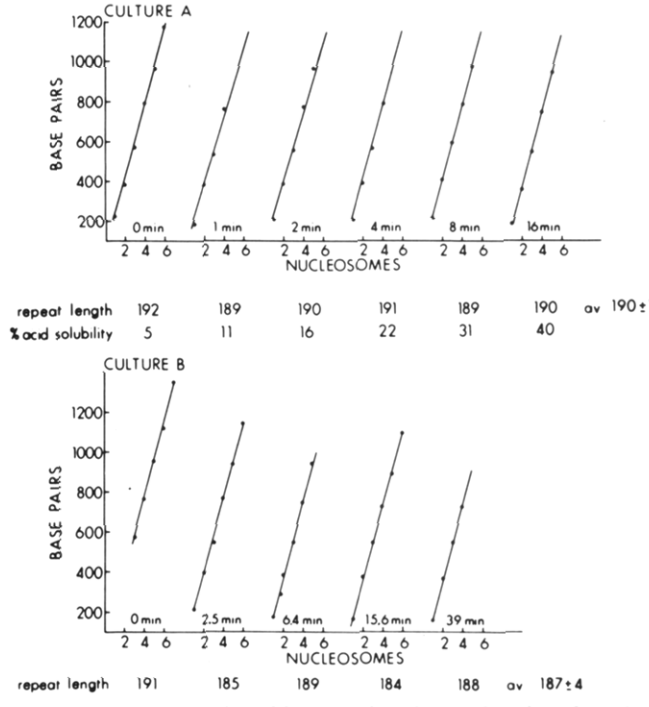

FIGURE 3: Representation of fragment lengths as a function of number of nucleosomes in each fragment for different cultures of two-cell embryos. Digestion times are indicated next to each straight line. Each calculated repeat length (slope of line) is indicated below graph.

conditions were found that could minimize the activity.

Linear least-squares analysis of the data in Figure 1 indicates very high correlation coefficients (greater than 0.99) for plots of DNA size vs. nucleosome number. When appropriate internal standards are included in all samples, the precision of this method is 1–2 base pairs under optimal circumstances (Sperling et al., 1980). However, its reliability is limited by

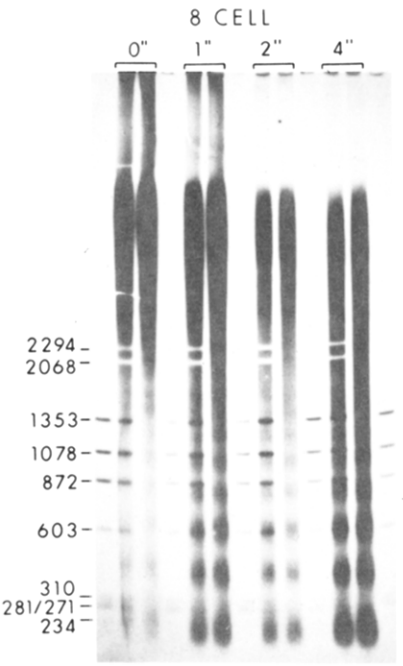

FIGURE 4: Fluorograph of agarose gel electrophoresis for a timed digest of nuclei isolated from eight-cell embryos that were grown from fertilization in [^{3}H]thymidine. Digestion times are indicated above lanes. White bands result from quenching due to inclusion of low specific activity restriction fragments of pBR 322 digested with *Eco*RI and *Pvu*I. Sharp dark bands are *Hae*III restriction fragments of ϕX174 (replicative-form) DNA.

the properties of the digested chromatin; not only is there a large dispersion in size for the fragments but also, as more digestion occurs, the higher oligomer bands finally disappear, resulting in a decrease of reliability at very long digestion times. For sea urchin chromatin at two-cell and eight-cell stages, as well as other premorula stages of development (not shown), this spreading (see Figures 2 and 4) of bands is considerably more pronounced than that for chicken erythrocyte, bovine thymus, and cell culture systems (Shaw et al., 1976; Todd & Garrard, 1977; Sperling et al., 1980), which may indicate less rigidity in the placement (or a propensity for sliding) of the core histones along the average 189 bp nucleosomal segment in two-cell-stage embryos. Nevertheless, the data acquired from the gels shown, as well as others not shown, indicate a two-cell nucleosome most probable average repeat of 189 ± 2 bp. Repeat lengths are in agreement for various isolations and different length (approximately 30 or 50 min) labeling periods. Analysis of the fluorograph of the DNA isolated from eight-cell embryos (Figure 4) indicates an increase from the 189-bp repeat size of two-cell embryos to 201 bp for the eight-cell stage.

Of special note is Figure 2—where the DNA was labeled for ~30 min, or one-third of the time for completion of the first cleavage. Although the repeat pattern of this agarose gel is nearly identical with that of longer labeling times, the presence of an additional DNA fragment of 308 bp at early times in digestion (cf. zero time) is quite pronounced. Fluorographs of DNA labeled for a longer (~50 min) period, during two-thirds of a division time (data not shown), display only a shoulder in this region—indicating a lower abundance of the 308-bp fragment relative to other labeled DNA, as would be expected for newly replicated DNA that has matured into nucleosomes. This band is not clearly resolved on acrylamide gels but appears to run with a mobility slightly lower than that of the monomer. Since the repeat lengths determined for both long and short pulses are the same within the statistical accuracy of these experiments and since there is no change in the apparent repeat length as a function of digestion time [in contrast to that reported for newly replicated chromatin by Jackson et al. (1981)], we are confident that the average repeat lengths determined here are not affected by small contributions from newly replicated DNA fragments.

After very long digestion (limit digest), most of the DNA in two-cell nuclei is cleaved into monomer-size fragments. The size distribution of this DNA is centered around 145 bp and presumably corresponds to the core nucleosomal DNA. Very little of the DNA is cleaved into fragments of less than ~120 bp even after long digestion. In view of the presumed openness of chromatin in early stage embryo nuclei, it is surprising that the core particle is so resistant to further DNA degradation. This stability of the core is also observed in nucleosomes of the eight-cell stage.

Discussion

We have presented the first reports of sizing of chromatin in two-cell embryos, which are difficult to study because of their fragile nuclei and the relatively small amount of DNA compared to endogenous proteins and yolk material. It is known that the size of the nucleosome subunit repeat of the sea urchin, *S. purpuratus*, varies during development; it is greatest in the larval stage where the late histone variant subtypes predominate and in the differentiated sperm cell, which has its own characteristic histone subtypes (Keichline & Wassarman, 1979; Arceci & Gross, 1980a,b). Only a few reports address the chromatin structure of embryos in the first few cleavages following fertilization [cf. Savic et al. (1981) and Shaw et al. (1981)]. From partial micrococcal nuclease digests, we have measured the average nucleosomal repeat lengths for two-cell and eight-cell embryos of *S. purpuratus*, labeled with [^{3}H]thymidine such that only the DNA synthesized since fertilization is monitored.

The 189 base pair repeat, which has been obtained in this paper for the average subunit repeat size of the nucleosome in the two-cell-stage embryo, is the shortest yet reported in sea urchin development. If one considers that the haploid sperm repeat is larger by nearly 60 base pairs, these results indicate that a dramatic rearrangement of chromosomal proteins must occur on sperm DNA chromosomes following fertilization so as to allow for the huge decrease in average nucleosome size observed after the first cleavage. It is interesting to note that CSH1 (the H1-like protein found in two-cell chromatin) is the largest H1 variant in sea urchins. If it were responsible for this small repeat, it would have a unique three-dimensional interaction that is worthy of further study. Savic et al. (1981) have reported that polyspermic embryos quickly replace sperm H1 with CSH1 without a concomitant change in the repeat length of the sperm component. Replication may be necessary to allow for the protein rearrangements that are necessary to produce large changes in the repeat size.

With two more cell divisions, the most probable average repeat size subsequently increases by 12 bp to about 201 bp. This is in reasonable agreement with the 196-bp value obtained for unlabeled eight-cell embryos (presumably run without internal lane standards) by Savic et al. (1981). Thus, the transition from a short repeat length at the two-cell stage to a longer repeat length of 220 bp at blastula (Keichline & Wasserman, 1979) and 230 bp at larval stages (Arceci & Gross, 1980) is a gradual developmental process. Whether lengthening of the average nucleosome repeat during embryogenesis is mediated by the amount of cleavage stage and later stage histone subtypes remains to be determined.

Since we have shown previously that nucleosomes exist in the earliest developmental stages and that the protein content of early stage nucleosomes differs from that of pluteus- and prism-stage embryos (Shaw et al., 1981), it is possible to conclude that the histone complements within the nucleosome vary during development in a manner coordinate with the new histone synthesis. This implies that stage-specific switches in histones may be a primary cause for the observed changes in repeat length of the nucleosome, although the similarity of the protein composition of two- and eight-cell chromatin (S. A. M. Chambers and B. R. Shaw, unpublished data) indicates that either additional conditions must be required to modulate these changes or that DNA repeat lengths are very sensitive to rather minor changes in the histone-variant complement.

Briefly digested two-cell chromatin indicated the presence of DNA fragments averaging about 308 bp (Figure 2). These fragments disappeared at increasing levels of digestion and were also less prominent in a digest of cells labeled for a longer period (culture A). Multimers of this size were not apparent in any of the digests, and the DNA did not conform to any integer band order. In view of its different mobility on acrylamide gels, this DNA may not have a typical double-stranded conformation [see Marini et al. (1982)]. Since normal nucleosomal core particles are resistant to inner cleavage, it is unlikely that this fragment arises from cleavages within assembled dimer nucleosomes. Because its presence is most pronounced in isolations having the shortest labeling period and if one considers the high degree of replication activity occurring at the two-cell stage, the ~308-bp fragment probably derives from a replication intermediate nucleoprotein complex such as that described by Levy & Jacob (1978, 1981) for blastula-stage sea urchins. Longer periods of labeling would be expected to, and do, reduce the relative intensity of such complexes, because more DNA has matured. Additional studies of this fragment will be necessary to ascertain its exact structure and origin.

There are certain problems with interpreting the results of all nucleosome-sizing studies, including those reported here [cf. Todd & Garrard (1977) and Sperling et al. (1980)]. A primary concern arises from the breadth or spreading of the bands, which presumably reflects different types of DNA packaging and accessibility in the nucleus. Digests of highly differentiated tissues usually yield sharper oligomeric bands than noncommitted, active tissues [cf. Lohr et al. (1977a,b)]. Band breadth may arise from a number of factors: (1) It may reflect an inherent heterogeneity in DNA length of the nucleosome repeating units (thus implying different types of packaging of the DNA) (Todd & Garrard, 1977, 1979) or sliding of nucleosomes during digestion. (2) It may reflect heterogeneity among the cell populations, each having its own repeat length size. (3) Cleavage by nuclease may occur nonrandomly and at more than one site between the core particles, thus broadening the bands (Lohr et al., 1977a; LaFond et al., 1981). (4) Restricted accessibility of the nuclease to certain chromosomal regions would also give rise to broadened bands, since at any one time a trimer nucleosome from a more exposed region would be shorter than a trimer (of the same original size) in a less exposed region, due to the higher probability of multiple cuts in the more exposed nucleosome. The latter gives good reason to examine the size of DNA at low levels of nuclease digestion. However, in systems that are rapidly dividing (e.g., the two-cell embryo), calculating the repeating unit at the earliest digestion times may yield primarily the unit size of the newly replicated chromatin or that of the most decondensed chromatin. Thus it is important to determine the average nucleosomal repeat size at different times in the digestion process so as to show that the calculated repeat lengths reflect the average spacings between nucleosomes of major chromatin domains in the nucleus. In our studies here, the average nucleosomal repeat shows no change from 5 to 40% digestion, suggesting that in these early cleavage stages the bulk of the DNA lacks the structurally dissimilar domains described in more highly differentiated systems [cf. Todd & Garrard (1977, 1979)].

Although no concensus on the relationship of the metabolic or genetic state of a cell with the repeat length of its chromatin is apparent in the literature, it is possible to corroborate some generalizations on the basis of our studies: (1) Long repeat lengths are generally associated with highly differentiated cells [cf. Sperling & Weiss (1980)]; the results presented here are consistent with that hypothesis. (2) Long repeat lengths are also thought to be characteristic of cells that are less active in cell division. The short repeat length of the two-cell chromatin is consistent with this hypothesis, too, since the mitotic rates of the new zygote and eight-cell embryo are considerably greater than those of the 32-cell and blastula-stage embryos.

The significance of repeat lengths remains elusive. Although a repeat length is an average size, Sperling & Weiss (1980) have demonstrated that different lengths characterize differentiated functions. Changes in the nucleosomal repeat may reflect an important process of development. Many reports indicate that phasing occurs on functionally important regions of DNA [for review, see Cartwright et al. (1982)] and that nucleosome placement on promoter sites profoundly affects the rate of RNA transcription in vitro (Wittig & Wittig, 1982). Changes in the repeat length might be developmentally necessary to bring nucleosomes into proper register during development. In this manner, phasing over functionally important regions of the genome could occur from nonphased chromatin in the early cell stages.

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Registry No. Micrococcal nuclease, 9013-53-0.

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Chromatin Structural Changes in Synchronized Cells Blocked in Early S Phase by Sequential Use of Isoleucine Deprivation and Hydroxyurea Blockade†

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ABSTRACT: We have investigated the loss of histone H1 from chromatin [D'Anna, J. A., Gurley, L. R., & Tobey, R. A. (1982) *Biochemistry 21*, 3991–4001] and the structure of chromatin from Chinese hamster (line CHO) cells blocked in early S phase by sequential use of isoleucine deprivation G_1 block and 1 mM hydroxyurea (HU) blockade. Measurements of H1 content in the cell and histone turnover indicate that H1 is lost from the cell and that there is negligible replacement synthesis of H1 during the period of the S-phase block. As H1 is lost, chromatin appears to undergo structural change. After 10 h of HU block, the new deoxyribonucleic acid (DNA) and a portion of the old DNA have measured nucleosome repeat lengths (37 °C digestion) which are less than those of controls and similar to those observed by Annunziato and Seale [Annunziato, A. T., & Seale, R. L. (1982) *Biochemistry 21*, 5431–5438] for new immature chromatin in the absence of HU. By 24 h of HU block, nearly all of the chromatin has assumed a pseudoimmature conformation in which the nucleosome cores appear to be more closely packed along the DNA chain, but the new DNA is slightly more resistant than old DNA to attack by micrococcal nuclease. Electrophoretic analysis of nucleoprotein particles produced by micrococcal nuclease digestion of nuclei indicates that (1) the distribution of mononucleosome species changes during HU block and (2) some mononucleosome species appear to be enriched in normally minor proteins which may determine the electrophoretic mobility of the nucleoprotein particles in agarose–acrylamide gels. The results raise the possibility that (1) during the early stages of replication (or prior to the passage of the replication fork), H1 is dissociated from initiated replicons and (2) H1 does not reassociate in a concerted fashion with the H1-depleted chromatin until the replication fork has passed and, perhaps, a substantial portion of the replicon has been replicated.

Release of synchronized G_1 cells into hydroxyurea (HU)[1] inhibits the accumulation of dATP pools (Walters et al., 1973) and greatly reduces the rate of DNA synthesis (Tobey & Crissman, 1972), but it does not prevent cells from entering S phase (Walters et al., 1976a). Autoradiography employing high concentrations of high specific activity radioactive thymidine shows that G_1 cells enter S phase at the same rate in the presence of HU as in its absence (Walters et al., 1976a). Furthermore, during the first 10 h of HU block, there is an accumulation of new small DNA (Walters et al., 1976a; Cress

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[1] Abbreviations: bp, base pairs of DNA; DNA, deoxyribonucleic acid; FCM, flow cytometry; HU, hydroxyurea; $NaDodSO_4$, sodium dodecyl sulfate; NP-40, Nonidet P-40 nonionic detergent; PCA, perchloric acid; Cl_3CCOOH, trichloroacetic acid; TCB, 15 mM Tris-HCl and 3 mM $CaCl_2$, pH 7.2; TCMB, 15 mM Tris-HCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.2; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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