Structure of Chromatin in Sea Urchin Embryos, Sperm, and Adult Somatic Cells†

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ABSTRACT: Electrophoretic analyses of DNA extracted from micrococcal nuclease partial digests of sea urchin embryo chromatin revealed a DNA repeat length of 222 base pairs for chromatin from morula, mesenchyme blastula, and pluteus stage embryos. No significant differences were observed in the electrophoretic patterns of DNA extracted from micrococcal nuclease limit digests of chromatin from sea urchin embryos at different stages of development; a core fragment containing 140 base pairs of DNA was seen at all stages of embryogenesis examined. Limit digest DNA patterns obtained with sea urchin embryo chromatin were virtually indistinguishable from those obtained with mouse liver chromatin. Furthermore, micrococcal nuclease partial and limit digest DNA patterns of adult sea urchin gut chromatin were identical with those obtained with sea urchin embryo and mouse liver chromatin. These findings suggest that the amount and arrangement of DNA in nucleosomes of sea urchin chromatin remain the same throughout the development of the sea urchin, from embryo to adult. Electrophoretic analyses of DNA extracted from micrococcal nuclease partial digests of sea urchin sperm chromatin revealed a DNA repeat length of 250 base pairs. Limit digest DNA patterns of sea urchin sperm chromatin differed somewhat from patterns obtained with sea urchin embryo and mouse liver chromatin in that they exhibited a major peak at 160 base pairs of DNA, with only a shoulder corresponding to the 140 base pair core fragment. Electrophoretic patterns of DNA extracted from DNase I digests of sea urchin sperm chromatin exhibited the same 10 base interval between DNA fragments as that seen with mouse liver chromatin on denaturing gels. However, while the DNA patterns of DNase I digests of liver chromatin displayed a prominent band at 80 nucleotides, sperm chromatin displayed prominent bands at 70 and 80 nucleotides. Similarly, a comparison of the electrophoretic patterns of DNA extracted from DNase II digests of sea urchin sperm chromatin with those from mouse liver chromatin revealed several significant differences on denaturing gels. These results suggest that the unique histones found in sea urchin sperm chromatin affect both the amount and arrangement of DNA in the nucleosomes of sperm chromatin.

Digestion of plant, animal, or yeast chromatin with micrococcal nuclease results in the production of DNA fragments which are multiples of the smallest fragment that accumulates during the early phase of the digestion. These fragments reflect the organization of DNA and histones in discrete particles containing about 200 base pairs of DNA, termed nucleosomes (Kornberg, 1977). Further digestion of chromatin with micrococcal nuclease results in the production of a "core particle" containing 140 base pairs of DNA and two each of the histones H2A, H2B, H3, and H4. Due to the interaction of DNA and histones, more extensive digestion ("limit digest") of the core particle gives rise to a characteristic array of DNA fragments (Axel, 1975; Sollner-Webb & Felsenfeld, 1975; Compton et al., 1976; Lohr et al., 1977). The size of the core particle appears to be conserved in all species examined thus far, whereas the length of DNA between particles ("linker") varies (Kornberg, 1977). Despite this variation, digestion of chromatin with pancreatic DNase I always gives rise to a characteristic pattern of DNA fragments on denaturing gels (Lohr et al., 1977; Noll, 1974; Sollner-Webb & Felsenfeld, 1977; Morris, 1976; Noll, 1976).

In a previous report we examined the kinetics and products of digestion of sea urchin embryo and sperm chromatin by micrococcal nuclease (Keichline & Wassarman, 1977). We found that, while the rate and extent of solubilization of chromatin DNA by micrococcal nuclease decreases as sea urchin development proceeds from the hatching blastula to the pluteus stage, nucleosomes remain the same size throughout this period of embryogenesis. Furthermore, we found that sea urchin sperm chromatin is composed of nucleosomes containing about 250 base pairs of DNA, thus far, the largest nucleosome reported for any source of chromatin (Kornberg, 1977). These results are of interest since the histone complement of sea urchin chromatin changes both qualitatively and quantitatively during early embryogenesis (Hill et al., 1971; Cohen et al., 1975; Arceci et al., 1976; Newrock et al., 1978) and since sea urchin sperm chromatin contains unique histones (Ozaki, 1971).

The results presented here extend our previous studies of the structure of sea urchin embryo and sperm chromatin (Keichline & Wassarman, 1977). Using a new method for the isolation of nuclei from sea urchin embryos, we have been able to show that, even as early as the morula stage of embryogenesis, sea urchin chromatin is composed of nucleosomes containing about 222 base pairs of DNA, as compared with about 199 base pairs for mouse liver chromatin. We have also found that sea urchin gut chromatin is composed of nucleosomes containing about 222 base pairs of DNA indicating that the size of nucleosomes remains the same throughout sea urchin development, from embryo to adult. The amount and arrangement of DNA in the core particle of sea urchin chromatin also remain the same throughout development. Finally, we have found that, although nucleosomes in sea urchin sperm chromatin are unusually large (250 base pairs of DNA), their substructure, as revealed by nuclease digestion, is similar to that of nucleosomes in chromatin from other sources. The differences observed here between the nuclease digestion products of sperm and liver chromatin are discussed in relation to the reported presence of a unique H1-like histone in sea urchin sperm chromatin (Ozaki, 1971).

Materials and Methods

Nucleases. Micrococcal nuclease (6000 units/mg) was obtained from Worthington Biochemical Corp., pancreatic

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DNase I from Sigma, and spleen acid DNase II from Worthington Biochemical Corp. The nucleases were used without further purification.

Preparation of Sea Urchin Embryo Nuclei. Gametes of *Strongylocentrotus purpuratus* (Pacific Biomarine, Venice, CA) were obtained by coelomic injections of a few milliliters of 0.5 M KCl. Eggs were washed several times in Millipore filtered sea water (MFSW)[1] and were suspended in MFSW at 15 °C and fertilized with an appropriate dilution of fresh sperm. Fertilization membranes were removed from eggs to be grown to early embryonic stages by addition of papain (Sigma) and cysteine hydrochloride to final concentrations of 0.5% and 2%, respectively, 45 s after fertilization. After 4 min the eggs were centrifuged very gently by hand and washed three times by hand centrifugation. The demembranated eggs were gently resuspended in MFSW containing 50 μg/mL of streptomycin and 100 U/mL of penicillin G.

Embryos at the desired stages were centrifuged and washed three times in Ca^{2+}, Mg^{2+}-free sea water (CMFSW) and then once in CMFSW containing 0.01% EDTA; this step and all succeeding steps were performed at 0–4 °C. The pelleted embryos were resuspended in 0.5 M sucrose, 10 mM Tris, pH 7.5, and the cells were dissociated by several passages of the Teflon pestle of a Potter-Elvehjem homogenizer. As few as three strokes were required for morula stage embryos, while 25 or more strokes were usually required for pluteus stage embryos. The primitive skeleton of pluteus stage embryos was removed nearly completely by a very brief low speed centrifugation after diluting the cell suspension with an equal volume of CMFSW. Resuspension of the pellet and recentrifugation diminished the loss of cells with the skeletons. Cells which had been dissociated were then filtered through Nitex mesh (pore size, 25 μm) which removed unfertilized eggs, cell clumps, and remaining skeletal spicules.

The suspension of cells in 0.5 M sucrose was adjusted to a concentration of 1:20 relative to packed cell volume. Cells were lysed by adding an equal volume of buffer containing 20 mM NaCl, 10 mM $MgCl_2$, 20 mM Tris, pH 7.5, 1% Triton X-100 with gentle mixing. The extent of cell lysis was monitored with a phase microscope. After a few minutes, when lysis was complete, the suspension of nuclei was centrifuged for 10 min at 600*g*. The pellet was gently resuspended in a few drops of a buffer containing 0.25 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris, pH 7.5. The volume was increased to several milliliters, and the suspension was centrifuged at 600*g* for 10 min; this washing procedure was repeated twice. Finally, the nuclei were resuspended in digestion buffer containing 0.25 M sucrose, 3 mM $MgCl_2$, 10 mM Tris, pH 7.5.

Preparation of Sea Urchin Sperm Chromatin. Freshly collected sperm were suspended in buffer containing 80 mM NaCl, 20 mM EDTA, pH 7.5, and were homogenized vigorously by hand in a Dounce homogenizer. The sperm were centrifuged for 10 min at 2000*g* and rehomogenized in the same buffer. This washing procedure was monitored by phase microscopy and removed most sperm tails and caused the outer sperm membrane to swell markedly. Sperm were then vigorously dounced in a solution of 80 mM NaCl, 20 mM EDTA, pH 7.5, and 1% Triton X-100; this removed the outer sperm membrane and any remaining tails. Sperm nuclei were pelleted by centrifugation and washed two times in the same solution without detergent. Lysis of nuclei and solubilization of chromatin was accomplished by placing the nuclei in distilled water and allowing the mixture to stand for 1–2 h. The nuclei began to lyse immediately under these conditions with the rate and extent of lysis being greatest at high dilutions. Occasional slow strokes with a Teflon pestle in a Potter-Elvehjem homogenizer facilitated homogeneity of the chromatin preparation. Sperm chromatin, which always formed an extremely viscous gel, was layered over 1.7 M sucrose, 5 mM EDTA, 10 mM Tris, pH 7.5, and spun at 105000*g* for 3.5 h. The pellet was resuspended in 10 mM Tris, pH 7.5, and the concentration of chromatin DNA adjusted to 2 mg/mL. Chromatin was mixed with an equal volume of digestion buffer (2×) in preparation for nuclease digestion.

Preparation of Mouse Liver Nuclei and Chromatin. Frozen mouse livers were minced and then homogenized in a Dounce homogenizer in buffer containing 0.25 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris, pH 7.5, and 0.5% Triton X-100. The homogenate was layered over 2.2 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris, pH 7.5, and the nuclei were pelleted by centrifugation for 1 h at 40000*g*. The nuclear pellet was gently resuspended and washed three times in the wash buffer used for embryo nuclei, and the nuclei were finally resuspended in digestion buffer.

Chromatin was prepared from mouse liver nuclei which had been washed three times in 80 mM NaCl, 20 mM EDTA, pH 7.5. Washed nuclei were lysed in distilled water and the lysate was centrifuged through 1.7 M sucrose, 5 mM EDTA, 10 mM Tris, pH 7.5, at 105000*g* for 3.5 h. The chromatin pellet was redissolved in 20 mM Tris, pH 7.5, at a DNA concentration of 2 mg/mL.

Preparation of Sea Urchin Gut Nuclei. Nuclei were prepared from fresh gut tissue obtained from adult sea urchins. The isolation procedure used was essentially the same as that used to prepare mouse liver nuclei.

Digestion of Chromatin. All digestions were carried out at a DNA concentration of 1 mg/mL, based either on the assumed value of 1.8 pg of DNA per nucleus in the sea urchin (Poccia & Hinegardner, 1975) or on the absorbance at 260 nm for solubilized chromatin preparations. Micrococcal nuclease digestions were carried out with intact nuclei resuspended at appropriate dilutions in 0.25 M sucrose, 3 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM Tris, pH 7.5. Isolated sperm chromatin digested with DNase I was suspended in a buffer containing 3 mM $MgCl_2$, 10 mM Tris, pH 7.5. DNase II digestions of sperm chromatin were carried out in a buffer containing 5 mM EDTA, 10 mM Tris, pH 7.5.

Termination of nuclease digestions was accomplished by chilling the reaction mixture and adding EDTA to 25 mM and $NaDodSO_4$ to 0.25%. Ribonuclease (40 μg/mL) was added and the samples were incubated for an additional 8 h, chloroform extracted, ethanol precipitated, and finally redissolved in a 1:10 dilution of electrophoresis buffer.

Electrophoretic Analysis of DNA Fragments. Analysis of the repeat distance of embryo, sperm, and liver chromatin was carried out in 2% agarose slab gels. The agarose was dissolved in a running buffer of 0.1 M Tris–borate, pH 8.3, containing 0.25 mM EDTA. Analysis of the core fragment and smaller fragments was carried out in 12% polyacrylamide gels using the same buffer as for agarose gels. Denaturing gels containing 12% polyacrylamide and 5 M urea were prepared with the same running buffer used in nondenaturing gels, but diluted 1:1 with water. Samples were prepared for denaturing gels by the addition of NaOH to 0.1 N and then an equal volume of 10 M urea which was made up in a solution of the tracking dyes, bromphenol blue and xylene cyanol. The samples were

[1] Abbreviations used: MFSW, Millipore filtered sea water; CMFSW, Ca^{2+}, Mg^{2+}-free sea water; $NaDodSO_4$; sodium dodecyl sulfate.

heated for 15 s in boiling water just prior to electrophoresis. Following electrophoresis the gels were stained for 1 h with ethidium bromide at 1 μg/mL for nondenaturing gels and 5 μg/mL for denaturing gels. The gels were photographed under UV light using a 23A red filter and photographic negatives of the gels were scanned with a Joyce Loebl microdensitometer. Agarose gels were calibrated with DNA fragments of known size from *Hae*III digests of SV40 DNA (Yang et al., 1976) or PMB9 DNA (D. Jolley, personal communication). The SV40 genome was taken to be 5224 base pairs of DNA (Fiers et al., 1978). The values for the DNA repeat length of sea urchin and liver chromatin were determined by subtracting the lengths of neighboring oligomers (determined directly from electrophoretic mobility) from one another and calculating the mean difference. Polyacrylamide gels of limit digests were calibrated with DNA fragments from a *Hae*III digest of PMB9 DNA, since it contains a greater number of fragments in the limit digest region than does a digest of SV40 DNA.

Results

Isolation of Sea Urchin Embryo Nuclei. The method initially tried for isolation of sea urchin embryo nuclei involved centrifugation of dissociated cells and resuspension in lysis buffer (Hogan & Gross, 1972). However, due to the presence of magnesium in the lysis buffer, clumping of cells was an aggravating problem. Attempts to dissociate clumps by repeated pipetting were not totally successful and also resulted in damage to nuclei. This problem was avoided by addition of 2× lysis buffer directly to the dissociated cells which were left suspended in 0.5 M sucrose without a centrifugation step. This method of isolation of nuclei from sea urchin embryos was satisfactory at all stages of development examined. Lysis of dissociated cells occurred in a very few minutes and included dissolution of the many granules in the cytoplasm. Use of Triton X-100 at a concentration of 0.5% was found to be sufficient for granule dissolution at all stages examined. Lower concentrations of Triton X-100 did not always yield clean nuclei, while higher concentrations did not substantially improve the appearance of the nuclei and increased the risk of damaging the nuclei. The hypotonicity of the lysis buffer aided cell lysis in the presence of detergent without necessitating vigorous homogenization. At the same time, sucrose helped to maintain the integrity of the nuclei under cell lysis conditions. The nuclei were generally quite stable in lysis buffer for periods of 60 min or more, although an effort was made to suspend the nuclei in detergent-free buffer as soon as possible after cell lysis. The nuclei produced by this method were spherical and free of cytoplasmic tags. The yield of nuclei with respect to intact cells initially present in suspension was approximately 30% for the morula stage and even higher for later embryonic stages.

Micrococcal Nuclease Partial and Limit Digests of Sea Urchin Embryo Chromatin. The DNA repeat length of sea urchin embryo chromatin was determined on 2% agarose gels using morula (32 cells), mesenchyme blastula, and pluteus stage embryos. A comparison of the electrophoretic patterns of DNA extracted from micrococcal nuclease partial digests of embryo chromatin and of mouse liver chromatin is made in Figure 1. A DNA repeat length of 222 (± 10) base pairs was determined for sea urchin embryo chromatin at all stages of development using the procedure described in Materials and Methods and this value is considerably larger than the 199 (± 6) base pair repeat length obtained for mouse liver chromatin.

The size distribution of DNA fragments extracted from extensive micrococcal nuclease digests (limit digests) of sea urchin embryo chromatin was determined on 12% polyacrylamide nondenaturing gels which resolve fragments in the range 40–140 base pairs in length. The electrophoretic patterns of limit digests of morula, mesenchyme blastula, and pluteus stage chromatin are compared with that of liver chromatin in Figure 2. The patterns obtained with the three embryonic stages are virtually indistinguishable from each other and from the pattern obtained with liver. A core DNA fragment is seen in each case in the region of 140 base pairs.

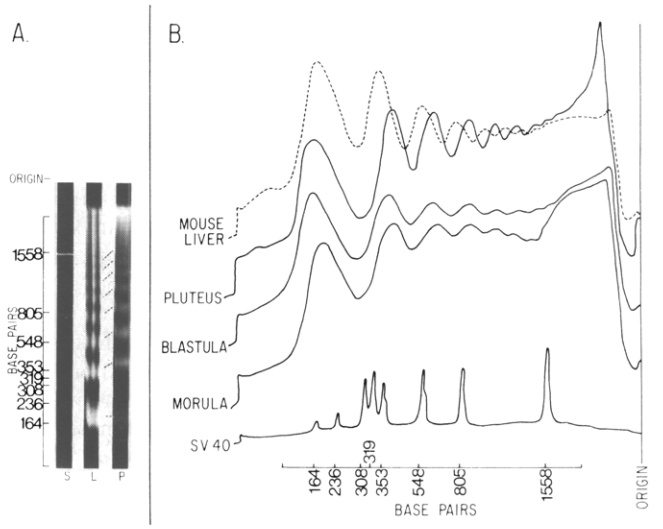

FIGURE 1: Electrophoretic analysis of DNA extracted from micrococcal nuclease partial digests of sea urchin embryo and mouse liver chromatin. DNA was extracted from nuclei exposed for 1 min to 100 U/mL of micrococcal nuclease and analyzed on 2% agarose slab gels as described in Materials and Methods. The SV40 genome was taken to be 5224 base pairs (Fiers et al., 1978) and a plot of log of DNA fragment size (number of base pairs) in a *Hae*III digest of SV40 against distance migrated was used to determine the sizes of the sea urchin embryo and mouse liver DNA bands. The lengths of neighboring oligomers were subtracted from one another and the mean difference was calculated to obtain DNA repeat lengths (size of the nucleosome). (A) Photographs of lanes of ethidium bromide stained gels containing SV40 *Hae*III DNA fragments as standards, S, mouse liver DNA, L, and sea urchin pluteus stage DNA, P. (B) Microdensitometer tracings of photographic negatives of gels containing SV40 DNA fragments as standards, mouse liver DNA, and sea urchin morula, mesenchyme blastula, and pluteus stage DNA.

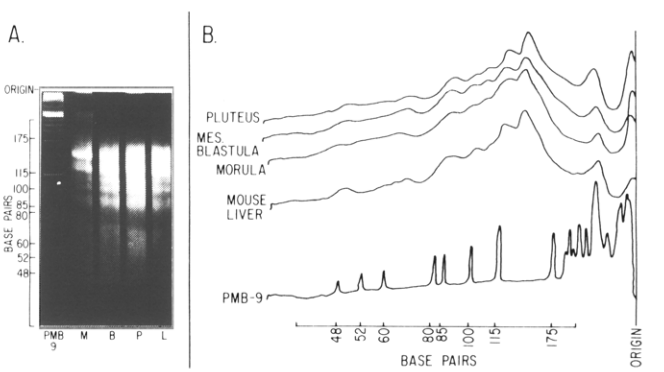

FIGURE 2: Electrophoretic analysis of DNA extracted from micrococcal nuclease limit digests of sea urchin embryo and mouse liver chromatin. DNA was extracted from nuclei exposed for 2 h to either 100 U/mL (embryo) or 500 U/mL (liver) of micrococcal nuclease and analyzed on 12% polyacrylamide nondenaturing slab gels as described in Materials and Methods. DNA fragments in a *Hae*III digest of PMB9 were used to calibrate the gel. (A) Photograph of an ethidium bromide stained gel containing *Hae*III DNA fragments of PMB9 as standards, mouse liver DNA, L, and sea urchin morula, M, mesenchyme blastula, B, and pluteus, P, DNA. (B) Microdensitometer tracings of a photographic negative of the gel shown in A.

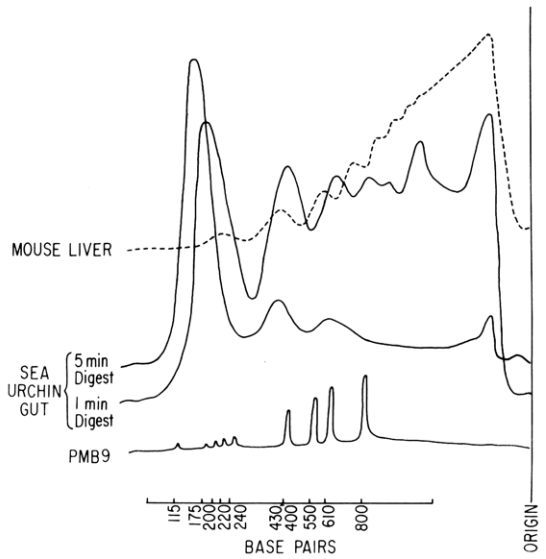

FIGURE 3: Electrophoretic analysis of DNA extracted from micrococcal nuclease partial digests of adult sea urchin gut and mouse liver chromatin. DNA was extracted from nuclei exposed for 1 min (gut and liver) or 5 min (gut) to 100 U/mL of micrococcal nuclease and analyzed on 2% agarose slab gels as described in Materials and Methods. DNA fragments in a *Hae*III digest of PMB9 were used to determine the DNA repeat lengths as described in the legend to Figure 1. Microdensitometer tracings of photographic negatives of ethidium bromide stained gels containing PMB9 DNA fragments as standards, mouse liver DNA, and sea urchin gut DNA are shown.

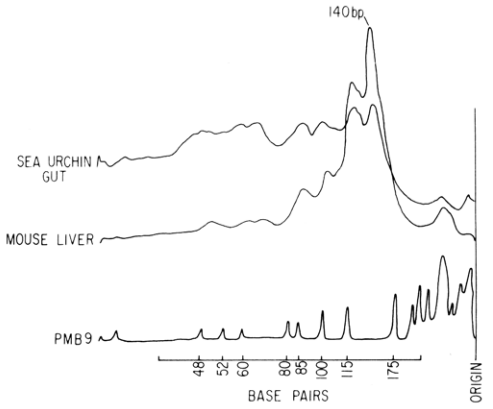

FIGURE 4: Electrophoretic analysis of DNA extracted from micrococcal nuclease limit digests of adult sea urchin gut and mouse liver chromatin. DNA was extracted from nuclei exposed for 2 h to 100 U/mL of micrococcal nuclease and analyzed on 12% polyacrylamide nondenaturing slab gels as described in Materials and Methods. DNA fragments in a *Hae*III digest of PMB9 were used to calibrate the gel. Microdensitometer tracings of a photographic negative of a gel are shown.

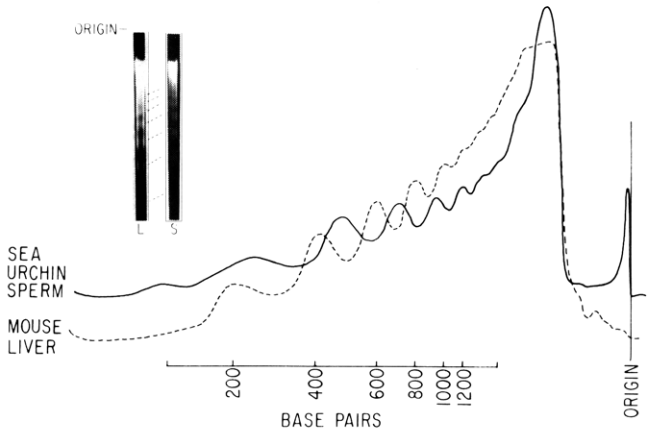

FIGURE 5: Electrophoretic analysis of DNA extracted from micrococcal nuclease partial digests of sea urchin sperm and mouse liver chromatin. DNA was extracted from nuclei exposed for 30 min (sperm) or 1 min (liver) to 100 U/mL of micrococcal nuclease and analyzed on 2% agarose slab gels as described in Materials and Methods. Gels were calibrated as described in the legend to Figure 1. Photographs of ethidium bromide stained gel lanes containing mouse liver DNA, L, or sea urchin sperm DNA, S, are shown together with microdensitometer tracings of photographic negatives of these lanes.

Therefore, although the DNA repeat length of sea urchin embryo chromatin is considerably larger than that of mouse liver chromatin, nucleosomes from both sources contain a core with approximately 140 base pairs of DNA.

Micrococcal Nuclease Partial and Limit Digests of Sea Urchin Gut Chromatin. Sea urchin embryo chromatin possesses an unusually long DNA repeat length as revealed in micrococcal nuclease partial digests. To determine whether this property is characteristic of sea urchin chromatin in general, or only of embryonic stages of development, micrococcal nuclease partial digests of adult sea urchin gut chromatin were analyzed as above. A comparison of the electrophorectic patterns of DNA extracted from micrococcal nuclease digests of sea urchin gut chromatin and mouse liver chromatin on 2% agarose gels shows that the sea urchin chromatin yields a series of more slowly migrating fragments (Figure 3). A DNA repeat length of 222 (± 12) base pairs is obtained for sea urchin gut chromatin using the procedure described in Materials and Methods. Therefore, the DNA repeat length of chromatin from adult sea urchin somatic cells is identical with that of chromatin from embryonic cells. Furthermore, as with DNA fragments extracted from micrococcal nuclease limit digests of embryo chromatin, the electrophoretic pattern of fragments from a limit digest of gut chromatin is similar to that obtained with mouse liver chromatin on 12% polyacrylamide nondenaturing gels (Figure 4). A core fragment containing 140 base pairs of DNA is observed and all other fragments align with those in the mouse liver digest.

Micrococcal Nuclease Partial and Limit Digests of Sea Urchin Sperm Chromatin. The DNA repeat length of sea urchin sperm chromatin was determined by electrophoretic analysis of DNA extracted from micrococcal nuclease partial digests on 2% agarose gels. A comparison of the electrophoretic pattern of DNA isolated from digests of sea urchin sperm chromatin with that of mouse liver chromatin shows that sperm chromatin yields a series of more slowly migrating fragments (Figure 5). A DNA repeat length of 250 (± 12) base pairs is obtained for sperm chromatin and 199 (± 6) for liver chromatin using the procedure described in Materials and Methods. This repeat length is considerably larger than the value of 222 base pairs obtained for sea urchin embryo and gut chromatin.

The electrophoretic pattern of DNA extracted from micrococcal nuclease limit digests of sea urchin sperm chromatin strongly resembles that of mouse liver chromatin on 12% polyacrylamide nondenaturing gels (Figure 6). Microdensitometer tracings of these gels show a peak in the region of the 140 base pair core DNA fragment and other peaks in the sperm chromatin digest align quite well with those in the liver chromatin digest. However, the electrophoretic pattern of sperm chromatin fragments is more diffuse than that obtained with the liver digest and, more importantly, the sperm chromatin digest exhibits a major peak at about 160 base pairs of DNA which persists even after exhaustive digestion of sperm chromatin with micrococcal nuclease. While a shoulder at 160 base pairs of DNA has been seen with liver chromatin, under conditions which did not yield a limit digest, this shoulder

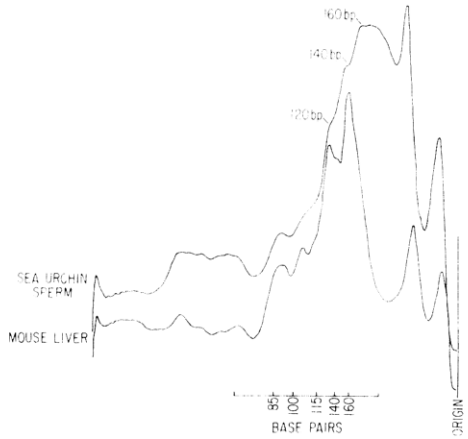

FIGURE 6: Electrophoretic analysis of DNA extracted from micrococcal nuclease limit digests of sea urchin sperm and mouse liver chromatin. DNA was extracted from isolated sperm chromatin and liver nuclei exposed for 2 h to 500 U/mL of micrococcal nuclease and analyzed on 12% polyacrylamide nondenaturing slab gels as described in Materials and Methods. Gels were calibrated as described in the legend to Figure 4. Microdensitometer tracings of a photographic negative of a gel are shown.

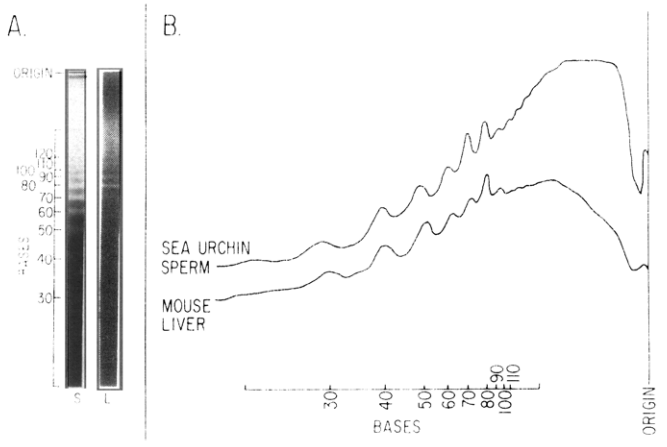

FIGURE 7: Electrophoretic analysis of DNA extracted from pancreatic DNase I digests of sea urchin sperm and mouse liver chromatin. DNA was extracted from isolated sperm and liver chromatin exposed for 1 h to 25 U/mL of pancreatic DNase I and analyzed on 12% polyacrylamide denaturing slab gels as described in Materials and Methods. Gels were calibrated using DNA fragments in a *Hae*III digest of PMB9. (A) Photographs of lanes of ethidium bromide stained gels containing sea urchin sperm DNA, S, or mouse liver DNA, L. (B) Microdensitometer tracings of photographic negatives of the lanes shown in A.

disappears when a limit digest is obtained.

DNase I and DNase II Digests of Sea Urchin Sperm Chromatin. When the DNA extracted from pancreatic DNase I digests of sea urchin sperm chromatin is subjected to electrophoresis on 12% polyacrylamide denaturing gels, a series of single-stranded fragments are observed at intervals of 10 nucleotides (Figure 7). Overall, this electrophoretic pattern is very similar to that obtained with mouse liver chromatin. However, while microdensitometer tracings of the liver chromatin pattern exhibit a prominent peak at 80 nucleotides, the sperm chromatin pattern has prominent peaks at both 70 and 80 nucleotides. The presence of these two prominent peaks in DNase I digests of sea urchin sperm chromatin has been observed at several different times of digestion and consistently on all gels used to analyze the DNA fragments.

Spleen acid DNase II has also been used to compare the structure of sea urchin sperm chromatin with that of mouse liver chromatin. The electrophoretic pattern of DNA extracted

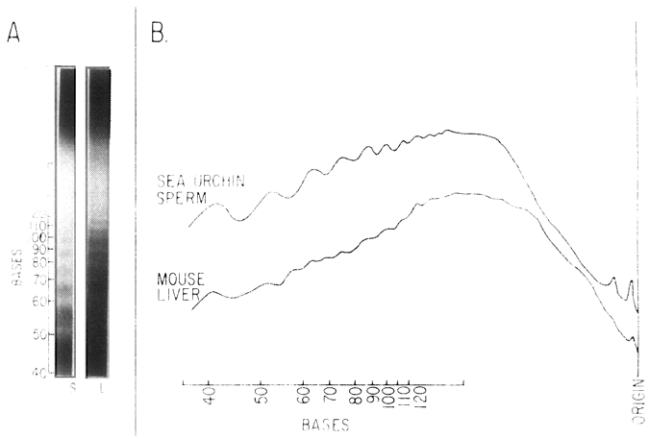

FIGURE 8: Electrophoretic analysis of DNA extracted from spleen acid DNase II digests of sea urchin sperm and mouse liver chromatin. DNA was extracted from isolated sperm and liver chromatin exposed for 2 h to 250 U/mL of porcine spleen DNase II and analyzed as described in the legend to Figure 7. (A) Photographs of lanes of ethidium bromide stained gels containing sea urchin sperm DNA, S, or mouse liver DNA, L. (B) Microdensitometer tracings of photographic negatives of the lanes shown in A.

from a DNase II digest of sperm chromatin exhibits the characteristic series of fragments at 10 base intervals on 12% polyacrylamide denaturing gels (Figure 8). Unlike DNA extracted from DNase I digests, prominent bands are not observed at 70 or 80 nucleotides. DNase II digests of mouse liver chromatin also exhibit a 10 base interval between DNA fragments; however, the electrophoretic pattern is more diffuse than that obtained with sperm chromatin when digestions are performed in the presence of EDTA and some intermediate bands are also present in the region of 55, 65, and 105 nucleotides.

Discussion

In attempting to study the structure of chromatin in nuclei isolated from sea urchin embryos, we considered the integrity of the nuclei used in these experiments of utmost importance. Such consideration is especially critical at the early stages of embryonic development (e.g., morula stage) when the nuclei are large and quite fragile. We did not wish to complicate our analyses of chromatin structure by comparing results obtained using damaged or broken nuclei with those obtained using intact nuclei. Since other methods for isolation of sea urchin embryo nuclei proved to be unsatisfactory, particularly for the early stages of development, we developed an isolation procedure which resulted in minimal damage to nuclei at any stage of embryonic development and should be applicable to other cell types which have large, fragile nuclei.

The results reported here show that the DNA repeat length of sea urchin chromatin, 222 base pairs, remains the same throughout development, from early embryo to adult. The size of the core particle, 140 base pairs, also remains the same throughout development and the arrangement of DNA within the core particle of adult or embryonic sea urchin chromatin cannot be distinguished from that of mouse liver chromatin in micrococcal nuclease limit digests. It is somewhat surprising to find that the structure of sea urchin chromatin, as revealed by nuclease digestion, remains the same throughout development. Several investigators have examined the histone composition of sea urchin chromatin during embryogenesis and have shown that the histone complement, including histone H1, changes dramatically during this period (Hill et al., 1971; Cohen et al., 1975; Arceci et al., 1976; Newrock et al., 1978). These changes in the histone complement of sea urchin embryo

chromatin occur as embryonic cells are undergoing alterations in their functional state (e.g., changes in cell cycle). It has been shown that the size of the DNA repeat length in chromatin from a variety of sources is not necessarily correlated with either the rate of cell division or the functional state of cells (Compton et al., 1976). On the other hand, Noll (1976) has suggested, on the basis of results obtained with chromatin from *Neurospora crassa*, that changes in the amino acid composition of histone H1 can affect micrococcal nuclease digestion of chromatin. Thus far, the only change we have been able to detect in sea urchin chromatin during embryogenesis is a decrease in the rate and extent of solubilization of chromatin DNA by micrococcal nuclease as development proceeds (Keichline & Wassarman, 1977).

Sea urchin sperm chromatin has a DNA repeat length of 250 base pairs, giving it the largest nucleosome reported for any source of chromatin thus far (Kornberg, 1977). This value is similar to that reported by Spadafora et al. (1976) for sperm chromatin from *Arbacia lixula*. Electrophoretic analyses of DNA extracted from micrococcal nuclease limit digests and DNase I digests of sperm chromatin indicate that its core particles contain 140 base pairs of DNA, but that an additional 20 base pairs are extremely resistant to digestion. These results are of interest in view of the presence of a unique histone in sperm chromatin which has a high molecular weight and a greater electric charge density than histone H1 due to a very high content of lysine and arginine residues (Ozaki, 1971). Various observations suggest that it is histone H1 that determines the spacing of adjacent nucleosomes in chromatin due to its interaction with the nonnucleosomal or "linker" regions of chromatin (Noll, 1977). The presence of a histone H1 with a lower than usual electric charge density has been used to explain the short DNA repeat lengths in *Neurospora* chromatin (170 ± 5 base pairs; Noll, 1976) and in *Aspergillus* chromatin (154 ± 9 base pairs; Morris, 1976). By analogous reasoning, the unique H1-like histone in sea urchin sperm chromatin may give rise to the unusually long DNA repeat length (250 ± 12 base pairs) due to its interaction with a linker region containing 110 base pairs of DNA.

The interaction between nucleosomes of sea urchin sperm chromatin and the unique H1-like histone also appears to be stronger than that between mouse liver nucleosomes and H1. It has been suggested that conversion of the 160 base pair DNA fragment of chromatin to the 140 base pair core by micrococcal nuclease is accompanied by the release of H1 (Noll, 1977). The interaction of H1 with a 20 base pair region is supported by the finding that micrococcal nuclease limit digests of H1-depleted chromatin exhibit no transient 160 base pair DNA fragment, but only the 140 base pair core fragment (Noll, 1977; Noll & Kornberg, 1977). Sea urchin sperm chromatin accumulates a 160 base pair DNA fragment in micrococcal nuclease limit digests which is only very slowly converted into the 140 base pair core. These results suggest that the unique H1-like histone in sea urchin sperm chromatin (Ozaki, 1971) interacts strongly with the 160 base pair DNA fragment and prevents its conversion into the core fragment with the concomitant release of the histone. The relationship of this interaction to the tight packing of chromatin in the mature sperm is not clear as yet.

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