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Modulation of nucleosome structure by histone subtypes in sea urchin embryos

(chromatin/*Strongylocentrotus purpuratus*/DNase I mapping/conformational changes)

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ABSTRACT **Switches of the types of histones synthesized and incorporated into chromatin occur during sea urchin embryogenesis. In an attempt to define the possible effects of these variant histones on chromatin structure, I have isolated and characterized nucleosome core particles from *Strongylocentrotus purpuratus* blastula (nearly 100% early histones) and pluteus (75% late histones). Both particles contain 146-base-pair lengths of DNA wrapped around an octamer of H2A, H2B, H3, and H4. Although sharing these similarities with the canonical core particle, the nucleosome structures have certain features that differ from those of typical adult tissues. Both the reversible and the irreversible conformational transitions occurring on heating core particles are destabilized in the embryonic particles vs. "typical" core particles. The blastula core particle unfolds more easily than pluteus (or other) nucleosomes under the stress of low ionic strength. The rate of DNase I digestion of pluteus core particles is about half that of particles from blastula; certain cutting sites differ in their susceptibility between the two embryonic particles and between these two and the canonical core particle. The data demonstrate that the variant histones synthesized during early embryogenesis have demonstrable effects on chromatin structure, even at this basic level.**

The question of the possible role of variant forms of histones in alteration of chromatin structure or function, or both, has been of interest since such subtypes of these generally highly conserved proteins were identified (1–4). During early embryogenesis in sea urchins, three distinct subtypes of histones H1, H2A, and H2B are synthesized at specific developmental stages. During the cleavage stages (before 16-cell), one set of these proteins is synthesized from stored maternal mRNA (4–6). Starting about 8-cell and persisting through blastula, a second set of these histones is synthesized and incorporated into chromatin; mRNA for these histones is derived from both stored maternal message and newly synthesized embryonic message (3, 7, 8). The third set, consisting of several protein variants, is then synthesized, continuing through the mature pluteus stage; this set is derived entirely from mRNA made with embryonic genetic information (7, 8). Sequence differences between the three sets of subtypes of H2A and H2B are not known; however, the different subtypes are generally only distinguishable by electrophoresis on Triton X-100/acid/urea gels (3, 9). In contrast to these variations, the proteins H3 and H4 are apparently constant during development, even though they are synthesized from different sets of histone genes that are expressed coordinately with the H1, H2A, and H2B subtypes (8).

The functional significance, if any, of such histone switches has not been defined. It could be suggested that the switches in histone subtypes reflect the need during development for vastly differing rates of histone synthesis; the developing embryo might synthesize histones from a highly reiterated set of genes early in development, when division rates are high, and then switch to a slightly amplified set of histone genes as the division rate slows. Alternatively, the different subtypes of histones present during development might create functionally different populations of nucleosomes, having characteristics appropriate for chromatin at the particular developmental stage. A wedding of these two disparate ideas—namely, that many histone genes code for proteins with characteristics appropriate for rapid replication of the genome early in development and that, later, fewer genes supply sufficient protein for a lower rate of division—with appropriate structural consequences in the nucleosome is also tenable.

Three previous studies have addressed the possible effects of histone switches on chromatin organization. Keichline and Wassarman (10, 11) reported a decrease in the rate and extent of solubilization of chromatin DNA by micrococcal nuclease as development proceeded from morula to pluteus. They found no change in the chromatin DNA repeat length during development. In contrast, Arceci and Gross (12) reported a steady increase in the repeat length during progression from morula to 16-day larva. Both the rate of digestion of chromatin by micrococcal nuclease and the nucleosome repeat length are (primarily) related to the type and amount of H1 present in the nucleus. It would seem likely that the core histone subtypes have only minor effects on these parameters.

We have demonstrated that the variant inner histones present in sea urchin sperm lead to a modulation of the structure of the chromatin core particle (13). Now I have isolated core particles from two stages of sea urchin embryos, containing different populations of the developmentally regulated inner histones, and have compared their static and dynamic structural properties to one another and to typical core particles from adult tissues. Significant variations in certain properties are described for both developmental stages. The data show that histone switching in early embryogenesis has structural consequences and suggest that these may have functional consequences.

EXPERIMENTAL SECTION

Strongylocentrotus purpuratus were obtained from Pacific Biomarine (Venice, CA) and maintained in the laboratory in artificial seawater (Instant Ocean, Eastlake, OH). Spawning was induced by intracoelomic injection of 0.55 M KCl. Eggs were collected in and washed several times with artificial seawater (14). Sperm was collected directly from the gonopores of male sea urchins. Eggs were suspended (10^7 eggs per liter) and fertilized with diluted sperm (0.25–0.5 ml of a 1:100 dilution is seawater per 10^7 eggs). All preparations utilized for the current studies demonstrated over 95% fertilization membrane eleva-

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Abbreviation: bp, base pair(s).

tion within 4 min after addition of sperm. Embryos were cultured at 15°C with gentle stirring in Bellco spinner flasks. Blastulae were collected after 16 hr by centrifugation. For further culture, embryos were diluted 5-fold with artificial seawater, and penicillin (50 units/ml) and steptomycin (50 mg/liter) were added. Pluteus-stage embryos were collected by filtration on 70-μm nylon mesh. Nuclei were prepared by methods described by Arceci and Gross (12); the only modification was a filtration of disaggregated pluteus preparations through 50-μm nylon mesh to remove skeletal elements.

Micrococcal nuclease (Worthington or P-L Biochemicals) digestions were in 10 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/1 mM $CaCl_2$/1 mM phenylmethanesulfonyl fluoride at 37°C. Reactions were terminated by addition of EDTA to a final concentration of 25 mM and cooling to 0°C. Core particles were isolated from the solubilized chromatin by using isokinetic sucrose gradient centrifugation on gradients with a meniscus concentration of 5% (wt/wt), containing 0.1 M NaCl (15).

Methods for analysis of histones and DNA by gel electrophoresis (16, 17), 5′-end labeling of core particles (17), thermal denaturation analysis, and analytical ultracentrifugation (13) were exactly as described elsewhere. Rates of DNase I digestion of core particles were determined by (*i*) digestion of 5′-end labeled particles for varying times with the nuclease, (*ii*) isolation and denaturation of DNA, and (*iii*) electrophoresis on denaturing 5% (wt/vol) polyacrylamide gels. After autoradiography, the fraction of the end label present as a 146-base fragment was determined by quantitative densitometry. Whereas cuts at 10 base pair (bp) from the 3′ end of the core particle are not scored, all other fragments are excluded from the undigested fraction, leading to a fairly accurate assessment of the rate of digestion of core particles in terms of the first DNase I cleavage.

RESULTS

Composition of Core Particles. Core particles were isolated from blastula and pluteus stages of *S. purpuratus* embryos. Gel electrophoresis of total DNA from samples at various stages of digestion was utilized to establish conditions producing a maximal yield of core particles. Optimal conditions for excision of core particles differed for the two stages; when digestions were performed for 8–10 min at a DNA concentration of 2 mg/ml, 300 units/ml was optimal for blastula nuclei, whereas 1000 units/ml served best for pluteus. These findings are consistent with the observations of others on rates of micrococcal nuclease digestion of sea urchin nuclei at different stages of embryogenesis (10, 12). Both sets of core particles contained DNA with a sharp cutoff length of 146 bp, as observed for all other core particles examined by ourselves and others (18). Some tailing to longer lengths was present in the particles employed for these studies; about 10% of the DNA for each preparation was longer than core particle length (but less than 170 bp). The length distribution was essentially identical for blastula and pluteus core particles.

Fig. 1 shows the proteins of blastula and pluteus core particles as separated by Triton X-100/acid/urea gel electrophoresis. This solvent system decreased the mobility of H2A markedly and that of H3 slightly, enabling clear resolution of the four inner histones and the embryonic variants (3, 9). Blastula core particles contained > 90% of H2A and H2B as the early proteins (α-subtype). In contrast, in pluteus core particles over 75% of these two histones consisted of the late subtypes, the β, γ, and δ families. H3 and H4 in the core particles at the two stages of development were electrophoretically indistinguishable. Both of these arginine-rich histones were present as acetylated derivatives and as the parent unmodified protein; the degree of modification of H3 and H4 was similar for the two sets of core particles. Quantitation of the amount of histones present in the two sets of core particles by electrophoresis in $NaDodSO_4$ gels was made by comparison of densitometric scans of the sea urchin samples and control samples from chicken erythrocyte core particles. This analysis demonstrated equimolar ratios of the four small histones for each sample. No degradation was detected, and the content of any proteins other than the inner histones was <5% of the total protein present.

Reversible Conformational Changes for Core Particles. Two reversible conformational changes have been described for isolated core particles, and the likely structural alterations in each have been defined (18); they are diagrammed in Fig. 2. Upon lowering ionic strength to less than 100 mM, the core

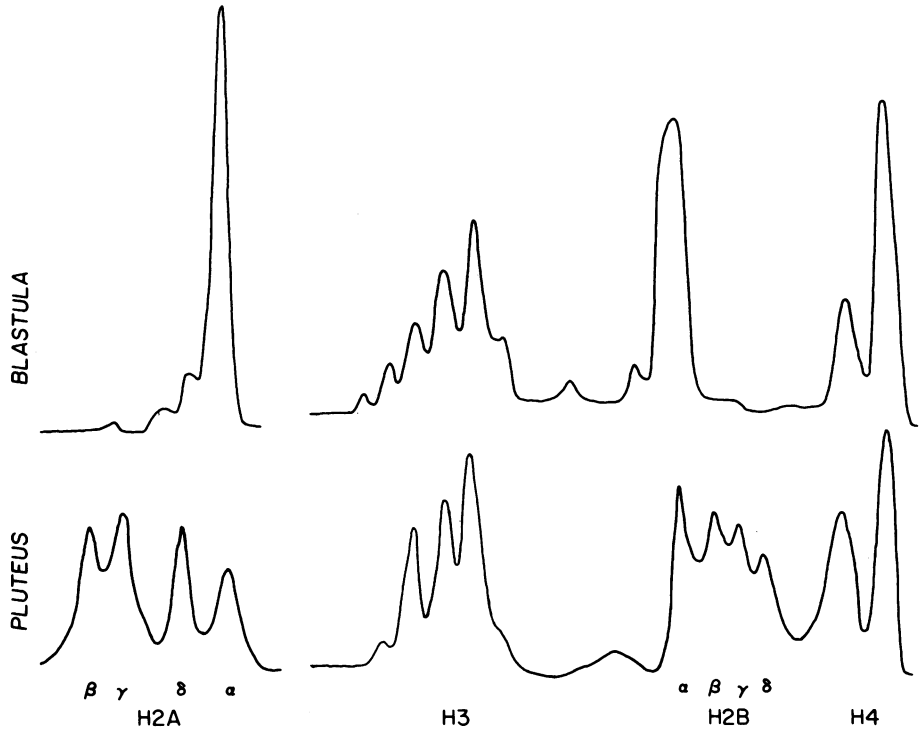

FIG. 1. Histone subtypes in sea urchin hatching blastula and pluteus core particles. Acid-extracted histones from sea urchin core particles were separated on Triton X-100/acid/urea gels (9), stained with Coomassie blue, and scanned. Due to the large separation between H2A and the other three histones, the scan is presented in two segments. Migration was from left to right. Three late putative H2B variants are seen in the pluteus sample and arbitrarily labeled β, γ, and δ, although the equivalence of any of these bands with the two variants described by Cohen *et al.* (3) is unknown.

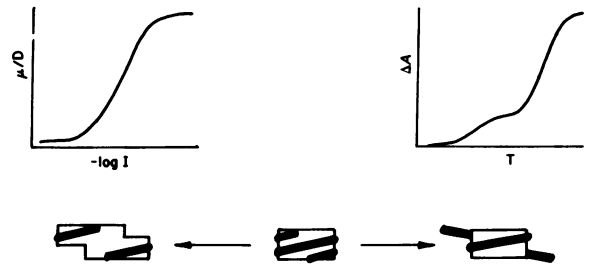

FIG. 2. Diagrammatic representation of two reversible conformational changes for nucleosome core particles. (*Upper Left*) Alteration in electric dichroism (μ/D) as a function of ionic strength (I). (*Upper Right*) Melting profile. *A*, absorbance; T, temperature. (*Lower*) A tightly folded core particle (in the center) is shown unfolding at low ionic strength [as modeled by Wu *et al.* (19)] to the left or unspooling partially in the reversible phase of melting (20, 21) to the right.

particle undergoes a shape change, leading to altered sedimentation, diffusion (22), and electric dichroism (19) properties. This shape change is blocked by formation of histone–histone crosslinks (19, 22, 23), indicating that disruption of the normal protein contacts in the core particle is likely involved in the conformational transition. Based on analysis of electric dichroism data, Wu *et al.* (19) modeled the state of the core particle at low ionic strength as an expanded disc, 5 nm in height and about 15 nm in diameter, surrounded by nearly one full turn of DNA (Fig. 2).

Sedimentation velocity analysis of the low ionic strength expansion for the two sets of embryonic core particles is shown in Fig. 3. Data for the pluteus particle follow very closely the curve determined for sperm core particles (13) which, in turn, agrees closely with the transition for calf thymus core particles (19). In contrast, core particles from blastula stage expanded under the stress of lowered ionic strength at a significantly higher salt concentration (Fig. 3). Thus, although both particles sedimented at 11 S at ionic strength 0.1 M at all lower ionic strengths, the blastula particle was more highly unfolded than the pluteus core particle. Analysis of the curves suggests midpoints for the transition being 1.2 mM and 3.6 mM for pluteus and blastula core particles, respectively.

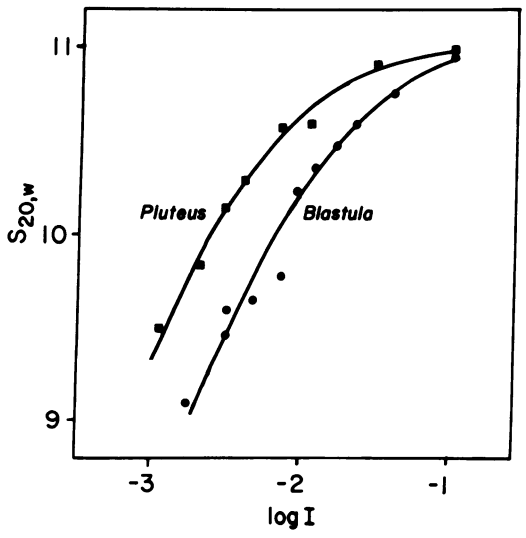

FIG. 3. Expansion of core particles with low ionic strength (I) stress. Analytical ultracentrifugation of blastula and pluteus core particles was performed at the indicated ionic strengths and at a DNA concentration of 40 μg/ml. The solid curve through the pluteus data is that previously determined for sperm core particles (13). $S_{20,w}$, corrected sedimentation coefficient.

The second reversible change in core particle structure occurred on heating. Melting of core particles occurs in (at least) two distinct phases (20). The phase occuring at about 60–65°C is reversible on lowering temperature and derives from melting of about 40 bp of DNA per particle. No changes in protein secondary structure occur during the reversible melting (20). The particle changes shape about 10°C below the melting temperature; spectroscopic measurements indicate that about 40 bp of DNA unspool from their position on the surface of the core particle at the same temperature as this shape change (20, 21, 24). S1 nuclease digestion of particles at the temperature of the first transition suggested that the reversible transition was unspooling and then melting 20–25 bp of DNA at each end of the core particle segment (Fig. 2) (21). The second transition in the melting results from total disruption of protein secondary structure and irreversible denaturation of the DNA (20).

Fig. 4 shows the derivative thermal denaturation profiles for sea urchin blastula and pluteus core particles. The two transitions noted above were present for both particles, and the magnitude of the reversible transition was nearly identical for the two nucleoproteins. The blastula core particle was less stable to thermal disruption of structure than was the canonical core particle (for example, that from chicken erythrocyte). Midpoints for both the reversible and the irreversible transitions were lower in the case of blastula core particles by about 6°C and 4°C, respectively, than for chicken erythrocyte core particles. Melting of the pluteus core particle was similar to that of the blastula particle. The main irreversible transition occurred at an identical temperature for both sets; the temperature for the reversible transition was about 3°C lower for pluteus core particles than for blastula core particles. The differences between sea urchin core particles and the chicken erythrocyte particle did not derive from differences in the melting properties of the DNAs *per se*. In 0.15 M NaCl/0.015 M sodium citrate, pH 7, melting temperatures for protein-free DNA from sea urchin and chicken were 86°C and 86.5°C, respectively (data not shown).

DNase I Cutting Site Frequency in Core Particles. Nuclease digestion of core particles labeled at the 5′ end with ^{32}P, fol-

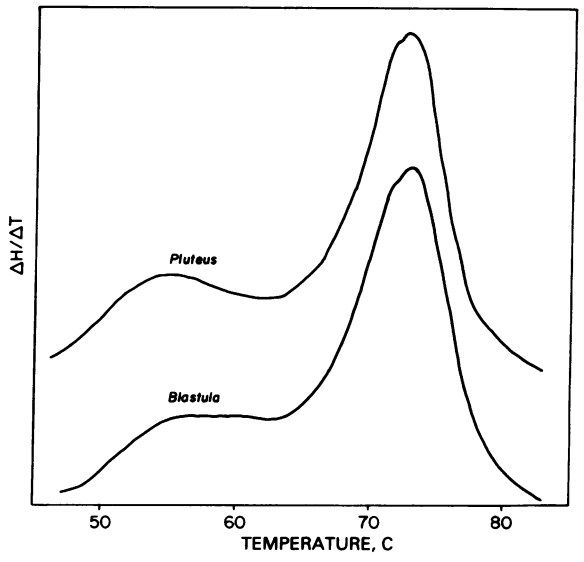

FIG. 4. Thermal denaturation of core particles from sea urchin blastula and pluteus. Melting was carried out in 0.25 mM EDTA (pH 7.0). The data are presented as derivative melting profiles, where the slope of the melting curve has been determined by linear least-square fitting to data over a 2°C range about each experimental point. Thus, the peaks in the derivative melt correspond to the midpoints of transitions observed in the direct melt (as in Fig. 2). ΔH/ΔT, change in hyperchromicity with temperature.

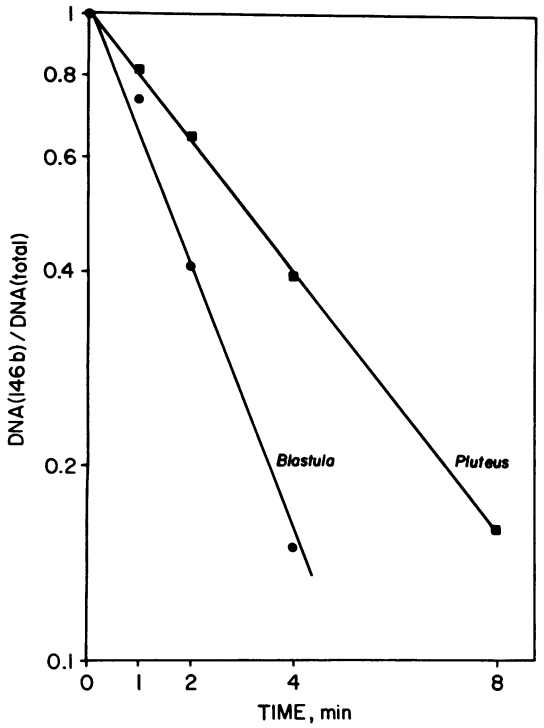

FIG. 5. Rate of digestion of blastula and pluteus core particle DNA by DNase I. 5'-End labeled core particles (0.5 μg for pluteus and 2 μg for blastula, both in the presence of 500 μg of unlabeled carrier chicken erythrocyte core particles) were digested for varying times with DNase I; DNA was isolated, denatured, and electrophoresed on 5% polyacrylamide/7 M urea gels. Quantitative densitometry of an autoradiogram of the gel was used to measure the fraction (146-base DNA/total DNA) of the DNA remaining uncut at each time point.

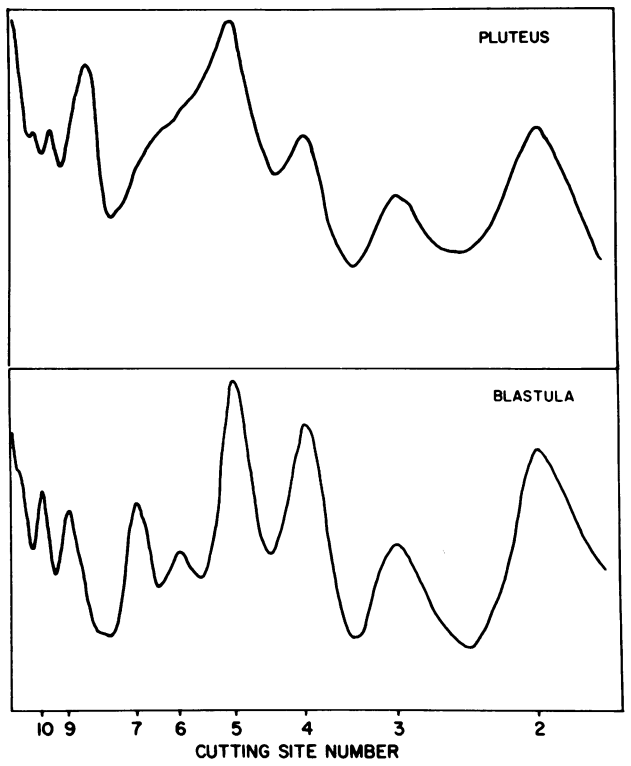

FIG. 6. DNase I cutting sites in blastula and pluteus core particles. Scans of autoradiograms of electrophoretic gels are shown for 5'-end-labeled core particles digested with DNase I for 4 min (blastula) or 8 min (pluteus). Note that the degree of digestion is essentially identical for the two sets of core particles (see Fig. 5).

lowed by isolation and electrophoresis of DNA and autoradiography, allows determination of the distances from the 5' end of the DNA where cutting occurs and estimation of the relative frequency with which certain sites are cleaved (17). Native core particles from various sources yield similar patterns with cutting at sites spaced about 10 bases from the end; sites about 30, 60, 80, and 110 bases from the ends are cut relatively infrequently by DNase I (17, 25, 26). This pattern is altered by different DNA base sequences (27), presence of H1 (28) or high mobility group proteins (29, 30), histone acetylation (31), and by the variant H2A or H2B (or both) of sea urchin sperm (13).

Fig. 5 shows rates of digestion, measured as disappearance of 146-base labeled DNA, of 5'-end labeled core particles from blastula and pluteus core particles. The rate of digestion of the blastula core particle was roughly twice that of the particle from pluteus.

Fig. 6 shows scans of labeled fragments from blastula and pluteus core particles at equivalent stages of digestion by DNase I. In general, they were quite similar to the cutting site maps of core particles from HeLa cells or chicken erythrocytes. However, compared to the erythrocyte, cutting at the sites about 30 and 70 bases from the 5' end was more frequent in the blastula core particle. Additionally, in the pluteus core particle, cutting at 40 and 100 bases from the end was quite reduced compared to the blastula particle, seen by comparison of the relative intensities of bands 4 and 5 or 9 and 10 for the two samples (Fig. 6). The pluteus core particle also had an increased (but heterogeneous) frequency of cutting about 60 bases from the 5' end.

DISCUSSION

Evolutionary conservation of histone sequences is a hallmark of these proteins (32). In adult metazoan organisms, sequences of H3 and H4 are essentially constant, and those of H2A and H2B vary only slightly. Correlated with this conservation is the observation that the core particle length of DNA and its basic wrapping are rigidly conserved structural features of chromatin (18). Further, thermal denaturation profiles, low ionic strength unfolding, and DNase I cutting site maps are nearly, if not exactly, identical for core particles from a variety of sources, such as chicken erythrocyte, calf thymus, HeLa cells, etc. Variant forms of H2A and H2B do exist, however, in the sperm of some animals and during embyrogenesis in a number of species. I have shown that sea urchin sperm histone variants modulate the structure of chromatin, even at the most basic level—the core particle of the nucleosome (13). Here, this observation has been extended to show that the histone switches occurring in early embryogenesis of sea urchins lead to demonstrable differences in core particle structure at different developmental stages, a suggestion initially made by Cohen and coworkers (3, 4). Thus, while both blastula and pluteus core particles share with the canonical nucleosome certain features (146-bp DNA length, an octamer of histones, and compact structure) other static and dynamic features of the embryonic nucleosomes differ from those of the canonical core particle.

Both blastula and pluteus core particles undergo the reversible and irreversible thermal transitions at temperatures below those required for the chicken erythrocyte core particle. These changes are not due to the degree of acetylation of H3 and H4 in the sea urchin particles, because core particles containing hyperacetylated histones (from butyrate treated HeLa cells) have lesser changes in melting properties than those observed currently for sea urchin particles, even though the degree of histone acetylation is greater for the HeLa core particles (31). Some feature of the urchin embryonic histones leads to a facilitation of the unspooling and melting of the ends of core particle DNA, the reversible transition, and total disruption of core

particle structure—the irreversible transition.

In contrast to their similarities in melting properties, the two embryonic core particles differ in unfolding at low ionic strengths. The earlier histones apparently interact with one another in a weaker fashion than the late histones, allowing expansion of the blastula core particle at higher ionic strengths than those required for the particle from pluteus.

Sequences of the early set of histones have been deduced from DNA sequences (33). Compared to calf thymus, sea urchin H2B shows a number of changes in protein sequence. Most of these occur in the amino-terminal third of the protein, long thought to be the primary site of histone–DNA interaction in chromatin. However, several differences are present in the remaining two-thirds of the protein sequence; some of these are not conservative replacements and thus, may, lead to significant changes in histone–histone interactions, thought to be the major role of this segment of the histone sequence (18). Sequence information for the late embryonic histones is not available for comparison with the early proteins.

Comparison of the biology of tissues with different core particle structures offers a clue to plausible functional effects of the histone variants. Consider changes which affect low ionic strength unfolding on the one hand and melting behavior and DNase I susceptibility on the other.

The core particle from sea urchin blastula unfolds at higher ionic strengths than those from pluteus, chicken erythrocyte, sperm, or calf thymus. This structural change requires partial disruption of the histone octamer (19, 22, 23). Biologically, the early sea urchin embryo is distinguished from the other tissues studied by a rapid rate of division; the first seven divisions occur in about 10–12 hr, and thereafter the division rate slows markedly (34). Although histone octamers are thought to remain conserved through replication (35), it is not unreasonable to assume that some degree of disruption of the protein core is required for events at the replication fork in eukaryotes.

Melting transitions and DNase I susceptibility are altered in different directions from properties of the canonical core particle by histone variants in sea urchins. Embryonic histones lead to lowered melting temperatures and increased accessability of some DNase I cutting sites (Figs. 4 and 6); the variants present in sea urchin sperm have the opposite effects (13). Biologic features of embryo, adult tissues, and sperm indicate that they vary in transcriptional activity, high in embryo and nonexistent in sperm, with adult tissues intermediate. Here, it is shown that core particle structure is modulated by the variant forms of inner histones; I suggest that these modulations may affect replication and transcriptional capability and access to the DNA by proteins.

In contrast to a generally assumed constancy of structure of the core particle, we now know of a number of features that alter demonstrably the structure of this most basic component of chromatin. To date, nucleosome structure has been shown to vary from the canonical model due to (*i*) variant forms of H2A and H2B (ref. 13; this work), (*ii*) presence of H1 (28), (*iii*) presence of nonhistone proteins (29, 30), (*iv*) modification of histones after synthesis (31), and (*v*) differing DNA sequences (27). Thus, variations in all components of chromatin have demonstrated structural effects at the level of the core particle. Overall, it is my impression that many types of nucleosomes can and do exist in chromatin; some will differ from others in a static fashion, and some will differ dynamically.

I realize that for no case do we know that the detected structural variations have any significance in terms of chromatin function during replication or transcription. However, given the generally conserved nature of the histones and the correlated conserved structure of the core particle, it is my bias that the presence of histones that differ from the usual (by modification or in sequence) alters chromatin structure in a biologically significant fashion. Changes in inner histones may, in addition, lead to alterations in chromatin structure at other levels that we have not yet examined. Note particularly the suggestion of McGhee *et al.* (36), based on several lines of evidence, that the amino-terminal regions of the histones might be involved in particle–particle interactions leading to stabilization of the next levels of DNA folding in chromatin.

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