Structure of Sea Urchin Sperm Chromatin Core Particle*

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Sea urchin sperm chromatin contains forms of H1, H2A, and H2B which differ from those present in adult tissues. We have delineated some effects of the variant H2A and H2B on chromatin by study of the structure of the core particle from Strongylocentrotus purpuratus sperm. The particle contains 145 base pairs of DNA and equal amounts of the four smaller histones. It sediments at 11 S and has a circular dichroism spectrum similar to that of particles containing more typical histones. The sperm core particle undergoes a shape change at low ionic strength, as observed for chicken erythrocyte particles. In contrast to these similarities, the melting profile of the sperm particle is quite different from that of erythrocyte; both the reversible transition and the irreversible denaturation of the core particle occur at higher temperatures. The sperm core particle is digested by DNase I more slowly than the core particle from chicken erythrocyte. Cutting site maps for the sperm core particle reveal the same basic organization of DNA by these histones; however, certain features of the map differ significantly from that for chicken erythrocyte, demonstrating a modulation of the canonical core particle structure by the unusual histones present in sea urchin sperm.

The core particle of the nucleosome is recognized as the fundamental building block for the first level of organization of nucleoprotein structure in nearly all eukaryotic cells. In a wide variety of tissues from numerous species, the core particle consists of 145 base pairs of DNA wrapped around an octamer of the four smaller histones, two each of H2A, H2B, H3, and H4 (for a review, see Refs. 1–3). With the exception of studies of core particles containing hyperacetylated histone (4), all currently available information suggests the essential constancy of the structure of the complex of the histone octamer and its associated 145 bp of DNA. For the case of hyperacetylated histones, small variations from properties of core particles containing unmodified histones were noted, a decrease in melting temperature of less than 1°C, and alterations in the nuclease susceptibility of the ends of the core particle DNA and the cutting site about 60 bp from the 5' end of the DNA (4).

At the level of chromatin, at least, the properties of portions of the DNA differ from those of the bulk DNA. In a now classic example, transcribed genes are present in nucleosomes (5) but are more rapidly degraded by DNase I than gene segments which are not active in a given tissue (6, 7). The presence of HMG 14 and 17 appears to be necessary for this differential DNase sensitivity of active genes (8). It is not known whether core particle structure per se differs for transcribed versus nontranscribed gene segments; the observation that HMG 14 and 17 restore DNase I sensitivity to chromatin or monomer nucleosomes suggests that other features (besides these proteins) of chromatin structure at this level of organization distinguish functionally distinctive gene segments (9).

In this context, it is of interest to assess the roles played by the various individual histones in packaging and stabilizing DNA in the core particle. Since histone sequences in most higher species have been conserved strongly (10), this too has been difficult. In contrast to this conservation, major variations in lysine-rich histones occur during early embryogenesis in sea urchin. Variant forms of H1, H2A, and H2B associate with DNA at the cleavage stage, others do so during the transition from the 8- to 16-cell embryo to mesenchyme blastula, and these eventually yield to the several adult forms at or before gastrulation (for a review, see Ref. 11). Variant forms of these histones are also present in sea urchin sperm nuclei (12). The H1-like molecule, H1m, is more basic than other H1's; it has nearly equal contents of lysine and arginine (about 20 mole % each) and a ratio of basic to acidic amino acids of nearly 10:1 (13). Strongylocentrotus purpuratus H2A, migrates more rapidly on sodium dodecyl sulfate gel electrophoresis than the adult form; the sequence of the histone for Psammachinus miliaris is known (10). More striking variants are the H2B's of Paracentrotus lividus, compared to calf H2B, these proteins are about 21 residues larger. Many of the additional residues are arginyls located in a highly basic, extended NH2-terminal region (14, 15).

Study of structure of chromatin of sea urchin sperm should detail any structural consequences for core particles derived from the variant H2A, and H2B. In addition, since sperm is a transcriptionally inactive cell, study of its chromatin structure might provide insight into the mechanisms used to preclude transcription of chromosomal DNA. We report here such an investigation of the structure of the nucleosomal core particle from sperm of S. purpuratus.

EXPERIMENTAL PROCEDURES

S. purpuratus were obtained from Pacific Biomarine, Venice, CA, and maintained at 14°C in artificial sea water (16). Spawning was induced by intracoelomic injection of 0.55 M KCl. Sperm were washed by low speed centrifugation in sea water and then in 80 mM NaCl, 20 mM EDTA, and 10 mM Tris/Cl, pH 8.0, and stored as a frozen pellet. Sperm nuclei were prepared as described by Reichline and Wasserman (17). Core particles were prepared by suspending nuclei at a
DNA concentration of 2 mg/ml in 10 mM NaCl, 1 mM CaCl₂, and 10 mM Tris/Cl, pH 8.0, warm to 37°C, and digesting with Staphylococcal nuclease (Worthington) using a concentration of 400 units/ml for 10 min. Digestion was terminated by addition of EDTA to 25 mM and cooling to 0°C. Nuclei were pelleted, washed once in 0.5 M NaCl and 25 mM EDTA, pH 7, and then centrifuged at 20,000 × g for 5 min. Sucrose gradients, isokinetic for a particle with density of 1.51 g/cm³ at 4°C, were prepared with a meniscus concentration of 15% (w/w) sucrose and contained 10 mM Tris/Cl and 1 mM EDTA, pH 8.0 (18). The gradients were overlaid with a shelf equal to the sample volume of 0.9 M NaCl, 4% (w/w) sucrose. Centrifugation in the SW 41 rotor was at 40,000 rpm for 22 hr. gradients were emptied through a flow cell in a Zeiss PM6 spectrophotometer. After dialysis to 0.25 mM EDTA, pH 7, particle preparations were adjusted to contain 0.1 M NaCl to precipitate internally cut nucleosomes, centrifuged, and the soluble material stored at 4°C. Chicken erythrocyte (Pel-Freez) core particles were prepared as previously described (19).

Histones were extracted from nuclei with 0.4 M H₃SO₄ at 4°C, dialyzed to water, and lyophilized. Gel analysis of histones from core particles was made by direct solubilization of the particles in sample buffer. Electrophoretic analysis was as described (20, 21). DNA was purified by precipitation and analyzed as previously described, using 5% polyacrylamide gels for resolution of native, double-stranded DNA and 12% polyacrylamide gels containing 7 M urea for resolution of denatured, single-stranded DNA fragments (22, 23). Sizing standards for 5% gels were an Hae III digest of φX174 RF DNA (Bethesda Research Laboratory).

When necessary, core particles were labeled at the 5' end using γ-³²P]ATP (New England Nuclear) and polynucleotide kinase (Miles). Observed velocities, analyzed as least square regression lines, the kinetics of digestion of total core particle DNA by DNase I, the rates of digestion and mapping of cutting site susceptibility were done as previously described (19, 23). For determination of the kinetics of digestion of total core particle DNA by DNase I, aliquots of a digestion mixture were precipitated at 0°C in HCIO₄, and the coefficient. Data were corrected to 0.2°C digestion mixture was terminated by addition of EDTA to 10 mM ATP (New England Nuclear) and polynucleotide kinase (Miles) and the chromatins of core particles was measured in #H1-like histones. While the early points in a digestion of sperm nuclei by nuclease yield a monomer DNA length of about 200 bp (mean size), with time, the size of the major portion of DNA in the phonomer band becomes about 145 bp (Fig. 2), equivalent to the DNA size in the core particle from other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues. Identity of core particle DNA lengths in sperm and other tissues.
contrast to nuclei from other cells we have studied, where addition of, or dialysis against, EDTA suffices to release core particles and their multimers after staphylococcal nuclease digestion, only non-sedimenting DNA and no histones are released from sea urchin sperm nuclei by such treatments. Addition of β-mercaptoethanol, mechanical homogenization, and even sonication did not effect nuclear lysis and release of core particles. Apparently H1, variant smaller histones, or non-histones maintain the integrity of the sperm nucleus even after extensive DNA digestion. To minimize the length of time, samples are exposed to the salt concentrations necessary for H1, dissociation, and to enhance resolution on the gradients, we have utilized a discontinuous gradient centrifugation method. Samples after digestion were lysed in 0.9 M NaCl, dissociating H1, but almost no other protein (in contrast to the results of others (28)). The sample is applied to a sucrose gradient at low ionic strength which is overlaid by a shelf of 0.9 M NaCl, 4% sucrose. The shelf allows chromatin particles to sediment away from H1, before entering the lower ionic strength of the gradient, where random association of these two species would be expected to occur. Resolution of such gradients is equal to that of conventional gradients used for preparation of more easily isolated core particles (Fig. 3). The core particles contain DNA of length about 145 bp, with some tailing to longer sizes, equal amounts of the four smaller sperm histones, and no H1, (Fig. 1). The circular dichroism spectrum of the sperm core particle in 0.1 M NaCl is closely similar to that of chicken erythrocyte core particles, with a maximum ellipticity at 282 nm of 1800 ± 150° cm²/dmol of phosphate. The sperm core particle is homogeneous on analytical ultracentrifugation and has a sedimentation coefficient of 11.0 S at ionic strength 0.1.

In order to assess the role of the variant histones in core particle stabilization, we have studied two conformational transitions for the sperm core particle. Both conformational changes, induced by lowering ionic strength or by heating, are reversible for other core particles and have been suggested as potential candidates for conformational changes which might accompany chromatin transcription (29-32).

As ionic strength is lowered to less than 30 mM, the core particles of chicken erythrocyte or calf thymus undergo a reversible conformational change, unfolding into a less compact structure. Gordon et al. found the unfolding to occur in two very sharp steps, centered at 7.5 and 1 mM, when studied by diffusion and sedimentation methods (29). In contrast, Wu et al. found a single transition, centered at 1.3 mM, using transient electric dichroism as an experimental method (30). Core particles cross-linked with formaldehyde (29) or dimethyl suberimidate (30) did not expand under the stress of lowered ionic strength, suggesting that partial disruption of the quaternary structure of the histone octamer was necessary for the conformational transition. Wu et al. fit their data for the core particle at low ionic strength with a model particle which is a 178-Å diameter disk, 60 Å high, with about one turn of DNA wrapped around the histones (30).

We have studied this conformational change in sperm core particles, using sedimentation velocity measurements to indicate changes in the particle shape. As ionic strength is lowered from 100 mM to less than 1 mM, the sedimentation coefficient of the sperm core particle decreases from 11.0 to 9.5 S (Fig. 4). The transition is smooth, with no indication of the abrupt transitions noted by Gordon et al. (29); rather the data resemble the transition observed by Wu et al. (30) by electric dichroism. Our data are fit well by a noncooperative transition from a particle with sedimentation coefficient of 11.0 S at higher ionic strength to one with sedimentation coefficient of 8.2 S at very low ionic strength, centered at an ionic strength of 1.2 mM. The exact nature of the core particle at very low ionic strength is not known; whatever it is in detail, the present data clearly show that the presence of variant forms of H2A and H2B in sperm core particles versus chicken or calf does not preclude the conformational change.

A second type of reversible conformational change occurs for core particles on heating. Weschet et al. showed that about 30% of the total hyperchromicity developed on denaturation of core particles occurred as a separate transition centered at about 60°C in 1 mM sodium cacodylate. The remainder of the DNA denatured at 75-76°C, concurrent with

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**Fig. 2. Digestion of S. purpuratus sperm nuclei with staphylococcal nuclease.** Sperm nuclei, prepared as described under "Experimental Procedures," were digested at 37°C with staphylococcal nuclease for the indicated product of time (min) × enzyme concentration (units/ml). DNA was isolated and electrophoresed on a 5% polyacrylamide gel.

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**Fig. 3. Sucrose gradient fractionation of a staphylococcal nuclease digest of sperm nuclei.** Sperm nuclei were digested with 400 units/ml of nuclease for 10 min at 37°C, lysed with 0.9 M NaCl, and centrifuged on the gradients described under "Experimental Procedures" for 22 h at 40,000 rpm and 5°C. Sedimentation is from right to left.
Sperm Core Particle

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The second and third, at temperatures of 68.5°C and 73°C, respectively, are due to melting of about 10% and 20% of the DNA. While their sum is similar in magnitude to the premelting transition observed for the erythrocyte core particle, the sperm transitions are displaced to temperatures about 10°C higher, closer to the temperature at which the entire structure of the core particle is disrupted.

We have investigated the effects of the variant H2A, and H2B, on this conformational change by melting chicken erythrocyte and S. purpuratus sperm core particles in 1 mM sodium cacodylate, pH 7.1 (Fig. 5). The thermal denaturation profile for the erythrocyte particle is quite similar to that previously reported by Weischet et al. (31). A broad transition, equivalent to melting of about 40 to 45 bp of core particles DNA, is centered at about 62°C. The remaining melt occurs at about 76°C; the magnitude of this phase is 70% of the total hyperchromicity, consistent with melting of 100 bp of core particle DNA. Comparison of this melting profile with that for sperm core particles (Fig. 5) reveals two major differences. First, the major melting temperature for the sperm core particle is about 2°C higher than that for the erythrocyte particle, being 78.5°C. Second, the reversible, first transition is either absent or shifted to higher temperatures for the sperm core particle. Thus, a significant portion of the total melt for the erythrocyte core particle has occurred at 60°C, while no increase in absorbance is observed at that temperature for the sperm core particle.

We have further analyzed the melting data for the sperm core particle by computing a derivative melting curve (Fig. 6) from the data shown in Fig. 5. The derivative curve is mostly simply resolved as the sum of four separate transitions (Table II). The first is small, occurring at 63.4°C and of a magnitude corresponding to melting of less than 2 bp of DNA per core particle. We assume that this is due to short tails of DNA on some of the core particles in our sample and will ignore it in further analysis. The second and third, at temperatures of 68.5°C and 73°C, respectively, are due to melting of about 10% and 20% of the DNA. While their sum is similar in magnitude to the premelting transition observed for the erythrocyte core particle, the sperm transitions are displaced to temperatures about 10°C higher, closer to the temperature at which the entire structure of the core particle is disrupted.

**Table II**

<table>
<thead>
<tr>
<th>Transition</th>
<th>Melting temperature</th>
<th>Fraction of total</th>
<th>Base pairs/core particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>68.5</td>
<td>0.10</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td>73.0</td>
<td>0.19</td>
<td>28</td>
</tr>
<tr>
<td>III</td>
<td>78.5</td>
<td>0.69</td>
<td>100</td>
</tr>
</tbody>
</table>

* Calculated assuming that the fraction of the total melting reflects denaturation of a given portion of the 145-bp core particle DNA in a set of totally homogeneous core particles.
The highest temperature transition occurs at 78.5°C, somewhat higher than that for the erythrocyte particle. As in the case of the erythrocyte core particle, this transition for sperm includes about 70% of the total hyperchromicity. It seems likely that this highest temperature transition reflects disruption of the structure of the histone octamer and melting of the 100 bp of DNA in the center of the core particle segment. A higher temperature for the melt in sperm versus erythrocyte signals greater stability of either histone-histone or histone-DNA interactions in the sperm core particle; we would expect histone-histone interactions to be similar in the two particles due to the closely similar protein sequences for the two species in the regions of the histones thought to be involved in protein-protein interaction (10).

Interpretation of the second and third phases of the sperm core particle melt is more difficult. Tentatively, we would suggest that these transitions reflect the same process that occurs in the premelt of other core particles, namely denaturation of about 20 to 25 bp of DNA at each end of the core particle. In the sperm core particle, the much more highly basic nature of the H2B, might lead to a greater stabilization of these DNA regions; more energy is required to disrupt their interactions with the histone octamer. Part of the attractiveness of this hypothesis arises from the presence in *S. purpuratus* of two forms of H2B, (Fig. 1). These are present in about a 2:1 ratio of the larger or more basic form to the smaller or slightly less basic form. This is the same ratio as the two phases of the premelting transition for the sperm core particle (Fig. 6, Table II). The two transitions at 68.5°C and 73°C for the sperm particle might result from two types of core particle end segment interactions with the two types of H2B, leading to different stabilizations of the end regions of core particle DNA.

Overall, the added stabilization of core particle structure by the variant histones in the sperm core particle is quite significant. It is very nearly equivalent in both melting temperatures and profiles to a 10-fold increase in ionic strength for the erythrocyte particle (cf. Fig. 6 with Fig. 5 of Ref. 31).

The two types of investigation of core particle structure detailed above are physical in nature and dynamic in terms of conformational alterations of core particle structure. The third type of study of the sperm core particle we describe differs in kind from the previous two. Here, we address the static interactions of histones and DNA by mapping the locations and relative susceptibilities of cutting sites for DNase I in the nucleosome core particle. Fig. 7 compares the autoradiograms resulting from such a mapping experiment for sperm and erythrocyte core particles. Fig. 8 is a scan of the autoradiograms at 2-min digestion time. General features of the cutting site susceptibilities for typical core particles previously reported (4, 19, 23, 33–38) are reproduced here for the erythrocyte particle. There is low frequency of cutting at sites about 30, 60, 80, and 110 bases from the 5’ end of the DNA and a general asymmetry in the cutting pattern (for convenience, we will denote distances from the 5’ end as multiples of 10; other studies have shown that the actual average number is nearer 10.4 and that there is an apparent variation in cutting site spacing in two domains of the core particle (34, 37, 38)). The map for the sperm core particle also shows cutting at sites spaced at multiples of about 10 bases from the 5’ end of the DNA. Again, low frequency of cutting is observed for the sites 30, 80, and 110 bases from the end. The site in the middle of the core particle, 70 bases from the 5’ end, which is moderately frequently cut in other core particles, is similar in its rate of cleavage in the sperm core particle. The sites 20 and 40 bases from the end, high frequency cutting sites in other core particles, are relatively infrequently cut in the sperm core particle, about as infrequently as the site 30 bases from the end (Fig. 8). The basic wrapping of DNA in the core particle, leading to DNase I nicking sites at 10 base intervals along the strands, appears to be common for sperm and other adult tissues previously studied. However, the details of the DNase I cutting site availability within the core particle suggest that the presence of H2A, and/or H2B, in the sperm core particle leads to a modulation of this structure compared to core particles from other tissues.

Comparison of the autoradiograms for sperm and erythrocyte core particles (Fig. 7) makes apparent the fact that the rate of disappearance of the 145-base DNA is slower and that there is less total radioactivity in the resolved bands for sperm than erythrocyte at any of the time points in the digestion. This indicates a general decreased susceptibility to the nuclease of the sperm core particle versus the erythrocyte core particle, but might also indicate an increased frequency of cutting at the site 10 bases from the 5’ end; fragments of this size are only marginally insoluble on ethanol precipitation and hence are underrepresented on electrophoresis of extracted samples. To examine this question, we have measured cutting rates at the site 10 bases from the 5’ end of core particle DNA for sperm and erythrocyte particles by determining the rate of solubilization of the label after digestion with DNase I and precipitation with perchloric acid/sodium chloride. Fig. 9 shows the results of the experiment. Initially, hydrolysis of the end label to acid solubility occurs about 2-fold more slowly for the sperm particle than for that from erythrocyte. Later in the digestion, rates become more closely similar (not shown). Thus, in addition to specific sites within the central region of the core particle being less susceptible to hydrolysis by DNase I for sperm versus erythrocyte, the site 10 bases from the end of nucleosomal DNA is also less accessible to this nuclease.
Sperm Core Particle

FIG. 8. DNase I map of cutting sites in S. purpuratus sperm and chicken erythrocyte core particles. Densitometric scans of the 2-min digested autoradiograms in Fig. 7 are displayed. The ordinate is linear with optical density.

FIG. 9 (left). Kinetics of DNase I cutting at the site 10 bases from the ends of core particles. 5'-3*P-Labeled core particles from chicken erythrocyte (---) and S. purpuratus sperm (---) were digested with 200 units/ml of DNase I for the indicated times and the fraction of the radiolabel made soluble in 5% CCl4COOH determined.

FIG. 10 (right). Kinetics of DNase I digestion of total DNA of core particles. Core particles from chicken erythrocyte (---) or S. purpuratus sperm (---) were digested for the indicated times with 200 units/ml of DNase I and the fraction of the total A260 made soluble in 0.5 M HClO4, 0.5 M NaCl determined.

Since H3 and H4 are essentially identical in sequence for the two types of particles, we assume that these differences result from differences in histones H2A and/or H2B between the two types of core particles.

Fig. 10 details the rates of digestion of bulk sperm and erythrocyte core particle DNA to acid solubility. At early phases of digestion, the sperm particle total DNA is solubilized about 3- to 4-fold slower than that from erythrocyte. This is expected in view of the decreased frequency of cutting at the site 10 bases from the end (Fig. 9), a cut which leads alone to acid-soluble DNA, and the decreased frequency of cutting at the 20- and 40-base sites (Fig. 7 and 8), cuts which lead to acid-soluble DNA when combined with cuts at the highly susceptible sites 10 or 50 bases from the end. Together, these alterations in cutting may explain the DNase I resistance of total DNA in the sperm core particle.

FIG. 11 (right). Kinetics of DNase I digestion of total DNA of core particles. Core particles from chicken erythrocyte (---) or S. purpuratus sperm (---) were digested for the indicated times with 200 units/ml of DNase I and the fraction of the total A260 made soluble in 0.5 M HClO4, 0.5 M NaCl determined.

DISCUSSION

In attempts to address the role played by different inner histones in organization of the structure of the core particle, most studies have involved reassembly of nucleoproteins containing partial complements of the histones, followed by analysis by physical or chemical means (26,27,39,40). While the information obtained in such studies must eventually be accommodated by models for nucleosome structure, they suffer from the obviously artificial nature of the complex under scrutiny. Study of the core particle from sea urchin sperm has advantages in assessing features of the role of H2A and H2B in nucleosome structure; the particle contains a full complement of the inner histones, bas H3 and H4 which differ from their counterparts in chicken erythrocyte by only a single amino acid residue each (41,42), and yet contains H2A and H2B which differ from their peers in adult urchin tissues or other species.

Since H3 + H4 are thought to form the overall structural framework for the nucleosome (26,27,39,40), it is not surprising that a number of features of the sperm core particle are similar to those of other tissues, specifically chicken erythrocyte. Superimposed on this framework of similarity with other core particles, some features of the structure of sperm core particles apparently are modulated by the presence in the histone octamer of the variant histones H2A and H2B. One of the most striking differences is the rate of digestion by DNase I of sperm core particles relative to that for erythrocyte particles. The initial rate of digestion of the sperm particle

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appears to be nearly 4-fold slower than that for erythrocyte. Digestion of core particle DNA to acid solubility by this nuclease requires nicking at two adjacent sites 10 bases apart, except at the site 10 bases from the ends. The site at 10 bases is one of the most frequently cut in studies of detailed susceptibilities of core particle DNA to DNase I (35, 36). The rate of digestion of end-labeled sperm particles by DNase I is 1.5 to 2 times less than the rate for erythrocyte particles. Hence, the regions near the ends of the core particle DNA are less accessible to the nuclease in the sperm particle than in the erythrocyte nucleosome. Since the rate differences are not the same for end label and total DNA, other sites within the core particle must also be less readily digested by this nuclease in the case of the sperm nucleosome. Inspection of the cutting site map (Figs. 7 and 8) confirms this supposition. Except for central region, 60 to 70 bp from the 5' end, all sites in the sperm particle are cut at lower frequency than in other core particles. Particularly, the sites 20 and 40 bp from the ends, highly accessible in most core particles, are much less frequently cut in the sperm particle. This is readily seen by comparison of the relative intensities of bands 2, 4, and 5 for sperm and erythrocyte; they are nearly equal in intensity for erythrocyte and differ markedly for sperm. While it is tempting to equate directly these differences in cutting site frequency with the differences in H2A and H2B for the two types of particles, the effects in the sperm particle are so global that direct interaction of the histone variants with the affected cutting sites may be a naive interpretation. A more general alteration in histone-DNA interactions or unknown features of nucleosome structure may underlie the observed differences. The differences in cutting site frequencies for sperm versus erythrocyte apparently do not arise from differences in the DNA for the two preparations, since maps obtained for complexes of these two sets of inner histones with poly(dA-dT)·poly(dA-dT) are closely similar to those for the native particles.  

Whatever the distinctive features of the sperm core particle that lead to differences in DNase I digestion from other core particles, they likely are due to the NH2-terminal regions of H2A, and H2B. For the known sea urchin sperm histone sequences the NH2-terminal regions differ from the corresponding calf histones; the COOH-terminal two-thirds of the molecules are highly conserved (10, 14, 15). Since these COOH-terminal segments are thought to be involved in the interactions which lead to the proper quaternary structure of the histone octamer (10), one expects the protein structure of the globular portion of the nucleosome core to be similar for sperm and other nucleosomes. Support for this expectation derives from the expansion of the sperm core particle at low ionic strength. Similar histone-histone interactions in the sperm and erythrocyte core particles may allow them to expand in similar fashion at low ionic strengths.

In contrast to the above conformational alteration in core particle structure, a second conformational transition for the nucleosome is markedly dissimilar for sperm and erythrocyte core particles. A reversible melting of 30% of the DNA occurs in heating core particles; at ionic strength about 1 M, sufficient to disrupt the structure of the entire core particle. This two portions of the first unwinding transition might then correspond to two types of stabilization of these end segments by the two different H2B, species. Certainly, the increased stability of histone-DNA interactions near the end regions of the core particle in sperm (versus erythrocyte) detected by melting analysis is very consistent with the differences in DNase I digestion rates of this region for the two particle types (Fig. 9).

There are two interesting parallels between certain properties of the sperm core particle and the chromatosome, a particle containing about 160 bp of DNA and, in addition to the inner histone octamer, a molecule of H1 or H5 (19). Both are more slowly degraded by DNase I than erythrocyte core particles. The chromatosome appears to totally lack the first transition conformational change on heating (19); in the sperm core particle, stable histone-DNA interactions lead to a significant increase in the energy required to disrupt the association of the ends of core particle DNA with the histone octamer. We have previously described features of a plausible mechanism for transcription of DNA bound to histones (19, 32, 44); one essential element of the proposal was unwinding of the ends of core particle DNA from the octamer to allow the beginning of transcription into a nucleosomal DNA segment. Such unwinding is apparently precluded in the chromatosome by the presence of H1 or H5; evidence suggesting that chromatin containing H1 may be transcriptionally inactive has been summarized previously (19). We now find that the unwinding conformational transition is much less easily achieved in sperm core particles than in others; sperm is essentially inactive as a template for RNA polymerase (45). While only correlutive, the data demonstrate two ways through which the unwinding conformational transition can be blocked or inhibited and suggest further studies which might elucidate the role of this change in core particle structure in the transcription of chromatin DNA.

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Sperm Core Particle