Electron Microscope Evidence for the Presence of Globular Structures in Different Sperm Chromatins

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ABSTRACT Dispersion of nuclear fibers of the spermatozoa of dogfish, man, and bull is made possible after treatment with a reducing and alkylating reagent coupled with an anionic detergent; the same detergent used at a low ionic strength dissociates the nuclear content of the rainbow trout sperm. Electron microscopy of such dispersed nuclear fibers has shown a beads-on-a-string configuration for these four types of sperm chromatin. These structures are morphologically similar to those described in somatic cell nuclei as nucleosomes, although in sperm chromatin the basic proteins associated with DNA were significantly different from histones.

The nucleus of the vertebrate spermatozoon is characterized by dense, tightly packed chromatin, highly resistant to disruption (4, 15, 16). This condensation of the nuclear content occurs during spermiogenesis and is the result of various biochemical changes in the basic proteins associated with DNA in chromatin. In vertebrate sperm, somatic histones are generally replaced by other typical, small, basic proteins whose nature and properties often differ from species to species (5, 6). These new molecules interact with DNA and are responsible for the high degree of condensation of sperm nuclei. In several cases the amino acid composition of these proteins is known and for some of them the sequence has been determined (1, 2, 5, 6).

On the other hand, works concerning the fine structure of sperm chromatin nucleofilaments are rather sparse and contradictory. Recently, we have shown the persistence of globular structures during all stages of spermiogenesis in the dogfish (20). In the present paper we have extended our investigations on three other types of spermatozoa whose chromatin contains basic nuclear proteins already known: bull (12), man (8, 18, 32, 36) and rainbow trout (1). A beads-on-a-string configuration was regularly found in the four species examined by electron microscopy.

MATERIALS AND METHODS

Preparation of Trout Sperm Chromatin

Small pieces of mature testis of rainbow trout (*Salmo irideus*) containing only spermatozoa were homogenized in a medium containing 150 mM KCl, 20 mM Tris-HCl buffer, pH 7.5 (medium I). After filtration through six layers of surgical gauze, this homogenate was centrifuged at 1,800 *g* for 10 min. The pellet was washed three times in the same medium. Then, it was resuspended in medium I containing 4% Tween (Atlas Powder Co., Wilmington, Del.) (vol/vol) and left for 30 min at 4°C. After two washings in medium I, sperm heads were suspended in medium I containing 2 M sucrose and centrifuged at 48,000 *g* for 1 h. The nuclei were washed three times in a solution consisting of 1 mM Tris, pH 7.5, 0.2 mM EDTA, and lysed by addition of 10 μl/ml of 15 % Sarkosyl (Geigy Industrial Chemicals, Div. Geigy Chemical Corp., New York, N. Y.) After 10 min, the lysate was centrifuged for 5 min at 500 *g* and the supernate was recovered and dialyzed against 1 mM Tris, pH 7.5, 0.2 mM EDTA.

Preparation of Bull, Human, and Dogfish Sperm Chromatin

Sperm samples after three washings in medium I were treated with 4% Tween as described above. Sperm heads were separated from middle pieces and flagella by sonication (Measuring and Scientific Equipment Ltd., London, England; 3 × 5 s at maximal power). Nuclei and chromatin were prepared as described before (20).

For the preparation of chromatin from the dogfish sperm depleted of its acid-soluble fraction (Z3), purified nuclei were treated overnight with 0.25 N HCl. After centrifugation at 1,800 *g* for 10 min, the nuclear pellet was washed in a medium containing 60 mM Tris-HCl, pH 8.8, and 2 mM EDTA; chromatin was then prepared as described before (14, 20).

Control of the Protein Composition of the Chromatin Preparations

1 N HCl was added to aliquots of the different sperm chromatins to give a final concentration of 0.25 N. After a 1-h extraction, the suspension was centrifuged at 1,800 *g* for 30 min. Acid-soluble proteins were precipitated by addition of trichloroacetic acid to a final concentration of 20%. After centrifugation, the pellet was washed with acidified acetone and acetone and dried under vacuum. Electrophoresis of nuclear proteins was performed in 17% polyacrylamide gels according to Panyim and Chalkley (31).

The protein content of chromatin samples was estimated according to Lowry et al. (26), using salmon protamine (Sigma Chemical Co., St. Louis, Mo.) as standard; DNA was detected with the diphenylamine method of Burton (7).

RESULTS AND DISCUSSION

In contrast to the conservative structure of histones associated with DNA in somatic cells, the basic proteins present in sperm

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chromatin show great diversity during evolution (1, 2, 4–6, 8). The compact structure of sperm heads is the result of DNA-protamine interactions that subsequently give rise to very strong cross-linking of nucleoproteins.

To study the fine structure of nucleofilaments in sperm chromatin, the solubilization of the chromatin requires the use of an anionic detergent or a reducing reagent coupled with the same detergent when disulfide cross-links are present in nucleoproteins (4, 15, 20, 37).

Under these conditions we have shown, for the first time, the persistence of globular structures in sperm chromatin of the dogfish (20). In this case, three basic protein fractions are

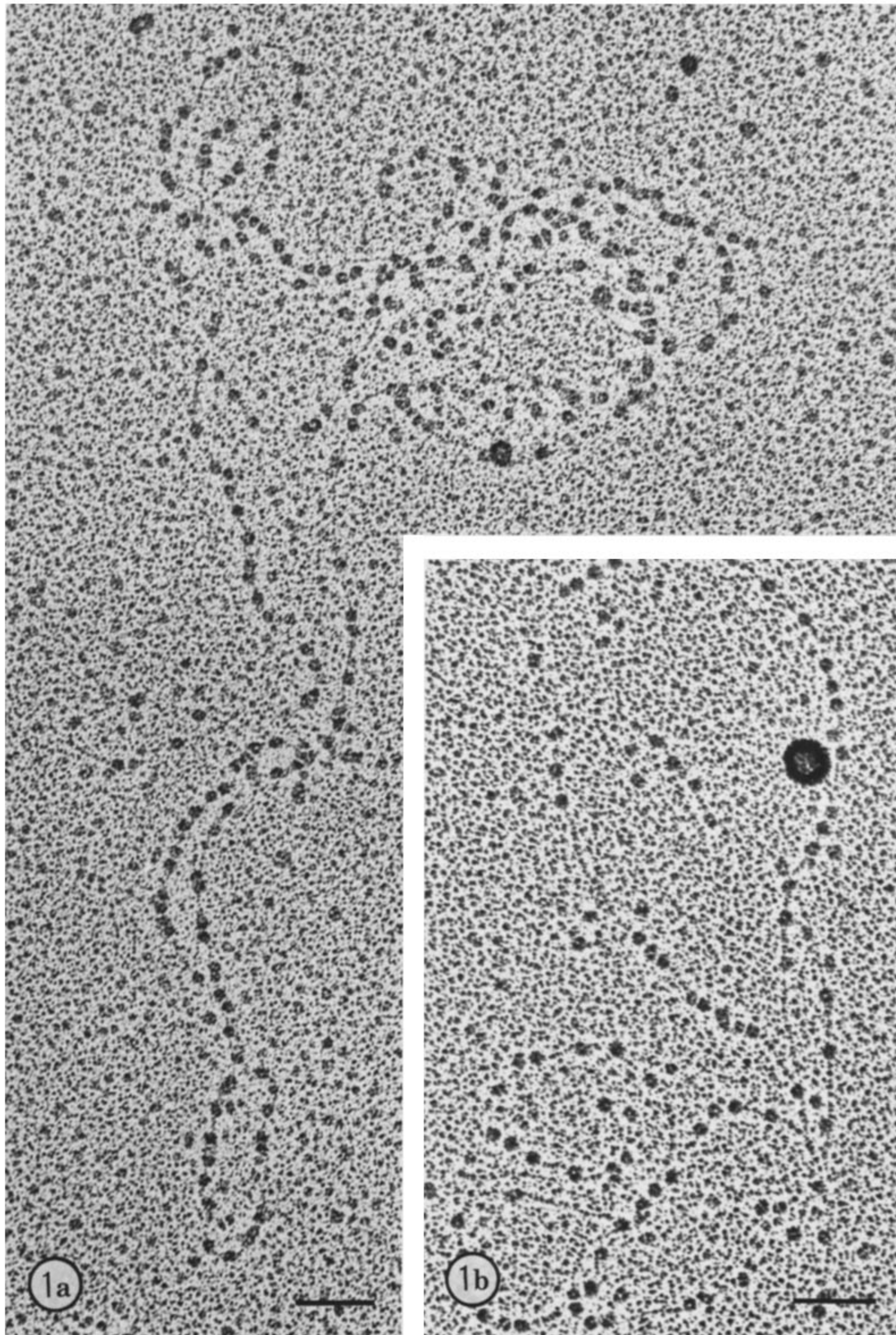

FIGURE 1 Structure of dogfish sperm chromatin: (*a*) chromatin depleted of the acid-soluble fraction by 0.25 N HCl; (*b*) total sperm chromatin. The samples for electron microscopy were processed as described in Material and Methods. Bar, 0.1 μm. × 120,000.

present in the sperm nucleus (19). One of these fractions is acid-soluble in 0.25 N HCl. The nucleosome-like structure in dispersed nucleofilaments is seen both in the presence of the three protein fractions (Figs. 1 *b* and 3 *a*) and when the acid-soluble fraction has been removed with 0.25 N HCl (Figs. 1 *a* and 3 *b* and *c*).

Intact chromatin showed a protein:DNA ratio of 3.09 (average of three preparations). In chromatin depleted of its acid-soluble fraction, this ratio was found to be 2.35 (average of three preparations).

Structure of Nucleofilaments in Bull and Human Sperm Chromatin

In these species, as in the case of the dogfish, the disruption and solubilization of sperm chromatin is possible only after disulfide bond reduction, an alkylation step (12.5 mM iodoacetamid), and use of Sarkosyl detergent. The necessity of using such reducing and alkylating reagents agrees well with the presence of cystine-rich protein fractions in sperm chromatin (4, 8, 12, 15, 18, 32). Under these conditions, electron micros-

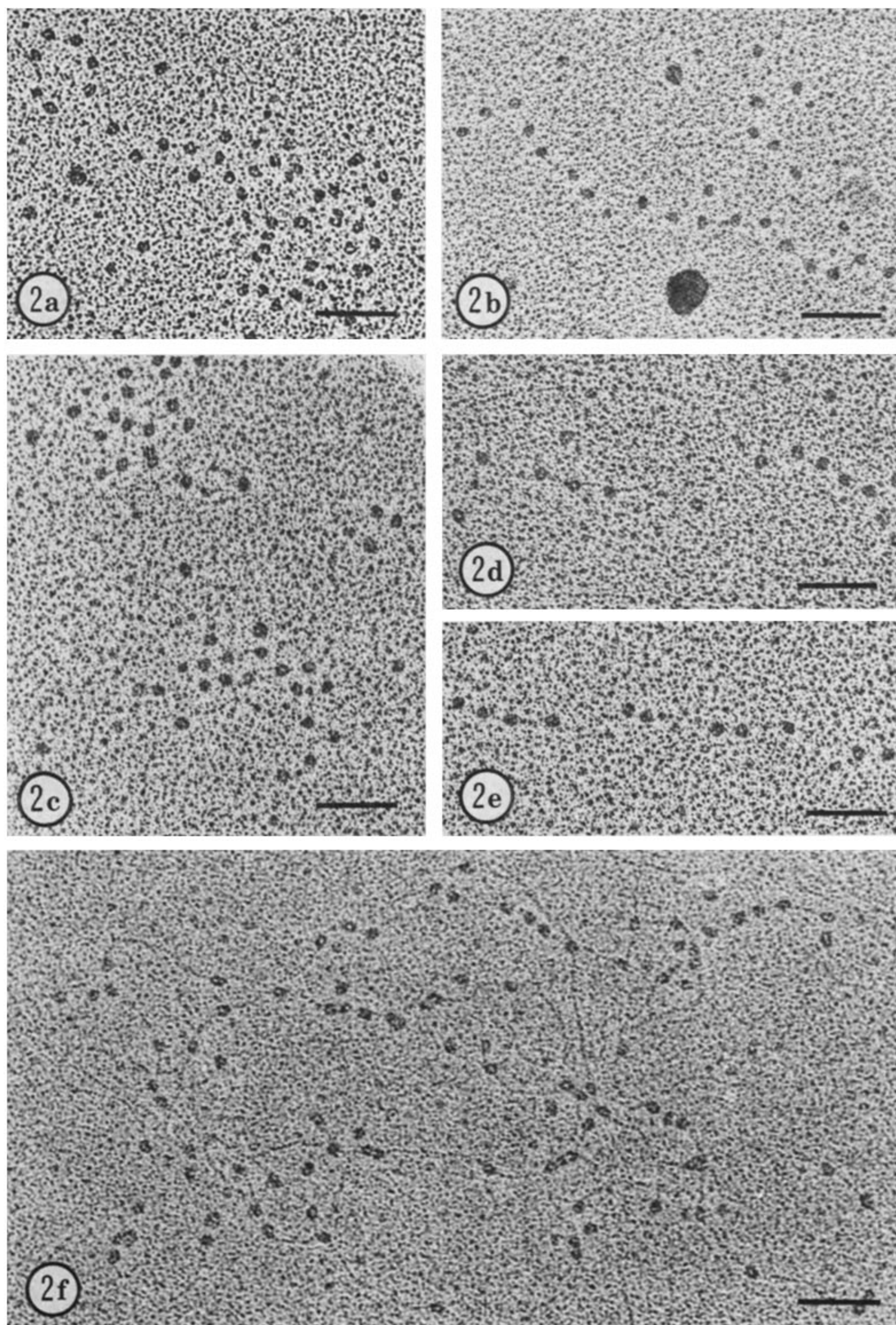

FIGURE 2 Structure of human (*a* and *b*), bull (*c*, *d*, and *e*) and trout (*f*) sperm chromatin. Bar, 0.1 μm. × 120,000.

copy has revealed the presence of globular nucleosome-like structures in the nucleofilament, which are regularly deposited along the DNA molecules. The mean diameter of these particles is regular and corresponds to 126 ± 8.9 Å and 147 ± 10 Å in human and bull sperm chromatin, respectively (Fig. 2*a–e* and Table I).

Samples of bull and human sperm chromatin were prepared as for their visualization at the electron microscope level and then extracted by 0.25 N HCl. The presence of protamines was verified by polyacrylamide gel electrophoresis (Fig. 4*b* and *c*).

Structure of Trout Sperm Chromatin

The nuclear sperm protein fraction was characterized by the presence of iridine (3 fractions) as determined by Ando and Watanabe (1) and corresponding to a typical arginine-rich protamine that is devoid of cysteine.

In this species, low ionic strength and an anionic detergent were found to be sufficient for solubilization of chromatin.

TABLE I

Dimensions of Chromatin Subunits from Various Sperm Nuclei

Type of sperm chromatin	Mean diameter of globular subunits after platinum shadowing
	Å
Rainbow trout	128.3 ± 7.9
Man	126 ± 8.9
Bull	146.7 ± 10
Dogfish	
Total sperm nuclei	140 ± 7.9
Sperm nuclei after extraction by 0.25 N HCl	139 ± 7.2

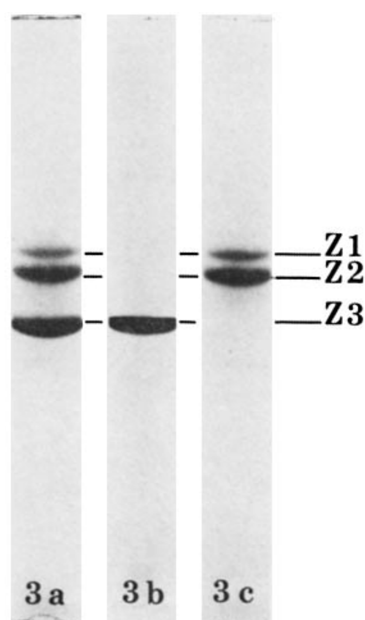

FIGURE 3 Electrophoresis pattern of dogfish nuclear proteins extracted from samples of dispersed sperm chromatin. Three protein fractions (*Z1*, *Z2*, and *Z3*) were obtained from total sperm chromatin (*a*), two from chromatin depleted of its acid-soluble fraction (*c*); (*b*) Z3 fraction obtained after extraction of nuclei with 0.25 N HCl.

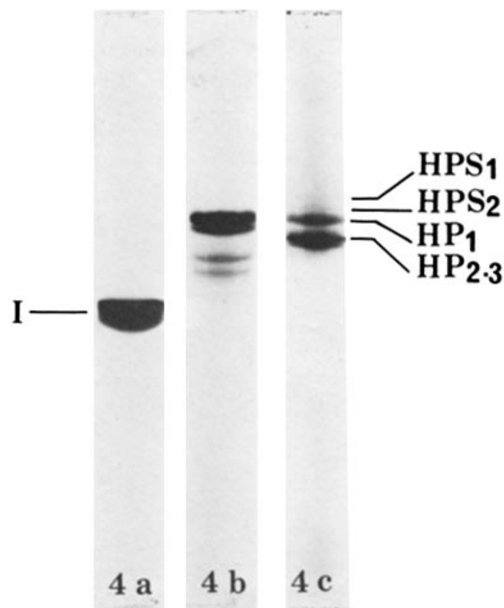

FIGURE 4 Protein pattern in aliquots of sperm chromatin samples of trout (*a*), bull (*b*), and man (*c*). (*a*) *I* corresponds to the typical protamine of *Salmo irideus* sperm chromatin. (*b*) The major band corresponds to the cystein-rich protamine of bull sperm nuclei. The electrophoretic pattern obtained looks like those obtained by Marushige and Marushige (28) and shows a major band and additional ones that correspond very probably to some proteolysis of the protamine-like fraction in the presence of a reducing reagent (11, 28, 38). (*c*) The five fractions of the human sperm chromatin HPS_1, HPS_2, HP_1, HP_2, and HP_3 (36) are present in the control.

Under these conditions the dispersed fibers of deoxyribonucleoprotamines appear to be in a beads-on-a-string configuration with globular particles (128.3 ± 7.9 Å) connected by a variable length of the DNA linker (Fig. 2*f*). An acid extraction of trout sperm chromatin prepared as for electron microscopy confirmed the presence of a typical protamine (Fig. 4*a*).

All our observations corresponded to the study of well-dispersed nucleofilaments resulting from nuclear disruption and dispersion of chromatin. However, we have no idea of the superorganization of these fibers inside sperm nuclei. If the presence of repeating subunits (nucleosomes [29] or nu-bodies [30]) of somatic cell chromatin is currently incontestable (reviews in references 10, 17, 27), similar observations on sperm chromatin fibers are contradictory.

Nucleosomes have been found only in Echinoderm sperm chromatin with various methods (13, 23, 34, 35), and these results are not surprising because sperm histones here are only slightly different from those present in somatic cell nuclei. In mature sperm where DNA is associated with protamines (*Salmo* type of Bloch [6]) or protamine-like proteins (Mammalia type of Bloch [6]), the absence of repeating subunits in sperm chromatin was demonstrated in the trout (21, 22), the house-cricket (25, 27), and the mouse (9, 24). This may be attributable to the research workers in these studies not using reducing and alkylating reagents or detergents permitting the dispersion of chromatin fibers. On the other hand, by using this technique, a beads-on-a-string structure of sperm chromatin has been already described in man (37) and dogfish (20), and we have obtained similar results with trout, bull, and human sperm chromatin.

Recent studies have shown that the DNA molecule is capable

of generating "nucleosome-like" or globular structures with proteins different from histones such as clupeine (3) or HU protein from *E. coli* (33). The observations reported here, which are based only on electron microscopy, should be extended by various biochemical studies that are now in progress. Currently, we are also engaged in the study of the interactions between DNA and small, highly basic nuclear proteins of sperm nuclei, using physicochemical methods that may reveal the significance of globular structures discovered in spermatozoan chromatin.

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