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# Remodeling of Sperm Chromatin After Fertilization Involves Nucleosomes Formed by Sperm Histones H2A and H2B and two CS Histone Variants

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**Abstract** The composition of nucleosomes at an intermediate stage of male pronucleus formation was determined in sea urchins. Nucleosomes were isolated from zygotes harvested 10 min post-insemination, whole nucleoprotein particles were obtained from nucleus by nuclease digestion, and nucleosomes were subsequently purified by a sucrose gradient fractionation. The nucleosomes derived from male pronucleus were separated from those derived from female pronucleus by immunoadsorption to antibodies against sperm specific histones (anti-SpH) covalently bound to Sepharose 4B (anti-SpH-Sepharose). The immunoadsorbed nucleosomes were eluted, and the histones were analyzed by Western blots. Sperm histones (SpH) or alternatively, the histones from unfertilized eggs (CS histone variants), were identified with antibodies directed against each set of histones. It was found that these nucleosomes are organized by a core formed by sperm histones H2A and H2B combined with two major CS histone variants. Such a hybrid histone core interacts with DNA fragments of approximately 100 bp. It was also found that these atypical nucleosome cores are subsequently organized in a chromatin fiber that exhibits periodic nuclease hypersensitive sites determined by DNA fragments of 500 bp of DNA. It was found that these nucleoprotein particles were organized primarily by the hybrid nucleosomes described above. We postulate that this unique chromatin organization defines an intermediate stage of male chromatin remodeling after fertilization. J. Cell. Biochem. 85: 851–859, 2002. 

**Key words:** chromatin; zygotes; fertilization; male pronucleus; sea urchins

Following fertilization, the condensed sperm nucleus is transformed into male pronucleus, which fuses with the egg pronucleus re-establishing the diploid condition of the embryo. Sperm chromatin condensation results from the interaction of DNA with a set of nuclear basic proteins, which are removed from sperm chromatin during male pronucleus formation [reviewed by Imschenetzky et al., 1999; Philpott et al., 2000]. In echinoderms, it has been postulated that sperm chromatin remodeling occurs following several interdependent states after fertilization. The initial step (step I) is characterized by a fully condensed conical shaped sperm pronucleus. The intermediate step (step II) is defined by an ovoid or swollen male pronucleus containing a partially decondensed chromatin. At the final step (step III), the male pronucleus becomes spherically shaped and contains a fully decondensed chromatin [Cothreen and Poccia, 1993]. The transformation from state I to II requires protein phosphorylation by factors that are absent in oocytes before maturation and may be blocked by protein kinases inhibitors. Progression from state II to III depends on the rise of intracellular pH that occurs at fertilization [Cothreen and Poccia, 1993; Cameron and Poccia, 1994]. From in vitro experiments, it has been concluded that the first step in male pronucleus formation involves the disassembly of sperm nuclear lamina which requires the phosphorylation of lamin B by protein kinase C. Then, the conical nucleus is transformed into a spherical pronucleus in a process that requires ATP, which promotes the

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binding of newly formed nuclear envelope vesicles to chromatin and of GTP, which induces the fusion of these vesicles to one another [reviewed by Collas, 2000].

Concerning histone transitions, it is well documented that sea urchin sperm chromatin is condensed by a set of sperm-specific histones (SpH) while the chromatin of unfertilized eggs is organized by cleavage-stage (CS) histone variants. These variants are also forming the embryonic chromatin after the fusion of male and female pronucleus and during the initial cleavage divisions [reviewed by Imschenetzky et al., 1980; Poccia, 1986]. These changes in histone composition are reflected by differences in chromatin organization that can be followed using micrococcal nuclease digestion. Sperm nucleosomes are packed by sperm histone variants (SpH) interacting with 220–240-bp DNA fragments, while unfertilized egg nucleosomes are organized by cleavage-stage histone variants (CS) that interact with 126 bp of DNA [Imschenetzky et al., 1989]. Such chromatin composition persists during the initial cleavage stages. As it was demonstrated previously in sea urchins, the chromatin of zygotes exhibits an organization, which is very similar to that found in unfertilized eggs [Imschenetzky et al., 1980; Savic et al., 1981; Shaw et al., 1981]. Under polyspermic conditions it was proposed that these protein transitions are completed at the end of the first cleavage [Green and Poccia, 1985], whereas, in normal zygotes, the complete set of sperm histones are released from male chromatin coincidentally with the first S phase of the initial cleavage. We have previously shown that in zygotes the initial release of the tetramer of sperm histones H3–H4 is followed by the dimers of histones H2A–H2B and H1 [Imschenetzky et al., 1991, 1996a]. Subsequently, we have postulated that the sperm specific histones are degraded during male chromatin remodeling by a cysteine-protease that is activated shortly after fertilization. This protease degrades sperm histones leaving the maternal CS histone variants intact [Imschenetzky et al., 1997]. The protection of CS histone variants against proteolysis is due to their extensive poly(ADP-ribosylation) [Morin et al., 1999a]. Alternatively, we have also demonstrated that sperm specific histones H1 and H2B are protected against this protease by their phosphorylation [Morin et al., 1999b]. This protection mechanism is consistent with the post-translational status of the SpH that are present at an intermediate stage of male chromatin decondensation. Previously, Poccia et al. [1990] have reported that SpH2B and SpH1 become phosphorylated shortly after fertilization.

Sea urchin sperm histones can be differentiated from the CS histone variants by immunobiochemical approaches. Thus, the fate of paternal and maternal histone variants can be followed after fertilization by antibodies against either sperm histones, or alternatively, against CS histone variants. By using this approach, it was found that at an intermediate stage of male chromatin decondensation, the chromatin is formed by hybrid nucleoprotein particles containing a subset of sperm histones, as well as, of maternal histone variants [Imschenetzky et al., 1996a]. This result may be interpreted according to two alternatives which are mutually exclusive. The first alternative implies that these hybrid polynucleosomes are the result of two distinct uncompleted nucleosome cores, one containing only the dimers of sperm specific histones H2A–H2B, while the other is organized only by a subset of CS histone variants. The second alternative, implies a unique and homogeneous type of particles, in which the sperm histones are combined with the CS histone variants, thus organizing an hybrid nucleosome core. If the second alternative appears to be the correct model, single nucleosomes should contain SpH2A–SpH2B dimers, as well as, a cohort of CS histone variants. To elucidate among these two alternatives, we have isolated nucleosome cores from male pronucleus and analyzed their protein composition.

## MATERIALS AND METHODS

### Gametes and Zygotes

Sea urchin, *Tetrapygus niger*, were collected from the bay of Concepción and maintained in an aquarium containing natural sea water under constant aeration. Unfertilized eggs, sperm, and zygotes were obtained as described previously [Imschenetzky et al., 1991].

### Nucleosome Isolation

Nuclei were purified from unfertilized eggs and zygotes harvested 10 min post fertilization by an aqueous ethanol/TritonX-100 procedure performed basically as described by Poccia et al. [1981]. To obtain nucleosomes, the isolated

nuclei were washed twice with 5 volumes of a buffer 0.01 M Tris-HCl at pH 7.6, 0.01 M NaCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$ (buffer Ca II–Mg II), then resuspended in 1 volume of the same buffer containing 72 U/ml of micrococcal nuclease (MNase) (Worthington, NJ), and incubated at 37°C for 15 min. The MNase digestion was stopped by adding an equal volume of a solution of 10 mM $Na_2EDTA$, pH 7.4, at 4°C for 30 min. Nuclear debris were removed by centrifugation at 3,000*g* for 10 min, and the supernatant containing the nucleosomal fraction was loaded on a sucrose density gradient 5–20% (w/v) in a buffer 10 mM Tris-HCl, pH 7.2, containing 0.7 mM $Na_2EDTA$ that was centrifuged for 18 h at 100,000*g* at 4°C in a Sorvall SW 28 rotor, as described by Shaw et al. [1981]. Two ml fractions were collected, and the absorbance at 230 and 260 nm was determined. The DNA fragments contained in each fraction were isolated as described by Savic et al. [1981] and further analyzed on a horizontal 2 % (w/v) agarose gels in 0.04 M Tris-acetate buffer, pH 8.0, containing 1 mM EDTA as described by Weintraub [1984]. Fractions enriched in single nucleosomes (mononucleosomes) were selected and subsequently used to isolate the nucleosomes derived from male pronucleus.

### Isolation of Nucleosomes From Male Pronucleus

The isolation of single-nucleosomes from male pronucleus was performed by the immunoadsorption of the single nucleosomes containing sperm histones (SpH). With this purpose, CNBr-activated Sepharose-4B (Sigma, St. Louis, MO) was used to bind polyclonal antibodies against SpH following the procedure described by Tijssen [1988]. Polyclonal antibodies against SpH were obtained and purified as described previously [Imschenetzky et al., 1991]. To couple the antibodies to the CNBr-activated Sepharose-4B beads, these antibodies were dialyzed overnight at 4°C against 0.5 M NaCl and 0.1 M $NaHCO_3$, pH 8.3 (coupling buffer), and incubated with CNBr-activated Sepharose overnight at 4°C, then transferred to 1 M ethanolamine at pH 8.0 for 2 h at room temperature to block the remaining active groups. Consecutive washes with coupling buffer were followed with 0.1 M acetate buffer at pH 4.0 containing 0.5 M NaCl to eliminate the excess of adsorbed proteins. The protein-Sepharose conjugate obtained was stored at 4°C. To immunoadsorbe the nucleosomes containing the SpH to the anti SpH-Sepharose conjugate, the fraction enriched in mono-nucleosomes obtained from the sucrose gradient was incubated with the anti SpH-Sepharose beads during 1 h at room temperature and then overnight at 4°C. The immunoadsorbed nucleosomes were eluted from the Sepharose conjugate with 0.1 M sodium acetate at pH 4.0. The proteins contained in these nucleosomes were further extracted with 0.25 N HCl, precipitated with 20% (w/v) trichloroacetic acid (TCA), washed with cold acetone as described previously, and then analyzed by SDS/PAGE [Imschenetzky et al., 1991]. The identification of sperm histones or alternatively of the CS histone variants was performed by Western blots. A negative control of the immunoadsorption procedure was performed by using nucleosomes obtained from unfertilized eggs of sea urchins, which were incubated under the same conditions as described above with the Sepharose–4B conjugate.

### Protein Analysis by Western Immunoblot

Sample preparation and electrophoresis in 18% PAGE–0.1% SDS were performed as described by Laemmli [1970]. After electrophoresis, the gel was transferred to nitrocellulose membranes and analyzed by Western immunoblots, as described by Towbin et al. [1979] and modified by Imschenetzky et al. [1991]. The immunodetection of proteins was performed with polyclonal antibodies against SpH, or alternatively against the CS histone variants that were obtained and purified as described [Imschenetzky et al., 1991]. As we have informed previously, the anti-SpH antibodies did not cross-react with the CS histone variants and alternatively, the anti-CS-histone variants antibodies did not cross-react with the SpH [Imschenetzky et al., 1991, 1996a].

We have demonstrated previously that the CS histone variants were extensively poly(ADP-ribosylated) in unfertilized eggs and zygotes [Imschenetzky et al., 1996b]. Therefore, to determine if the histone variants that are forming the nucleosomes isolated from male pronucleus were modified, the Western blots were incubated as well, with antibodies directed against ADP-ribose polymers (BIOMOL, PA). To remove the primary and secondary antibodies, after each incubation, the nitrocellulose membrane was stripped with 100 mM

2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7 (stripping buffer), for 30 min at 50°C. The detection was performed using a ECL chemiluminescence kit (Amersham Pharmacia Biotech, UK).

## RESULTS

### Isolation of Nucleosomes From Male Pronucleus

The strategy used to separate nucleosomes from male pronucleus was based on our previous results, which indicated that the antibodies directed against histone variants from sperms did not cross-react against the CS variants from unfertilized eggs. Therefore, the anti-SpH antibodies were a useful tool that could be applied to discriminate among the nucleosomes derived from male and female pronucleus. We have previously reported that at an intermediate stage of male chromatin decondensation, the nucleosomes contained SpH2A and SpH2B, therefore, it was predictable that such nucleosomes may be retained by Sepharose beads that are covalently bound to anti-SpH antibodies, whereas those derived from the female pronucleus should not be retained.

Following this rationale, we initially purified nuclei from zygotes at an intermediate stage of male pronucleus decondensation (10 min p.i.) and subsequently obtained nucleoprotein particles (NP-ps) by micrococcal nuclease digestion. The mononucleosomes present in these Np-ps were further purified on a sucrose density gradient, and the DNA fragments derived from the fractions of this gradient were isolated and analyzed by agarose gels, as described in Materials and Methods. As shown in Figure 1A, this gradient successfully separates polynucleosomes (Fig. 1A, arrows 1–3 and Fig. 1B, lanes 1–3) from mono-nucleosomes (Fig. 1, arrow 4 and Fig. 1B, lane 4). In addition, the analysis of the DNA fragments reveals that discrete nucleoprotein particles (Np-ps) were generated after MNase digestion exhibiting a periodicity of 500 bp of DNA, thus reflecting a particular organization of the chromatin (Fig. 1B, lanes 1–3).

Subsequently, to separate the nucleosomes derived from male pronucleus from those derived from female pronucleus, the fractions

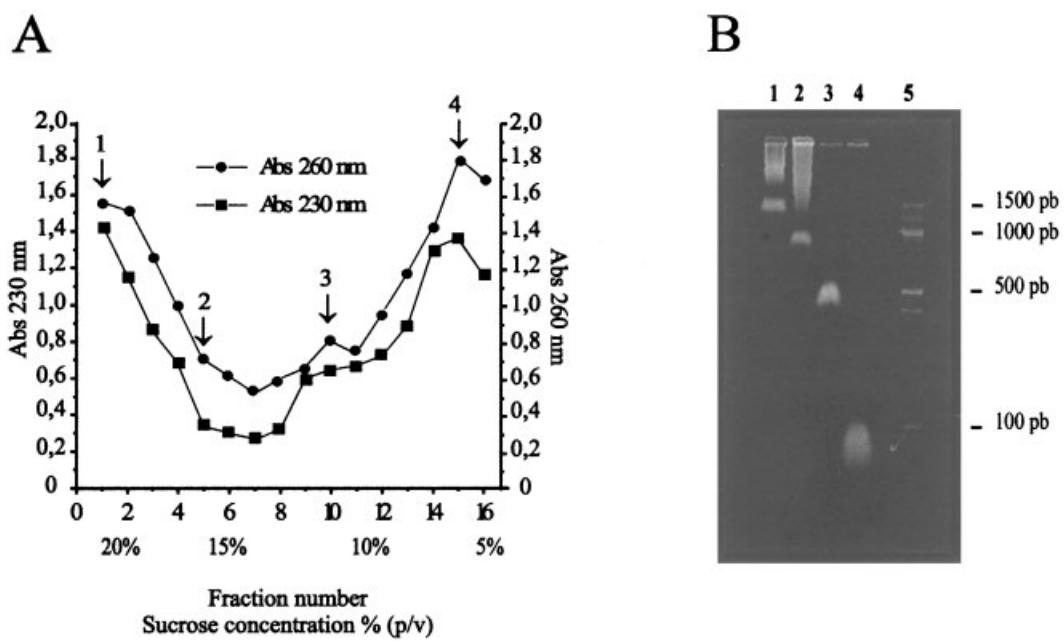


**Fig. 1.** Isolation and analysis of nucleosomes derived from male pronucleus. Nucleosomes were isolated from zygotes by nuclease digestion and separated by a sucrose density gradient as described in Materials and Methods. **A**: Fractionation profile of nucleosomes on sucrose density gradient. Fraction corresponding to a sucrose concentration of 20% (w/v) (arrow 1), to a sucrose concentration of 15% (w/v) (arrow 2), to a sucrose concentration of 10% (w/v) (arrow 3), and to a sucrose concentration of 6% (w/v) (arrow 4). The DNA fragments contained in the sucrose gradient fractions indicated by arrows (arrows 1–4) were purified and further analyzed in 2% agarose gels and stained with ethidium bromide. **B**: DNA fragments extracted from the fraction signalized by the arrows 1 (**lane 1**), arrow 2 (**lane 2**), arrow 3 (**lane 3**), and arrow 4 (**lane 4**). **Lane 5** contains 0.5 μg of a DNA ladder containing fragments that are multiples of 100 bp of DNA that were run in the same gel.

from the sucrose gradient containing the mononucleosomes (Fig. 1A, arrow 4) were collected and then immunoadsorbed to beads of Sepharose-4B bound to polyclonal antibodies against the SpH (anti-SpH-Sepharose). The specificity of such an immunoadsorption procedure was investigated in parallel with nucleosomes derived from unfertilized eggs that are known to be formed exclusively by CS histone variants [Imschenetzky et al., 1989, 1996a]. Therefore, similarly to the nucleosomes derived from zygotes, single nucleosomes from unfertilized eggs were isolated by sucrose gradient and incubated with the anti-SpH-Sepharose beads as described above. As shown in Figure 2, the fraction marked by an arrow was confirmed to contain the mononucleosomes by analyzing the DNA fragments (2B) and subsequently adsorbed to anti-SpH-Sepharose in parallel with the mononucleosomes isolated from zygotes. The results, summarized in Table I, indicate that the nucleosomes from unfertilized egg were not adsorbed to the anti-SpH-Sepharose, while those obtained from zygotes were partially adsorbed. Based

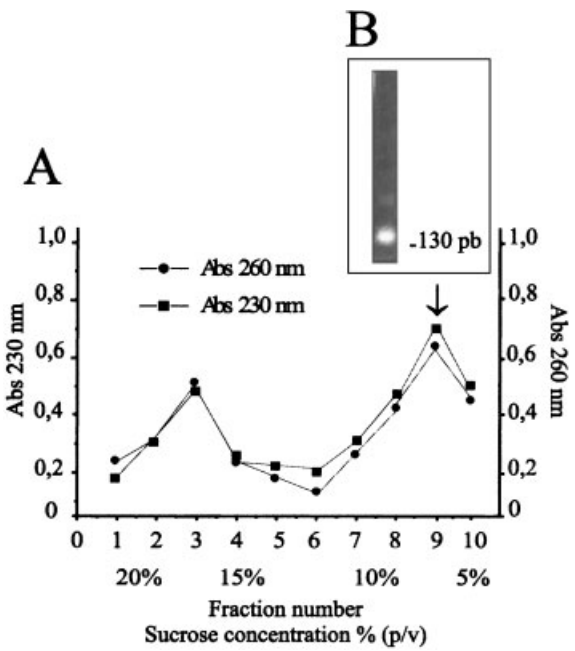


**Fig. 2.** Isolation and analysis of nucleosomes derived from unfertilized eggs. Nucleosomes were obtained from unfertilized eggs by nuclease digestion and separated by sucrose density gradient as described in Materials and Methods. The fraction corresponding to a sucrose concentration of 6% (w/v) that is enriched in mononucleosomes is indicated by an arrow. **A**: Fractionation profile of sucrose density gradient. The DNA fragments that were extracted from the fraction signaled by an arrow were analyzed in 2% (w/v) agarose gel and stained with ethidium bromide (**B**).

**TABLE I. Immunoadsorption of Nucleosomes With Anti-SpH-Sepharose Beads**

| | Male Pronucleus Nucleosome | Unfertilized Egg Nucleosomes | A (nm) |
|---|---|---|---|
| Total Fraction | 0.543 | 0.931 | 230 |
| | 0.404 | 0.729 | 260 |
| Not Immunoadsorbed | 0.390 | 0.906 | 230 |
| | 0.285 | 0.690 | 260 |
| Eluate | 0.118 | 0.000 | 230 |
| | 0.097 | 0.003 | 260 |

Nucleosomes were obtained from unfertilized eggs or from zygotes by nuclease digestion followed by sucrose density gradients. The fractions of the gradient containing single nucleosomes were collected and incubated with Sepharose 4B conjugated to the antibodies against sperm histones (anti-SpH-Sepharose). The proteins contained in the nucleosomes that were adsorbed to the anti-SpH-Sepharose were eluted and quantified. All these procedures are described in Materials and Methods.

on these results, we have concluded that the mono-nucleosomes that were adsorbed in the anti-SpH-Sepharose beads were those derived exclusively from the male pronucleus. This result is consistent with the lack of cross-reactivity between the histone variants from sperms (SpH) and those derived from unfertilized eggs (CS) [Imschenetzky et al., 1991, 1996a].

To analyze the protein composition of the nucleoprotein particles associated to DNA fragments of 500 bp, the fraction marked by arrow 3 in Figure 1A was collected and analyzed by PAGE/SDS (Fig. 3). The electrophoretic migration of the protein components of these particles were consistent with the histones either from sperms or from unfertilized eggs. As shown (Fig. 3B, lanes 3, 4) these particles contained proteins comigrating with both the SpH (Fig. 3B, lane 2) and the CS histone variants (Fig. 3, lane 5) that become evident after the silver staining of this gel. Several bands that were located above histones position were also observed in the Coomassie blue stained gels (Fig. 3A, lanes 3, 4).

### Protein Composition of the Nucleosomes Isolated From Male Pronucleus

To determine the protein composition of the nucleosomes derived from the male pronucleus isolated at an intermediate stage of remodeling, we isolated the nucleosomes that were retained on the Sepharose-4B conjugated resins as described in Materials and Methods. The histone variants forming these nucleosomes were then extracted with 0.25 N HCl, separated by PAGE/

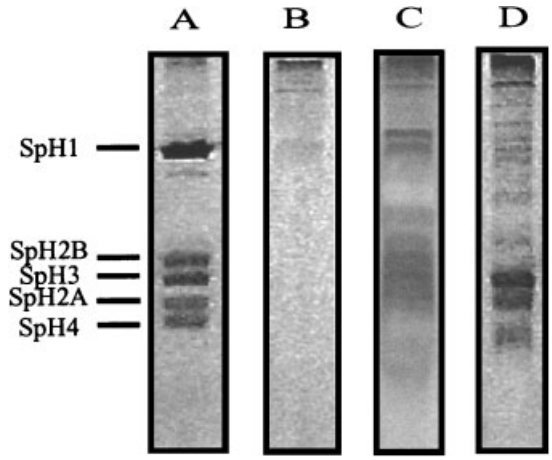


**Fig. 3.** Protein composition of the nucleoprotein particles organized by DNA fragments of 500 bp. Total proteins were isolated from the fractions of the sucrose gradient that were enriched in nucleoprotein particles formed by DNA fragments of 500 bp (signaled by arrow 3 in Fig. 1), and analyzed by SDS/PAGE. Histone variants from sperms and from unfertilized eggs were analyzed in the same gel. Whole histones isolated from sperms and stained with Coomassie blue (**lane a**). Proteins isolated from the nucleoprotein particles defined by DNA fragments of 500 bp were stained with Coomassie blue (**lane b**) and with silver (**lane c**). CS histone variants isolated from unfertilized eggs stained with Coomassie blue (**lane d**).

SDS, and further analyzed by Western blots that were revealed with polyclonal antibodies against either SpH or CS variants. The eventual poly(ADP-ribosylation) of these proteins was analyzed in parallel using polyclonal antibodies against ADP-ribose polymers. The results shown in Figure 4, indicate that the nucleosomes retained were formed by four major and several minor components (Fig. 4B, lane c). Two of these components were recognized by the anti-SpH antibodies and exhibited an electrophoretic migration consistent with SpH2A and SpH2B (Fig. 4B, lane d). SpH2B migrated slightly above the position of this histone as it is isolated from sperms, suggesting that it could represent a post-translationally modified isoform. This possibility is consistent with previous reports indicating that SpH2B is phosphorylated shortly after fertilization [Green and Poccia, 1985]. The maternally-derived histone variants were found as two major and several minor bands (Fig. 4B, lanes a, b). Interestingly, some of these bands correspond to poly(ADP-ribosylated) forms of CS histone variants, as shown by the Western blots that were revealed by using the anti-poly(ADP-ribose) antibodies (Fig. 4B, lane b). This result confirms previous reports indicating that the CS variants are extensively poly(ADP-ribosylated) in unfertilized eggs and during the initial cleavage cycles of sea urchin development [Imschenetzky et al., 1993, 1996b].

Taken together our results indicate that the male chromatin, at an intermediate stage of decondensation, is organized by an homogenous population of nucleosomal particles including a subset of CS variants, SpH2A, and SpH2B. The CS histone variants were found to be poly(ADP-ribosylated), while SpH2B seems to be phosphorylated.

## DISCUSSION

Single nucleosomes derived exclusively from male pronucleus of sea urchins were isolated post-fertilization at an intermediate stage of male chromatin remodeling. It was determined that these nucleosomes are formed by the sperm core histones SpH2A–SpH2B, as well as, by two major CS histone variants derived from maternal pools. These CS histone variants were found to be poly(ADP-ribosylated). Due to the electrophoretic heterogeneity of the CS variants induced by this post-translational modification, it is difficult to define without ambiguities each one of the individual CS histone. However, we found that one of this major forms migrates in the position of histone H3, while the other one migrates in that of histone H4. This nuclesomal composition is consistent with a previous report where we found that SpH3–H4 disappeared early from male pronucleus chromatin, whereas SpH2A–H2B remained [Imschenetzky et al., 1991]. Hence, the nucleosome core organization described here further defines the molecular organization of the hybrid nucleoprotein particles at the intermediate stage of male chromatin decondensation in sea urchins [Imschenetzky et al., 1996a].

We have also shown that this partially decondensed chromatin exhibits a specialized structure formed by repetitive units including DNA fragments of 500 bp that become evident following micrococcal nuclease digestion. These results suggest a tight and stable association between several nucleosomal particles, thus defining very specific nuclease sensitive sites.

The interaction between dimers of sperm histone variants Sp(H2A–H2B) with two major CS histone variants indicates that they are organized in a structure that resembles the tetramer previously formed by Sp(H3–H4).

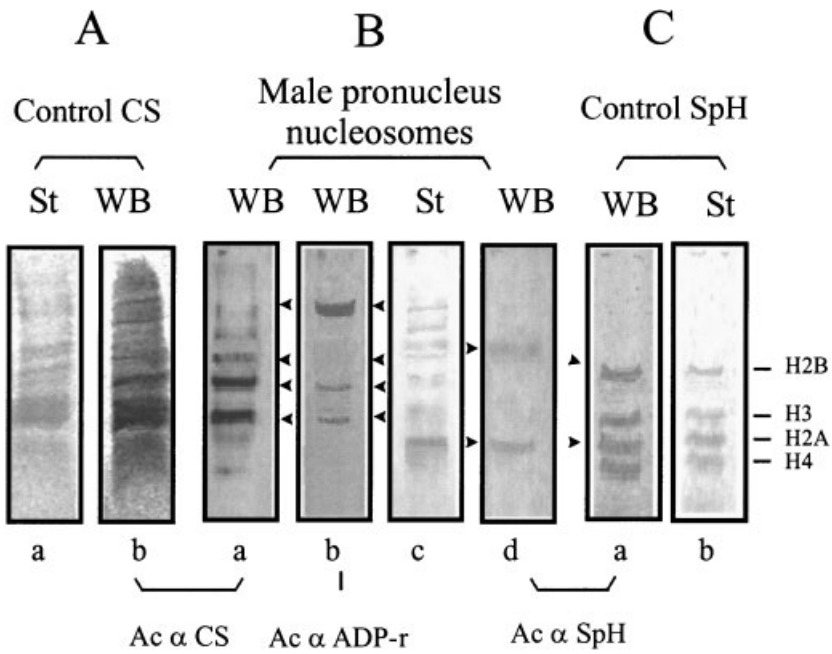


**Fig. 4.** Protein composition of nucleosomes isolated from male pronucleus. Single nucleosomes were isolated from male pronucleus as described in Materials and Methods, the proteins contained in these nucleosomes were separated by SDS–PAGE, transferred to a nitrocellulose membrane and revealed with antibodies against the CS histone variants, subsequently with antibodies against polymers of ADP-ribose and finally with antibodies against sperm histones (SpH), as described in Materials and Methods. Whole histone variants isolated from sperms or alternatively, from unfertilized eggs, were analyzed in parallel, as controls. **A**: Nitrocellulose membranes containing 30 μg of CS variants stained with Ponceau-S (**lane a**) revealed with antibodies against CS variants (**lane b**). **B**: 30 μg of proteins extracted from the nucleosomes isolated from male pronucleus, revealed with antibodies against CS variants (**lane a**), revealed with antibodies against ADP-ribose polymer (**lane b**), stained with Ponceau-S (**lane c**), and revealed with antibodies against SpH (**lane d**). **C**: Nitrocellulose membranes containing 25 μg of SpH: revealed with antibodies against SpH (**lane a**) and stained with Ponceau-S (**lane b**).

This result suggests that the CS variants should possess architectural motifs that are compatible with the histone fold domains that are fundamental for both the core octamer assembly and the DNA compactation [Arents and Moudrianakis, 1995]. Therefore, this suggestion is consistent with previous findings indicating that the CS histone variants are functional histones, despite the significant differences in their primary sequence with that of sperm specific histones or histones from other sources [Mandl et al., 1997].

The histone protein composition found in single nucleosomes during sperm chromatin remodeling is in agreement with previous reports that described the phosphorylation of H2B [Green and Poccia, 1985] as an early event in male chromatin decondensation. Moreover, we found that the migration of SpH2B derived from the sperm pronucleus at intermediate stage of decondensation is decreased compared with that of the non-phosphorylated SpH2B present in sperms. This decreased electrophoretic mobility is consistent with its phosphorylation. This result supports as well the protective role of SpH2B phosphorylation against the degradation by the SpH-protease at intermediate stage of sperm chromatin decondensation [Morin et al., 1999b].

It is yet unknown whether the activity of SpH-protease is coordinated with additional chromatin remodeling factors, or with histone chaperones that may promote the removal of the sperm histones before their degradation. The hybrid composition of the nucleosomes described in this report suggest that the release of SpH3 and SpH4 from the nucleosomal particles is concomitant to the assembly of the CS histone variants. Therefore, it may be speculated that the chromatin remodeling factors that are regulating the decondensation of sperm chromatin after fertilization may also be participating in the assembly of the CS variants that are replacing the SpH3–SpH4 octamer in these nucleosomes. In batracians, hyperphosphorylated nucleoplasmin has been postulated to function as a chaperone during the assembly of nucleosome cores, by adding dimers of

histones H2A–H2B to the tetramers of histones H3–H4. The tetramers of H3–H4 were postulated to be primarily organized by two other chaperone proteins N1 and N2. Nucleoplasmin depleted *Xenopus* egg cell free extracts are unable to carry out sperm chromatin decondensation, and this property is restored by the addition of pure nucleoplasmin, further indicating its role in male chromatin remodeling [reviewed by Philpott et al., 2000]. It has been postulated that the unpackaging of *Xenopus* male chromatin results from the binding of nucleoplasmin to the sperm specific basic proteins (SSBP) with higher affinity than that displayed by nucleoplasmin for histones H2A–H2B [Ohsumi and Katagiri, 1991; Katagiri and Ohsumi, 1994; Ito et al., 1996]. Hence, during sperm chromatin decondensation nucleoplasmin removes SSBP and deposits H2A–H2B dimers in a coordinated manner, this process is followed by histones phosphorylation and the final assembly of the H1-like linker histone B4 into chromatin [Dimitrov et al., 1994].

A chromatin remodeling activity, homologous to yeast SW12/SNF2 and to the human BRG1 containing complexes, has been recently identified in *Xenopus* oocytes. This protein complex is expressed throughout oogenesis and embryogenesis and appears to be involved in chromatin remodeling during transcriptional activation. Whether this complex also has a role in sperm chromatin remodeling that follows fertilization remains to be established [Gelius et al., 1999]. Otherwise, in the cytoplasm of sea urchin eggs, an ATP-dependent chromatin-remodeling activity has been recently found [Medina et al., 2001]. This chromatin remodeling complex was postulated to participate in sperm chromatin decondensation following fertilization. In addition, the potential contribution of sperm histone post-translational modifications following fertilization should also be considered. It has been shown that histone acetylation and poly(ADP-ribosylation) promotes nucleosome decondensation in vitro [García Ramírez et al., 1995; D'Amours et al., 1999]. Thus, it appears that several coordinated mechanisms may contribute to the remodeling of sperm chromatin following fertilization.

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