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Nucleoplasmin-Mediated Decondensation of *Mytilus* Sperm Chromatin. Identification and Partial Characterization of a Nucleoplasmin-like Protein with Sperm-Nuclei Decondensing Activity in *Mytilus californianus*†

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ABSTRACT: We have been able to induce sperm nuclear decondensation in the mussel *Mytilus californianus* (mollusc) using either egg extracts or pure nucleoplasmin from *Xenopus* (amphibian). The nuclear decondensation involves removal of the sperm nuclear basic proteins (SNBPs) which bind to nucleoplasmin. An attempt has been made to isolate an ooplasmic factor from *Mytilus* with a similar sperm-chromatin decondensing activity. An acidic, thermostable protein with a molecular mass of 58 000 has been purified and partially characterized.

With the exception of some decapod crustaceans, sperm chromatin is usually highly condensed and tightly packed within the sperm nucleus. The way in which such condensation takes place is achieved by the interaction with DNA of a structurally and compositionally heterogeneous set of sperm nuclear basic proteins (SNBPs)[1] (Bloch, 1969; Kasinsky, 1989). They range from sperm-specific histones to the highly specialized protamines such as those found in the sperm of mammals, in a phylogenetic manner (Ausio, 1995). Regardless of the protein composition, male chromatin becomes decondensed immediately after fertilization in a reaction that involves the removal of these sperm-specific nucleoproteins (Longo, 1985; Poccia, 1986). The molecular mechanisms involved in these processes have long remained obscure.

The search for an ooplasmic factor responsible for the removal of the SNBPs in amphibians led to the discovery of nucleoplasmin involvement (Ohsumi & Katagiri, 1991; Philpott et al., 1991).

Nucleoplasmin is a thermostable acidic pentameric protein (Earnshaw et al., 1980) that was first isolated from *Xenopus* (Laskey et al., 1978). It represents the most abundant protein present in the oocyte nucleus of this species (Mills et al., 1980; Krohne & Franke, 1980). The protein was initially shown to have the ability to mediate *in vitro* nucleosome assembly (Laskey et al., 1977, 1978), and evidence was later provided in support of a similar role *in vivo* (Kleinsdunidt et al., 1985). Experimental evidence for the dual role of nucleoplasmin (SNBP disassembly and nucleosome reassembly) in the remodeling of sperm chromatin immediately after fertilization has been obtained (Philpott & Leno, 1992; Itoh et al., 1993; Katagiri & Ohsumi, 1994).

In addition to the above, nucleoplasmin was shown to have the ability to disassemble sperm chromatin in a heterologous

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[1] Abbreviations: SNBP, sperm nuclear basic proteins; PAGE, polyacrylamide gel electrophoresis.

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system consisting of human sperm nuclei and different amphibian egg extracts or purified nucleoplasmin (Brown et al., 1987; Ohsumi et al., 1988; Itoh et al., 1993). The sperm-chromatin disassembling activity of amphibian nucleoplasmin in heterologous systems raises the possibility of a universal role for this molecule in this process. In fact, a heat-stable, p22, protein has been recently isolated from *Drosphila* embryos, which is able to mediate the decondensation of *Xenopus* sperm (Kawasaki et al., 1994). Also, interestingly enough, a nucleoplasmin-like molecule was recently isolated from the oocytes of the surf clam *Spisula solidissima* (Herlands & Maul, 1994).

In the present paper we show that amphibian nucleoplasmin as well as amphibian egg extracts can decondense the sperm chromatin from the mussel *Mytilus californianus*. In favor of this being a universal role, we have also identified a nucleoplasmin-like protein from the mussel oocytes with a similar nuclear sperm decondensing activity.

MATERIALS AND METHODS

Preparation of Mytilus Sperm Nuclei. Following sperm collection as in Ausio (1988), the sperm suspension in sterile filtered sea water was centrifuged at 1500*g* for 10 min at 4 °C. The pellet thus obtained was resuspended in 150 mM NaCl, 20 mM Tris-HCl pH 7.5, 0.2 mM PMSF, and 20 μg/mL TLCK (buffer A) and centrifuged again under the same conditions. The pellet thus obtained was homogeneized in buffer A containing 0.5% Triton X-100, using a polytron homogeneizer. After incubation for 10 min on ice, the suspension was centrifuged as in the previous steps. The pellet was then washed (resuspended and centrifuged) in buffer A, the final pellet was resuspended in buffer A containing 40% glycerol, and the nuclei thus obtained were stored at −80 °C.

Preparation of Xenopus Egg Extracts. "High-speed" *Xenopus* egg extracts were prepared as described previously (Itoh et al., 1993).

"Heated extracts" were prepared as follows. The proteins of the high-speed extract were heat denatured at 80 °C in a water bath for 10 min. After being cooled on ice, the extract was spun down at 10000*g* at 4 °C for 10 min. The supernatant was kept, and the pellet was resuspended in a half-volume of EM buffer (100 mM KCl, 2 mM $MgCl_2$, and 10 mM Tris-HCl, pH 7.4) to recover the thermostable fraction trapped in the pellet (~30%). This was centrifuged again as above, and the two supernatants were then combined and centrifuged at 150000*g* for 60 min at 4 °C and filtered through a 0.2-μm filter.

Preparation of Mytilus and Spisula Egg Extracts. The clams, *S. solidissima*, were obtained from the Marine Biological Laboratory (MBL), Department of Animal Resources, Woods Hole, MA. Mussels, *M. californianus*, were collected at Point-No-Point (Sooke) on Vancouver Island, British Columbia.

The mature female ovaries were submerged at room temperature in filter-sterilized sea water containing 40 mM KCl in order to parthenogenetically activate the oocytes (Allen, 1953; Herlands & Maul, 1994). After a few gonadal incisions and upon mild agitation, the eggs that started being spontaneously released were filtered through eight layers of sterile cheese cloth and collected in centrifuge tubes. After centrifugation at 480*g* for 10 min, the pellets containing the oocytes were suspended in 1 M glycerol and 20 mM phosphate buffer (Na_2HPO_4/KH_2PO_4, pH 8.0) to remove the vitelline layer (Maul, 1980). After 3 min of incubation the suspension was pelleted again at 480*g*, and the pellet was resuspended in EM buffer and processed as in Itoh et al. (1993) to obtain the high-speed extract. Heated extracts were prepared as described for *Xenopus*. $MgCl_2$ was omitted from all the buffers in the preparation of *Spisula* egg extract because of the reported low solubility of *Spisula's* nucleoplasmin-like protein (Herlands & Maul, 1994).

Isolation and Purification of Nucleoplasmin. Xenopus nucleoplasmin was basically prepared as described by Sealy et al. (1986, 1989) with a few minor modifications. The fractionation of the heated extract was carried out on an FPLC system (Pharmacia LKB Biotechnology, Uppsala, Sweden).

For the first fractionation, Q-Sepharose (Pharmacia LKB) was used instead of DEAE. The fractions that contained sperm-nuclei decondensing activity (see next section) were pooled and loaded onto a phenyl-Superose column.

Mytilus and *Spisula* nucleoplasmin-like proteins were fractionated in a similar way but using a DEAE (Bio-Rad Laboratories Inc.) HPLC column for the first fractionation step.

To estimate the protein concentration, an extinction coefficient of $A_{276} = 0.85$ for 1 mg/mL solution in a cuvette with a 1-cm path length was routinely used.

Incubation of Sperm Nuclei in Oocyte Extracts and in the Presence of Purified Protein Fractions. Mytilus or *Spisula* nuclei (2×10^6 nuclei) obtained by Triton X-100 treatment (see Materials and Methods) were resuspended in 150 μL of high speed egg extract and incubated for different periods of time at room temperature. Nuclear decondensation was followed by light microscopy. To this purpose, 2-μL aliquots were withdrawn at different times and mixed with 4 μL of 100 μg/mL bisbenzimide (Hoechst 33258) dye as described by Ohsumi et al. (1988).

In order to monitor the displacement of SNBPs from the sperm chromatin by egg extracts, aliquots of 2×10^6 nuclei were resuspended in 150 μL each of high speed extracts and incubated for different times at room temperature (5×10^6 sperm nuclei correspond to an average of 21 μg of sperm DNA). At the selected times the nuclear suspension was centrifuged at 16000*g* for 10 min at 4 °C in an Eppendorf microfuge.

The nuclear pellets thus obtained were extracted with 50 μL of 0.4 N HCl at 37 °C for 10 min. After centrifugation at 16000*g* for 10 min as above, the HCl extracts were precipitated with 6 volumes of cold acetone at −20 °C for 1 h. The protein precipitate was recovered by centrifugation, and the pellet was dried in a speed vac.

Sperm-nuclei decondensation by pure nucleoplasmin fractions was carried out as follows. Nuclei (1.5×10^6 or 3.0×10^6) or equivalent amounts of sperm chromatin were incubated at room temperature in the presence of 70 μL of nucleoplasmin (1 mg/mL) for different times. SNBP binding to nucleoplasmin was assessed by western blot analysis using an antibody against PL-II* from *Mytilus* (Carlos et al., 1993a). To this purpose, after incubation of the sperm samples in the presence of nucleoplasmin for 60 min at room temperature, the nuclear suspension was centrifuged at

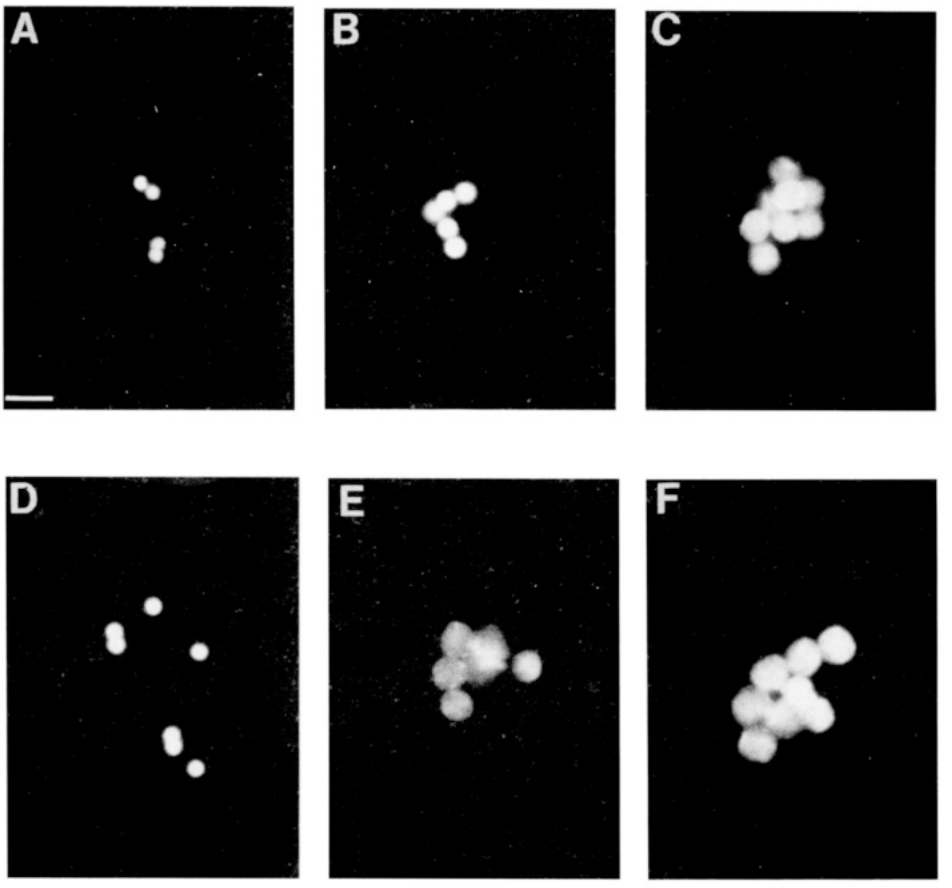

FIGURE 1: Decondensation of *Mytilus* (B, C) and *Spisula* (E, F) sperm nuclei by *Xenopus* high-speed egg extract upon incubation for 60 min at room temperature. (A and D) *Mytilus* and *Spisula* sperm nuclei incubated in the absence of extract. The bar is 10 μm.

10000*g* for 10 min at 4 °C, and aliquots of the supernatant were subsequently loaded onto "native" protein gels (see below).

Gel Electrophoresis. Acetic acid (5%)−urea (2.5 M) polyacrylamide gel electrophoresis (PAGE) was carried out as described previously (Jutglar et al., 1991).

SDS−PAGE was carried out according to Laemmli (1970).

Native PAGE using a 3% polyacrylamide stacking 7% polyacrylamide separation gel was carried out according to Laemmli (1970) except that SDS was omitted from all the solutions. Mini gels (100 × 70 × 0.75 mm) were run at room temperature and 100 V until bromophenol blue (used as tracking dye) ran out of the gel.

Protein (SDS−PAGE) standards were obtained from Bio-Rad (Bio-Rad Laboratories Inc.).

Western Blots. After electrophoresis in native gels the proteins were transferred to an Immobilon-P (PVDF) transfer membrane (Millipore, MA) at 90 V for 15 min at 4 °C using a Bio-Rad Mini Trans-blot cell (Bio-Rad Laboratories Inc.). The protein detection was then carried out as described elsewhere (Carlos et al., 1993a).

Analytical Ultracentrifuge. Analytical ultracentrifuge analysis was carried out in a Beckman XL-A analytical ultracentrifuge (Beckman Instruments Inc., Fullerton, CA) using an An-60 Ti rotor and double sector (aluminum-filled epon) centerpieces. Scans were taken at 230 nm.

Sedimentation velocity experiments were routinely carried out at 44 000 rpm and 20 °C. The scans were analyzed by the method of van Holde and Weischet (1978) using the Ultrascan data analysis software (Borries Demeler, San Antonio, TX). Apparent sedimentation coefficients were converted to standard conditions, $s_{20,w}$, as described elsewhere (Ausio et al., 1993).

The partial specific volume of *Mytilus* nucleoplasmin-like protein was estimated from its amino acid composition (see Table 1) according to Perkins (1986) and was determined to be 0.726 cm^3/g.

Amino Acid Analysis. Amino acid analyses were carried out on an ABI Model 420A derivatizer analyzer system (Applied Biosystems, Foster City, CA) as described elsewhere (Carlos et al., 1993a). The analyses were carried out at the Protein Microchemistry Center of the University of Victoria, Victoria, BC.

RESULTS AND DISCUSSION

Amphibian nucleoplasmin has been shown to induce sperm-nuclei decondensation in both homologous and heterologous vertebrate systems (Itoh et al., 1993; Katagiri & Ohsumi, 1994). Therefore, it seemed of interest to check whether amphibian nucleoplasmin could also induce (and thus be responsible for) a similar nuclear decondensation in invertebrate organisms. Interestingly, Longo et al. (1994) have recently reported that the sperm nuclei of *S. solidissima* undergo an important expansion when incubated in the presence of oocyte extracts from the same organism. Using electron microscopy, it was also shown that the expansion of the sperm nuclei observed *in vitro* resembled chromatin decondensation *in vivo* (Longo et al., 1994).

Figure 1 shows the nuclear expansion experienced by *Mytilus* and *Spisula* sperm nuclei when exposed to *Xenopus*

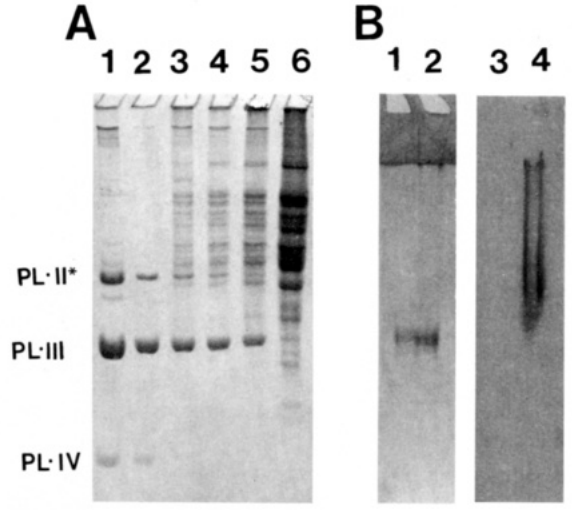

FIGURE 2: (A) Urea−acetic acid PAGE (2.5 M urea, 5% acetic acid) of the proteins from *Mytilus* sperm nuclei (extracted with 0.4 N HCl) after incubation with high-speed egg extract for (lanes 1 and 2) 0 min (lane 1 contains a double amount of protein), (lane 3) 5 min, (lane 4) 15 min, (lane 5) 30 min, and (lane 6) 60 min at room temperature. PL-II*, PL-III, and PL-IV are the three major *Mytilus* SNBP. (B) (Lanes 1 and 2) Native PAGE of (1) *Xenopus* nucleoplasmin and (2) *Xenopus* nucleoplasmin incubated for 90 min in the presence of *Mytilus* sperm nuclei; (lanes 3 and 4) western blot analysis of (1) and (2), using a polyclonal antibody against PL-II*. It should be noted that, under these conditions, the positively charged SNBPs by themselves (unassociated with nucleoplasmin) would not migrate into the gel.

high-speed egg extracts. We consistently found two different populations of swollen nuclei in *Mytilus* (Figure 1B,C) with average diameters 4.6 ± 0.36 and 6.4 ± 0.77 μm compared to 2.6 ± 0.3 μm for the control sperm nuclei. The relative proportions of these two populations were found to be variable between different oocyte extract preparations. After a 60-min incubation at room temperature, approximately 64% of the nuclei corresponded to the smaller diameter while the remaining 36% exhibited the larger diameter. To compare these results with those obtained by Longo et al. (1994), we also exposed *Spisula* sperm nuclei to the same *Xenopus* extracts (see Figure 1D−F). We again saw two different populations of swollen nuclei with average diameters of 7.4 ± 0.7 and 8.7 ± 0.2 μm when compared to 3.0 ± 0.17 μm for the control nuclei.

The results obtained with *Spisula* are very similar to the results obtained by Longo et al. (1994). Longo et al. (1994) demonstrated that different populations of swollen nuclei could be obtained depending on the time of parthenogenetic activation. Three different populations were described with sperm nuclear areas of 18, 37, and 61 μm^2, the last one corresponding to maximum activation. The last two values are almost identical to the area values, 43.0 and 59.5 μm^2, for the two populations observed in our case. This suggests that the two populations observed in our case for both *Spisula* and *Mytilus* are most likely the result of incomplete activation by the *Xenopus* extracts used. It is also important to notice that the absolute extent of nuclear expansion is different in *Mytilus* when compared to *Spisula*. This might reflect the different sperm-chromatin organization existing in these two species of bivalve molluscs, most likely as a result also of their different SNBP composition (Ausio & Subirana, 1982a,b). It could also arise from the different size of their genomes or perhaps from a combination of both.

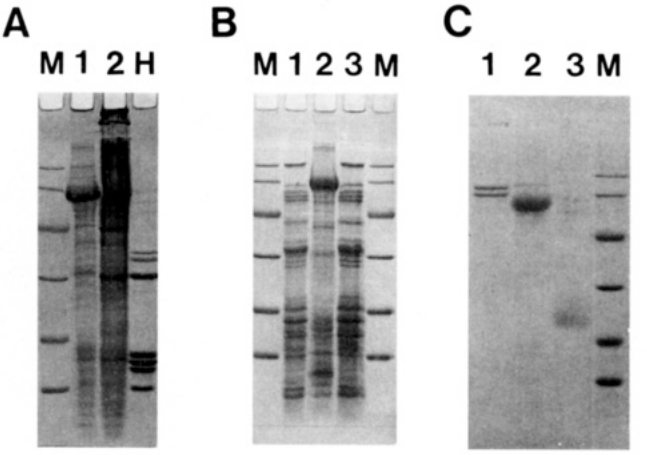

FIGURE 3: (A) SDS−PAGE of (1) proteins of the heated egg extract and (2) proteins of the high-speed egg extract from *Mytilus*. (B) Comparison of the proteins from *Xenopus* boiled egg extract (1 and 3) and from *Mytilus* egg extract (2). (C) Purified nucleoplasmin-like proteins from (1) *S. solidissima*, (2) *M. californianus* and (3) nucleoplasmin from *X. laevis*. H = chicken erythrocyte histones. M = protein standard used as a marker. The protein markers from top to bottom are rabbit muscle phosphorylase *b*, 97 kDa; bovine serum albumin, 66.2 kDa; hen eggwhite ovalbumin, 45 kDa; bovine carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; and hen egg white lysozyme, 14 kDa.

Although in the paper by Longo et al. (1994) it was clear that the nuclear expansion was the result of an extensive chromatin reorganization (decondensation), the detailed molecular mechanisms involved were not determined. Figure 2A shows an acetic acid−urea PAGE of HCl extracts from *Mytilus* sperm nuclei incubated for different times in the presence of high-speed *Xenopus* egg extract. As it can be seen, upon incubation the sperm nuclei lose all the SNBP. There does not seem to be an apparent preference for any of the three protamine-like proteins (PL-II*, PL-III, and PL-IV) (SNBP) which are present in the sperm of *Mytilus* despite their compositional and structural differences (Ausio & Subirana 1982a; Carlos et al., 1993a,b; Ausio, 1995). This is not surprising, however, considering the ability of *Xenopus* nucleoplasmin to bind and displace SNBP as structurally and compositionally different as human protamines and the SNBP from *Xenopus* (Itoh et al., 1993; Katagiri & Ohsumi, 1994). However, the process of SNBP removal seems to proceed more slowly in the *Mytilus* case.

It was previously shown that the SNBP removing activity of the *Xenopus* egg extracts resides in nucleoplasmin (Ohsumi & Katagiri, 1991). In fact, we have been able to reproduce the same results in Figure 1 and 2A using pure *Xenopus* nucleoplasmin (data not shown). When a similar experiment to that described in Figure 2A was carried out with purified *Xenopus* nucleoplasmin, it was possible to show using western blot analysis (see Figure 2B) that indeed the SNBPs removed from *Mytilus* sperm nuclei were bound to this molecule, thus confirming the results of Ohsumi and Katagiri (1991).

All these results raised the interesting possibility that a protein molecule with similar characteristics to those of *Xenopus* nucleoplasmin could also be present in the oocytes of *Mytilus*. In fact, a nucleoplasmin-like protein has recently been identified in the oocytes of the bivalve mollusc *S. solidissima* (Herlands & Maul, 1994). Unfortunately, however, the relationship of this molecule to the processes of nuclear expansion and chromatin decondensation observed

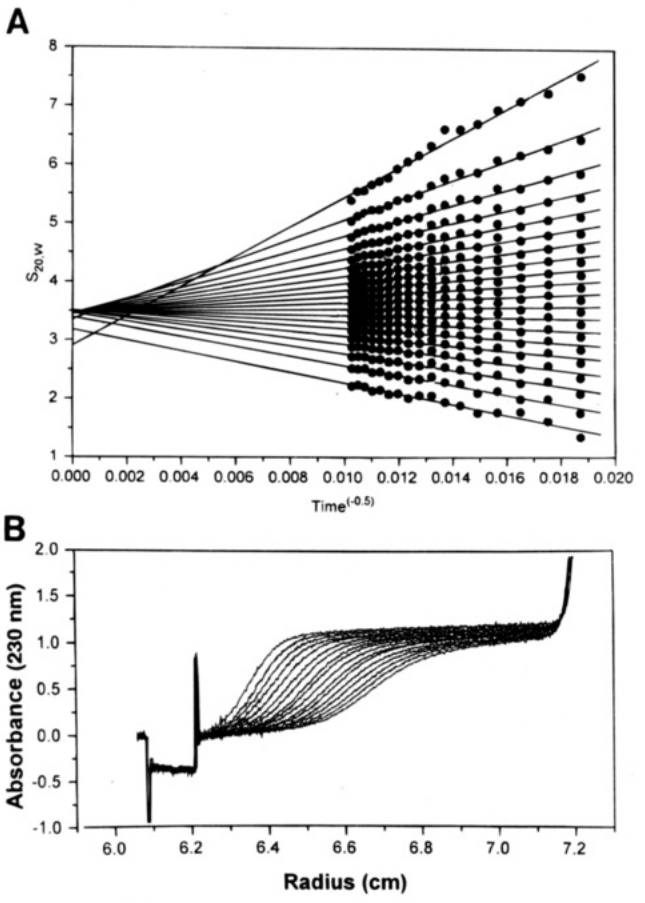

FIGURE 4: (A) Sedimentation velocity analysis of *Mytilus* nucleoplasmin-like protein. In this kind of analysis the number of lines converging toward a common $s_{20,w}$ value is proportional to the fraction of sample represented (van Holde & Weischet, 1978). (B) Scans (at 230 nm) of the sedimenting boundaries used in the sedimentation analysis shown in (A). The starting absorbance of the sample was $A_{230} = 1.2$, the speed was 44 000 rpm, and the temperature was 20 °C.

by Longo et al. (1994) was not established.

Following a procedure similar to that used for the preparation of *Xenopus* egg extracts (Itoh et al., 1993), we prepared high-speed and heated extracts from *Mytilus* oocytes. Figure 3A shows the protein composition of these extracts. As it can be seen in Figure 3A (lane 1), the heated extracts contain a major protein with an apparent molecular weight of 58 200 which is thermostable. This protein is also present in significant abundance in the high-speed extract (see Figure 3A, lane 2) before heat denaturation. In fact, as it can be seen in this lane, the protein is present in the same abundance as the histones from the egg, which can be clearly distinguished. Thus, as in the case of *Xenopus* nucleoplasmin, the main thermostable protein of the *Mytilus* egg extracts is an abundant protein. Comparison of the heated egg extracts from *Mytilus* and *Xenopus* (see Figure 3B) indicates that the 58 kDa protein from *Mytilus* is present in larger amount.

Using ionic exchange and hydrophobic interaction fractionation, we have been able to purify the 58 kDa protein to a high extent (see Figure 3C). The extent of homogeneity of the protein purified in this way can also be assessed from the sedimentation velocity analysis (see Figure 4). The molecule sediments as a monodisperse protein with a sedimentation coefficient $s_{20,w} = 3.5$ S. The purified protein also has the same sperm-nuclei decondensing activity observed in the case of *Xenopus* extracts and/or nucleoplasmin (see Figure 5). As in this later case (see Figure 1B,C), a bimodal distribution for the extent of nuclear expansion was observed with diameters of 3.8 ± 0.4 μm (Figure 5B) and 5.7 ± 0.2 μm (Figure 5C).

Figure 3C shows a comparative electrophoretic analysis of the heat-stable proteins isolated and purified from egg extracts of *Xenopus*, *Mytilus*, and *Spisula*. The amino acid composition of these proteins is shown in Table 1. As it can be seen there, all these proteins are highly enriched in acidic amino acids (Asp + Glu, 27−32%) and exhibit very similar amino acid compositions despite their different origins. This fact, together with the ability of *Xenopus* nucleoplasmin to decondense sperm nuclei in heterologous systems, suggests that these proteins may have been evolutionarily conserved at least at the compositional and functional level. At the structural level each one of these proteins exhibits different molecular weights.

From the polyacrylamide−SDS gels such as that shown in Figure 3C the apparent molecular weights determined by this technique were found to be 24 000 (*Xenopus* nucleoplasmin) 58 200 (*Mytilus*), and 67 700−75 000 (double band of *Spisula*). The 24 000 value obtained for *Xenopus* nucleoplasmin is in good agreement with that of 22 152 established from its primary structure (Dingwall et al., 1987). However, there seems to be an important difference between the apparent molecular weights reported here and those previously reported of 43 000 and 49 000 using the same electrophoretic analysis (Herlands & Maul, 1994). This is despite the fact that in both cases the protein appears to be

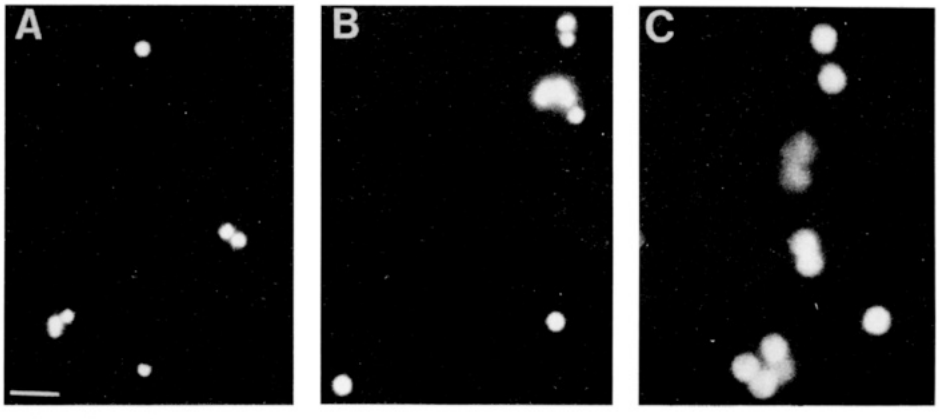

FIGURE 5: Decondensation of *Mytilus* sperm nuclei (B, C) by *Mytilus* nucleoplasmin-like protein (ca. 1 mg/mL) upon incubation for 90 min at room temperature. (A) Sperm nuclei incubated in the absence of the nucleoplasmin-like protein. The bar is 10 μm.

Table 1: Amino Acid Composition (mol %) of *Mytilus* (M) Nucleoplasmin-like Protein in Comparison to Nucleoplasmin from *Xenopus* (NP) and to a Nucleoplasmin-like Protein of the Oocytes from *Spisula* (S)

	NP[a]	NP[b]	M	S[c]	S[d]
Lys	11.3	12.5	5.0	8.7	7.4
His	2.2	1.0	1.8	0.7	0.8
Arg	2.2	2.5	1.7	1.6	1.5
Asx	9.2	7.0	9.9	9.4	10.7
Thr	5.4	5.5	6.3	3.4	6.3
Ser	6.7	6.5	14.1	8.4	7.7
Glx	20.0	20.5	16.6	23.4	22.1
Pro	7.3	6.0	4.0	5.4	7.4
Gly	8.2	7.0	8.1	8.0	7.3
Ala	7.2	9.0	7.3	9.6	6.0
$^1/_2$-Cys		1.5	1.5	nd[e]	
Val	6.2	5.5	5.3	5.1	4.7
Met	1.0	2.0	4.6	1.7	4.4
Ile	3.2	3.5	3.2	3.0	1.8
Leu	6.2	6.0	5.3	7.3	7.2
Tyr	2.1	1.0	3.0	2.2	6.8
Phe	2.3	2.0	2.5	2.1	4.2
Trp		1.0			

[a] From Earnshaw et al. (1980). [b] From the cDNA sequence (Dingwall et al., 1987). [c] This work. [d] From Herlands and Maul (1994). [e] nd = not determined.

a double band, and it exhibits an almost identical amino acid composition (see Table 1). This suggests that the disagreement might be of a technical nature, perhaps an incorrect assignment of the molecular weight standards. Alternatively, the differences in gel mobility could be accounted for by the presence of extensive differences in the levels of phosphorylation such as those observed between nucleoplasmin from eggs and oocytes in *Xenopus* (Sealy et al., 1986). Despite this discrepancy, it is clear that, in the case of the marine invertebrates, the size of the molecules is 2 to 3 times larger than *Xenopus* nucleoplasmin. However, *Xenopus* nucleoplasmin in its native conformation exists in a pentameric form (Earnshaw et al., 1980) of approximate molecular mass 110 000. Our laboratory is presently involved in the physical characterization (sedimentation equilibrium and circular dichroism) of the marine invertebrate nucleoplasmin-like proteins and the analysis of their native oligomerization state. This may help to explain the apparent structural diversity existing between the vertebrate–invertebrate systems as well as to account for the discrepancies on the apparent molecular masses reported for the *Spisula* nucleoplasmin-like protein.

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REFERENCES

Allen, R. D. (1953) *Biol. Bull. 105*, 213–230.
Ausio, J. (1986) *Comp. Biochem. Physiol. 85B*, 439–449.
Ausio, J. (1995) in *Advances in Spermatozoal Taxonomy and Phylogeny* (Jamieson, B. G. M., Ausio, J., & Justine, J. L., Eds.) Memoires de Muséum National d'Histoire Naturelle, Paris (in press).
Ausio, J., & Subirana, J. A. (1982a) *Exp. Cell. Res. 141*, 39–45.
Ausio, J., & Subirana, J. A. (1982b) *J. Biol. Chem. 257*, 2802–2805.
Ausio, J., van der Goot, G. F., & Buckley, J. T. (1993) *FEBS Lett. 333*, 296–300.
Bloch, D. P. (1969) *Genetics (Suppl.) 61*, 93–110.
Brown, D. B., Blake, E. J., Wolgemuth, D. J., Gordon, K., & Ruddle, F. H. (1987) *J. Exp. Zool. 242*, 215–231.
Carlos, S., Jutglar, L., Borrell, J. I., Hunt, D. F., & Ausio, J. (1993a) *J. Biol. Chem. 268*, 185–194.
Carlos, S., Hunt, D. F., Rocchini, C., Arnott, D. P., & Ausio, J. A. (1993b) *J. Biol. Chem. 268*, 195–199.
Dingwall, C., Dilworth, S. M., Black, S. J., Kearsey, S. E., Cox, L. S., & Laskey, R. A. (1987) *EMBO J. 6*, 69–74.
Earnshaw, W. C., Honda, B. M., Laskey, R. A., & Thomas, J. O. (1980) *Cell 21*, 373–383.
Herlands, L., & Maul, G. G. (1994) *Dev. Biol. 161*, 530–537.
Itoh, T., Ohsumi, K., & Katagiri, C. (1993) *Dev. Growth Differ. 35*, 59–66.
Jutglar, L., Borrell, J. I., & Ausio, J. (1991) *J. Biol. Chem. 266*, 8184–8191.
Kasinsky, H. E. (1989) in *Histones and Other Basic Nuclear Proteins* (Hnilica, L. S., Stein, G. S., & Stein, J. L., Eds.) pp73–163, CRC Press, Boca Raton, FL.
Katagiri, C., & Ohsumi, K. (1994) *J. Dev. Biol. 38*, 209–216.
Kawasaki, K., Philpott, A., Avillion, A. A., Berrios, M., & Fisher, P. A. (1994) *J. Biol. Chem. 269*, 10169–10176.
Kleinschmidt, J. A., Fortkamp, E., Krohne, G., Zentgraf, H., & Franke, W. (1985) *J. Biol. Chem. 260*, 1166–1176.
Krohne, G., & Franke, W. W. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1034–1038.
Laemmli, U. K. (1970) *Nature 227*, 680–685.
Laskey, R. A., Mills, A. D., & Morris, N. R. (1977) *Cell 10*, 237–243.
Laskey, R. A., Honda, B. A., Mills, A. D., & Finch, J. T. (1978) *Nature 275*, 416–420.
Longo, F. J. (1985) in *Biology of Fertilization* (Mets, C. B., & Monroy, A., Eds.) Vol. 3, pp 251–298, Academic Press, New York.
Longo, F. J., Matthews, L., & Palazzo, R. E. (1994) *Dev. Biol. 162*, 245–258.
Maul, G. G. (1980) *Exp. Cell. Res. 129*, 431–438.
Mills, A. D., Laskey, R. A., Black, P., & De Robertis, E. M. (1980) *J. Mol. Biol. 139*, 561–568.
Ohsumi, K., & Katagiri, C. (1991) *Dev. Biol. 148*, 295–305.
Ohsumi, K., Katagiri, C., & Yanagimachi, R. (1988) *Gamete Res. 20*, 1–9.
Perkins, S. J. (1986) *Eur. J. Biochem. 157*, 169–180.
Philpott, A., & Leno, G. H. (1992) *Cell 69*, 759–767.
Philpott, A., Leno, G. H., & Laskey, R. A. (1991) *Cell 65*, 469–478.
Poccia, D. (1986) *Int. Rev. Cytol. 105*, 1–65.
Sealy, L., Cotten, M., & Chalkley, R. (1986) *Biochemistry 25*, 3064–3072.
Sealy, L., Burgess, R. R., Cotten, M., & Chalkley, R. (1989) *Methods Enzymol. 170*, 612–630.
van Holde, K. E., & Weischet, W. O. (1978) *Biopolymers 17*, 1387–1403.

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