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Cytoplasm of Sea Urchin Unfertilized Eggs Contains a Nucleosome Remodeling Activity

Ricardo Medina, José Gutiérrez, Marcia Puchi, María Imschenetzky, and Martin Montecino*

Departamento de Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepcion, Concepcion, Chile

Abstract After fertilization the sea urchin sperm nucleus transforms into the male pronucleus which later fuses with the female pronucleus re-establishing the diploid genome of the embryo. This process requires remodeling of the sperm chromatin structure including the replacement of the sperm histones by maternally derived cleavage stage histone variants. In recent years, a group of protein complexes that promote chromatin-remodeling in an ATP-dependent manner have been described. To gain understanding into the molecular mechanisms operating during sea urchin male pronuclei formation, we analyzed whether chromatin-remodeling activity was present in unfertilized eggs as well as during early embryogenesis. We report that in the sea urchin *Tetrapygus niger*, protein extracts from the cytoplasm but not from the nucleus, of unfertilized eggs exhibit ATP-dependent nucleosome remodeling activity. This cytosolic activity was not found at early stages of sea urchin embryogenesis. In addition, by using polyclonal antibodies in Western blot analyses, we found that an ISWI-related protein is primarily localized in the cytoplasm of the sea urchin eggs. Interestingly, SWI2/SNF2-related proteins were not detected neither in the nucleus nor in the cytoplasm of unfertilized eggs. During embryogenesis, as transcriptional activity is increased an ISWI-related protein is found principally in the nuclear fraction. Together, our results indicate that the cytoplasm in sea urchin eggs contains an ATP-dependent chromatin-remodeling activity, which may include ISWI as a catalytic subunit. J. Cell. Biochem. 83: 554–562, 2001.

Key words: nucleosome remodeling; sea urchin

Sea urchin sperm chromatin is a tightly packaged structure formed by the interaction of sperm-specific histone proteins with the DNA within the sperm nucleus [Ausio, 1995]. Following fertilization, the sperm nucleus decondenses and transforms into the male pronucleus, which later fuses with the female pronucleus to re-establish the diploid genome of the embryo. This process requires chromatin-remodeling and involves the removal of sperm basic nuclear proteins and their replacement by histone proteins of maternal origin [Poccia, 1986]. It has been indicated that sperm chromatin-associated proteins are degraded by proteolytic activities present in the unfertilized egg and that this degradation is required for male pronuclei formation and subsequent amphimixis [Cameron and Poccia, 1994; Imschenetzky et al., 1997]. However, it has not been established whether this degradation takes place while the proteins remain associated to the sperm chromatin or after they are released from it.

It has been described that protein transitions during male pronuclei formation are promoted by nucleoplasmin, a widely distributed acid protein that functions as a chaperone [Philpott et al., 2000]. Hyperphosphorylated nucleoplasmin stimulates the assembly of nucleosome cores by facilitating the addition of histone

Abbreviations used: ISWI, *Drosophila* imitation SWI protein; DNase I, deoxyribonuclease I; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether) N, N, N′, N′-tetraacetic acid; HEPES, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]; kb, kilobase(s); PMSF, phenylmethylsulfonyl fluoride; TPCK, N-tosyl-L-phenylalanine chloromethyl ketone; PAGE, polyacrylamide gel electrophoresis; OC, osteocalcin.

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*Correspondence to: Dr. Martin Montecino, Departamento de Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepcion, casilla 160-C, Concepcion, Chile. E-mail: mmonteci@udec.cl

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H2A/H2B dimers to preformed histones H3/H4 tetramers. In addition, the involvement of the chaperones N1 and N2 in the disassembly of nuclear sperm-specific basic proteins in *Xenopus laevis* has been postulated [Leno et al., 1996].

In recent years, a group of protein complexes that promote changes in chromatin structure have been described [Peterson, 2000; Vignali et al., 2000]. The catalytic subunit in all of these complexes is an ATPase and requires ATP hydrolysis to promote alterations in the nucleosomal organization. Most chromatin-remodeling complexes identified are classified as those containing homologues of the yeast SWI2/SNF2 ATPase subunit, including the BRG1 and BRM complexes [Tamkun et al., 1992; Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996] or those containing homologues of the *Drosophila* imitation SWI (ISWI), including NURF, CHRAC, and ACF complexes [Tsukiyama et al., 1995; Ito et al., 1997; Varga-Weisz et al., 1997].

Multiple ISWI-containing chromatin-remodeling complexes have been recently identified in *X. laevis* [Guschin et al., 2000]. In addition, a BRG1-containing complex was isolated and characterized from *X. laevis* nuclear extracts and found to be present throughout oogenesis (stages I–VI) and embryogenesis [Gelius et al., 1999]. Interestingly, Wolffe and colleagues have reported that cytoplasmic extracts from *X. laevis* eggs contain a chromatin-remodeling activity that includes ISWI as a catalytic subunit. This remodeling activity promotes incorporation of proteins involved in pronuclei formation (e.g., linker histone B4 and nucleoplasmin) to a transplanted somatic nuclei in an energy-dependent manner [Kikyo et al., 2000]. However, the role of chromatin-remodeling complexes in male pronuclei formation has not been established.

To gain insight into the molecular mechanism operating during sea urchin male pronuclei formation, we have analyzed whether unfertilized eggs contain chromatin-remodeling activity. We have found that cytoplasmic, but not nuclear, protein extracts isolated from eggs of the sea urchin *Tetrapygus niger* exhibit ATP-dependent nucleosome remodeling activity. In addition, using specific polyclonal antibodies, we detected significantly higher concentrations of ISWI, but not BRG1, in the cytoplasm of these unfertilized eggs. Taken together, our results show for the first time that the cytoplasm from sea urchin eggs contains an ATP-dependent chromatin-remodeling activity, which may include ISWI as a catalytic subunit.

MATERIALS AND METHODS

Cell Culture

Sea urchin *T. niger* were collected from the Bay of Concepcion and maintained at 15°C in natural seawater. Eggs were collected by gently shaking the female gonads into filtered natural seawater, and rinsed several times by filtering through a 100 μm plankton net. Sperm cells were collected on a Petri dish. Embryos were grown at 15°C with gentle agitation and aeration to the desired stage of development.

Preparation of Nuclear and Cytoplasmic Extracts

Protein extracts from the cytoplasm or nucleus of sea urchin eggs and embryos were prepared according to Dignam et al. [1983] with minor modifications. All procedures were performed at 4°C. Eggs or embryos were collected by centrifugation at 50*g* for 5 min, washed in a solution containing 1.5 M dextrose, 10 mM HEPES pH 7.5, and 0.1 mM EDTA and centrifugated at 100*g* for 5 min. The eggs or embryos were then resuspended in 5 volumes of complete buffer A [buffer A (10 mM HEPES, pH 7.5, 10 mM KCl, 0.2 mM EDTA) supplemented with 0.75 mM spermidine, 0.15 mM spermine, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 100 μg/ml TPCK, 1 μg/ml leupeptin, 1 μg/ml pepstatin] and kept on ice for 10 min. This procedure was repeated twice centrifugating at 270*g* for 5 min each time. The cell suspension was then homogenized in the same buffer using a Dounce homogenizer (Pestle A) with 5–15 strokes. The integrity of nuclei was microscopically assessed following Trypan blue staining. The lysed cell suspension was filtered through a 55 μm plankton net to retain intact cells and centrifugated at 1,000*g* for 15 min. The cytosolic supernatant was supplemented with 0.3 volumes of 80% glycerol, frozen in liquid nitrogen, and stored at −80°C until use. The nuclear pellet was washed in 5 volumes of complete buffer A and then centrifugated at 17,000*g* for 10 min. The pellet was then extracted for 30–60 min under constant agitation in 1 volume of high salt complete buffer C [buffer C (20 mM HEPES, pH 7.5, 420 mM KCl, 25% glycerol,

0.2 mM EDTA) supplemented with 0.75 mM spermidine, 0.15 mM spermine, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 100 μg/ml TPCK, 1 μg/ml leupeptin, 1 μg/ml pepstatin]. Following centrifugation at 19,000*g* for 15 min, the supernatant containing the nuclear extract was separated and then rapidly frozen in liquid nitrogen and stored at −80°C until use.

Nucleosome Reconstitution

Labeled DNA fragments of about 150 bp containing either the sequence −161 to −10 from the rat osteocalcin (OC) gene promoter or −163 to −10 from the sea urchin early histone gene promoter [Medina et al., 2001], were generated by PCR using specific primers, one of which had been previously end-labeled with polynucleotide kinase (New England Biolabs, Beverly, MA) and [γ-^{32}P]ATP (New England Nuclear, Boston, MA). The labeled fragments were purified using G-50 Quick Spin Columns (Boehringer Mannheim, Indianapolis, IN) and then reconstituted into nucleosomes by the histone octamer transfer method or mock reconstituted (negative control) as previously described [Gutierrez et al., 2000]. Successful nucleosome reconstitution was verified by analyzing reconstituted samples by electrophoresis in native polyacrylamide gels and autoradiography. In addition, the cleavage pattern generated by incubation of these mononucleosomes with DNase I, micrococcal nuclease and specific restriction enzymes was determined. Specific primers used in the PCR reactions: for the OC promoter, PR1-pOC3.4B (5′-CCTTCGCCCCGGCAGC-3′) and PR2-pOC3.4B (5′-CTGTTCTGCAATACCTTTTATATCC-3′). For the early histone H3 promoter, PR1-pp1TnH3 (5′-CACTCTCTGCTTTGA-GATCC-3′) and PR2-pp1TnH3 (5′-CAAATGCGGTGCGTATTTAT-3′).

Nucleosome Remodeling Analysis

Nucleosome remodeling was analyzed either by testing changes in the DNase I digestion pattern or by evaluating increases in the restriction enzyme accessibility. For the DNase I analyses, reactions were performed in a 40 μl total volume containing 10 μg of nuclear extract and 40 fmol of labeled reconstituted mononucleosomes in the buffer 12 mM HEPES pH = 7.5, 60 mM KCl, 7 mM $MgCl_2$, 2 mM DTT, 13% glycerol, 80 μg/ml BSA, and 60 μM EDTA. Following incubation for 40 min at 30°C, the reaction mix was supplemented with 1 mM $CaCl_2$ and digestions with DNase I (Worthington, Freehold, NJ) (0.02 U for mock reconstituted DNA and 0.2 U for the nucleosomal DNA) were performed for 2 min at room temperature (18°C). The digestions were stopped by addition of a solution containing 92% ethanol, 7 μg/ml tRNA, and 0.7 M ammonium acetate. The DNA fragments were fractionated on a 6% sequencing gel and detected by autoradiography. For the restriction enzyme accessibility assays, reactions were performed in a 20 μl total volume of buffer (12 mM HEPES pH = 7.5, 60 mM KCl, 7 mM $MgCl_2$, 2 mM DTT, 13% glycerol, 80 μg/ml BSA, 60 μM EDTA), containing 10 μg of protein extract and 5 fmol of labeled reconstituted mononucleosomes. The protein extracts were depleted of ATP by gel filtration using G-25 columns (Boehringer Mannheim). Following 40 min of incubation at 30°C, the reaction mix was supplemented with 3 mM $MgCl_2$ and 20 μg/ml BSA, and then digested with 10 U of *Hha* I during 30 min at 30°C. Reactions were stopped by addition of 20 mM EDTA, 0.5% SDS, and 50 μg/ml of Proteinase K and then digested for 60 min at 37°C. After phenol extraction and ethanol precipitation, the samples were analyzed by electrophoresis on a 5% polyacrylamide gel (29:1) and visualized by autoradiography.

Western Blot Analysis

Protein extracts were electrophoresed in a 7.5% SDS–PAGE gel and blotted onto a nitrocellulose membrane (BIO-RAD, Hercules, CA). Immunodetection was performed using a 1:1,000 or 1:2,000 dilution of either anti human BRG1 or anti ISWI primary rabbit antisera, respectively (kindly donated by Dr. Anthony Imbalzano, University of Massachusetts Medical School and by Dr. Patrick Varga-Weisz, Marie Curie Research Institute, UK), and by using the Renaissance Chemiluminescence Plus reagent (NEN Life Science, Boston, MA).

RESULTS

Nucleosome Remodeling Activity is Found in the Cytoplasmic Fraction of Unfertilized Sea Urchin Eggs

Following fertilization, the sea urchin sperm nucleus decondenses and transforms into the male pronucleus, which later fuses with the female pronucleus to re-establish the diploid

genome of the embryo. To gain insight into the molecular mechanisms operating during sea urchin male pronuclei formation, we investigated whether sea urchin eggs contained chromatin-remodeling activity. We evaluated the presence of this activity in either cytosolic or nuclear protein fractions and determined their requirement for ATP hydrolysis to function.

As a source of chromatin for the remodeling assays, we reconstituted DNA segments of about 150 bp into histone H1-free mononucleosomes by the histone octamer transfer method as described in materials and methods. Successful and efficient nucleosomal reconstitution was verified analyzing samples by electrophoresis in native polyacrylamide gels as well as by assessing resistance to digestion enzymes or micrococcal nuclease digestion. As shown in Figure 1, our reconstitutions showed a high degree of packaging (above 75%) and resistance to cleavage by the restriction endonuclease *Hha* I (Fig. 1A). Similarly, the reconstituted mononucleosomes were micrococcal nuclease-resistant as compared with the mock-reconstituted DNA fragments, which resulted completely digested at significantly lower enzyme concentrations (Fig. 1B, compare lanes 4 and 8).

We next evaluated nucleosome remodeling activity by analyzing if proteins present in either partially purified cytoplasmic or nuclear fractions could alter the periodic cutting pattern every 10 bp, characteristic of nucleosomal DNA cleaved with DNase I [Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996]. It was found that protein fractions isolated from the cytoplasm of sea urchin eggs contained nucleosome remodeling activity (Fig. 2A). Moreover, this activity was found to be ATP-dependent as it was not detected in the absence of ATP (Fig. 2A, compare lanes 1 and 2).

Interestingly, we did not find nucleosome remodeling activity when the nuclear extracts from unfertilized sea urchin eggs were tested

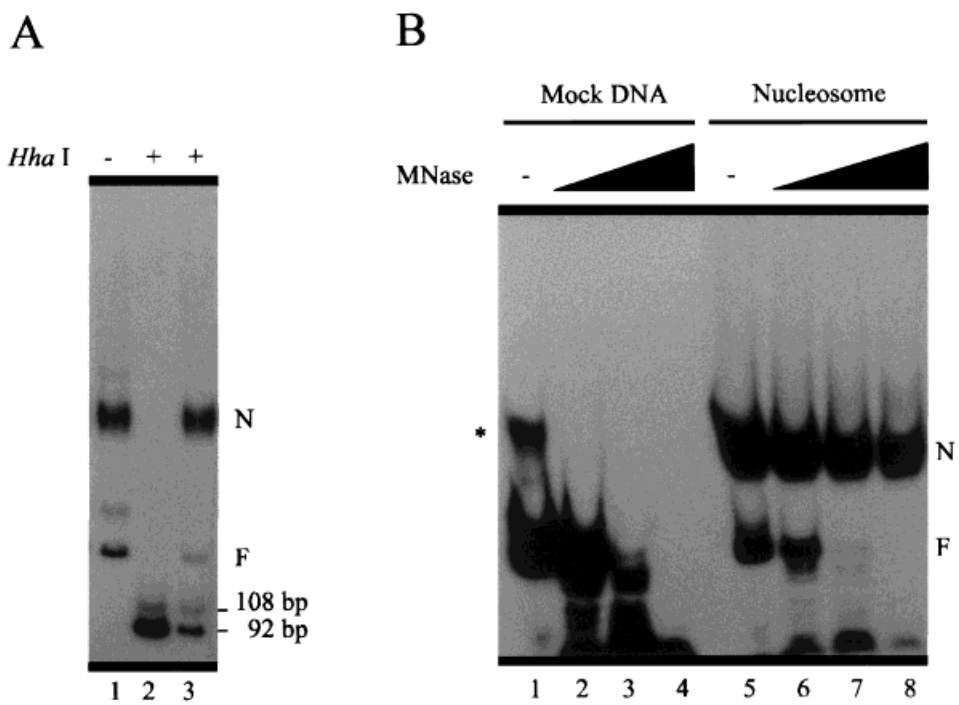

Fig. 1. Reconstitution of the nucleosomal particles. End-labeled DNA segments containing sequences −163 to −10 from the sea urchin early histone H3 gene promoter (A) and −161 to −10 from the rat osteocalcin (OC) gene promoter (B) were reconstituted as mononucleosomes by the histone octamer transfer method as described [Gutierrez et al., 2000]. Successful nucleosomal reconstitution was then evaluated by assessing resistance of the particles to nuclease digestion. **A**: Resistance to *Hha* I cleavage for 30 min at 30°C. **Lane 1**: Reconstituted nucleosome alone; **lane 2**: mock-reconstituted DNA cleaved with *Hha* I; **lane 3**: reconstituted nucleosome cleaved with *Hha* I. The position of the labeled cleavage product (92 bp), the nucleosome (N), and the free DNA (F) is marked at the right side of the gel. **B**: Incubation with increasing concentrations of micrococcal nuclease (MNase) for 5 min at 37°C. **Lanes 1–4**: Mock reconstituted DNA segments, and **lanes 5–8**: reconstituted mononucleosomes. The position of the nucleosome (N) and the free DNA (F) is marked at the right side of the gel. The asterik (*) marks the presence of a non-specific labeled product generated during the PCR amplification.

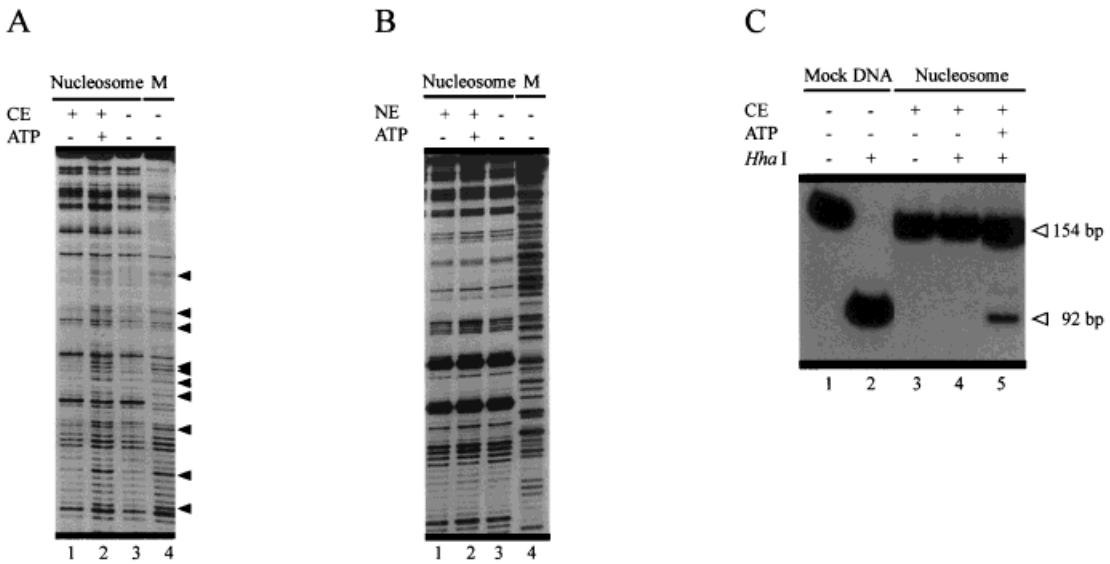

Fig. 2. The cytoplasm of sea urchin eggs contains an ATP-dependent nucleosome remodeling activity. The presence of nucleosome remodeling activity in the cytosolic (CE) or nuclear (NE) extracts of sea urchin eggs was assessed as described in materials and methods. **A**: Cytosolic extracts (10 μg) were incubated with end-labeled mononucleosomes in the presence (**lane 2**) or absence (**lane 1**) of ATP (also marked at the top of the gel). The samples were then digested with DNase I and the cleavage products analyzed in a sequencing gel and autoradiography. **Lane 4**: Mock-reconstituted DNA (M) digested with DNase I. The arrowheads at the right of the gel, mark the subbands that represent the loss of DNase I periodic cutting every 10 bp. **B**: Nuclear extracts (10 μg) assayed for nucleosome remodeling activity as described in A. **C**: Following incubation with cytosolic extracts (10 μg), the nucleosomes were digested with *Hha* I as described in materials and methods. Then, the purified DNA samples were fractionated by electrophoresis on a 5% polyacrylamide gel, dried, and visualized by autoradiography. The components added to each of the samples are summarized at top of the gel.

(Fig. 2B). The addition of an excess of ATP or further purification of these nuclear extracts did not change this result (data not shown).

To further confirm the presence of chromatin-remodeling activity in the cytoplasm of sea urchin eggs, we evaluated whether incubation of reconstituted nucleosomes with this extract resulted also in accessibility to restriction enzymes. As shown in Figure 2C, we found significantly higher cleavage with *Hha* I only in the mononucleosomes pre-incubated with cytosolic extract and ATP (Fig. 2C, lane 5). Thus, because the cutting sequence for *Hha* I is localized internal into the nucleosome core particle (92 bp from the 5′-end and 62 bp from the 3′end of the 154 bp DNA segment), it requires a previous ATP-dependent remodeling step to become accessible for cleavage.

Taken together, our findings indicate that unfertilized sea urchin eggs contain an ATP-dependent chromatin-remodeling activity localized primarily in the cytoplasm of the egg. In contrast, the nuclear fraction is devoid of significant levels of this activity.

Cytoplasm of Sea Urchin Embryos at Early Stages of Development Does not Contain Chromatin-Remodeling Activity

We investigated whether the chromatin-remodeling activity detected in the cytoplasm of sea urchin eggs could still be found following fertilization, throughout the initial stages of embryogenesis.

After fertilization in vitro, sea urchin embryos were cultured until they reached either the 32- or the 128-cell stage. The embryos were then harvested, cytosolic protein extracts prepared, and the presence of nucleosome remodeling activity tested. As shown in Figure 3, we did not find chromatin-remodeling activity in the cytoplasm of embryos at the 32- or 128-cell stage. Addition of an excess of ATP did not change this result (data not shown).

Hence, these results indicate that in contrast to unfertilized eggs, sea urchin embryos at early stages of development do not contain nucleosome remodeling activity in the cytoplasm.

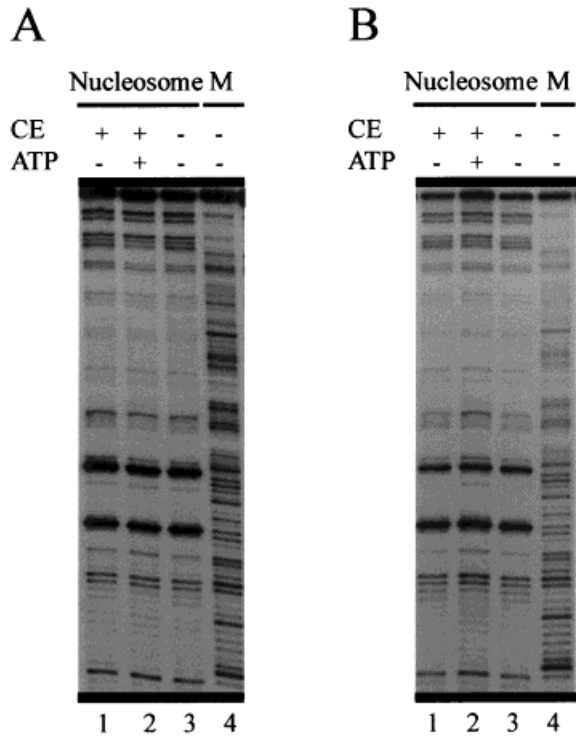

Fig. 3. The cytoplasm of sea urchin embryos does not contain nucleosome remodeling activity. Cytosolic protein extracts (CE) (10 μg) isolated from sea urchin embryos at the 32 (**A**) and 128 (**B**) cell stages of development, were analyzed for nucleosome remodeling activity as described in materials and methods. For both gels, **lane 1**: nucleosome + CE; **lane 2**: nucleosome - + CE + ATP (4 mM); **lane 3**: nucleosome alone; **lane 4**: mock-reconstituted DNA (M) digested with DNase I.

ISWI is Located Primarily in the Cytoplasm of Sea Urchin Eggs

Most chromatin-remodeling complexes identified are classified as those containing homologues of the yeast SWI2/SNF2 ATPase subunit, including BRG1 and BRM complexes [Tamkun et al., 1992; Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996] and those containing homologues of the *Drosophila* ISWI, including NURF, CHRAC, and ACF complexes [Tsukiyama et al., 1995; Ito et al., 1997; Varga-Weisz et al., 1997].

To determine whether BRG1 or ISWI homologues were present in the nuclear or cytosolic fractions of sea urchin eggs, we performed western blot analyses using specific polyclonal antibodies directed against the mammalian isoforms of these nucleosome remodeling factors. As shown in Figure 4A, we found that a 130 kDa sea urchin ISWI homologue was enriched in the cytosolic fraction of sea urchin eggs, although significantly lower concentrations were also detected in the nuclear fraction. We neither detected a BRG1-related (Fig. 4B) nor a BRM-related (data not shown) subunit within the cytosolic or nuclear fractions of sea urchin eggs.

It was also found that the concentration of the ISWI-related protein in the cytoplasm was decreased following fertilization (Fig. 4C), especially after the 4-cell stage of embryogenesis (Fig. 4C, compare lanes 2 and 3). Interestingly, this ISWI homologue remained in the nucleus during early embryogenesis (Fig. 4D), suggesting the presence of nuclear chromatin-remodeling activity containing ISWI as catalytic subunit. The preferential nuclear localization of ISWI correlates with the increase in transcriptional activity observed during sea urchin development [Lee et al., 1999; Medina et al., 2001].

Taken together, these results indicate that in sea urchin eggs, the chromatin-remodeling activity localized in the cytoplasm might contain an ISWI-related protein, but not BRG1, as a catalytic subunit. Our findings also suggest that following fertilization, and correlating with the enhancement in transcriptional activity during sea urchin development, the ISWI subunit is preferentially localized in the nucleus.

DISCUSSION

Sea urchin sperm chromatin is a tightly packaged structure formed by the interaction of sperm-specific histone proteins with the DNA within the sperm nucleus [Ausio, 1995]. Following fertilization the sperm chromatin is remodeled in a process that involves the removal of sperm basic nuclear proteins and their replacement by histone proteins of maternal origin [Poccia, 1986]. Here, we have investigated the presence of chromatin-remodeling activities that might be facilitating the removal of the sperm-specific proteins and their subsequent replacement process.

We determined that an ATP-dependent chromatin-remodeling activity is present in the cytoplasm but not in the nucleus, of unfertilized eggs from the sea urchin *T. niger*. Moreover, using specific polyclonal antibodies that recognize the mammalian ISWI ATPase, we determined that an ISWI-related sea urchin protein is present in significantly higher concentrations in the cytoplasm of unfertilized eggs. Surprisingly, we did not find BRG1 homologues in the

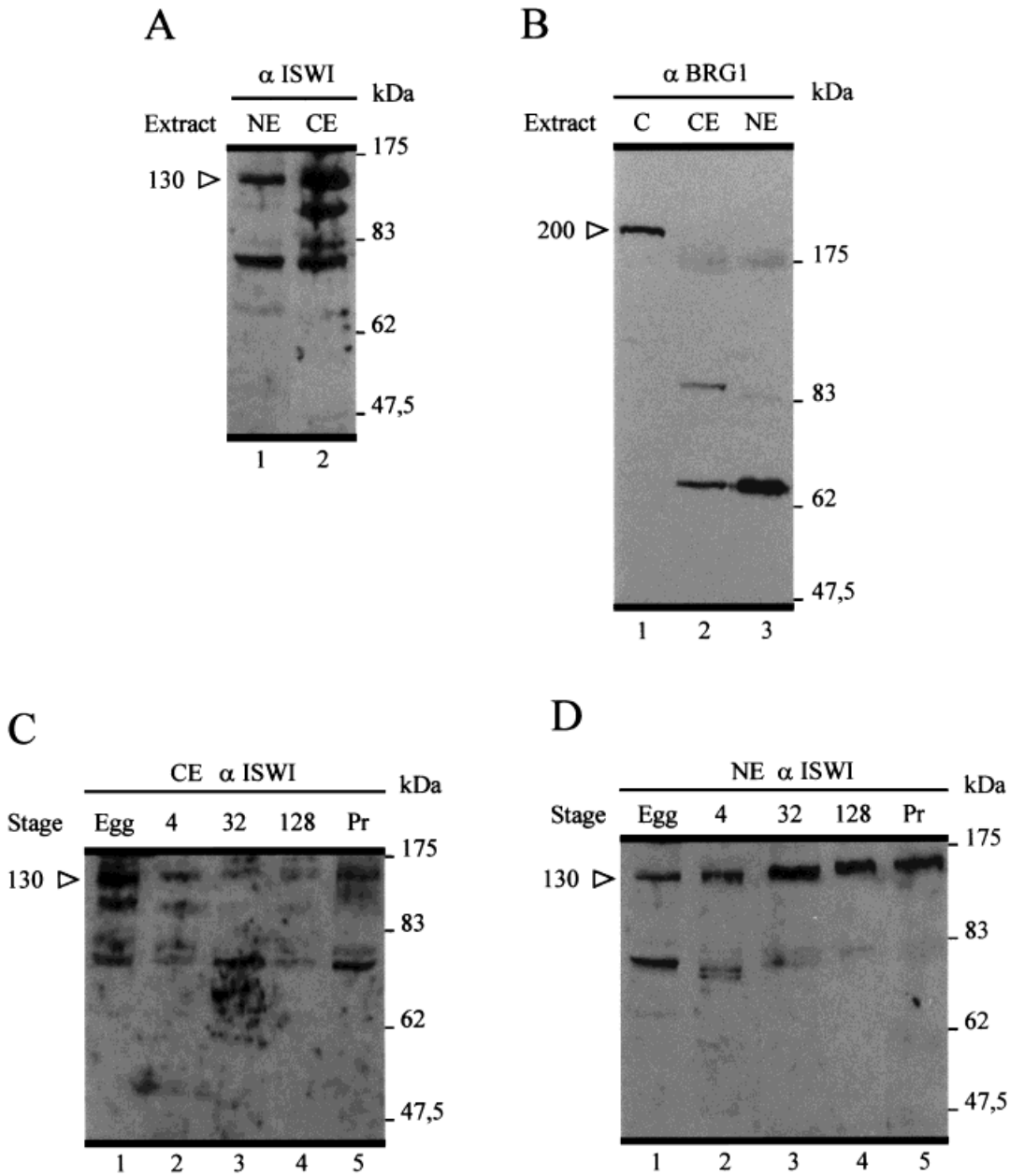

Fig. 4. An ISWI-related protein is localized in the cytoplasm of sea urchin eggs. The presence of ISWI and BRG1 homologues in the protein extracts isolated from sea urchin eggs and embryos was assessed by Western blot using polyclonal antobodies raised against the mammalian isoforms of these chromatin-remodeling factors as described in materials and methods. **A**: 15 μg of nuclear (NE) or cytosolic (CE) extracts were loaded and revealed with the anti ISWI antiserum. **B**: 20 μg of nuclear extract from the rat-derived ROS 17/2.8 cell line (**lane 1**) used as a control and 35 μg of sea urchin egg CE (**lane 2**) or NE (**lane 3**) were loaded and revealed with the anti BRG1 antiserum. **C**: CE (20 μg) obtained from sea urchin eggs (lane 1) or embryos at the 4-cell (**lane 2**), 32-cell (lane 3), 128-cell (**lane 4**), and prisma larvae (**lane 5**) stages (marked also at the top of the gel) were loaded and revealed with the anti ISWI antiserum. **D**: NE (20 μg) obtained from sea urchin eggs (lane 1) or embryos at the 4-cell (lane 2), 32-cell (lane 3), 128-cell (lane 4), and prisma larvae (lane 5) stages (marked also at the top of the gel) were loaded and revealed with the anti ISWI antiserum. In all the gels, the position of either the 130 kDa band representing the ISWI homologue or the 200 kDa band corresponding to BRG1, is marked at the left. Also, the position of the molecular size markers is marked at the right of the gels.

cytosolic or nuclear protein fractions of these eggs, suggesting that the nucleosome remodeling activity detected in the cytoplasm may contain an ISWI-related subunit as a catalytic component.

This chromatin-remodeling activity was not detected in the cytoplasm from sea urchin embryos during early stages of development. Similarly, we found that during embryogenesis, the cytosolic concentration of the ISWI-related protein was significantly decreased and its nuclear localization increased, suggesting that following fertilization ISWI-containing chromatin-remodeling activity localize predominantly in the nuclei. Interestingly, this result correlates with the increased transcriptional activity found in these embryos. We have reported that in *T. niger*, the mRNA levels for the early histone genes which are one of the most abundantly transcribed genes following fertilization [Lee et al., 1999], are increased at the 32-cell stage [Medina et al., 2001].

Multiple ISWI- and BRG1-containing chromatin-remodeling complexes have been also recently identified in *X. laevis* [Gelius et al., 1999; Guschin et al., 2000]. Interestingly, it was found that cytoplasmic extracts from *X. laevis* eggs contain a chromatin-remodeling activity that includes ISWI as a catalytic subunit. This remodeling activity promotes the incorporation of proteins involved in pronuclei formation to transplanted somatic nuclei in an energy-dependent manner [Kikyo et al., 2000]. Although the authors do not establish a specific link between the presence of this activity and the male pronuclei remodeling process, its localization in the cytoplasm of this unfertilized *X. laevis* eggs, suggest a function unrelated to transcription.

It has been found that following fertilization, sea urchin sperm chromatin-associated proteins are degraded by proteolytic activities present in the unfertilized egg. This degradation is required for male pronuclei formation and subsequent amphimixis [Cameron and Poccia, 1994; Imschenetzky et al., 1997; Imschenetzky et al., 1999]. However, it has not been established whether this degradation takes place while the proteins remain associated to the sperm chromatin or after they are released from it. One attractive possibility is that the tightly packaged sperm chromatin may be remodeled as a result of the sequential activity of ATP-dependent chromatin-remodeling complexes and chaperones such as nucleoplasmin [Philpott et al., 2000], N1, and N2 [Leno et al., 1996] facilitating then the subsequent degradation of the chromatin-associated proteins. Experiments to test this mechanism are in progress.

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REFERENCES

Ausio J. 1995. Histone H1 and the evolution of the nuclear sperm-specific proteins. In: Jamieson BGM, Ausio J, Justine J-L, editors. Advances in spermatozoal taxonomy and phylogeny. Mem Mus Natl Hist Nat Paris 166:447–462.

Cameron LA, Poccia DL. 1994. In vitro development of the sea urchin male pronucleus. Dev Biol 162:568–578.

Cote J, Quinn J, Workman JL, Peterson CL. 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science 265:53–60.

Dignam JD, Lebovitz RM, Roeder RG. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11:1475–1489.

Gelius B, Wade P, Wolffe A, Wrange O, Ostlund Farrants AK. 1999. Characterization of a chromatin remodelling activity in Xenopus oocytes. Eur J Biochem 262:426–434.

Guschin D, Geiman TM, Kikyo N, Tremethick DJ, Wolffe AP, Wade PA. 2000. Multiple ISWI ATPase complexes from xenopus laevis. Functional conservation of an ACF/CHRAC homolog. J Biol Chem 275:35248–35255.

Gutierrez J, Sierra J, Medina R, Puchi M, Imschenetzky M, van Wijnen A, Lian J, Stein G, Stein J, Montecino M. 2000. Interaction of CBF alpha/AML/PEBP2 alpha transcription factors with nucleosomes containing promoter sequences requires flexibility in the translational positioning of the histone octamer and exposure of the CBF alpha site. Biochemistry 39:13565–13574.

Imbalzano AN, Kwon H, Green MR, Kingston RE. 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature 370:481–485.

Imschenetzky M, Diaz F, Montecino M, Sierra F, Puchi M. 1997. Identification of a cysteine protease responsible for degradation of sperm histones during male pronucleus remodeling in sea urchins. J Cell Biochem 67:304–315.

Imschenetzky M, Puchi M, Morin V, Diaz F, Oliver MI, Montecino M. 1999. Potential involvement of post-translational modifications as a mechanism modulating selective proteolysis after fertilization. J Cell Biochem Suppl 32(33):149–157.

Ito T, Bulger M, Pazin MJ, Kobayashi R, Kadonaga JT. 1997. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell 90:145–155.

Kikyo N, Wade PA, Guschin D, Ge H, Wolffe AP. 2000. Active remodeling of somatic nuclei in egg cytoplasm

by the nucleosomal ATPase ISWI. Science 289:2360–2362.

Kwon H, Imbalzano AN, Khavari PA, Kingston RE, Green MR. 1994. Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature 370:477–481.

Lee Y-H, Huang GM, Cameron RA, Graham G, Davidson EH, Hood L, Britten RJ. 1999. EST analysis of gene expression in early cleavage-stage sea urchin embryos. Development 126:3857–3867.

Leno GH, Mills AD, Philpott A, Laskey RA. 1996. Hyperphosphorylation of nucleoplasmin facilitates Xenopus sperm decondensation at fertilization. J Biol Chem 271:7253–7256.

Medina R, Paredes R, Puchi M, Imschenetzky M, Montecino M. 2001. Developmentally-regulated interaction of a transcription factor complex containing CDP/cut with the early histone H3 gene promoter of the sea urchin *Tetrapygus niger* is associated with changes in chromatin structure and gene expression. Gene 272:237–248.

Peterson CL. 2000. ATP-dependent chromatin remodeling: going mobile. FEBS Lett 476:68–72.

Philpott A, Krude T, Laskey RA. 2000. Nuclear chaperones. Semin Cell Dev Biol 11:7–14.

Poccia D. 1986. Remodeling of nucleoproteins during gametogenesis, fertilization, and early development. Int Rev Cytol 105:1–65.

Tamkun JW, Deuring R, Scott MP, Kissinger M, Pattatucci AM, Kaufman TC, Kennison JA. 1992. Brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell 68:561–572.

Tsukiyama T, Daniel C, Tamkun J, Wu C. 1995. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell 83:1021–1026.

Varga-Weisz PD, Wilm M, Bonte E, Dumas K, Mann M, Becker PB. 1997. Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature 388:598–602.

Vignali M, Hassan AH, Neely KE, Workman JL. 2000. ATP-dependent chromatin-remodeling complexes. Mol Cell Biol 20:1899–1910.

Wang W, Cote J, Xue Y, Zhou S, Khavari PA, Biggar SR, Muchardt C, Kalpana GV, Goff SP, Yaniv M, Workman JL, Crabtree GR. 1996. Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J 15:5370–5382.