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FAST TRACK

Nuclear Cysteine-Protease Involved in Male Chromatin Remodeling After Fertilization Is Ubiquitously Distributed During Sea Urchin Development

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Abstract Previously we have identified a cysteine-protease involved in male chromatin remodeling which segregates into the nuclei of the two blastomeres at the first cleavage division. Here we have investigated the fate of this protease during early embryogenesis by immunodetecting this protein with antibodies elicited against its N-terminal sequence. As shown in this report, the major 60 kDa active form of this protease was found to be present in the extracts of chromosomal proteins obtained from all developmental stages analyzed. In morula and gastrula the 70 kDa inactive precursor, which corresponds to the major form of the zymogen found in unfertilized eggs, was detected. In plutei larvas, the major 60 kDa form of this enzyme was found together with a higher molecular weight precursor (90 kDa) which is consistent with the less abundant zymogen primarily detected in unfertilized eggs. As reported here, either the active protease or its zymogens were visualized in most of the embryonic territories indicating that this enzyme lacks a specific pattern of spatial-temporal developmental segregation. Taken together our results indicate that this protease persists in the embryo and is ubiquitously distributed up to larval stages of development, either as an active enzyme and/or as an inactive precursor. These results suggest that this enzyme may display yet unknown functions during embryonic development that complement its role in male chromatin remodeling after fertilization. J. Cell. Biochem. 101: 1–8, 2007.

Key words: cysteine-protease; male pronucleus; embryogenesis; chromosomal proteins; sea urchins

Following fertilization the sperm nucleus is transformed into the male pronucleus that fuses with the female pronucleus reestablishing the diploid embryonic genome. In this event male chromatin remodeling is a fundamental step in which sperm histones are lost from zygote chromatin and replaced by cleavage stage (CS) histone variants that are recruited from maternal stores [Poccia et al., 1981; Imschenetzky et al., 1991].

We have previously postulated that the loss of SpH is associated to SpH degradation catalyzed by a nuclear cysteine protease that degrades sperm histones, leaving the maternally derived CS histones intact. Due to its selectivity this enzyme was initially designated SpH-protease [Imschenetzky et al., 1997]. We have further shown that the selectivity of this enzyme is modulated by post-translational modification of its substrates (Morin et al., 1999a,b). Poly (ADP-ribosylation) protects female CS variants from degradation in zygotes, while phosphorylation protects SpH1 and SpH2B from being degraded at intermediate steps of male pronucleus decondensation, [Oliver et al., 2002, reviewed by Imschenetzky et al., 2003]. We had purified this protease to homogeneity, determined its N-terminal amino acid sequence and used this information to obtain antibodies against this enzyme. Since these antibodies specifically recognize the SpH-protease, they were previously used as an experimental tool to follow

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the fate of the SpH-protease after fertilization. We had reported that it is present in unfertilized eggs as clusters that are distributed near the nuclear membrane. Shortly after fertilization it was localized in the female, as well as, in the male pronucleus. At the time of the first S phase of the initial zygotic cell cycle it was visualized as a compact signal in the central region of the nucleus. At the first mitosis it was co-localized with the α-tubulin that was organizing the mitotic spindle, then after cytokinesis this protease segregated into the nucleus of the initial blastomeres [Concha et al., 2005a]. Since the potential persistence of this protease during embryogenesis is unknown, we had investigated if this enzyme is still present in the nucleus during sea urchin early developmental stages that were followed-up to the larval stages of development. In this context, we had immunolocalized this protease in embryos that were harvested at different developmental stages and further determined its presence in nuclei that were isolated form these embryos by Western blots. As described in this report, we found that this nuclear protease, as well as it precursors, are present during early sea urchin embryogenesis. The 60 kDa form of this enzyme was predominant in all the developmental stages analyzed, while the higher molecular weight inactive precursors were mostly detected after hatching.

MATERIALS AND METHODS

Gametes and Embryos

Gametes were collected from the sea urchin *Tetrapygus niger* that were obtained from the Bay of Concepción, Chile. Insemination was performed and development took place in sea water at room temperature under aeration. Embryos were harvested at different times after insemination as indicated in each experiment. Developmental stages were followed by light microscopy.

Anti-Protease Antibodies

Polyclonal antibodies against a synthetic peptide (TPGNLQIPDTVDWRD-) corresponding to the N-terminal sequence of the protease were obtained and purified by affinity chromatography on protein A-Sepharose 4 B (Sigma Chemical Company, St. Louis, MO) as described previously [Concha et al., 2005a]. A titer of 1:10,000 was achieved as determined by ELISA.

Chromatin Isolation, Protease Detection by Immunoblots and SDS–PAGE Zymography

Chromatin was purified from embryos harvested at different stages of development as described previously [Imschenetzky et al., 1990]. For immunoblots the chromosomal proteins isolated from different embryonic stages were analyzed on 12% (w/v) SDS–PAGE [Imschenetzky et al., 1997], transferred to nitrocellulose membranes and incubated with the anti-protease antibodies diluted 1/1,000 (w/v) in a solution of PBS containing 0.05% (v/v) Tween-20 complemented with 5% (w/v) skim milk. The procedures followed for the electrophoretic transfer, blotting and immunodetection were performed as described previously [Concha et al., 2005a]. Final detection was performed using an ECL chemiluminescence kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

The activity of this protease was analyzed by SDS–PAGE zymography that was performed essentially as described by Vafa and Nishioka [1995]. Twelve percent (w/v) SDS–PAGE was polymerized in the presence of 0.1% (w/v) gelatin (Sigma Chemical Company) before standard electrophoresis was carried out. After electrophoresis the gel was incubated in 2.5% Triton X-100 for 1.5 h to remove SDS and restore proteolytic activity, rinsed twice in distilled water, and incubated for 24 h at 37°C in 0.05 M buffer phosphate pH 7.5 containing 1 mM DTT, 1 mM EDTA, and 0.5% Triton X-100 to allow proteolysis. The gels were stained in 1% (w/v) Amido black 10 B (Merck, Darmstadt, Germany) in methanol/acetic acid/water (500:100:400) overnight and destained in the same solvent. Protease activity is visualized as clear band in a blue background wherever digestion of the copolymerized gelatin occurred.

Immunolocalization of the Protease

T. niger embryos were harvested at the times indicated in each experiment and processed as described by Picard et al. [1988]. The anti-protease antibodies were affinity purified on epoxy 1–6 diamino hexane-sepharose coupled to the N-terminal peptide of the protease (Pharmacia) and used for the protease localization as primary antibodies (diluted 1/100).

Binding of anti-protease antibodies was detected with FITC conjugated anti-rabbit IgG antibodies (Sigma Chemical Company) diluted 1/1,000. The specimens were analyzed in a confocal microscope Zeiss, LSM 410 invert (Axiovert 100), using incidental light, given by a wavelength of 488 nm of an argon laser, with an emission filter BP 510–525 nm. A pinhole diameter of 1.5 μm was used for the optical sectioning. The final images were obtained by the integrated and depth coding of all optical sections, using a LSM 3.95 Zeiss software. As controls, the anti-protease antibodies were pre-incubated with the competing antigenic peptide prior to incubation and the images obtained were compared with the same specimens stained with 1 μg/ml DAPI in order to visualize the nuclear position in these embryos in an epifluorescence Nikon Eclipse E-600 microscope using a filter B-2E/C [Monardes et al., 2005].

RESULTS

Protease Screening in Sea Urchin Embryogenesis

As we reported previously this cysteine-protease is present as an inactive zymogen in unfertilized eggs, which is activated by cleavage to the 60 kDa form of this protease after fertilization. Following the first cleavage division the 60 kDa active form of this enzyme segregates into nuclei of the two initial blastomeres [Concha et al., 2005a]. To investigate its presence during the different developmental stages, we have analyzed by SDS–PAGE chromosomal protein extracts isolated from embryos harvested at stages of morula (8 h post-insemination (p.i.), late gastrula (30 h p.i.) and pluteus larvas (72 h p.i.). The detection of the protease and its precursors was performed by Western blots revealed with anti-protease antibodies as described in Materials and methods. Since this protease was previously found in the nuclear fraction from unfertilized eggs and two cell embryos, we have compared whole nuclei extracts obtained from these cells (Fig. 1A) with those from embryos harvested at the stages of morula, gastrula, and pluteus larvas (Fig. 1B). As shown in Figure 1, the 60 kDa active enzyme is the major form of this enzyme found in all the embryonic chromosomal extracts analyzed. Interestingly in the two cell embryos only the active form of the enzyme was found (Fig. 1A) while in morula and gastrula a 70 kDa zymogen was observed in addition to the active form of the enzyme. This 70 kDa zymogen is consistent with the most abundant form of the precursor found in unfertilized eggs (Fig. 1B). In pluteus, a 90 kDa zymogen was found to coexist with the active form of this enzyme. This zymogen correlates perfectly to the less abundant form of the precursor found in unfertilized eggs (Fig. 1B). Either the 60 kDa active form of the protease or its 70 and 90 kDa zymogens were undetectable when the primary antibodies were pre-incubated with the competing antigenic peptide, therefore confirming the specificity of these antibodies (data not shown). The presence

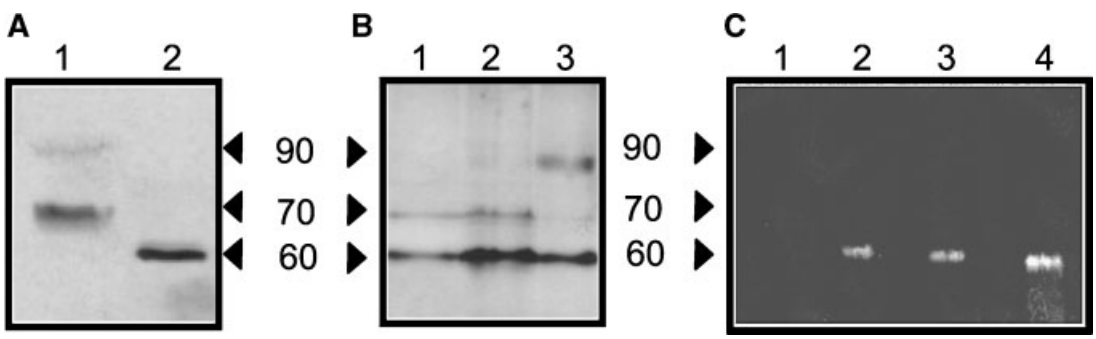

Fig. 1. Detection of the protease by immunoblotting and SDS–PAGE zymogram. Whole nuclear extracts were obtained from eggs and embryos harvested at different times p.i. and analyzed by immunoblotting. The protease was detected with antibodies generated against a peptide corresponding to the N-terminal sequence of the protease. MW markers were run in the same gel. **A**: Nuclear extracts obtained from *T. niger* eggs (**lane 1**) and two cell embryos (**lane 2**). **B**: Nuclear extracts from embryos harvested at the stage of morula (**lane 1**), late gastrula (**lane 2**) and pluteus (**lane 3**). **C**. SDS–PAGE zymogram of the protease obtained from unfertilized eggs and embryos harvested at different times p.i. Fifty microgram of proteins were analyzed in a SDS–PAGE zymogram gel as described in Materials and methods. MW markers were run in the same gel. Extracts of chromosomal proteins obtained from unfertilized eggs (**lane 1**), morula (**lane 2**), gastrula (**lane 3**) and pluteus (**lane 4**). The position of the active form of the protease is observed in the gel as a clear band against a dark background.

of 60 kDa active form of this protease in the chromatin of morula, gastrula, and pluteus was confirmed by SDS–PAGE zymography that was performed as described in Materials and methods. As shown in Figure 1C in unfertilized eggs no activity was detected confirming that only the precursors of this protease were present. In morula and gastrula a faint activity band was found which migrates at the 60 kDa position of the activity gel, thus confirming that the 70 kDa form of this protease shown in the Western blot is an inactive precursor of this protease. In pluteus a band migrating from 60 kDa downwards was found, suggesting that these extracts contain products derived from the proteolysis of this major 60 kDa form as well, together with the inactive 90 kDa zymogen. The presence of these protease degradation products are not surprising, since we had reported previously that this enzyme undergoes auto-proteolytic degradation in vitro [Concha et al., 2005a]. Taken together these results indicate that the 60 kDa form of this protease obtained from the chromatin of morula, gastrula, and pluteus, respectively, correspond to the active protease, while the 70 and 90 kDa forms represent the inactive precursors of this enzyme. These results are consistent with our previous reports, in which we had described the presence of the protease precursors in unfertilized eggs and identified the 60 kDa active protease in zygotes and early cleavage cells [Imschenetzky et al., 1997; Concha et al., 2005a]. Taken together these results demonstrate that the protease detected in embryos harvested at different developmental stages is associated to the chromatin of the embryos and that the active 60 kDa protease present during all steps of embryonic development, coexists with zymogens that appears to be specific to the developmental stage analyzed.

Developmental Distribution Pattern of the Protease

To define the spatial-temporal localization pattern of this protease, the embryos were harvested at different times after insemination and the positioning of the protease into the different embryonic territories was immunodetected with the anti-protease antibodies as described in Materials and Methods. Consistent with the biochemical evidence, which indicated the persistence of the protease up to larval stages of development, the protease was clearly detected in all the developmental stages analyzed. As shown in the depth images obtained by confocal microscopy of the embryos immunostained with the anti-protease antibodies, the protease was localized rather ubiquitously in morulas (Fig. 2A), late gastrulas, (Fig. 2B) and pluteus larvas indicating that it exhibits a nonspecific pattern of distribution up to the larval stage of development (Fig. 2C). These fluorescent signals were undetectable when the primary antibodies were pre-incubated with the competing antigenic peptide prior to the immunodetection assay as shown in Figure 3 (panels B, D, and F) in which the images obtained with the pre-adsorbed antibodies were compared with the same specimens stained with DAPI to visualize the cell nuclei position in these embryos (Fig. 3 panels A, C, and E). These results provides additional support in favor of the persistence of this cysteine-protease during

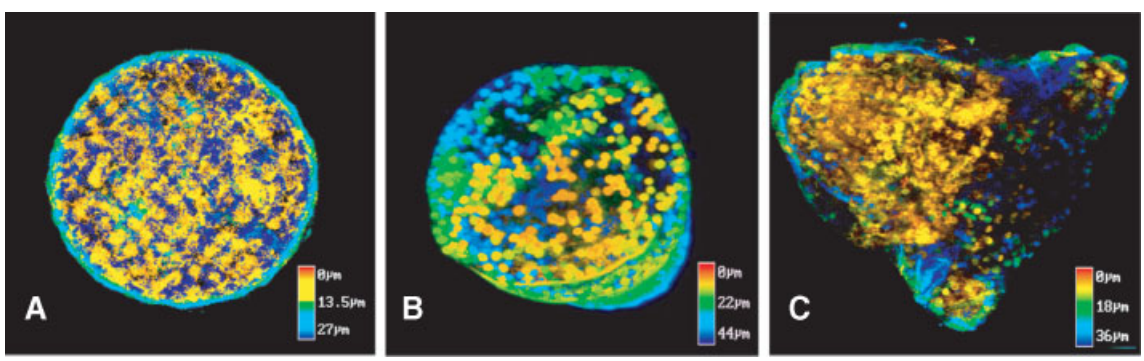

Fig. 2. Developmental distribution pattern of the protease. The visualization of the protease was performed in *T. niger* embryos harvested at different times p.i. The images corresponds to a depth coding of integration (10 or more) optical sections obtained after incubation with Fluorescein (FITC) second antibody. Depth coding integration of images derived from morula (**A**), late gastrula, (**B**) and pluteus larva (**C**).

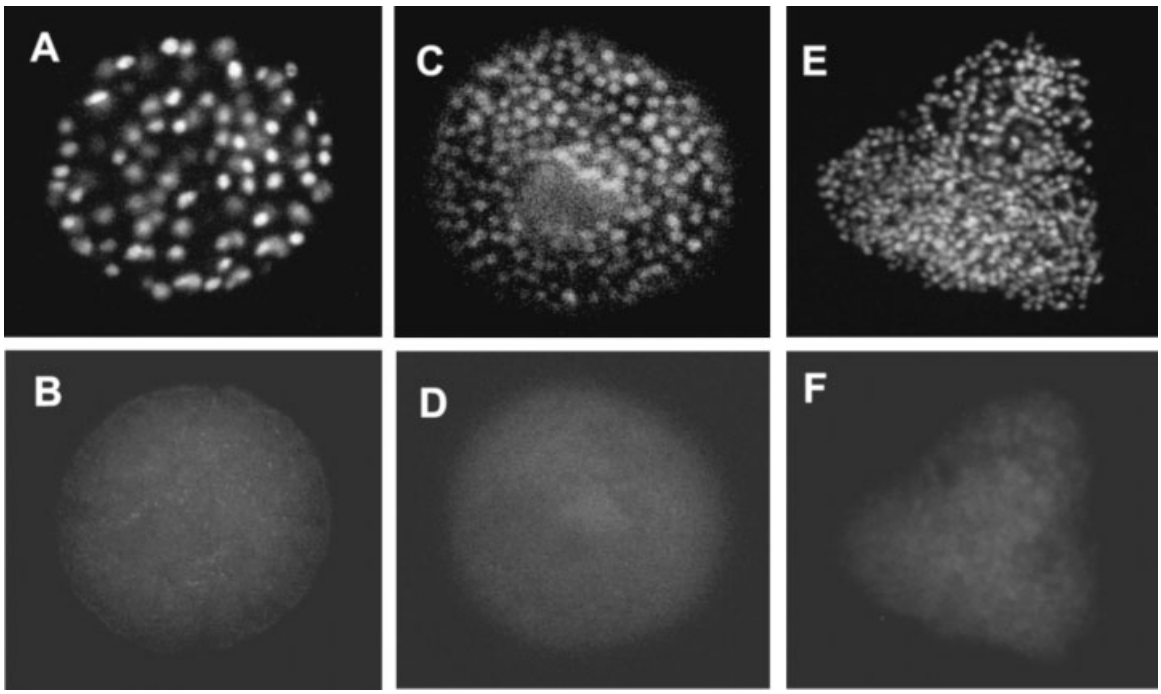

Fig. 3. Specificity of the anti-protease antibodies. The primary anti-protease antibodies were pre-incubated with the competing antigenic peptide prior to the immunodetection assay as described in Materials and methods (**panels B**, **D**, and **F**). Comparison of the images obtained with the pre-adsorbed antibodies with the same specimens stained with DAPI to visualize the nuclear position in the embryos (**panels A**, **C**, and **E**). Morula harvested at 8 h p.i.: DAPI stained (panel A), incubated with pre-adsorbed antibodies (panelB), Gastrula harvested 32 h p.i.: DAPI stained (panel C), incubated with pre-adsorbed antibodies (panel D). Pluteus harvested at 72 h p.i.: DAPI stained (panel E), incubated with pre-adsorbed antibodies (panel F), (bar 50 μm).

sea urchin embryogenesis and further defines its localization in the majority of the territories of the embryos until a larval stage of development.

DISCUSSION

In this report we demonstrated that the cysteine-protease which plays a fundamental role during male chromatin remodeling by degrading sperm specific histones [Imschenetzky et al., 1997], persists all through sea urchin embryogenesis. In all the developmental stages analyzed, the 60 kDa active form of the enzyme was detected, thus indicating that this enzyme is active during sea urchin embryogenesis and remains associated to chromatin. In addition, we have defined herein that the isoform of this enzyme is specific to the development stage analyzed. In unfertilized eggs, the 60 kDa active form of the enzyme was absent, but two precursors were detected, a less abundant form of 90 kDa and a more abundant form of 70 kDa. This result is consistent with our previous report indicating that the protease is inactive in the nuclear fraction of unfertilized eggs and may be activated by limited proteolysis in vitro [Imschenetzky et al., 1997]. Interestingly, mainly the 70 kDa precursor was evidenced in morulas and late gastrulas, while in plutei larvas, only the 90 kDa precursor was found. These results suggest that the activation of this protease in vivo may involve two steps, in the first the 90 kDa precursor is partially cleaved to the 70 kDa zymogen which is finally processed to give rise to the 60 kDa active form of this protease. Unfortunately it is impossible to interpret without ambiguities if the active enzyme and its precursors co-localize in different embryonic territories: As reported herein the unique antibodies available to detect this protease recognize the active protease, as well as, its precursors. Consequently, this matter should be investigated in the future.

We also demonstrate that, either the active protease or/and its precursors, are present in most of the embryonic territories before and after hatching. The ubiquitous distribution of this enzyme in the embryos suggests that this protease does not participate in a specific

regulatory network for development. In contrast, fundamental roles for other proteases in sea urchins embryogenesis are well documented at present. In this context SpAN, a metalloprotease related to BMP1 and tolloid from other systems, was shown to be differentially regulated in its transcription along the animal-vegetal axis by a maternally initiated mechanism that involve SpSox B1 and CBF (CCAAT binding factor) regulatory sites [Kenny et al., 2001]. For this type of metallo-protease it was reported that a down regulation of SpSoxB1 by beta-catenin-dependent gene products, like the transcription repressor SpKr1, are required for patterning and morphogenesis of the sea urchin embryos. It was further postulated that such tight regulation activates target genes required for gastrulation and specifies endoderm and mesenchyme cell fates [Kenny et al., 2003].

Similarly, matrix metalloproteases (MMPs) play essential roles in a variety of processes that require extracellular matrix remodeling and degradation. In sea urchins, a gelatinase that is specific to later stages of development was previously characterized from pluteus larvas [Robinson, 1997]. More recently, two MMPs genes (SpMMP 14 and SpMMP16) that exhibit a differential developmental regulation in the sea urchin *Strongylocentrotus purpuratus* have been described. These genes have the conserved cysteine switch, zinc binding, and hemopexin domains. They also contain consensus furin cleavage sites and putative transmembrane domains indicating that these are membrane type MMPs. SpMMP14 is expressed in all cells from eggs until the mesenchyme blastula stage embryo, then its expression becomes predominant in the cells derived from the animal pole in late gastrulas. SpMMP 16 has a low expression in unfertilized eggs, becoming predominant in the cells derived from the vegetal pole at the blastula stage and finally is found in the pigment cells later in development [Ingersoll and Pendharkar, 2005]. In agreement with results derived from sea urchins, MMPs have been also shown to be differentially expressed during *Xenopus laevis* development [Yang et al., 1997; Harrison et al., 2004].

From the ubiquitous spatial-temporal pattern of distribution of the protease described in this report, as well as, from its biochemical characterization, in particular its N-terminal sequence, substrate selectivity and specific association to chromatin, it is obvious that this protease is unrelated to the other types of proteases involved in embryogenesis mentioned above.

As previously reported the N-terminal amino acid sequence of the cysteine-protease responsible of sperm histones degradation after fertilization, exhibits a significant degree of homology with the cathepsin L family of proteases, which are normally related to the final degradation of proteins in lysosomes. This homology is further confirmed by the presence of cathepsin L typical consensus regions in the gene that encodes this protease (unpublished results). Its potential role in sea urchin embryogenesis remains unknown, however the information reported thus far by our laboratory seems to link this protease more likely to embryonic cell division. As reported recently, its inhibition in vivo, arrests the S phase of the first cell cycle, disrupts the first mitosis, blocks the initial cleavage cycle and ultimately aborts the development of the embryo [Concha et al., 2005b; Puchi et al., 2006]. Thus far, the evidence concerning other potential roles of cathepsin L, or cathepsine L variants, in reproduction is rather scarce. It was reported that it exhibits a hormone-regulated expression during ovulation. In the ovary, cathepsin L is expressed in granulosa cells of follicles at different stages of growth in rats [Robker et al., 2000], suggesting that this protease may play diverse roles in this tissue. It was also reported that endocrine cells like Sertoli cells of the testis, placental trophoblasts, as well as, certain tumors secrete cathepsin L [Ishidoh and Kominami, 1998], indicating that this protease functions at both intracellular and extracellular sites. Thus far it is unknown if the protease analyzed in this report resides only in the nucleus or may be translocated to other intracellular positions at particular stages of the cell cycle, or if it remains in the nucleus in cells that are not dividing. We had reported, that this protease localizes in the mitotic furrow at the first mitosis, but we had also visualized this enzyme at the cell cortex at the first cleavage division, indicating that it may be translocated to extranuclear positions at specifc stages of the cell cycle [Concha et al., 2005a]. In this context, we do not know yet if this protease may be secreted to extracellular domains. However there is increasing evidence that cathepsins secreted by macrophages, osteoclasts, fibroblasts, and transformed cells exert functions that impact angiogenesis and tumor

progression [Jean et al., 2002]. More directly, related to cell division, the potential role of proteases that are members of the cathepsin family was suggested previously for other systems. A strong repression of cell proliferation induced by MENT (myeloid and erythroid nuclear termination stage specific protein), an endogenous nuclear inhibitor of cathepsines K, L, and V was reported [Irving et al., 2002]. Furthermore, a nuclear variant of cathepsine L was identified and postulated to be involved in cell cycle control. This cathepsine L variant, described for a non embryonic system, catalyses the limited proteolysis of the CDP/cux transcription factor at the G_1/S transition of the cell cycle, modulating its activity as a transcription factor relevant for the expression of several genes involved in cell cycle control, such as DNA polymerase α, dihidrofolate reductase (DHFR), dihydroorotase (CAD) and cyclin A [reviewed by Goulet and Nepveu, 2004]. Thus far, it is not known if the cathepsin L-like isoform that has been analyzed in this report, may be related to the cathepsin L nuclear isoform identified in other cells.

However, the puzzling persistence of this protease during early embryogenesis, its association to chromatin and its ubiquitous distribution in most of the embryonic territories, suggests other potential roles yet unknown for this enzyme which we think is additive to its crucial role in male chromatin remodeling after fertilization and merits to be further investigated.

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REFERENCES

Concha C, Morin V, Bustos P, Genevière AM, Heck MM, Puchi M, Imschenetzky M. 2005a. Cysteine-protease involved in male chromatin remodeling after fertilization colocalizes with alpha-tubulin at mitosis. J Cell Physiol 202(2):602–607.

Concha C, Monardes A, Even Y, Morin V, Puchi M, Imschenetzky M, Genevière AM. 2005b. Inhibition of cysteine protease activity disturbs DNA replication and prevents mitosis in the early mitotic cell cycles of sea urchin embryos. J Cell Physiol 204(2):693–703.

Goulet B, Nepveu A. 2004. Complete and limited proteolysis in cell cycle progression. Cell Cycle 3(8):986–989.

Harrison M, Abu-Elmagd M, Grocott T, Yates C, Gavrilovic J, Wheeler G. 2004. Matrix metalloproteinase genes in *Xenopus* development. Develop Dynam 231:214–220.

Imschenetzky M, Puchi M, Merino V, Zepeda P. 1990. Microheterogeneity of late histones in larval stages of sea urchin development. Comp Biochem and Physiol 97B(3): 467–469.

Imschenetzky M, Puchi M, Pimentel C, Bustos A, Gonzales M. 1991. Immunobiochemical evidence for the loss of sperm specific histones during male pronucleus formation in monospermic zygotes of sea urchins. J Cell Biochem 47:1–10.

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(3):304–315.

Imschenetzky M, Puchi M, Morín V, Medina R, Montecino M. 2003. Chromatin remodeling during sea urchin early development: Molecular determinants for pronuclei formation and transcriptional activation. Gene 322:33–46.

Ingersoll EP, Pendharkar NC. 2005. Characterization of two matrix metalloproteinase genes during sea urchin development. Gene Expr Patterns 6:727–732.

Irving JA, Shushanov SS, Pike RN, Popova EY, Bromme D, Coetzer TH, Bottomley SP, Boulynko IA, Grigoryev SA, Whisstock JC. 2002. Inhibitory activity of a heterochromatin-associated serpin (MENT) against papain-like cysteine proteinases affects chromatin structure and blocks cell proliferation. J Biol Chem 277:13192–13201.

Ishidoh K, Kominami E. 1998. Gene regulation and extracellular functions of procathepsin L. J Biol Chem 379:131–135.

Jean D, Guillaume N, Frade R. 2002. Characterization of human cathepsin L promoter and identification of binding sites for NF-Y, Sp1 and Sp3 that are essential for its activity. Biochem J 361:173–184.

Kenny AP, Angerer LM, Angerer RC. 2001. SpSoxB1 serves an essential architectural function in the promoter SpAN, a tolloid/BMP1-related gene. Gene Expr 9(6): 283–290.

Kenny AP, Oleksyn DW, Newman LA, Angerer RC, Angerer LM. 2003. Tight regulation of SpSoxB factors is required for patterning and morphogenesis in sea urchin embryos. Dev Biol 261(2):412–425.

Monardes A, Iribarren C, Morin V, Bustos P, Puchi M, Imschenetzky M. 2005. During male pronuclei formation chromatin remodeling is uncoupled from nucleus decondensation. J Cell Biochem 96(2):235–241.

Morin V, Díaz F, Montecino M, Fothergill-Gilmore L, Puchi M, Imschenetzky M. 1999a. Poly(ADP-ribosylation) protects maternally derived histones from proteolysis after fertilization. Biochem J 343:95–98.

Morin V, Acuña P, Díaz F, Inostroza D, Martínez J, Montecino M, Puchi M, Imschenetzky M. 1999b. Phosphorylation protects sperm-specific histones H1 and H2B from proteolisis after fertilization. J Cell Biochem 76: 173–180.

Oliver MI, Concha C, Gutiérrez S, Bustos P, Montecino M, Puchi M, Imschenetzky M. 2002. Remodeling of sperm chromatin after fertilization involves nucleosomes

formed by sperm histones H2A and H2B and two CS histone variants. J Cell Biochem 85:851–859.

Picard A, Harricane MC, Labbe JC, Doree M. 1988. Germinal vesicle components are not required for the cell cycle oscillator of the early starfish embryo. Dev Biol 128:121–128.

Poccia DL, Salic J, Krystal G. 1981. Transitions in histone variants of the male pronucleus following fertilization and evidence for a maternal store of cleavage stage histone in sea urchin eggs. Dev Biol 82:287–296.

Puchi M, Quiñones K, Iribarren C, Bustos P, Morin V, Geneviève AM, Imschenetzky M. 2006. Microinjection of an antibody against the cysteine-protease involved in male chromatin remodeling blocks the development of sea urchin embryos at the initial cell cycle. J Cell Biochem 98:335–342.

Robinson J. 1997. Characterization of metalloproteinase: A late stage specific gelatinase activity in sea urchin embryo. J Cell Biochem 66:337–345.

Robker RL, Russell DL, Espey LL, Lydon JP, O'Malley BW, Richards JS. 2000. Progesterone-regulated genes in the ovulation process: ADAMTS-1 and cathepsin L proteases. Proc Natl Acad Sci (USA) 97:4689–4694.

Vafa O, Nishioka D. 1995. Developmentally regulated protease expression during sea urchin embryogenesis. Mol Reprod Dev 40:36–47.

Yang M, Murray M, Kurkinen M. 1997. A novel matrix metalloproteinase gene (XMMP) encoding vitronectin-like motifs is transiently expressed in *Xenopus laevis* early embryo development. J Biol Chem 272(21):13527–13533.