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Potential Involvement of Post-translational Modifications as a Mechanism Modulating Selective Proteolysis After Fertilization

Maria Imschenetzky,* Marcia Puchi, Violeta Morin, Fredy Diaz, M. Isabel Oliver, and Martin Montecino

Department of Molecular Biology, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Abstract The role of proteolysis during fertilization has been investigated only to a very limited extent as compared with its role on the control of cell cycle progression. In this report, we discuss briefly the proteases involved in fertilization, their relevance in the egg-sperm interaction and in the chromatin remodeling that occurs before the reestablishment of the diploid condition of the zygote. We further emphasize how the post-translational modifications of target proteins modulate these proteolytic events. J. Cell. Biochem. Suppls. 32/33:149–157, 1999. © 1999 Wiley-Liss, Inc.

Key words: fertilization/proteolysis; phosphorylation; ubiquitination; poly(ADP-ribosylation)

Fertilization is a crucial event in bisexual reproduction whereby two cells fuse together creating a new individual that inherits the genetic potentials of both parents. The initial step involves a specific recognition of both gametes followed by the entry of the sperm into the egg. This event promotes the activation of egg metabolism, which is in a quiescent state before fertilization. The consequence of fertilization is the reestablishment of the diploid condition, which precedes the initiation of the developmental program of the resulting zygote.

Proteolysis plays essential roles in the sperm-egg initial interaction, in egg activation, and in the establishment of the diploid condition of the zygotes. In the initial steps of fertilization, proteolysis is required for triggering the acrosomal reaction and sperm penetration of the egg. In a variety of animal species, meiosis in oocytes is arrested at prophase of the first meiotic cell cycle. Reinitiation of meiosis is evidenced by the dissolution of the nuclear envelope, signaled as germinal vesicle breakdown (GVBD), followed by chromosome condensation and the extrusion of the first polar body. GVBD is triggered by the maturation promoting factor (MPF). MPF contains a 34-kDa cyclin-dependent protein kinase (p34cdc2) and cyclin B. Further transition to anaphase is concurrent with the rapid degradation of cyclin B, thus inactivating the MPF. Cyclin B is poly-ubiquitinated by a cyclin-specific ubiquitination cascade of reactions, thus committing this protein to be degraded by the ubiquitin/proteasome pathway. This pathway is highly conserved in eukaryotes and represents the selective cellular degradation mechanism better understood at present. In other species, meiosis is arrested at the end of metaphase I or II, in such condition its reinitiation is triggered by sperm penetration. Finally there are other species, such as sea urchins and sand dollars, in which the final stage of oocyte maturation occurs before fertilization. Consequently, the haploid state is attained before insemination and these eggs remain arrested in the G1 phase of the cell cycle before fertilization.

After fertilization, the sperm nucleus decondenses and transforms into male pronucleus, which moves toward the egg pronucleus and fuses at amphimixis, thus reestablishing the diploid condition of the future embryo. Sperm chromatin is condensed by a set of nuclear basic proteins that interact with DNA within the sperm nucleus. Sperm chromatin decondensation is accompanied by the removal of sperm

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*Correspondence to: Maria Imschenetzky, Department of Molecular Biology, Universidad de Concepción, Casilla 160-C, Concepcion, Chile. E-mail: mimschen@udec.cl

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basic nuclear proteins and their replacement by maternal histones variants that are recruited from pools preexisting in unfertilized eggs. Proteins N1 and N2 and nucleoplasmin have been postulated to chaperone histones transitions that occur during male chromatin remodeling. Since these chaperones do no exhibit an associated proteolytic activity, the degradation of sperm nuclear proteins after their disassembly from sperm chromatin emerges as a fundamental event in male chromatin remodeling. The participation of proteases in male chromatin remodeling, as well as, the modulation of the degradation of nuclear basic proteins by post-translational modifications are discussed in this report.

PROTEOLYTIC EVENTS PARTICIPATING IN SPERM-EGG INTERACTION: INVOLVEMENT OF THE UBIQUITIN/ PROTEASOME PATHWAY

Among the proteolytic mechanisms mediating protein turnover in eukaryotes the ubiquitin/ proteasome-dependent pathway is fairly well understood at present. The 20S (700-kDa) proteasome is a multicatalytic proteinase complex formed by a core of 28 well-conserved subunits that may associate to a 19S (2,000-kDa) regulatory complex, forming the 26S proteasome that degrades ubiquitinated proteins [reviewed by Bochtler et al., 1999]. The ubiquitination of proteins targeted for degradation is catalyzed by a cascade of enzymatic reactions, resulting in the covalent binding of ubiquitin, a 76-amino acid well-conserved polypeptide, to a target protein. The cascade is initiated by an ATP-dependent activation of the ubiquitin C-terminus by the ubiquitin activating enzyme (E1), followed by a thioester transfer of this polypeptide to a ubiquitin conjugating enzyme (UBC or E2) that functions in a concerted manner with the ubiquitin protein-ligase (E3) to establish a covalent binding of the ubiquitin to ϵ-amino group of internal lysine residues in the substrate protein. As a result, the formation of either mono- or poly-ubiquitinated proteins are obtained [reviewed by Laney and Hochstrasser, 1999]. The release of ubiquitin from protein adducts is catalyzed by de-ubiquitinating enzymes (ubiquitin C-terminal hydrolases, UCH) whose functions may be summarized as follows: release of ubiquitin from remanents of proteasome substates, liberation of monomeric ubiquitin from fusion protein precursors, disassembly of polyubiquitin to recycle monomeric units of this polypeptide, and editing of inappropriately ubiquitinated proteins [reviewed by Wilkinson and Hochstrasser, 1998].

During fertilization, proteasome mediated proteolysis plays essential roles in triggering the acrosomal reaction, as well as, in sperm penetration throughout the vitelline coat. In ascidians, indirect evidence based on the effects of specific inhibitors indicates that a 20S proteasome is involved in sperm binding to the egg vitelline coat, whereas an atypical 930-kDa proteasome participates in sperm penetration by digesting the vitelline coat. Inhibitors of trypsin-like proteinases also block fertilization, suggesting the participation of other proteases like acrosin and spermosin in the digestion of the vitelline coat [Sawada and Someno, 1996]. In sea urchins, the acrosomal reaction is induced by contact with the jelly coat of the egg. Although the mechanism mediating proteasome activation is not fully understood, it has been suggested that products derived from the hydrolysis of sperm phospholipids catalyzed by phospholipase A_2 (PLA_2) which is transiently activated by the egg jelly may participate in proteasome activation [reviewed by Mykels, 1998]. A summary of role of proteasome in fertilization is illustrated in Figure 1A.

Genetic evidence obtained in mammalians supports the essential role of the ubiquitin/ proteasome pathway in mice reproduction; as reported, the phenotype of mutants of the ubiquitin-conjugating enzyme (hHR6B homologues in mice) causes male sterility [Roest et al., 1996].

Unfortunately, the identification of specific protein targets that are committed to degradation by the ubiquitin/proteasome pathway during fertilization is almost totally lacking. Consequently, the precise events that participate in sperm-egg initial interactions remain basically unknown.

A full-grown oocyte remains metabolically quiescent until fertilization. Some species are arrested at prophase of the first meiosis of the primary oocytes, this step is equivalent to the G2/ M transition of a mitotic cell cycle. In response to hormones, these oocytes complete meiosis and mature. In other species, the unfertilized egg remains arrested at the end of metaphase I or II of the meiotic cell cycle. Reinitiation of meiosis is determined by sperm penetration of the egg, which promotes the activation of the maturation promoting factor

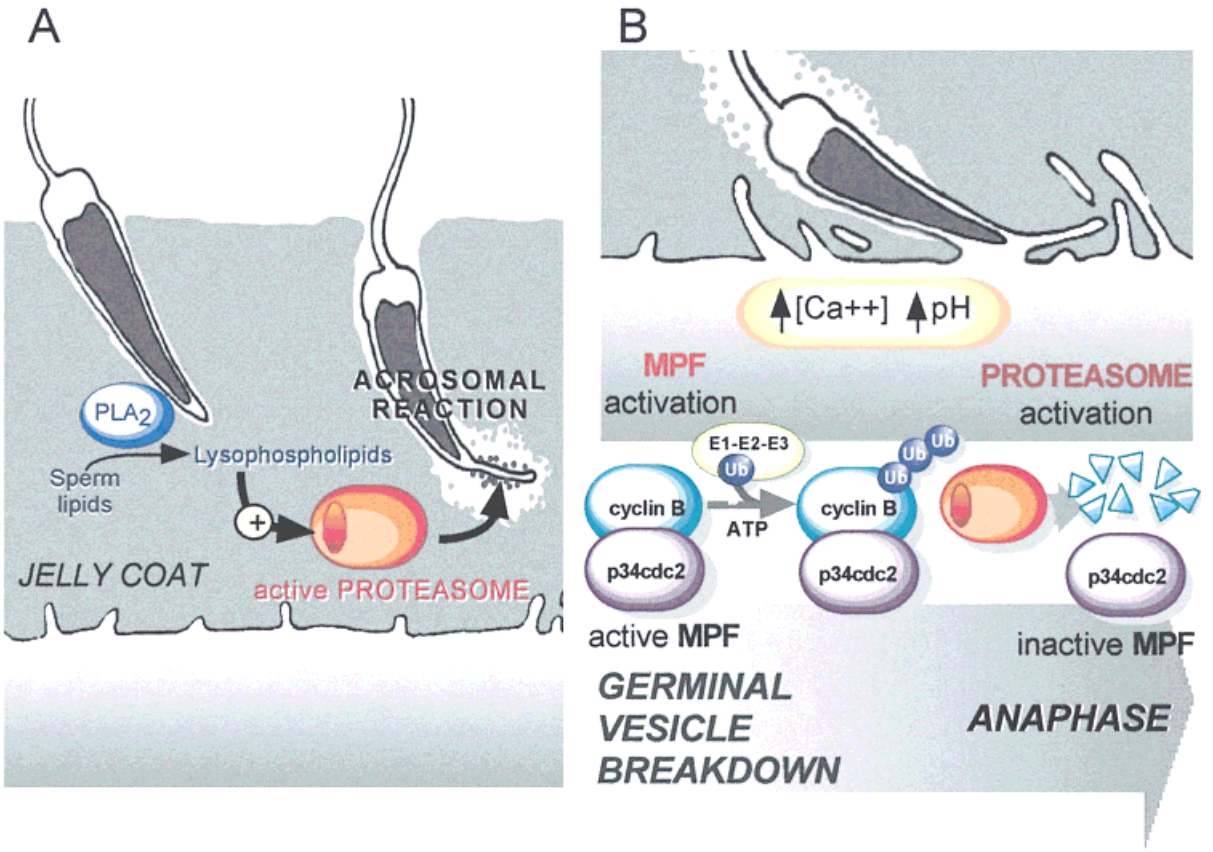

Fig. 1. Summarized scheme of the fundamental proteolytic events involved in the initial sperm-egg interaction, based in different experimental models. **A:** Model illustrating proteasome activation stimulated by sperm binding to the egg jelly coat in sea urchins. Proteasome is activated by lysophospholipids. resulting from the degradation of phospholipids by phospholipase A2 (PLA_2). Activated proteasome promotes acrosomal reaction triggering the sperm entry into the egg. **B:** Sperm penetration of the egg induces an increase of intracellular Ca^{2+} and pH. In meiosis arrested oocytes, the maturation promoting factor (MPF) is activated triggering the germinal vesicle breakdown (GVBD). This event determines the chromosome condensation and the extrusion of the first polar body. These ionic changes also activate an intracellular proteasome that degrades poly-ubiquitinated cyclin B, inactivating the MPF and promoting egg transition to anaphase. Poly-ubiquitination of cyclin B is catalyzed by an ATP-dependent cyclin-specific ubiquitination cascade of reactions involving three enzymes (E1-E2-E3).

(MPF). This factor contains a cyclin-dependent protein kinase (p34cdc2) and cyclin B. Further transitions from metaphase to anaphase involves a ubiquitin-mediated cyclin degradation, thereby inactivating the MPF [reviewed by Mykels, 1998; Koepp et al., 1999].

The ubiquitination of cyclin B that targets the degradation of this protein, occurs in a conserved 9-amino acids motif, located at the N-terminus of this protein, called the destruction box (D box). Cyclin degradation, as well as its relevance in the control of cell cycle check points has been reviewed recently [Laney and Hochstrasser, 1999, Koepp et al., 1999].

Proteasome activation during oogenesis has been reported in many species. In echinoderms, proteasome activation during oogenesis has been linked to oocyte maturation in response to 1-methyladenine (1-Me-Ad) [reviewed by Mykels, 1998]. Thus far, it is unknown how the proteasome activates the MPF, but injection of anti-proteasome antibodies blocks oocyte maturation, as defined by the germinal vesicle breakdown (GVBD) [Sawada et al., 1997].

In ascidians, the oocytes remain arrested at metaphase of meiosis I, and meiosis reinitiation is triggered by sperm penetration. Oocyte activation may be simulated by Ca^{2+} ionophore A23187. A transient increase of a 26S proteasome activity is observed coincidentally with oocyte activation. It has been postulated that this increased 26S proteasome activity results from the assembly of the 20S proteasome core to the PA 700 regulatory complex, which is

modulated by the increase of intracellular Ca^{2+} that follows sperm penetration. Similarly, a Ca^{2+} stimulated proteasome activation had been reported to occur in oocytes from batracians that are arrested at metaphase of meiosis II, at the moment of fertilization. Alternatively, it has been reported that the 26S proteasome activation in sand dollars is dependent on the rise of intracellular pH that occurs at fertilization [Chiba et al., 1999]. It has been suggested that a Ca^{2+}/calmodulin protein kinase (Ca M kinase) may be involved in proteasome activation. Otherwise, it was reported that proteasome subunits may be phosphorylated by protein kinases, such as, PKA, PKC, and CK2 [reviewed by Mykels, 1998]. A summary of the participation of the ubiquitin/proteasome pathway in oocyte maturation which is triggered by sperm entry is illustrated in Figure 2B. Although the precise mechanism that regulates the activation of the 26S proteasome complex remains unknown, proteasome-phosphorylation emerges as a potential mechanism that may be involved in this process. In other biological systems, such as K562 human myelogenous leukemia cells, nuclear proteasome activation is catalyzed by poly(ADP-ribose) polymerase (PARP); thus, the active proteasome is found in a poly(ADP-ribosylated) form [Ullrich et al., 1999]. Unfortunately, the eventual poly(ADP-ribosylation) of the proteasomes participating in fertilization has not yet been investigated, but PARP activation mediated by its phosphorylation, coincides with the activation of the MPF in *Xenopus laevis* eggs [Aoufouchi and Shall, 1997].

CHROMATIN REMODELING UPON FERTILIZATION

At fertilization, the zygote is a unique cell formed by two sets of chromatin that coexists as two separate entities. Maternal chromatin is organized into nucleosomes, whereas paternal chromatin is highly compacted by the interaction of a set of sperm specific nuclear basic proteins with DNA, thereby determining the repression of the male genome. A variety of nuclear basic proteins have been described in different species, ranging from cysteine-rich protamines in mammals to sperm-specific histones in sea urchins [rewieved by Ausio, 1995].

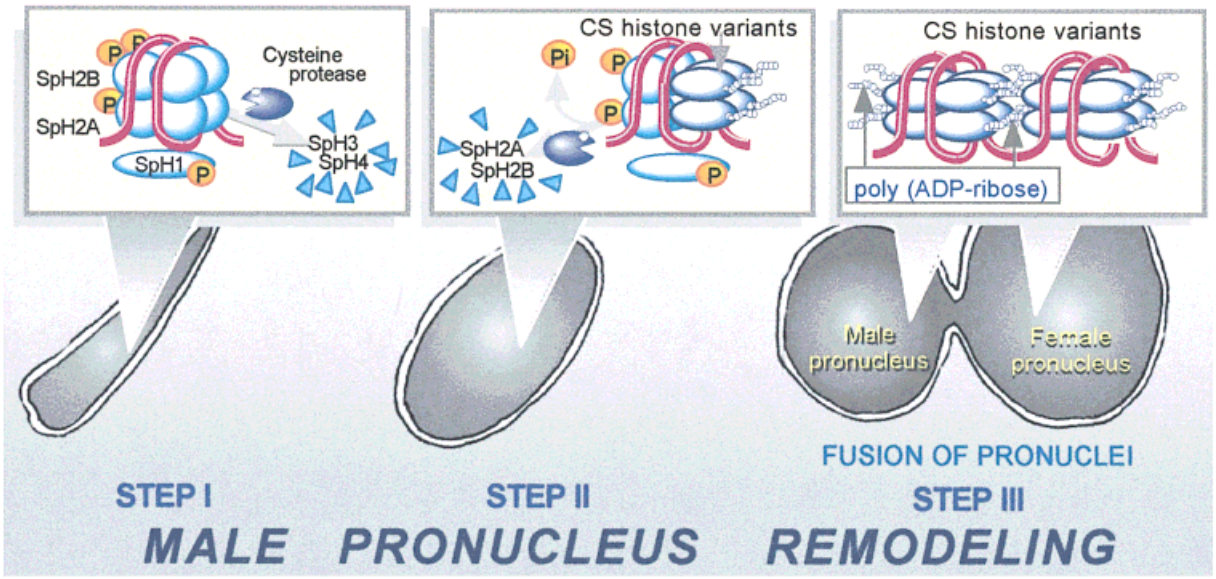

Fig. 2. Hypothetical model illustrating the different steps involved in male chromatin remodeling that follows fertilzation in sea urchins. Step I: Conically shaped male pronucleus containing a fully condensed sperm chromatin. Sperm histones H1 (SpH1) and H2B (SpH2B) are phosphorylated. Step II: Ovoid or swollen male pronucleus containing a partially decondensed chromatin. Chromatin is formed by hybrid nucleosomes containing SpH2A, phosphorylated SpH2B and SpH1, as well as, a subset of poly(ADP-ribosylated) cleavage stage histone variants (CS) that were recruited from maternal pools. During the transition from step I to step II, sperm histones H3 (SpH3) and H4 (SpH4) are degraded by the SpH cystein-protease. Step III: Spherically shaped male pronucleus. The fully decondensed male chromatin fuses with the female pronucleus. Both male and female chromatin, are organized by poly(ADP-ribosylated) CS histone variants. During the transition from step II to step III sperm histones H1 (SpH1) and H2B (SpH2B) are dephosphorylated by an unknown phosphatase and then degraded by the SpH cysteine-protease, while the poly (ADP-ribosylated) CS histone variants remain intact.

To initiate a successful developmental program after fertilization, the male chromatin must undergo decondensation, to establish a compatibility, in terms of protein composition, with female chromatin. Such relaxation of male chromatin must be completed at the moment of the fusion of male and female genomes. The decondensation of male chromatin that follows fertilization results from the removal of sperm basic nuclear proteins and their replacement by histones from maternal origin [reviewed by Poccia, 1986].

It was initially postulated that these protein transitions are promoted by nucleoplasmin, a thermostable pentameric acid protein, that lacks species specificity and is widely distributed in vertebrates, as well as, in invertebrates. Hyperphosphorylated nucleoplasmin has been postulated to act as a chaperone in the assembly of nucleosome cores, by adding dimmers of histones H2A-H2B to the tetramers of histones H3-H4 that are primarily organized by a couple of two additional histone-chaperone proteins, namely N1 and N2. The involvement of nucleoplasmin and proteins N1 and N2 in the disassembly of nuclear sperm-specific basic proteins has been postulated [reviewed by Laskey et al., 1993, Leno et al., 1996]. Alternatively a chromatin remodeling activity which is homologous to yeast SW12/SNF2 and also to human BRG1 chromatin remodeling protein complex, has been identified recently in *Xenopus* oocytes. This protein complex is involved in chromatin remodeling during transcriptional activation. It is expressed throughout oogenesis and embryogenesis; however, its participation in sperm chromatin remodeling after fertilization is uncertain [Gelius et al., 1999].

In echinoderms, it has been postulated that sperm chromatin remodeling occurs by several interdependent states after fertilization. The initial step (step I), is characterized by a fully condensed conical shaped sperm pronuclei. The intermediate step (step II), is defined by an ovoid or swollen male pronucleus containing a partially decondensed chromatin. At the final step (step III), which coincides with amphimixis, the male pronucleus becomes spherically shaped and contains a fully decondensed chromatin [Cameron and Poccia, 1994]. The transformation from state I to state II requires protein phosphorylation by factors that are not present in oocytes before maturation and may be blocked by protein kinases inhibitors such as staurosporine. Progression from state II to state III is promoted by the rise of intracellular pH that occurs at fertilization [Cameron and Poccia, 1994]. Concerning histone transitions, sea urchins sperm chromatin is condensed by a set of sperm-specific histones (SpH) and the chromatin of unfertilized eggs is organized by cleavage-stage (CS) histone variants. These variants are also forming the embryonic chromatin during the initial cleavage divisions [reviewed by Poccia, 1986]. These changes in histones composition are reflected in differences in chromatin organization that can be followed by micrococcal nuclease digestion. Sperm nucleosomes are packed by sperm histone variants (SpH) interacting with 220- to240-bp DNA fragments, while unfertilized egg nucleosomes are organized by cleavage-stage histone variants (CS) that protect 126 bp of DNA [reviewed by Poccia, 1986, Imschenetzky et al., 1989]. Such chromatin composition persists during the initial cleavage stages. Afterward two additional sets of histone genes are sequentially expressed in a developmentally regulated manner, the early α variants that are assembled into chromatin from middle cleavage stages (16 blastomers) until hatching and the late variants that are predominant from blastula stage onward [Busslinger et al., 1985].

Sea urchin sperm histones may be differentiated from the CS histone variants by immunobiochemical approaches. As reported, the fate of sperm histones can be followed after fertilization by two-dimensional western blot analysis using polyclonal antibodies against either sperm proteins or against CS histone variants. These two sets of polyclonal antibodies do not cross-react between each other. On the basis of this approach, it was found that at an intermediate stage of male pronucleus remodeling, the chromatin is formed by nucleoprotein particles containing a subset of sperm-specific histones, as well as maternally derived CS histone variants. At the final stages of male pronucleus decondensation, the organization of chromatin is similar to that of the female pronucleus exhibiting a shortened DNA repeat length, as compared with sperm nucleosomes. Such chromatin organization persists during the initial cleavage divisions [Imschenetzky et al., 1996a]. Consequently, as described for most species, in sea urchins, sperm basic proteins (SpH) are lost from chromatin before the establishment of the diploid condition

POST-TRANSLATIONAL MODIFICATIONS OF HISTONES AT FERTILIZATION

The information concerning histone post-translational modification that occurs before the establishment of the diploid condition is derived mainly from sea urchins, although there are some reports concerning histones acetylation in relation to zygotic genome activation. In mouse, neither male nor female chromatin is acetylated before fertilization. After fertilization, hyperacetylated histone H4 associates with paternal but not maternal chromatin and such differential pattern of H4 acetylation persists before amphimixis. Thus, it has been suggested that histones acetylation may be involved in the exchange of protamine to histone during male pronucleus formation [Adenot et al., 1997]. By contrast, to these results, it has been reported that in *Xenopus* the inhibition of histone acetylation does not affect embryonic development until gastrulation [Almouzni et al., 1994]. Alternatively, genetic studies in *Drosophila* embryo, indicate an essential role for CBP (CREB binding protein) histone acetyltransferase activity, a general genome activator that has been implicated in chromatin decondensation. CBP has been proposed to interact with the Dorsal transcription factor in early embryos. In mature eggs, Dorsal is located in the cytoplasm and upon fertilization it migrates to nuclei located in the ventral regions of the embryo. Mutations of the CBP homologue (*majire*) result in a failure to activate the gene twist, a Dorsal target, which participates in mesoderm differentiation [Akimaru et al., 1997].

In sea urchin sperm, the five histones are found in an unmodified form. By contrast , the CS histone variants are extensively poly(ADP-ribosylated) in unfertilized eggs [Imschenetzky et al., 1996b]. Shortly after fertilization, sperm histone variants SpH1 and SpH2B become phosphorylated. The phosphorylation target sites involve serine/threonine residues that are located in the N-terminal region of SpH2B variants and at both the N-terminal and C-terminal regions of SpH1. It has been postulated that these phosphorylation occurs at target sequences corresponding to SPKK motifs that are clustered in N-terminal regions of SpH1 and SpH2B variants which become reversibly phosphorylated during spermatogenesis [Poccia and Green, 1992]. The phosphorylation of SpH1 and SpH2B weakens the binding capacity of these histones to linker DNA, inducing chromatin relaxation [Green et al., 1993]. However, information about the level of phosphorylation of SpH2B variants and SpH1, as well as the precise sequences modified in vivo during male chromatin remodeling is still lacking, and should not be homologued to the reversible phosphorylation that occurs during spermatogenesis. Immunobiochemical data derived from our laboratory indicate that, during the intermediate stage of male pronucleus development, SpH1 and SpH2B coexist within nucleoprotein particles with maternally derived CS histone variants. After amphimixis, the complete set of sperm histones is lost, and the chromatin is composed exclusively by CS histone variants. The specific protein kinase responsible for the phosphorylation of SpH1 and SpH2B is unknown and warrants future investigation.

In contrast with sperm histones, the CS histone variants that organize the chromatin in sea urchin unfertilized eggs and zygotes and during the initial cleavage divisions are extensively poly(ADP-ribosylated). It was found that the poly(ADP-ribosylation) of CS histone variants changes in a cell cycle-dependent manner and is required for zygotic DNA replication [Imschenetzky et al., 1993; 1996b]. This post-translational modification is catalyzed by the poly(ADP-ribose) synthetase (PARP), a nuclear enzyme found in a wide variety of eukaryotic cells. PARP transfers the ADP moiety of NAD^+ to chromosomal proteins to form either oligo- or poly(ADP-ribosyl) linear or branched homopolymers. This enzyme contains two zinc-binding domains and participates in the recognition of DNA strand breaks. Its activity is strictly dependent on DNA single- or double-stranded interruptions. The removal of these polymers or oligomers from acceptor proteins is catalyzed by two enzymes the poly(ADP-ribose) glycohydrolase and the poly(ADP-ribose) protein lyase, yielding ADPR-ribose that is further hydrolyzed to AMP and ribose phosphate. The coordinated action of these enzymes induces a transient modification of the chromosomal proteins involved in the regulation of DNA recombination or DNA replication/DNA repair [reviewed by de Murcia et al., 1994].

HISTONE POST-TRANSLATIONAL MODIFICATIONS MODULATE PROTEOLYSIS AFTER FERTILIZATION

In most species, the decondensation of sperm chromatin which occurs during male pronucleus formation is accompanied by the loss of sperm basic nuclear proteins [reviewed by Poccia and Green, 1992]. Since the participation of nucleoplasmin and proteins N1 and N2 are insufficient to explain the disappearance of sperm basic nuclear protein from the fertilized eggs, the proteolytic cleavage of proteins associated with the sperm chromatin emerges as a complementary fundamental event that should be activated concomitantly with other fertilization-related events. Once again, the major lines of evidence in this regard are derived thus far from experimental data obtained from sea urchin embryos. Cameron and Poccia [1994] found by using protease inhibitors that degradation of nonhistone chromosomal proteins is required for male pronucleus formation. Following this rationale, a novel 60-kDa cysteine protease was found in our labaratory that is present as a proenzyme in unfertilized eggs and is activated shortly after fertilization [Imschenetzky et al., 1997]. At pH 8.0, the condition found post insemination of sea urchin eggs [Whitaker and Steinhardt, 1985], this enzyme specifically degrades the five SpH, leaving the maternally derived CS histone variants intact. Consistently, the inhibition of this enzyme blocks the normal degradation of the five SpH that occurs in vivo during male pronucleus remodeling [Imschenetzky et al., 1997].

Owing to this strict substrate selectivity, it has been postulated that this cysteine-protease plays a predominant role in the degradation of sperm-specific histones. Consequently this protease was named SpH-cysteine protease. It has been further demonstrated that the puzzling proteolytic selectivity displayed by the SpH-protease is determined by the poly(ADP-ribose) moiety of cleavage-stage variants, which protects the modified proteins from degradation [Morin et al., 1999a]. More recently it was demonstrated that the phosphorylation of SpH1 and SpH2B histone variants protects these histones from degradation by the nuclear SpH-protease [Morin et al., 1999b]. In summary, the SpH-protease postulated to participate in male chromatin remodeling exhibits a unique modulation of its activity, namely, its inhibition by post-translational modification of its substrates, either by its poly(ADP-ribosylation) or by its phosphorylation.

The biochemical properties of the SpH cysteine protease are consistent with its role in the progression through the different steps that are involved in male pronucleus remodeling described by Cameron and Poccia [1994]. As mentioned above, the transformation from initial conical condensed state of sperm nucleus (state I) to an ovoid shaped male pronucleus (state II) may be inhibited by N-ethylmaleimide, a compound that blocks sulfydryl groups. This requirement for thiol groups may be correlated with the participation of the cysteine protease in the first step of male pronucleus formation. Progression from state II to state III is promoted by the rise of intracellular pH that occurs at fertilization [Cameron and Poccia, 1994]. Therefore, the activity of the SpH cysteine protease appears to be modulated by this rise in intracellular pH, thus participating in the progression from state II to state III. Furthermore, the presence of the inactive form of the SpH-cysteine protease in unfertilized eggs, is in agreement with the independence of male pronucleus formation from new proteins synthesis after fertilization [reviewed by Poccia, 1986]. A model that summarizes these events, which are potentially involved in male chromatin remodeling, is illustrated in Figure 2.

Alternatively, the involvement of a 28-kDa protease that specifically degrades the SPKK motif in histones, thereby cleaving potential phosphorylation target sites in SpH1 has been postulated by Suzuki et al., [1990]. In common with the SpH cysteine protease, the SPKK-protease was found to be sensitive to leupeptin inhibition, suggesting that the SPKK-protease may also be a member of the cysteine type proteases, although no formal evidence was provided to enable the unequivocal classification of the enzyme as a cysteine protease. Undoubtedly, however, the SPKK-protease is distinct from the SpH- cysteine protease both in its apparent molecular mass and in its restricted specificity. As reported by Suzuki et al., [1990], the SPKK protease promotes a limited proteolysis only of sperm histones H1 and H2B. Consequently, even though the participation of the SPKK-protease in sperm histone degradation during male pronucleus formation can not

be denied, its role appears more limited than that displayed by the SpH-cysteine protease described above.

This strict substrate selectivity of the SpH-cysteine protease clearly defines it as unique and distinct from all the proteases described previously. The eventual relationship of the SpH- protease with the family of cysteine proteases participating in other cellular responses is unknown. It is also unknown whether similar proteolytic enzymes are present in the nucleus of female gametes from other species, consequently these questions remain open and should be investigated in the future.

SUMMARY

Proteolytic events during fertilization regulate the initial sperm-egg interactions, activation of unfertilized eggs, and remodeling of chromatin, which determines the successful establishment of the diploid state of the future embryo. Protein-ubiquitination, is at present the most generalized positive signal for specific protein degradation. By following this pathway, it should be possible to define the specific degradation of protein targets at the initial sperm-egg interactions. At this step, these proteins that are targeted for degradation are basically unknown. In addition, the precise molecular events that determine proteasomes activation are also lacking thus far. Upon fertilization, ubiquitin targeted cyclin B degradation determines the progression through the meiotic cell cycle. The major unresolved question concerns the mechanism that determines specific cyclin ubiquitination in a time-ordered manner. Whether specific protein-phosphorylation determines a time-dependent subsequent ubiquitination is a matter that should be resolved in the future. Finally, the issue related to male chromatin decondensation after fertilization, is as one of the major challenges related to the mechanisms that govern a successful reestablishment of the diploid state of the zygote. In this context, histone poly(ADP-ribosylation) and phosphorylation emerge as negative signals against proteolysis. As discussed above, these post-translational modifications inhibit histone degradation catalyzed by a cysteine-protease that is activated after fertilization. It is unknown whether these post-translational modifications represent a more general mechanism related to chromatin decondensation in other biological systems. Poly(ADP-ribosylation) of nuclear proteins is a very general post-translational modification of nuclear proteins in eukaryotes. This modification promotes chromatin relaxation and has been involved in DNA repair events affecting cell survival and cell death pathways. Cell death pathway, is associated to the activation of cysteine-proteases belonging to the very abundant caspase family of proteases. Consequently, the potentiality of poly(ADP-ribosylation) of nuclear proteins as a more general protection mechanism against proteolysis emerges as a problem that warrants investigation.

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