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FAST TRACKS

Conservative Segregation of Maternally Inherited CS Histone Variants in Larval Stages of Sea Urchin Development

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Abstract Three sets of histone variants are coexisting in the embryo at larval stages of sea urchin's development: the maternally inherited cleavage stage variants (CS) expressed during the two initial cleavage divisions, the early histone variants, which are recruited into embryonic chromatin from middle cleavage stages until hatching and the late variants, that are fundamentally expressed from blastula stage onward. Since the expression of the CS histones is confined to the initial cleavage stages, these variants represent a very minor proportion of the histones present in the plutei larvae, whereas the late histone variants are predominant. To determine the position of these CS in the embryonic territories, we have immunolocalized the CS histone variants in plutei larvas harvested 72 h post-fertilization. In parallel, we have pulse labeled the DNA replicated during the initial cleavage cycle with bromodeoxyuridine (BrdU) and its position was further determined in the plutei larvas by immunofluorescence. We have found that the CS histone variants were segregated to specific territories in the plutei. The position in which the CS histone variants were found to be segregated was consistent with the position in which the DNA molecules that were replicated during the initial cleavage divisions were localized. These results strongly suggest that a specification of embryonic nuclei occurs at the initial cleavage divisions which is determined by a chromatin organized by CS histone variants. J. Cell. Biochem. 88: 643–649, 2003.

Key words: histones; embryogenesis; chromatin; nucleus; echinoderms

During early developmental stages of sea urchins three sets of histones are sequentially expressed. Initially, the maternally inherited cleavage stage variants (CS) are translated during the two initial cleavage stages, then the early histone variants are expressed from middle cleavage stages (16 blastomeres) until hatching and finally the late histone variants become predominant from blastula stage onward [reviewed by Giudice, 1999].

It is well documented at present that the CS histone variants are very distinctly related to those encoding early or late histone variants [Brandt et al., 1997]. In contrast to other histone genes known thus far, CS histone genes contain introns, polyadenylation addition signals, and long untranslated sequences. Although, it may be deduced from the DNA sequence information that the genes encoding CS histone variants are independent of DNA replication [Mandl et al., 1997], their translation appears to be contemporaneous with the S phase of the initial cleavage cell cycles [Imschenetzky et al., 1995].

In contrast to the CS histone variants, the genes encoding the early histone variants are clustered, tandemly arranged, and are highly repetitive [reviewed by Giudice, 1999]. Transcription of these genes reaches a peak at the 128 cells stage and then gradually decreases until complete inhibition at blastula stage. The control of accumulation and proper temporal expression of these genes reside in specific regulatory elements located at the promoters of these genes. The interaction of these regulatory elements with its cognate transcription factors, which have being only partially characterized

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thus far, have been correlated with specific changes in chromatin structure along the promoter and coding sequences of these genes [reviewed by Giudice, 1999; Melfi et al., 2000; Medina et al., 2001].

The family of late histone genes, present in 6–12 copies per genome is found in a nontandem arrangement. These late genes neither contain introns nor polyadenylation signals. Their expression occurs in a cell-cycle dependent manner due to the presence of a terminal palindrome located at their 3′end. Their transcription is regulated by a combined action of transcription factors and enhancers that are interacting with regulatory elements found in their promoter regions. These factors and regulatory elements have being only partially characterized [reviewed by Giudice, 1999; Li and Childs, 1999, Mancini et al., 2001].

The differences between these three sets of histone variants determine distinct chromatin organizations since the CS histone variants organize atypical nucleosomes, while late histones organize conventional nucleosomes [Savic et al., 1981, Imschenetzky et al., 1989]. Since the CS histone variants are still present at larval stages of development, it is predictable that distinct chromatin conformation should be found in distinct cells in the plutei larvas. Based on the lack of significant immune cross-reactivity between the CS variants and the late histones, we have immunolocalized the CS histone variants in larvas harvested 72 h post-insemination. In parallel, we have labeled the DNA molecules that were replicated during the initial cleavage division with bromodeoxyuridine (BrdU) and further determined its position in the larvas. The results shown in this report are consistent with a conservative type of distribution in which the CS histone variants and the DNA replicated at the initial cleavage division segregate together during early developmental stages of sea urchins.

MATERIALS AND METHODS

Fertilization and Embryonic Cultures

Gametes were collected from the sea urchins *Tetrapygus niger*, insemination was performed and development took place in sea water at room temperature, under aeration. The different stages of development were followed by microscopy and the swimming plutei larvas were filtered through a 100 μm pores plankton netting and washed several times with sterile sea water [Imschenetzky et al., 1990].

Isolation of Histone Variants and Western Immunoblots

Chromatin was purified from unfertilized eggs and the CS variants were obtained as described by Imschenetzky et al. [1989]. Plutei larvas were harvested at 72-h post-insemination (p.i.) and the histones were isolated from the chromatin [Imschenetzky et al., 1990]. Subsequently, the histone variants isolated either from unfertilized eggs or from the plutei larvas, were separated by electrophoresis in one-dimensional 18% (w/v) polyacrylamide gels containing sodium dodecyl sulfate (SDS–PAGE). After electrophoresis, these histones were transferred to nitrocellulose membranes, and immuno detected with antibodies anti-CS histone variants that were obtained as described previously [Oliver et al., 2002]. Since native CS histone variants were previously found to be poly(ADP-ribosylated) [Imschenetzky et al., 1996], the anti-CS variants antibodies were incubated with (ADP-ribose) polymers covalently bound to Sepharose 4B to remove the fraction of antibodies that may recognize the polymers of ADP-ribose. A final titer of the antibodies against CS histone variants of 1:12,800 was achieved as determined by ELISA.

Monitoring DNA Replication and DNA Labeling

To label the DNA replicated during the initial cleavage divisions, 10^{-3} M BrdU was added to cultures of embryos from 90 min until 105 min p.i. Then the embryos were extensively washed with sea water to remove the BrdU and cultured in sea water until 72 h p.i. to collect the labeled plutei larvas.

To determine the incorporation of ^{3}H-thymidine into DNA, these zygotes were incubated continuously from 3 to 180 min after insemination in ^{3}H-thymidine (NEN, Boston, MA, Sp. act. 108 Ci/mmol) at concentration of 1 μCi/ml. At different times after insemination 1 ml samples of the zygote suspension were processed as previously described [Imschenetzky et al., 1991]. In parallel, 10^{-3} M BrdU was added to the cultures of zygotes and the samples were subsequently processed, essentially as described by Picard et al. [1996]. For BrdU detection, larvas plutei were fixed in 4 N HCl for 2 h and

post-fix in methanol 100% for 30 min. Subsequently, the embryos were washed twice in buffer A + Tween (150 mM NaCl, 50 mM Tris-HCl pH 7.4, 0.05% (v/v) Tween-20) and blocked with 3% (p/v) bovine serum albumin (BSA) in buffer A + Tween. Monoclonal anti-BrdU antibodies (Boehringer Manheim, Germany) diluted 1/10 in 0.5% (p/v) BSA in buffer A + Tween were used as primary antibodies and as secondary antibody a fluoresceine-linked sheep anti-mouse IgG diluted 1/500 in 0.5 % (p/v) BSA in buffer A + Tween. Green FITC fluorescent signals were observed in an epifluorescence Nikon Eclipse E-600 microscope using a filter B-2E/C.

Immunolocalization of CS Histone Variants

For immunolocalization of CS histone variants, the larvas plutei were processed essentially as described by Moreau et al. [1998]. The larvas were harvested at 72-h p.i. permeabilized in 10 mM sodium ethyleneglycol-bis(β-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid (NaEGTA) 25 mM, 2-(*N*-morpholino)ethanesulfonic acid (MES) pH 6.8, 0.55 mM $MgCl_2$, 25% (v/v) glycerol, and Nonidet P-40 (NP-40) 1% (v/v) for 2 h at room temperature, subsequently fixed in 75% (v/v) methanol, 25% (v/v) glycerol, and finally blocked overnight in 3% (p/v) of BSA in buffer A (150 mM NaCl and 50 mM Tris-HCl pH 7.4). Polyclonal affinity-purified anti-CS antibodies diluted 1/1000 in 0.3% (p/v) BSA in buffer A were used as primary antibody and Alexa Fluor 594 goat anti-rabbit IgG (Molecular Probes, Oregon) diluted 1/500 in 0.3% (p/v) BSA in buffer A as second antibody. Alexa Fluor fluorescent signals were observed in an epifluorescence Nikon Eclipse E-600 microscope using a filter G-2E/C.

Merged images in which the red signals corresponding to immunolocalization of CS histone variants and the green signals corresponding to the BrdU incorporated into DNA are colocalized and were observed in an epifluorescence Nikon Eclipse E-600 microscope using a dual filter F-T.

RESULTS

Western Blots of Plutei Histones

Whole histones isolated from plutei larvas harvested 72 h post-insemination were separated by PAGE–SDS and further analyzed by Western blots that were revealed with anti-CS variants antibodies as described in Materials and Methods. As shown in Figure 1 (lane A), late histones are represented by H1 and the nucleosomal core histones identified as H3, a closely migrating doublet of H2A-H2B and histone H4. The CS histone variants, shown in Figure 1 (lane C) are very heterogeneous and cannot be unambiguously identified according to their electrophoretic migration, as reported previously this heterogeneity is due to the extensive poly(ADP-ribosylation) of each one of these histones [Imschenetzky et al., 1996]. The Western blots shown in Figure 1 (lanes B–D), reveal that the antibodies obtained against the CS variants did not cross-react to a significant extent with the late histone variants. The late histone H3 showed a very minor cross-reaction which becomes evident only when the membranes were over exposed (Fig. 1, lane B). Alternatively, the majority of the CS histone variants were recognized by the anti-CS antibodies, as mentioned above the identification of these variants according to their electrophoretic migration is not feasible due to the extensive poly(ADP-ribosylation) of each one of these variants.

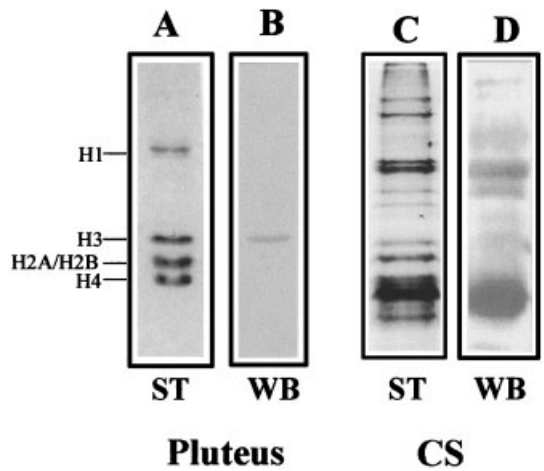

Fig. 1. Immune cross reactivity of histones from plutei larvas and CS histone variants. Whole histones were isolated from larvas harvested 72-h post-insemination (p.i.) as described in Materials and Methods, and separated by SDS–PAGE, transferred to a nitrocellulose membrane, and revealed with antibodies against the CS histone variants. Total CS histone variants isolated from unfertilized eggs were analyzed in parallel, as controls. Nitrocellulose membranes containing 20 μg histones from plutei larvas stained with Ponceau-S **(lane A)** revealed with antibodies against CS variants **(lane B)**, 30 μg of CS variants transferred to nitrocellulose membranes and stained with Ponceau-S **(lane C)** revealed with antibodies against CS variants **(lane D)**.

Labeling of the DNA During the Initial Cleavage Division

To investigate the fate of the DNA molecules that was replicated during the initial cleavage divisions in the distinct territories of the larvas, we have incorporated BrdU into DNA during the initial cleavage cycles and further localized the labeled DNA molecules in plutei larvas as described in Materials and Methods. Unfortunately, a long incubation of embryos in BrdU was toxic and the pluteus stage was not attained. Thus different incubation intervals with BrdU were assayed, in order to achieve the labeling of DNA with BrdU to an immuno detectable level, without interfering with the normal development (results not shown). The best results were achieved by incubating in BrdU from 90 min until 105 min p.i. As shown in Figure 2, this interval corresponds to the initial part of the second S phase. In *T. niger*, similarly to other sea urchins, the first S phase occurs from 30 to 50 min p.i., while the second S phase occurs between 90 and 120 min p.i. The first cleavage is observed at 90 min p.i. while the second cleavage occurs at 140 min p.i. As shown in Figure 2, the incubation with BrdU described above was sufficient to label the DNA replicated during the initial cleavages in order to be immunodetected in the four cleavage stage and normal plutei larvas were successfully achieved (Fig. 2; panels A and B). The specificity of the immunofluorescent detection of the BrdU incorporated into DNA was further confirmed with control plutei larvas that were treated with the set of anti-BrdU antibodies as described in Materials and Methods in which no fluorescent signals were observed (results not shown). To confirm the normal progress of replication cycles during initial cleavage divisions, the incorporation of ^{3}H thymidine into DNA was followed in the zygotes labeled with BrdU, as described in Materials and Methods. As shown in Figure 2, the initial cell cycles were not altered as may be judged by timing of the ^{3}H thymidine incorporation into DNA.

Colocalization of CS Histone Variants and the DNA Replicated at the Initial Cleavage Cyles in Plutei larvas

The immunolocalization of CS histone variants was performed in plutei larvas that were harvested 72-h p.i. and detected with the anti-CS histones antibodies, and the fluorescent signals were observed in an epifluorescence microscope as described in Materials and Methods. In parallel, the plutei larvas labeled with BrdU, obtained as described above, were also processed and further observed by epifluorescence microscopy as described in Materials and Methods. It was found that the CS variants are present fundamentally in the cells that were predominantly located in the oral territory of the larvas and around the gut of the larvas (Fig. 3; panel A). As shown, the territory in which CS histone variants were located in the plutei larvas appears consistent with the region

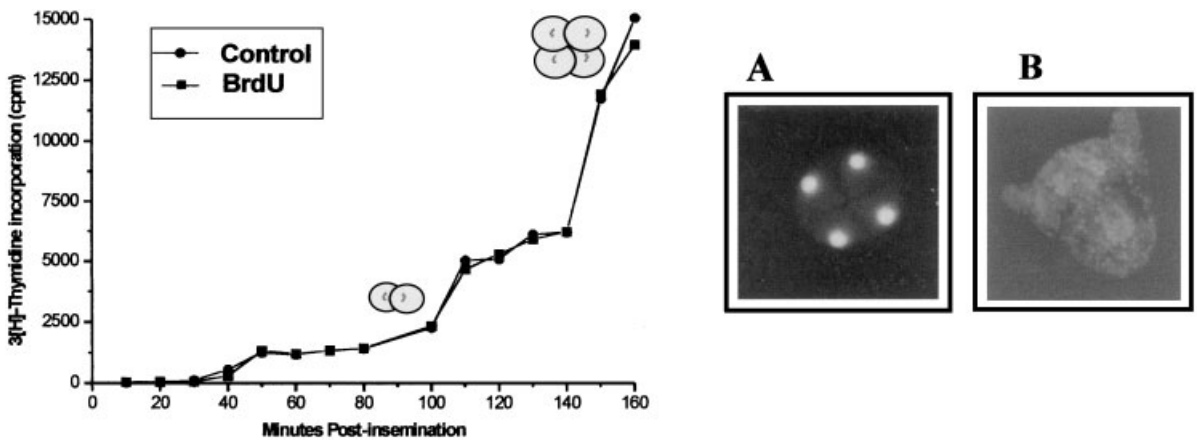

Fig. 2. Labeling DNA with BrdU and monitoring the early developmental stages. DNA replication was followed during the initial cleavage cycles by measuring the incorporation of $^{3-}$H thymidine into DNA, BrdU was added from 90 min until 105 min p.i. and each sample was processed as described in Materials and Methods. As a control an experiment was run in parallel in the absence of BrdU (Fig. 2). Four cleavage stage embryos and plutei larvas harvested 72-h p.i. were collected and processed as described in Materials and Methods to detect the fluorescent signals derived from the incorporation of BrdU into DNA. **Panel A**: Four cleavage stage embryos (**panel B**) plutei larvas.

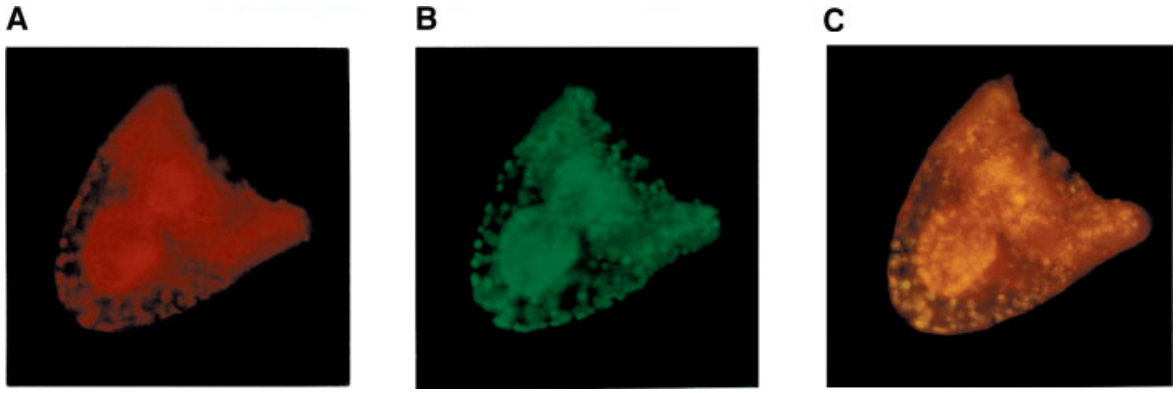

Fig. 3. Colocalization of CS histone variants and the DNA replicated at the initial cleavage cycles in plutei larvas. BrdU was incorporated into DNA from from 90 min until 105 min p.i. and detected in plutei larvas harvested at 72-h p.i. The plutei larvas were processed for double-label immuno fluorescent signals. Variants were as described in Materials and Methods. The CS histone variants that were detected using antibodies against CS histone variants are shown in red in **panel A**. The BrdU incorporated into DNA was detected with antibodies against BrdU is shown in green in **panel B**. The merged image (yellow) showing colocalization of CS histone variants (red) and incorporation of BrdU into DNA (green) is shown in **panel C**.

in which the DNA molecules replicated during the initial cleavage division were found (Fig. 3; panel B). As shown in Figure 3 panel C, these images are further supported by the colocalization of both fluorescent signals, that derived from the positioning of CS histone variants and that correspond to the DNA molecules replicated during the initial cleavage division. These results indicate that the CS histone variants become segregated to specific territories during early developmental stages of sea urchins, which predominantly are located in the oral region of the larvas. This segregation is coincident with the position in which the DNA molecules that were replicated in the initial cleavage cycle were localized in the plutei.

DISCUSSION

This report demonstrates that the CS histone variants are confined into specific territories during early development of sea urchins. This segregation is correlated to the embryonic positioning of the DNA molecules that were replicated during the developmental interval in which these variants were fundamentally expressed, this is the initial cleavage stage. This conclusion is experimentally based on the lack of significant immune cross-reactivity of the antibodies generated against the CS variants that are expressed at initial stages of development, with other histone variants that are expressed afterwards. As informed the CS histone variants are proteins very distantly related to the other two sets of histones expressed afterwards in development [Mandl et al., 1997]. Otherwise, the confined and clustered signals related to the positioning of the CS variants found in larval stages of development provide additional support to an almost negligible cross-reactivity between the CS variants and the families of histones expressed later in development.

The conservative developmental segregation of CS histone variants described in this report may specify early embryonic cells in terms of its basic chromatin structure. As it is shown in this report, such specification persists as clusters of cells localized in determined territories at larval stages of development. In this context, it is provocative to speculate, that such cells may represent a wider spectrum of developmental potentialities than most other cells in the embryos that have followed a specific pattern of differentiation. The sea urchin early development, is one of the models that has being extensively studied to define the mechanisms that participate in the formation of axes that govern the embryonic pattering mechanisms. In this model the determination of the initial animal-vegetal (AV) axis is maternally inherited and becomes morphologically evident at the 16 cells stage. Such initial specification mechanisms have being correlated with the unequal distribution of distinct sets of animalizing and vegetalizing transcription factors activities which are combined with zygotically produced factors [Davidson et al., 1998; reviewed by Angerer, 2000]. In contrast to the specification that characterizes the 16 blastomeres stage, the four initial blastomeres are plastic in terms of

its developmental potentials, as it has been proved in the pioneer work performed at the dawn of experimental embryology by Hans Driesch [1892]. The interpretation performed by Driesch, based on the equal partition of animal and vegetal territories, remains fundamentally valid at present, and may be correlated with the gradients of animalizing and vegetalizing transcription factors that are present in the eggs at fertilization [Angerer, 2000; reviewed by Davidson 2002]. Concomitantly with the gradients determined by specific transcription factor activities, this report strongly suggests that chromatin organization should play a fundamental role as well, in the time and space dependent pattering of embryonic structures during development. The specification of chromatin in particular embryonic cells may facilitate or inhibit the binding of transcription factors that will be promoting the activation or repression of specific sets of genes in a spaciotemporal dependent manner during development. Interestingly, in batracians an oocyte-specific histone variant, encoded in the H1M (B4) gene, has being described as closely related to the CS1 histone variant from sea urchins. This B4 batracian histone variant resides in the linker region of the chromatin [Dimitrov et al., 1994, Dimitrov and Wolffe, 1996]. More recently, it has being isolated in mouse a gene encoding a 34-kDa histone variant that displays a substantial homology with oocyte-specific variant from the frog and CS1 from sea urchins. It was postulated that this mammalian histone variant, named H1oo, may play a critical role in the control of gene expression presumably through the perturbation of chromatin structure [Tanaka et al., 2001]. In addition, an histone variant nominated H2A.Z which exhibits a conserved function that differs from the major H2A variants known thus far, has being described as well [Jackson and Gorovsky, 2000]. This variant is crucial for *Drosophila melanogaster* and also for mammalian development. Furthermore, it has being postulated that the H2A.Z variant plays a pivotal role in establishing the chromatin structures required for the complex patterns of gene expression essential for normal mammalian development [Faast et al., 2001]. The role of H2A.Z in chromatin organization has being inferred to the creation of specific chromatin domains poised for transcriptional activation [Fan et al., 2002]. It is unknown thus far if all these histone variants that have being correlated to the progress of embryonic development in other species (B4 in batracians, H1oo in mammals and H2A.Z), resides in specific embryonic territories similarly to the CS variants of sea urchins. Otherwise, the potential link between the presence of these variants and the developmental plasticity that characterizes the initial cleavage stages of development remains to be investigated in the future.

REFERENCES

Angerer L, Angerer R. 2000. Animal-vegetal axis patterning mechanisms in the early sea urchin embryo. Dev Biol 218:1–12.

Brandt W, Schawger S, Rodrigues J, Busslinger M. 1997. Isolation and amino acid sequence analysis reveal an ancient evolutionary origin of the cleavage stage (CS) histones of the sea urchin. Eur J Biochem 247:784–791.

Davidson E, Cameron A, Ransick A. 1998. Specification of cell fate in the sea urchin embryo: Summary and some proposed mechanisms. Development 125:3269–3290.

Davidson EH, Rast JP, Oliveri P, Ransick A, Calestani C, Yuh CH, Minokawa T, Amore G, Hinman V, Arenas-Mena C, Otim O, Brown CT, Livi CB, Lee PY, Revilla R, Rust AG, Pan Z, Schilstra MJ, Clarke PJ, Arnone MI, Rowen L, Cameron RA, McClay DR, Hood L, Bolouri H. 2002. A genomic regulatory network for development. Science 295(5560):1669–1678.

Dimitrov S, Wolffe AP. 1996. Remodeling of somatic nuclei in *Xenopus laevis* egg extracts: Molecular mechanism for the selective release of histones H1 and H1(0) from chromatin and the acquisition of transcriptional competence. EMBO J 15(21):5897–5906.

Dimitrov S, Dasso MC, Wolffe AP. 1994. Remodeling of sperm chromatin in Xenopus egg extracts: The role of core histone hyposphorylation and linker histone B4 in chromatin assembly. J Cell Bio 126:591–601.

Driesch H. 1892. The potency of the first two cleavage cells in echinoderm development. Experimental production of partial and double formations. In: Willier BH, Oppenheimer JM editors. Foundations of experimental embryology. Hafner, New York.

Faast R, Thonglairoam V, Schulz TC, Beall J, Wells JR, Taylor H, Matthaei K, Rathjen PD, Tremethick DJ, Lyons I. 2001. Histone variant H2AZ is required for early mammalian development. Curr Bull 11(15):1183–1187.

Fan JY, Gordon F, Luger K, Hansen JC, Tremethick DJ. 2002. The essential histone H2AZ regulates the equilibrium between different chromatin conformational states. Nat Struct Biol 9(3):172–176.

Giudice G. 1999. Genes and their products in sea urchin development. Curr Top Dev Biol 45:41–116.

Imschenetzky M, Puchi M, Gutiérrez S, Massone R. 1989. Analysis of supranucleosomal particles from unfertilized eggs of sea urchin. Exp Cell Res 182:436–444.

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

Imschenetzky M, Montecino M, Puchi M. 1991a. Poly(ADP-ribosilation) of atypical CS histone variants is required for the progression of S phase in early embryos of sea urchins. J Cell Biochem 46:234–241.

Imschenetzky M, Wilhem V, Puchi M, Medina R. 1995. In sea urchin embryos the translational of cleavage stage histone variants is confined to S phase and partially uncoupled from DNA replication. Biol Res 28:131–140.

Imschenetzky M, Morin V, Carvajal N, Montecino M, Puchi M. 1996. Decreased heterogeneity of CS histones variants after hydrolysis of the ADP-ribose moiety. J Cell Biochem 61:109–117.

Jackson JD, Gorovsky MA. 2000. Histone H2AZ has a conserved function that is distinct from that of the major H2A sequence variants. Nucleic Acid Res 28(19):3811–3816.

Li Z, Childs G. 1999. Temporal activation of the sea urchin late H1 gene requires stage-specific phosphorylation of the embryonic transcription factor SSAP. Mol Cell Biol 19(5):3684–3895.

Mancini P, Dentice M, Aniello F, Branno M, Piscopo M, Pulcrano G, Fucci L. 2001. The replacement H3.3 histone gene in Paracentrotus lividus sea urchin: Structure and regulatory elements. Biochim Biophys Acta(1–2):39–45.

Mandl B, Brandt W, Superti-Furga G, Graninger P, Birnstiel M, Busslinger M. 1997. The five Cleavage-Stage (CS) histones of the sea urchin are encoded by a maternally expressed family of replacement histone genes: Functional equivalence of the CS H1 and frog H1M (B4) proteins. Mol Cell Biol 17:1189–1200.

Medina R, Gutiérrez J, Puchi M, Imschenetzky M, Montecino M. 2001. Cytoplasm of sea urchin unfertilized eggs contains a nucleosome remodeling activity. J Cell Biochem 83:554–562.

Melfi R, Palla F, Di Simone P, Alessandro C, Cali L, Anello L, Spinelli G. 2000. Functional characterization of the enhancer blocking element of the sea urchin early histone gene cluster reveals insulator properties and three essential cis-acting sequences. J Mol Biol 304(5):753–763.

Moreau JL, Marques F, Abdelhamid B, Schatt P, Lozano JC, Peaucellier G, Picard A, Genevière AM. 1998. Cdk2 activity is dispensable for the onset of DNA replication during the first mitotic cycles of the sea urchin early embryo. Dev Biol 200:182–197.

Oliver MI, Concha C, Gutiérrez S, Bustos A, Montecino M, Punchi M, Imschenetzky M. 2002. Remodeling of sperm chromatin after fertilization involves nucleosomes formed by sperm histones H2A and H2B and two CS histones variants. J Cell Biochem 85:851–859.

Picard A, Galas S, Dorèe M. 1996. Newly assembly cyclin B-cdc2 kinase is required to supress DNA replication between meiosis I and meiosis II in starfish oecytes. EMBO J 15:3590–3598.

Savic A, Richman P, Williamson P, Poccia D. 1981. Alterations in chromatin structure during early sea urchin embryogenesis. Proc Natl Acad Sci USA 78: 3706–3710.

Tanaka M, Hennebold J, Macfarlane J, Adashi E. 2001. A mammalian oocyte-specific linker histone gene H1oo: Homology with the genes for the oocyte-specific cleavage stage histone (cs-H1) of sea urchin and the B4/H1M histone of the frog. Development 128(5):655–664.