Chromatin remodeling during early developmental stages of sea urchins

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Different sets of histone genes are sequentially expressed in early developmental stages of sea urchins: the CS variants are found in chromatin of unfertilized eggs and at initial cleavage stages, then the early variants (E) are expressed from middle cleavage stages (16 blastomeres) till hatching, afterwards the late variants (L) are predominant from blastula stage onward (Newrock et al, 1977; Busslinger et al, 1985). In this report a comparison is established between the chromatin formed by CS variants isolated from unfertilized eggs and that organized by somatic type histones obtained from larval stages of development (plutei, harvested 72 hours post insemination). Two major aspects are compared: characterization of histone variants and structural organization of chromatin. From the results presented herein it is concluded that larval histones are proteins unrelated to the CS variants, both in their aminoacid composition and in their immunological determinants. These changes in histones composition lead to a distinct structural chromatin organization represented by nucleoparticles exhibiting different sensibilities to nucleases digestion.

Key words: *Embryogenesis, equinoderms, histones, nucleosomes.*

INTRODUCTION

In the scope of the present knowledge, the differentiated state of a given cell can be viewed as the permanent repression of certain genes, combined with the activation either constitutive or inducible of other genes. In eukaryotes the structural and functional organization of chromatin are defined by interactions of chromosomal proteins with DNA regulatory sequences, as well as, by the ordered and organized packing of DNA by histones to permit these interactions. Thus, the differences in genome activity during embryonic development can be achieved by changes in chromatin composition sequentially determined by chromosomal proteins during embryonic development. Further support of the potential role of chromosomal proteins in embryonic differentiation, instead of a permanent modification of DNA, is added by the results reported by Gurdon (1968), indicating that the differentiated state of a given cell can be reversed to the initial totipotent embryonic state if their nuclei are transferred into an egg.

In contrast to the increasing amount of information available on DNA regulatory sequences and of its interactions with specific regulatory factors, the knowledge of structural details of chromatin packing that may determine eukaryotic genome functionality, is far from understood (von Holt, 1985; von Holt *et al*, 1989; Travers, 1989; Pabo and Sauer, 1992).

The basic packing unit that determines chromatin structure is the nucleosome: an ectomere formed by four histones, namely H2A, H2B, H3 and H4, wrapped by 146 base

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pairs (b.p.) of DNA. A fifth histone H1 is located in the DNA internucleosomal regions, or linker DNA. H1 interacts with each other and to the nucleosomal core, providing the basis for a higher ordered folding of chromatin (Kornberg, 1977). Histones have previously been considered as highly conserved proteins, not exhibiting significant variations among species, cells or different physiological states of a given cell (Isenberg, 1979). At present, a more detailed study of these proteins has demonstrated that histones are a family of closely related proteins. For instance, in the sea urchin *Parechinus angulosus* at least 24 different histone variants can be found, as defined by their primary structure (Brandt *et al*, 1979; Schwager *et al*, 1983; Old and Woodland, 1984; von Holt *et al*, 1989; Poccia and Green, 1992). These studies, as well as others, based on the isolation of histone gene products have led to the conclusion that in sea urchins the expression of different batteries of histone variants occurs in a developmentally regulated manner. In unfertilized eggs and early cleavage stages the atypical CS variants are the only basic proteins present in chromatin, then the early variants are found from 16 blastomeres till hatching, afterwards the late variants are predominant (Newrock *et al*, 1977; Busslinger and Barberis, 1985; Imschenetzky *et al*, 1986, 1989 a) The organization of genes encoding CS variants has not yet been established. The genes coding for early histone variants are clustered tandemly arranged and highly repetitive and the late histone genes are present in 6 to 12 copies per genome in a non tandem arrangement (Maxson *et al*, 1983; Busslinger and Barberis, 1985; Kaumeyer and Weinberg, 1986). The differential expression of these distinct sets of histones is correlated with different nucleosome populations, detectable by micrococcal nuclease digestion of chromatin (Savic *et al*, 1981; Shaw *et al*,1981; Richards and Shaw, 1984).

Thus far, the sea urchin *Tetrapygus niger* is the only species in which the major CS histone variants have been isolated from chromatin. Seven major cleavage stage histone bands were obtained by electroelution from polyacrylamide urea/acetic acid gels. The electrophoretic migration of CS variants was consistent with histones from other sources, but their aminoacid composition was totally different (Jenson *et al*, 1980; Imschenetzky *et al*, 1984). Due to the inconsistencies of their aminoacid compositions with classical histones, no parallel in their nomination was established and these basic chromosomal proteins were nominated CSA to CSG according to their decreasing electrophoretic mobility in urea/acetic acid gels (Imschenetzky *et al*, 1986). Further studies had demonstrated that the nucleoprotein particle organized by CS variants differs from classical nucleosomes, since the minimal DNA repeat length protected from nuclease digestion is of 123 b.p. as opposed to 146 b.p. found in typical nucleosomes (Imschenetzky *et al*, 1989b).

Late histone genes are represented by different sets of genes coding for distinct H1, H2A and H2B variants but identical histones H3 and H4 (Childs *et al*, 1982; Maxson *et al*, 1983; Kemler and Busslinger, 1986; Kaumeyer and Weinberg, 1986; Knowles *et al*, 1987). The transcription rates, the steady state levels and the rates of decay of single histone genes products increase from early to late blastulas declining in larval stages (plutei 72 hours p.i.). The protein characterization of larval histones revealed that they are electrophoretically heterogeneous and that they differ from those present in sperms of the same species, as well as, from late histone variants that were isolated previously by others from blastulas of a different species (Brandt *et al*, 1979; von Holt *et al*, 1984; Imschenetzky *et al*, 1989a, 1990). In general, the larval histones were very similar to somatic type histones as compared to histones from calf thymus, considered to be representative of somatic type histones (Jenson *et al*, 1980; Imschenetzky *et al*, 1989a). The shifts in electrophoretic heterogeneity of each histone determined previously during larval stages of development are due to post-translational modifications, since the aminoacid composition of the whole complement of histones is invariable from early to late plutei stages (72 to 120 hours p.i.) (Imschenetzky *et al*, 1990).

The comparison of CS histone variants with larval histones presented in this report,

demonstrates that the chromatin from unfertilized eggs and early cleavage stages of the sea urchin *Tetrapygus niger* is packed by basic proteins that are not related to the somatic type histones that are predominant in larval stages of development. Evidence is provided that the differential chromatin packing determines a distinct sensibility of chromatin to nuclease digestion. These results indicate that the chromatin of early embryonic stages has a structural organization that differs from the predominant chromatin packing found in larval stages of development.

MATERIALS AND METHODS

Eggs and plutei larvas

Sea urchins *Tetrapygus niger*, were collected from the bay of Concepción and maintained at room temperature in aquarium containing natural sea water under constant aeration. Unfertilized eggs, and the plutei larvas that were harvested 72 hours post-insemination, were obtained as described previously (Imschenetzky *et al*, 1986, 1989a).

Histones isolation and electrophoretic analysis

Chromatin isolation and histone obtainment was performed as described previously (Imschenetzky *et al*, 1986, 1989a). The bulk CS histone variants and the total histone complement isolated from the plutei larvas was subjected to one-dimension in 18% (w/v) polyacrylamide gel electrophoresis containing sodium dodecylsulfate (PAGE/SDS) and to two-dimensional analysis in 15% (w/v) polyacrylamide gels containing Triton DF-16, urea and acetic acid (PAGE/TAU) in the first dimension and SDS in the second dimension. The gels were prepared as described previously (Laemmli, 1970; Alfageme *et al*, 1974; Imschenetzky *et al*, 1989a, 1991b).

Amino acid analysis

Individual CS histone variants purified from unfertilized eggs and the major histones purified from plutei larvae were hydrolyzed in 5.7 N HCl and analyzed in a Beckman Model 119 CL amino acid analyzer as described previously (Imschenetzky *et al*, 1986, 1989a). Tryptophan was determined by spectrofluorometry in an Aminco Bowman spectrofluorometer (Brand and Witholt, 1967).

Preparation of antisera and Western immunoblots analysis

Histones isolated from plutei larvas were used as antigen. Briefly, an initial dose of 1.2 mg of histones in 1 ml of buffer 0.1 M sodium phosphate pH 7.4 were emulsified with 1 ml of complete Freund adjuvant and used to immunize rabbits. Inoculations were given biweekly with the same dosis of antigen, but dissolved in incomplete Freund adjuvant. The titre of the serum was determined by ELISA using an alkaline phosphatase antibody conjugate, essentially as described by Voller and Bidwell (1986). When the titre of the antiserum was 1/640, was diluted 1/50 in a Tris buffered saline solution (TBS) containing 0.02 M Tris pH 7.5, 0.5 M NaCl and 1% (w/v) gelatin to incubate the nitrocellulose membranes for the Western immunoblot analysis. The procedures followed for the electrophoretic transfer, blotting and immunodetection were essentially those described by Towbin *et al*, (1979), modified as described previously (Imschenetzky *et al*, 1988a, 1991b). Because of differences in the transfer time of histones to nitrocellulose membranes, two nitrocellulose membranes were analyzed separately, the first containing H1 and the second the histones H3, H2A, H2B and H4.

Nucleus isolation, digestion with nucleases and electrophoresis of supranucleosome particles

Whole nuclei were isolated from unfertilized eggs and from plutei larvas respectively, by an aqueous ethanol/ Triton X-100 procedure described by Poccia *et al* (1981). The isolated nuclei were subsequently digested with micrococcal nuclease by following the procedure described by Savic *et al* (1981).

After digestion, the nuclei were pelleted by low speed centrifugation in cold buffer containing 10 mM Tris-HCl pH 7.4, 1 mM EDTA, the nuclear debris was removed and the soluble chromatin obtained was electrophoresed on 1% agarose gels to analyze the supranucleosome particles as described previously (Weintraub, 1984; Imschenetzky *et al*, 1989b).

Isolation of DNA fragments and electrophoresis of DNA

The isolation of DNA fragments was performed according to the procedure described previously by Savic *et al* (1981). Electrophoretic analysis of DNA fragments derived from nuclease digestion was carried out by horizontal 1.9% agarose gels - 0.1% SDS as described by Weintraub (1984).

RESULTS

Transition of the histone complement during early developmental stages: a comparison between initial cleavage and larval stages of development.

To compare the set of histone that are packing the chromatin of early cleavage cells with those that are predominant in larvas of the plutei stage of development, the whole histones complement was isolated from chromatin purified from unfertilized eggs and plutei larvas, respectively, and analyzed by electrophoresis in one and two-dimensional polyacrylamide gels. The patterns of migration of the set CS histone variants are compared with larval histones in Figure 1. No clear differences in the electrophoretic migration could be established. As shown in Figure 1, larval H1 comigrates with two bands of CSC isolated from cleavage cells, H3, H2A and H2B are located in CSF region and CSG migrates as H4. The slots containing the set CS histone variants contain additional bands that are not observed among the larval histones: CSA and CSB migrating ahead of H1, CSD-E between H1 and H3 and four other bands that will not be analyzed in these report since these proteins were found to be more related to HMG chromosomal proteins (Oñate *et al*, 1992). The two-dimensional pattern of electrophoretic migration reveals that both batteries of histone variants are heterogeneous in all their components. From the two-dimensional comparison, no clear differences in migration were observed between the major histones found in larval stages and those from cleavage cells, except for the lack of a CS variant located in the region of larval H2A that is shifted in its migration in the first dimension in TAU/PAG gels. However, the high micro-heterogeneity of the proteins compared in Figure 1 makes an accurate interpretation very difficult. Thus the electrophoretic evidence is not conclusive.

To define more precisely differences between CS histone variants and larval histones, the aminoacid compositions of the individual larval histones are compared to the CS histone variants which exhibit similar electrophoretic migration. As shown in Tables I and II, the larval histones corresponds to the well defined somatic type (Isenberg, 1979; Jenson *et al*, 1980), whereas, those of CS variants are different. With the exception of the lack of tryptophan and a low content of aromatic aminoacid residues in both sets of histone variants, no other significant similarity was observed. No parallel to known histones can be established with CS variants, since the seven CS variants have a lower amount of basic aminoacid residues and a higher amount of acidic aminoacid residues. Differently from larval histones any of the CS variants is rich in lysine and alanine as H1, or in arginine as H3 and H4. From these results it could be expected that the CS histone variants and the larval histones are non related proteins in terms of their primary structure.

To confirm that the CS histone variants and the larval histones are non related proteins in terms of their sequence, the immunological relatedness between both set of proteins was determined by Western blots. Both sets of histone variants, the CS variants and the larval histones were electrophoresed in SDS gels and transferred to nitrocellulose membranes that were subsequently tested

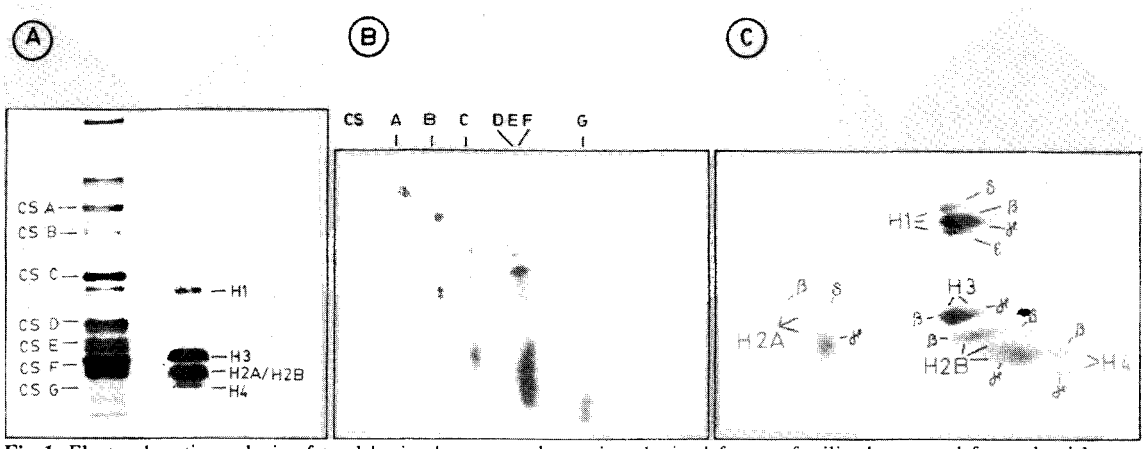

Fig 1. Electrophoretic analysis of total basic chromosomal proteins obtained from unfertilized eggs and from plutei larvae. Acid soluble proteins were obtained from chromatin and electrophoresed under different denaturing conditions. **A:** Analysis in 18% polyacrylamide gels containing 0.1% SDS. **B:** and **C:** Two dimensional electrophoretic analysis in 15% polyacrylamide/ TAU gels used as first dimension and 18% polyacrylamide/ 0.1% SDS gels as the second dimension. **(1B)** CS variants from unfertilized eggs and **(1C)** histones from plutei larvae.

TABLE I

Amino acid composition (mol/100 mol)

	EGG CSC	PLUTEI H1
Ssx	9.6	2.9
Thr	4.2	4.8
Ser	6.8	4.0
Glx	11.8	5.5
Pro	6.9	6.0
Gly	11.2	6.3
Ala	8.0	27.8
Val	6.3	3.8
Met	–	0.9
Ile	3.8	2.3
Leu	6.7	3.4
Tyr	2.4	0.4
Phe	2.0	0.8
Trp	–	–
His	2.4	0.6
Lys	10.0	26.3
Arg	7.9	3.0

against rabbit sera raised against the larval histones. The results shown in Figure 2 demonstrate clearly that the CS variants are proteins unrelated immunologically with larval histones, both to H1 and to the histones that are forming the nucleosomal core, since non cross-reaction could be observed between both groups of proteins.

Transitions in nucleoprotein particles

The supranucleosomal particles derived from unfertilized egg nuclei after digestion with endogenous and micrococcal nuclease were compared with those derived from the nuclei isolated from pluteus larvas. The products of digestion were electrophoresed in 1% agarose gels and the electrophoretic migrations of the nucleoparticles from both sources were determined by comparison with the migration of DNA fragments derived from ØX174 digested with HaeIII restriction nuclease that were run in parallel (Fig. 3). As shown in Figure 3 a broad and uniform distribution of supranucleosomal particles was obtained both from unfertilized eggs, as well as from pluteus larvas. Two particles were derived from unfertilized eggs that were located in the region of the gel corresponding to DNA fragments of 3.4 x 10^3 kDa to 1.95 x 10^3 kDa. Similarly, from larvas pluteus two less defined particles were obtained located from 3.4 x 10^3 kDa to 0.56 x 10^3 kDa.

The DNA contained in the supranucleosomal particles was isolated and analyzed electrophoretically on 1.9% agarose, 0.1% SDS gels, ØX174 digested with restriction nuclease Hae III was used as DNA size markers. From the supranucleosomal particles obtained from unfertilized eggs a periodic distribution of

TABLE II

Amino acid composition (mol/100 mol)

	EGG			PLUTEI			
	CSE	CSF	CSG	H2A	H2B	H3	H4
Asx	8.0	6.9	7.2	5.8	4.6	4.9	4.8
Thr	5.5	4.8	5.1	4.2	6.4	7.2	4.8
Ser	5.8	8.8	5.8	6.9	8.2	5.5	5.4
Glx	9.5	11.0	10.1	9.8	8.0	12.3	8.5
Pro	3.8	2.2	3.5	4.2	3.6	4.7	1.4
Gly	8.2	14.8	7.6	14.3	9.6	8.3	18.1
Ala	9.1	6.5	9.0	14.4	13.3	15.6	9.6
Val	8.2	4.5	6.2	6.7	6.8	5.7	7.1
Met	–	–	–	0.6	0.2	0.5	0.7
Ile	4.0	2.2	3.8	3.5	4.3	4.3	4.1
Leu	5.4	6.4	8.6	8.9	5.2	7.1	5.8
Tyr	2.8	2.8	2.7	0.4	1.1	1.1	0.7
Phe	2.7	2.6	1.8	1.9	1.6	2.7	1.7
Trp	–	–	–	–	–	–	–
His	2.7	6.0	2.7	1.8	1.7	1.5	1.9
Lys	14.3	13.6	15.1	10.0	12.5	10.4	9.1
Arg	10.2	7.0	10.2	8.6	8.8	11.7	10.0

DNA fragments was observed. These DNA fragments were located between 250 and 1500 b.p. of DNA and some preferential cutting sites that are indicated by arrows were detected. In contrast to the supranucleosomal particles obtained from larvas pluteus a broad distribution of DNA fragments were obtained, these fragments were located between 354 b.p. and 140 b.p.

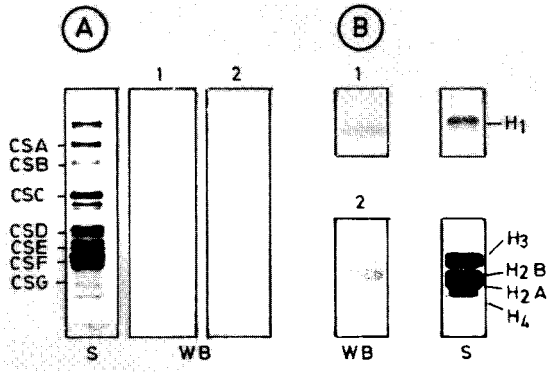

Fig 2. Western blot analysis of CS variants isolated from unfertilized eggs using antibodies to larval histones. CS variants and larval histones were electrophoresed in 18% acrylamide gels containing 0.1% SDS, then transferred to nitrocellulose membranes for 30 min (lanes 1A and B) and 60 min (lanes 2A and B), the nitrocellulose membranes were revealed using rabbit sera anti-larval histones. A: CS variants stained with Coomasie blue (S). Lanes 1 and 2 show the corresponding Western blots (WB). B: Larval histones stained with Coomasie blue (S) and the corresponding Western blots (WB).

DISCUSSION

Although the sets of histone variants from cleavage cells and from plutei larvas compared in this report, are not clearly distinguishable in their electrophoretic migrations, the difference in the amino acid compositions and their immunological unrelatedness clearly demonstrates that both sets of proteins are different. From these results it may be deduced that the genes coding for the CS histone variants are not homologous to late histone genes, or to somatic histone genes from other sources. Such conclusion could be taken as an explanation of the failure in the isolation of the genes encoding CS histone variants that remain still unknown. Opposed to the CS variants are the sea urchins early and late histone genes that are related to histones from other sources and therefore are very

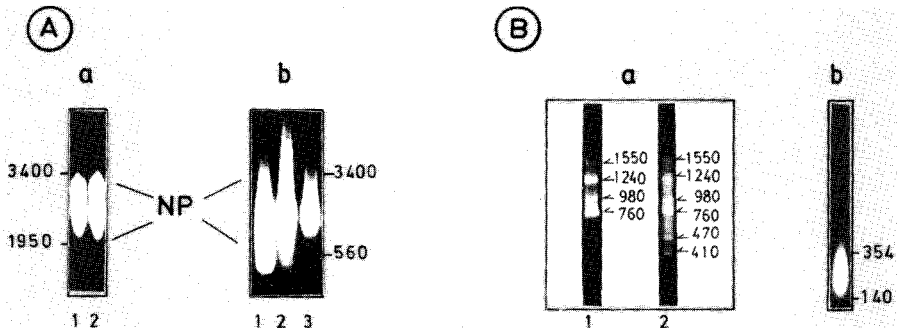

Fig 3. A: Supranucleosomal particles and DNA fragments obtained by digestion of nuclei with nucleases. (a) Nuclei from unfertilized eggs were digested for 10 min with endogenous (lane 1) and micrococcal nuclease (lane 2). (b) Nuclei from plutei larvas were digested for 10 min with endogenous nuclease (lane 1) and with micrococcal nuclease for 3 min (lane 2) and 10 min (lane 3), respectively. The DNA fragments obtained after digestion with nucleases were deproteinized and analyzed in 1.9% agarose gels B: (a) DNA fragments obtained from unfertilized eggs, (b) DNA fragments obtained from plutei larvas.

well characterized at present (Poccia, 1986). From these results it may also be deduced that the electrophoretic migration of proteins is dangerous as a unique criterion to define similarities or differences between proteins, since it can lead to erroneous interpretations.

The results on larval histones are consistent with previous reports indicating that the predominant larval histones exhibit general characteristics of somatic histones. Here it was demonstrated that larval histones will determine a chromatin formed predominantly by classical nucleosomes (Imschenetzky *et al*, 1989b). These results indicate that in sea urchins, starting from larval stages of development onward, the embryonic chromatin is structurally organized as in somatic cells. Such suggestion is in agreement with the decreasing heterogeneity of the mRNA species coding for each histone class reported previously (Halsell *et al*, 1987; Ito *et al*, 1988).

More controversial is the set of CS histone variants, characteristic of cleavage stages of development, since their molecular characterization indicates that these are a group of proteins unrelated from all the histones now known. If their general compositional characteristics are taken as a criterion of defining histones, the CS variants should be considered as chromosomal proteins different from histones. However, if the functional criterion is considered, histones could be responsible for the ordered packing of chromatin, then the CS variants are homonymous to histones from other sources. The functionality of CS variants as histone homonymous is sustained by the following lines of evidence. The basic nucleoparticles that form the chromatin at initial stages of sea urchin development are organized by the CS histone variants, so these variants interact with each other and with DNA to assemble embryonic chromatin (Imschenetzky *et al*, 1989b). During initial cell cycles of embryonic development, CS variants are subjected to post-translational modifications as poly(ADP-ribosylation) and phosphorylation, as well as typical histones (Green and Poccia, 1985, 1989; Wu *et al*, 1986; Boulikas *et al*, 1990; Imschenetzky *et al*, 1991a, 1992). Similar to cell cycle dependent histones, the CS variants are synthesized contemporary with DNA replication and are immediately recruited into chromatin (Wilhelm, 1987). Tacking together all this evidence we propose that despite of the compositional differences of the CS chromosomal proteins and typical histones, the CS histone variants should be considered as histone homonymous.

Besides the sea urchin, which is the organism that has received the more accurate attention, developmentally regulated switches of histone variants have been also described for other organisms, such as the mud snail *I. obsoleta* (Mackay and Newrock, 1982), chicken (Urban and Zweidler, 1983; Engle, 1984), mouse (Zweidler, 1984; Graves *et al*, 1985) and wheat (Rodrigues *et al*, 1985), but were not

found in others, such as the *X. laevis* (Woodland, 1980), *Drosophila* (Anderson and Lengyel, 1984) and *U. caupo* (Franks and Davis, 1983). At present, it is unclear if the failure to detect such switches in these species is due to the lack of histone variants that are particular of very early stages of development or to the unfitness of the methods used for their detection, since DNA probes coding for CS histone variants are not yet available, and the electrophoretic studies are not conclusive as it was demonstrated herein.

The second aspect analyzed in this report concerns the effect of the developmentally regulated changes of histones composition on the structural organization of chromatin. Based on both, the nucleoprotein particles and the DNA fragments that are contained in these particles, it can be concluded that the chromatin packing of supranucleosomal particles that is predominant in larvas pluteus, are different from those found in unfertilized eggs and early cleavage stages of development. It can also be concluded that the chromatin in larval stages of development is organized into nucleoparticles that are more accessible to nucleases digestion, similarly to the chromatin of somatic cells. Differently from larval stages, the chromatin from early cleavage cells is more protected from digestion and is organized into supranucleosomal complexes that are stabilized by a network of proteins. The precise structural organization of the supranucleosomal particles organized by CS histone variants remains to be determined in the future. The biological meaning of the chromatin organization found at initial stages of development could be related to the very high replication rate that is particular to cleavage stages of development or/and alternatively, it may also determine the cellular totipotent condition of these cells related to genome expression.

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REFERENCES

ALFAGEME CR, ZWEIDLER A, MAHOWALD A, COHEN LH (1974) Histones of Drosophila embryo. J Biol Chem 249: 3729-3736

ANDERSON KV, LENGYEL JA (1984) In: STEIN G, STEIN L, MARZLUFF F (eds) Histone Genes. NY: Wiley. pp 135-162

BUSSLINGER M, BARBERIS A (1985) Synthesis of sperm and late histone cDNA of the sea urchin with a primer complementary to the conserved 3' terminal palindrome: evidence for tissue-specific and more general histone gene variants. Proc Natl Acad Sci USA 82: 5676-5680

BOULIKAS T, BASTIN B, BOULIKAS P, DUPUIS G (1990) Increase in histone poly (ADP-ribosylation) in mitogen activated lymphoid cells. Exp Cell Res 187: 77-84

BRAND L, WITHOLT B (1967) Fluorescence measurements. Meth Enzymol 11: 776-856

BRANDT WF, STRICKLAND WH, STRICKLAND M, CARLISLE L, WOODS D, VON HOLT C (1979) A histone programme during the life cycle of the sea urchin. Eur J Biochem 94: 1-10

CHILDS G, NOCENTE-MC GRATH C, LIEBER T, VON HOLT C, KNOWLES J (1982) Sea urchin (*Lytechinus pictus*) late stage histone H3 and H4 genes: characterization and mapping of a clustered but nontandemly linked multigene family. Cell 31: 383-393

ENGLE JD (1984) Organization and expression of chicken histone genes. In: STEIN G, STEIN L, MARZLUFF F (eds) Histone Genes. NY: Wiley. pp 263-280

FRANKS RR, DAVIS FC (1983) Regulation of histone synthesis during early *Urechis caupo (Echiura)* development. Dev Biol 98: 101-109

GRAVES RA, MARZLUFF WF, GIEBELHAUS DH, SCHULTZ GA (1985) Quantitative and qualitative changes in histone gene expression during early mouse embryo development. Proc Natl Acad Sci USA 82: 5685-5689

GREEN GR, POCCIA DL (1985) Phosphorylation of sea urchin sperm H1 and H2B histones precedes chromatin decondensation and H1 exchange during male pronuclear formation. Dev Biol 108: 235-245

GREEN GR, POCCIA DL (1989) Phosphorylation of sea urchin histone CS H2A. Dev Biol 134: 413-419

GURDON JB (1968) Transplanted nuclei and cell differentiation. Sci Am 219: 24-35

HALSELL S, ITO M, MAXSON R (1987) Differential expression of early and late embryonic histone genes in adult tissues of the sea urchin *Strogylocentrotus purpuratus*. Dev Biol 119: 268-274

IMSCHENETZKY M, PUCHI M, OYARCE A, MASSONE R, INOSTROZA D (1984) A comparative study of the histones isolated from sperm of the sea urchin *Tetrapygus niger*. Comp Biochem Physiol 78 B: 393-399

IMSCHENETZKY M, PUCHI M, MASSONE R (1986) Characterization of cleavage stage chromosomal proteins from zygotes of the sea urchin *Tetrapygus niger*. Comp Biochem Physiol 84 B: 23-27

IMSCHENETZKY M, PUCHI M, MASSONE R, ROCCO M, GAMBOA S (1988a) The fate of sperm specific

non-histone chromosomal proteins after fertilization in sea urchins. Arch Biol Med Exp 21: 241-245

IMSCHENETZKY M, PUCHI M, MASSONE R, OYARCE AM, ROCCO M (1988b) Remodeling of chromatin during male pronucleus formation in the sea urchin *Tetrapygus niger*. Arch Biol Med Exp 21: 409-416

IMSCHENETZKY M, PUCHI M, MASSONE R, ZEPEDA P, INOSTROZA D, VEGA I (1989a) Characterization of histones from plutei larvae of the sea urchin *Tetrapygus niger*. Comp Biochem Physiol 94B: 241-244

IMSCHENETZKY M, PUCHI M, GUTIERREZ S, MASSONE R (1989b) Analysis of supranucleosome 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 97 B: 467-469

IMSCHENETZKY M, MONTECINO M, PUCHI M (1991a) Poly(ADP ribosylation) 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, PUCHI M, PIMENTEL C, BUSTOS A, GONZALES M (1991b) Immunobiochemical evidence for the loss of sperm specific histones during male pronucleus formation in monospermic zygotes of sea urchin. J Cell Biochem 47: 1-10

IMSCHENETZKY M, MONTECINO M, PUCHI M (1992) Temporally different poly(adenosine diphosphate-ribosylation) signals are required for DNA replication and cell division in early embryos of sea urchins. J Cell Biochem 51: 198-205

ISENBERG I (1979) Histones. Annu Rev Biochem 48: 159-191

ITO M, BELL J, LYONS G, MAXSON R (1988) Synthesis and turnover of late H2B histone mRNA in developing embryos of the sea urchin *Strongylocentrotus purpuratus*. Dev Biol 129: 147-158

JENSON G, CHIN-LIN P, GERBER-JENSON B, LITMAN G (1980) Structurally unique basic protein coextracted with histones from calf thymus chromatin. Proc Natl Acad Sci USA 77: 1389-1393

KAUMEYER JF, WEINBERG ES (1986) Sequence, organization and expression of late embryonic H3 and H4 histones genes from the sea urchin *Strongylocentrotus purpuratus*. Nucleic Acid Res 14: 4557-4576

KEMLER I, BUSSLINGER M (1986) Characterization of two non-allelic pairs of late histone H2A and H2B genes of the sea urchin: differential and tissue-specific expression in the adult. Mol Cell Biol 6: 3746-3754

KNOWLES J, LAI Z, CHILDS G (1987) Isolation, characterization and expression of the gene encoding the late histone subtype H1 of the sea urchin. Mol Cell Biol 7: 478-485

KORNBERG RD (1977) Structure of chromatin. Annu Rev Biochem 46: 931-954

LAEMMLI UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond) 227: 680-685

MACKAY S, NEWROCK KM (1982) Histone subtypes and switches in synthesis of histone subtypes during *Ilyanassa* development. Dev Biol 93: 430-437

MAXSON R, MOHUN T, GORMEZANO G, CHILDS G, KEDES L (1983) Distinct organization and patterns of expression of early and late histone gene sets in the sea urchin *Stongylocentrotus purpuratus*. Nature (Lond) 301: 120-125

NEWROCK RM, ALFAGEME CR, NARDI RV, COHEN LH (1977) Histone changes during chromatin remodeling in embryogenesis. Cold Spring Harb Symp Quant Biol 42: 421-431

OLD RW, WOODLAND HR (1984) Histone genes: not so simple after all. Cell 38: 624-626

OÑATE E, MEDINA R, PUCHI M, INOSTROZA D, GAMBOA S, GUTIERREZ S, IMSCHENETZKY M (1992) Proteinas de alta movilidad electroforética (HMG) en óvulos de *Tetrapygus niger*. VI Reun Soc Biol Cel Chile

PABO C, SAUER R (1992) Transcription factors: structural families and principles of DNA recognition. Annu Rev Biochem 61: 1053-1095

POCCIA D, 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 the sea urchin eggs. Dev Biol 82: 287-296.

POCCIA D (1986) Remodeling of nucleoproteins during gametogenesis, fertilization, and early development. Intl Rev Cytol 105: 1-65

POCCIA D, GREEN GR (1992) Packaging and unpackaging the sea urchin sperm genome. Trends Biochem Sci 17: 223-227

RICHARDS RG, SHAW BR (1984) Temporal changes in nucleosome populations during sea urchin early development. Biochemistry 23: 2095-2102

RODRIGUES J, BRANDT WF, VON HOLT C (1985) The amino acid sequence of wheat histone H2A. A core histone with a C-terminal extension. Eur J Biochem 150: 499-506

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

SHAW B, COGNETTI G, SHOLES WM, RICHARDS R (1981) Shift in nucleosome populations during embryogenesis: Microheterogeneity in nucleosomes during development of the sea urchin embryo. Biochemistry 20: 4971-4978.

SCHWAGER S, BRANDT WF, VON HOLT C (1983) The isolation of isohistones by preparative gel electrophoresis from embryos of the sea urchin *Parechinus angulosus*. Biochim Biophys Acta 741: 315-321

TOWBIN H, STAEHELIN T, GORDON J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76: 4350-4354

TRAVERS A (1989) DNA conformation and protein binding. Annu Rev Biochem 58: 427-452

URBAN MK, ZWEIDLER A (1983) Changes in nucleosomal core histone variants during chicken development and maturation. Dev Biol 95: 421-428

VOLLER A, BIDWELL D (1986) Enzyme-linked immunoadsorbent assay. In: ROSE N, FRIEDMAN H, FAHEY J (eds) Manual of Clinical Laboratory Immunology. Washington, DC: American Society for Microbiology. pp 99-109

VON HOLT C, DE GROOT P, SCHWAGER S, BRANDT WF (1984) The structure of sea urchin histones and considerations on their function. In: STEIN G, STEIN J (eds) Histone Genes. NY: John Wiley & Sons. pp 65-105

VON HOLT C (1985) Histones in perspective. BioEssay 3:120-123

VON HOLT C, BRANDT WF, GREYLING HJ, LINDSAY GG, RETIEF JD, RODRIGUES J, SCHWAGER S, SEWELL BT (1989) Isolation and characterization of histones. Meth Enzymol 170: 431-523

WEINTRAUB H (1984) Histone H1 dependent chromatin superstructures and the suppression of gene activity. Cell 38: 17-27

WILHELM V (1987) Síntesis de proteínas cromosomales y su posible rol en la regulación de la replicación del genoma. Tésis, Univ Concepción

WOODLAND HR (1980) Histone synthesis during the development of *Xenopus*. FEBS Lett 121: 1-7

WU RS, PANUSZ HT, HATCH CL, BONNER WM (1986) Histones and their modifications. CRC Crit Rev Biochem 20: 201-263

ZWEIDLER A (1984) Core histone variants of the mouse: primary structure and differential expression. In: STEIN G, STEIN L, MARZLUFF F (eds) Histone Genes. NY: Wiley. pp 339-372