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Histone H1c decreases markedly in postreplicative stages of chicken spermatogenesis

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ABSTRACT The relative proportions of four major chicken histone H1 subtypes (referred to as H1a, H1b, H1c and H1d) change markedly in different chicken tissues. The relative amount of H1c is higher in nonreplicating somatic tissues, such as liver, than in replicating immature testis. The proportion of H1c sharply decreases as spermatogenesis proceeds, being much lower in mature than in immature testis. It has been proposed that the relative increment of H1c correlates with low rates of cell division in chicken tissues. It was assumed that the sharp decrease in H1c observed during maturation of chicken testis was a consequence of the intensification of proliferative activity in spermatogonia (Berdnikov *et al.*, 1976). Our results, however, clearly show that the decrease of H1c during maturation is due to the low levels of this protein in postreplicative stages of spermatogenesis, where H1c is barely detectable. These results suggest that the presence of the arginine-rich H1c subtype would neither be compatible with the relaxed structure of acetylated chromatin present in active replicating cells nor with the hyperacetylated chromatin characteristic of postreplicative late spermatids undergoing the nucleohistone nucleoprotamine transition.

KEY WORDS: *histone H1 variants, H1c, chromatin, spermatogenesis*

Introduction

Histones are involved in the packaging of DNA in the eukaryotic nuclei. Whereas histones H2A, H2B, H3 and H4 play a fundamental role in the formation of nucleosomes, histone H1 participates in all levels of DNA packaging: 1) Sealing off the two turns of nucleosomal DNA, and thereby stabilizing the nucleosomal structure; 2) inducing the salt-dependent compaction of the nucleosomal strand; 3) folding the nucleosomal cores into a compact 30 nm fiber, and 4) aggregating the chromatin *in vitro* in a way that might be similar to the heterochromatic clumps observed in electron micrographs (McGhee and Felsenfeld, 1980; Losa *et al.*, 1984).

In all species studied histone H1 is heterogeneous (Cole, 1987). In the chicken, six histone H1 subtypes and their corresponding genes have been sequenced (Coles et *al.*, 1987; Shannon and Wells, 1987). Though little is known about the physiological role of histone H1 subtypes, it has been suggested that chromatin might be viewed as a mosaic of aggregation-resistant and aggregation-prone regions which differ in H1 content both qualitatively and quantitatively (Cole, 1987).

During spermiogenesis chromatin undergoes one of the most dramatic changes observed in eukaryotes (Mezquita, 1985). The euchromatic and heterochromatic nuclear regions are replaced in elongating spermatids by fibers of uniform appearance. The chromatin of elongated spermatids contains hyperacetylated histones and undergoes a structural change with a marked increment in DNA binding sites, followed by protamine deposition and the subsequent histone removal (Mezquita and Teng, 1977a, b; Oliva and Mezquita, 1982; Oliva and Mezquita, 1986; Oliva et *al.*, 1987).

The marked structural and functional transitions of chromatin during spermatogenesis offer an ideal system to study the involvement of histone H1 subtypes in these processes. We report here the characterization of histone H1 complement in postreplicative stages of spermatogenesis in comparison with the complement of replicative immature testis enriched in spermatogonia. The complement of chicken testis cells has also been compared with nonreplicating somatic tissues, such as liver, or erythrocytes.

Results

Changes in the relative proportions of H1 subtypes in different chicken tissues

We have determined the relative proportions of histone H1 subtypes in different chicken tissues: immature testis, mature testis, liver, erythrocytes and reticulocytes. The four major histone H1 subcomponents quantified in this paper correspond to the main electrophoretic bands previously described in chick embryo and chicken testis cell nuclei at different stages of spermatogenesis

Abbreviations used in this paper: SDS, sodium dodecylsulfate; DNase I, deoxyribonuclease.

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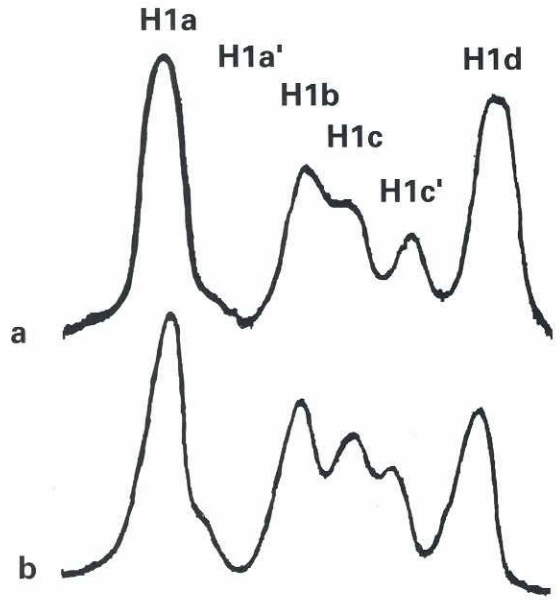

Fig. 1. Polyacrylamide gel electrophoresis of histone H1 proteins from chicken reticulocytes (a) and chicken erythrocytes (b). *Histone H1 proteins were electrophoresed on an acid/urea 18% polyacrylamide gel at 150 V for 72 h. The resolved bands are labeled H1a, H1a', H1b, H1c, H1c' and H1d, from left to right, in order of increasing mobility on the gel. Densitometer scans were obtained with a Hoefer densitometer.*

(Mezquita and Teng, 1977a). The major histone H1 subtypes obtained from chicken tissues have been characterized by electrophoretic mobility in acetic/urea/polyacrylamide gels, SDS-gels, two-dimensional electrophoresis and peptide maps with the protease V8 and chymotrypsin (Boix, 1987). Using a modification of the Panyim and Chalkley gel system, Shannon and Wells (1987) have been able to resolve two additional components. The six chicken histone H1 subtypes, labeled H1a, H1a', H1b, H1c, H1c' and H1d, in order of increasing mobility on acid/urea polyacrylamide gels, have been sequenced (Coles et al., 1987) and assigned to their corresponding genes (Shannon and Wells, 1987). The resolution obtained only permits the quantification of four major H1 bands: H1a (H1a+H1a'), H1b, H1c (H1c+H1c'), and H1d (Fig. 1). The relative proportions of the four major chicken histone H1 subtypes change markedly in different chicken tissues (Fig. 2). The relative amount of H1c is higher in nonreplicating somatic cells, such as liver, than in replicating immature testis (Table 1).

Changes in the relative proportions of four major chicken histone H1 subtypes during chicken spermatogenesis

The proportion of H1c sharply decreases from immature to mature chicken testis (Table 1). It was proposed that the decrease in H1c observed during maturation of chicken testis correlated with the intensification of proliferative activity in spermatogonia (Berdnikov *et al.*, 1976). To test whether or not the decrease in H1c was due to an increase in cell division or to structural changes of chromatin occurring in postreplicative stages of chicken spermatogenesis we determined the relative proportion of H1 subtypes in cells at successive stages of spermatogenesis separated by centrifugal elutriation (Fig. 3). Our results show that the decrease of H1c during maturation is due to the low levels of this protein in postreplicative stages of spermatogenesis, particularly in nonreplicating, transcriptionally inactive late spermatids, where H1c is barely detectable (Fig. 2). As spermatogenesis proceeds H1c decreases and H1b, a histone H1 subtype containing less arginine residues at the carboxyterminal domain (Fig. 4) becomes the predominant histone H1 in the relaxed hyperacetylated chromatin characteristic of the final stages of spermatogenesis during the nucleohistone nucleoprotamine transition (Fig. 3).

To eliminate the possibility that the change in the electrophoretic pattern of chicken testis H1 in relation to chicken liver H1 was due to differential degradation during sample preparation, H1 histones were extracted from a mixture of testis and liver cells. The resulting pattern is additive as shown in Fig. 5, indicating that differential

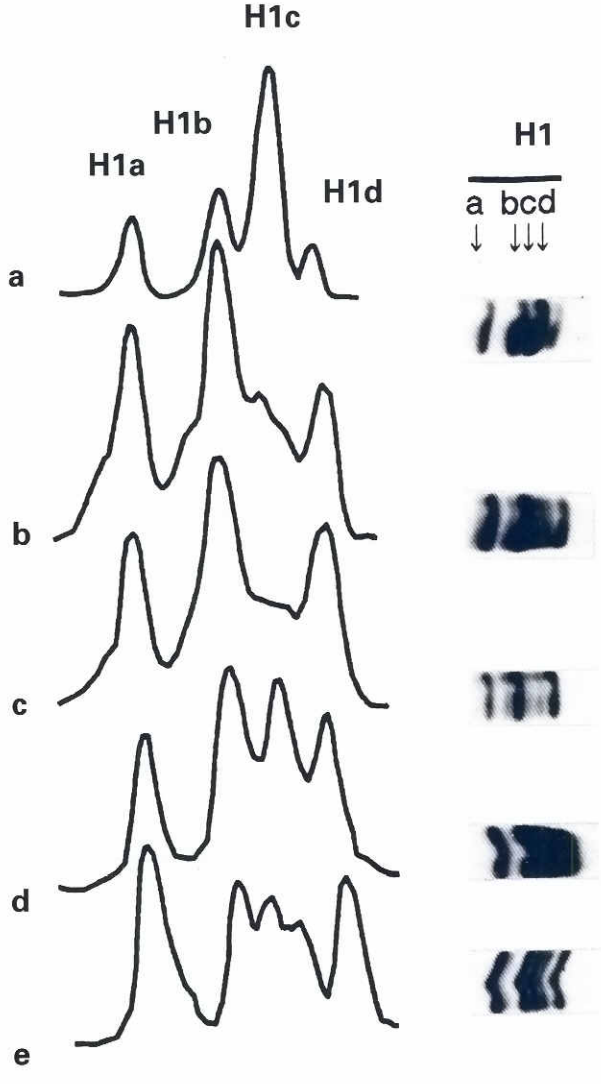

Fig. 2. Comparison of histone H1 subtypes obtained from different chicken tissues. *Histone H1 proteins were electrophoresed, scanned and labeled as indicated in the legend for Fig. 1. Chicken tissues: liver* **(a)**, *mature testis* **(b)**, *elongated spermatids* **(c)**, *immature testis* **(d)**, *erythrocytes* **(e)**.

TABLE 1

CHANGES IN THE RELATIVE PROPORTIONS OF H1 PROTEINS IN DIFFERENT CHICKEN TISSUES

Chicken tissues or cells	H1a (%)	H1b (%)	H1c (%)	H1d (%)
Liver	18±3	25±3	48±5	10±3
Immature testis	19±3	34±3	29±4	18±3
Mature testis	24±2	43±4	13±5	19±5
Erythrocye	33±2	20±1	26±1	20±1
Reticulocyte	32±2	18±1	20±1	26±2

H1 proteins were extracted from each tissue and electrophoresed in acetic acid/urea polyacrylamide gels. H1 proteins were quantified from the gel scans or by dye elution from the excised bands, as described in the Materials and Methods section. The resolution obtained permits the quantification of four major H1 bands: H1a (H1a+H1a'), H1b, H1c (H1c+H1c') and H1d. Reticulocytosis was induced by injection of phenylhydrazine (Rapoport, 1986).

proteolysis in both samples is not the cause of the observed patterns.

Postranslational modifications of histone H1 subtypes, such as phosphorylation or poly ADP-ribosylation, can also be responsible for the observed differences in the electrophoretic patterns. To eliminate this possibility H1 histones were digested with alkaline phosphatase and phosphodiesterase as described in the Materials and Methods section. Neither qualitative nor quantitative differences were observed in the electrophoretic patterns after digestions (results not shown).

Chicken histone H5, but not histone H1c, reacts with an antibody against rat H1°

In mammals the H1 subtype H1° accumulates in nondividing cells and in terminally differentiated cells (Lennox and Cohen, 1983; Piña *et al.*, 1987). The similarity between the regulation of H1c synthesis during chicken myogenesis and H1° synthesis in mammals, led to the proposal that these proteins may be functionally homologous. We have tested a possible cross-reactivity between rat H1° and H1c to rat H1° antibody. The antibody cross-reacted with chicken erythrocyte histone H5 but not with H1c or any other perchloric acid soluble protein extracted from chicken cells (Fig. 6).

Discussion

We have determined the relative proportions of histone H1 subtypes in different chicken tissues: testis, liver and erythrocytes. The four major histone H1 subcomponents quantified in this paper correspond to the main electrophoretic bands previously described in chicken testis cell nuclei at successive stages of spermatogenesis (Mezquita and Teng, 1977a) and in different chicken tissues (Berdnikov *et al.*, 1976; Winter *et al.*, 1985a,b).

The major changes in the proportions of histone H1 subtypes observed mainly concern the H1c (H1c+H1c') subtype. This protein is abundant in nonreplicating tissues, such as liver, and markedly decreases in immature chicken testis enriched in actively replicating spermatogonia. This observation is consistent with the correlation previously reported between H1c levels and low rates of cell division in different chicken tissues (Berdnikov *et al.*, 1976; Winter *et al.*, 1985a,b).

During chicken myogenesis the H1c subtype presents unique properties: 1) The level of H1c mRNA is completely uncoupled to DNA replication in contrast to the level of the other mRNA's encoding H1a, H1b and H1d that are more tightly coupled. 2) Histone H1c is the most stable subtype, the other subtypes showed shorter half-lives. 3) Both facts, the selective synthesis of H1c after DNA replication and its higher stability, determine the accumulation of H1c in nondividing muscle cells (Winter *et al.*, 1985a,b). The relative amount of H1c also increases in culture chick embryo fibroblasts as cell division decreases following density-dependent inhibition of growth (Smith *et al.*, 1981). H1c is the major component in nondividing chick embryonic lens fibers (Teng *et al.*, 1974).

During maturation of chicken testis a sharp decrease in the relative amount of H1c was observed (Berdnikov *et al.*, 1976). In line with other observations that indicate a negative correlation between H1c levels and replicating activity, the decrease in H1c during chicken testis maturation was interpreted as a consequence of the intensification of proliferation of spermatogonia. Our results show however that as spermatogenesis proceeds the transition from replicating spermatogonia to nonreplicating spermatids is accompanied by a drastic decrease in H1c instead of the expected increase characteristic of other nondividing cells.

Two nonreplicating terminally differentiated chicken cells — late spermatids and lens fibers — possess a very different chromatin

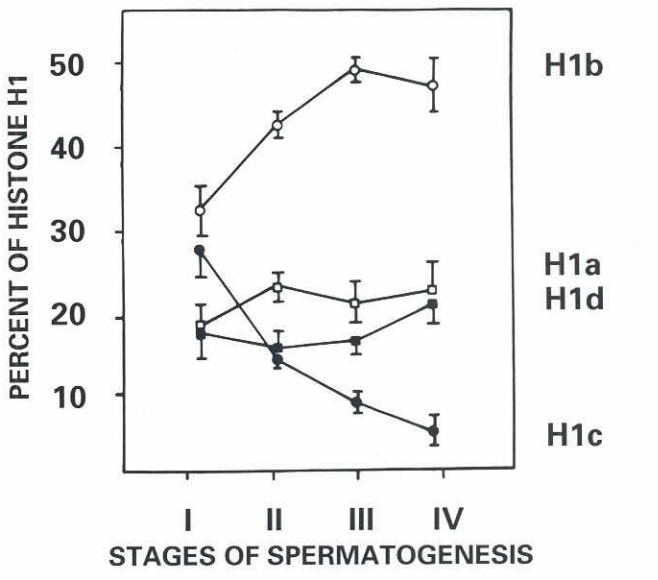

Fig. 3. Changes in the relative proportions of H1 proteins obtained from chicken testis cells at successive stages of spermatogenesis. *Chicken testis cells were separated by centrifugal elutriation and H1 proteins were extracted from each fraction and electrophoresed in acetic acid/urea polyacrylamide gels. H1 proteins were quantified from gel scans or by dye elution from the excised bands, as described in the Materials and Methods section. The resolution obtained permits the quantification of four major bands: H1a (H1a+H1a') (❑), H1b(○), H1c (H1c+H1c') (●), and H1d (■).*

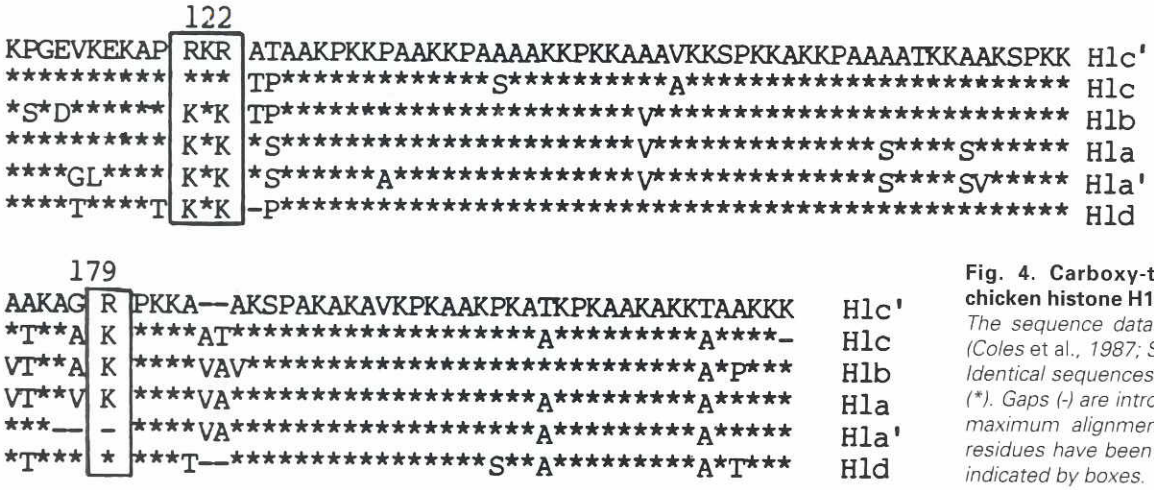

Fig. 4. Carboxy-terminal sequences of chicken histone H1 derived from the genes. *The sequence data are from the references (Coles* et al., *1987; Shannon and Wells, 1987). Identical sequences are depicted by asterisks (*). Gaps (-) are introduced into sequences for maximum alignment. Regions where lysine residues have been replaced by arginines are indicated by boxes.*

structure, highly relaxed in spermatids (Mezquita and Teng, 1977a, b) and highly condensed in lens fibers (Sanwal et *al.*, 1986). In spermatids, H1c is barely detectable, whereas in lens fibers H1c is the major H1 subtype. We propose that the presence of histone H1c might not be compatible with the relaxed structure of the hyperacetylated chromatin of chicken spermatids. In a similar way the presence of H1c might interfere with the acetylated and relaxed chromatin structure of replicating cells. H1c (H1c+H1c') possesses a higher content of arginine residues than the other H1 subtypes at the C-terminal end of the molecule required for chromatin folding (Shannon and Wells, 1987). This fact is consistent with the proposed role of H1c in heterochromatinization.

The enrichment of H1c in nonreplicating tissues and its decrease during spermiogenesis agree with the presence of abundant stable polyadenylated H1c transcripts in quiescent and differentiated tissues and the absence of polyadenylated transcripts in chicken spermatids (Kirsh *et al.*, 1989).

Two families of lysine-rich histones, H5 and H1°, are present in terminally differentiated cells, and may be responsible for the structure of chromatin regions that are compacted more tightly than active chromatin. Although the role played by chicken H1c in chromatin condensation might be similar, we have shown that this H1 protein does not cross-react with an antibody to rat H1° that recognizes chicken H5. Differences in the sequence of these proteins, particularly in the number of arginine residues present in the carboxyl-terminal domain, may confer specific properties to the condensed chromatin in different cells.

In mammalian spermatogenesis it also has been postulated that specific patterns of H1 subtypes may be responsible for the marked changes in chromatin structure observed in premeiotic, meiotic and postmeiotic cells (Seyedin and Kisler, 1980; Lennox and Cohen, 1984; Meistrich et *al.*, 1985).

Materials and Methods

Animals

Sexually immature (6-weeks-old) and sexually mature (25-50 weeks-old) Hubbard White Mountain chickens were used in the experiments.

Separation of chicken testis cells by centrifugal elutriation

Chicken testis cells were prepared and separated by centrifugal elutriation essentially as described in Roca and Mezquita (1989). Mature testes were decapsulated and minced finely with scissors. The minced tissue was gently suspended in 10 vol. of minimum essential medium (Eagle) containing 0.1% (w/v) trypsin and 2µg of DNase I/ml. The suspension was incubated at 31°C for 30 min with gentle stirring in a water bath. After incubation, the cell suspension was filtered through four layers of surgical gauze and centrifuged for 20 min at 1500g in a JE-7.5 Beckman rotor. The sample was resuspended in 50 ml of Ca^{++}/Mg^{++}-free phosphate buffered saline containing 0.02% (w/v) soybean trypsin inhibitor, 0.1% bovine serum albumin,

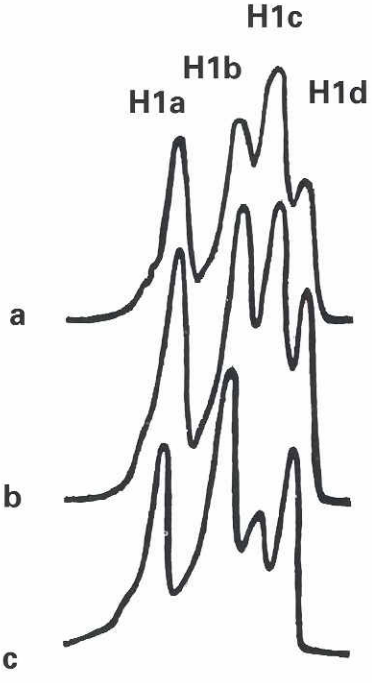

Fig. 5. Comparison of the electrophoretic pattern of histone H1 proteins obtained from chicken liver (a), chicken testis (c) and a mixture of both tissues (b). *Similar amounts of liver and mature testis or a mixture of both tissues were used to isolate H1 proteins. Electrophoresis was performed in acetic acid/urea polyacrylamide gels and the H1 subtypes were labeled as H1 a-d from left to right, in order of increasing mobilities.*

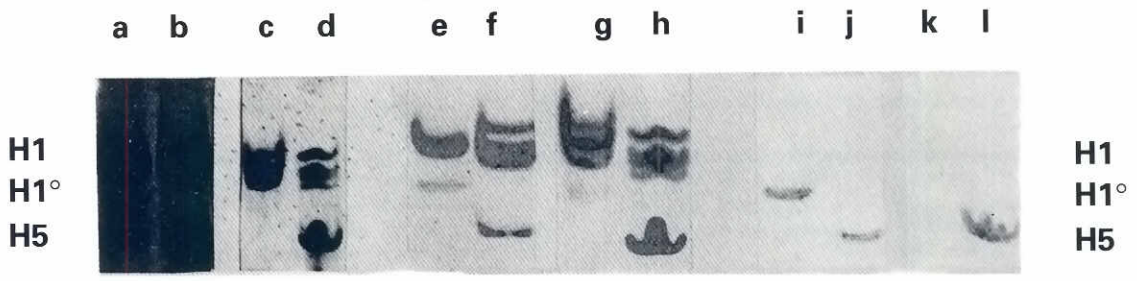

Fig. 6. Reaction of 5% perchloric acid soluble proteins, obtained from rat and chicken tissues, with antiserum to rat H1°. *Histone H1 proteins and other 5% perchloric acid soluble proteins obtained from rat and chicken tissues were electrophoresed in 15% acetic acid/urea polyacrylamide gels, transferred to nitrocellulose paper and reacted with rat H1° antiserum. Bound antibodies were detected with peroxidase-conjugated anti-rabbit IgG, using 4-chloronafthol and H_2O_2 to visualize the complex.* **(Lanes a-d)**, *Coomassie Brilliant Blue stained gels.* **(Lanes e-h)**, *proteins transferred to nitrocellulose paper.* **(Lanes i-l)**, *proteins transferred to nitrocellulose paper and reacted with anti-rat H1°. Tissues: rat liver* **(a, e, i)**, *chicken liver* **(b, f, j)**, *chicken testis* **(c, g, k)**, *chicken erythrocyte* **(d, h, l)**.

and 0.1% glucose. The cell suspension was diluted in Ca^{++}/Mg^{++}-free phosphate buffered saline to a final concentration of 25-30x10^6 ml. A cell suspension of 20 ml was loaded into a JE-6 Beckman elutriator rotor and separations were performed with speeds of 3000 rpm and flow rates of 3-100 ml/min. Fractions of 125 ml were collected. The following cell types were obtained: testicular spermatozoa and residual bodies (3 ml/min flow rate); elongated spermatids (11 ml/min flow rate); round spermatids (20 ml/min flow rate); meiotic and premeiotic cells and multinucleate cells (37 ml/min flow rate).

Isolation and analysis of histone H1

Histone H1 was extracted from chicken cells with 0.74 N $HClO_4$ (Johns, 1977). Electrophoreses were performed in acetic acid/ urea/polyacrylamide gels (Panyim and Chalkley, 1969; Shannon and Wells, 1987). Band intensities were quantified by gel scanning with a Hoefer densitometer and by dye elution from the excised bands (Tal et al., 1985).

Alkaline phosphatase and Phosphodiesterase digestion of H1

Perchloric acid extracts from chicken testis cells were digested either with alkaline phosphatase (Sigma) essentially as described by Lennox *et al.* (1982), or with snake venom phosphodiesterase from *Crotalus durissus* (Boehringer). Aliquots containing 30-60 µg of protein were digested with 2-15 µg of alkaline phosphatase or 0.025-0.050 units of snake venom phosphodiesterase, for 1 h at 37°C in 50 mM Tris/HCl, 2 mM ß-mercaptoethanol, 100 mM Na_2SO_4 and 10 mM $MgCl_2$, pH 7.5, containing Trasylol (100 units/ml) to inhibit proteolytic activity.

Electrophoretic transfer from polyacrylamide gels to nitrocellulose sheets and immunological detection of proteins

Histone H1 subtypes were separated on SDS gels or in acetic acid/urea gels and electrophoretically transferred to nitrocellulose paper (Towbin et *al.*, 1979). Rabbit anti-rat histone H1° serum was kindly provided by Dr. P. Suau and Dr. P. Martínez (Autonomous University of Barcelona). The antibody binding was detected by a peroxidase conjugated antirabbit IgG, using 4-chloro-1-naphthol as colorimetric substrate of the peroxidase reaction.

Acknowledgments

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References

BERDNIKOV, V.A., GOREL, F.L., ARGUTINSKAYA, V.A., CHEREPANOVA, V.A. and KILEVA, E.V. (1976). Study of the relationship between the subfraction composition of histone F1 and the type of tissue in birds. *Mol. Biol. 10:* 887-896.

BOIX, J. (1987). Changes in H1 complement during chicken spermatogenesis. Ph. D. Thesis, University of Barcelona.

COLE, R.D. (1987). Microheterogeneity in H1 histones and its consequences. *Int. J. Pept. Protein Res. 30:* 433-449.

COLES, L.S., ROBINS, A.J., MADLEY, L.K. and WELLS, J.R.E. (1987). Characterization of the chicken histone H1 gene complement. Generation of a complete set of vertebrate H1 protein sequences. *J. Biol. Chem. 262:* 9656-9663.

JOHNS, E.W. (1977). The isolation and purification of histones. In *Methods in Cell Biology* Vol. 16 (Eds G. Stein, J. Stein and L.J. Kleinsmith). Academic Press, N.Y., pp. 183-203.

KIRSH, L.A., GROUDINE, M. and CHALLONER, P.B. (1989). Polyadenylation and U7 snRNP-mediated cleavage: alternative modes of RNA 3' processing in two avian histone H1 genes. *Genes Dev. 3:* 2172-2179.

LENNOX, R.W. and COHEN, L.H. (1983). The histone H1 complements of dividing and non-dividing cells of the mouse. *J. Biol. Chem. 258:* 262-268.

LENNOX, R.W. and COHEN,L.H. (1984). The histone H1 complements of dividing and non-dividing cells of the mouse. *Dev. Biol. 103:* 80-84.

LENNOX, R.W., OSHIMA, R.G. and COHEN, L.H. (1982). The H1 histones and their interphase phosphorylated states in differentiated and undifferentiated cell lines derived from murine teratocarcinomas. *J. Biol. Chem. 257:* 5183-5189.

LOSA, R., THOMA, F. and KOLLER, T. (1984). Involvement of the globular domain of histone H1 in the higher order structures of chromatin. *J. Mol. Biol. 175:* 529-551.

McGHEE, J.D. and FELSENFELD, G. (1980). Nucleosome structure. *Annu. Rev. Biochem. 49:* 1115-1156.

MEISTRICH, M.L., BUCCI, L.R., TROSTLE-WEIGE, P.K. and BROCK, W.A. (1985). Histone variants in rat spermatogonia and primary spermatocytes. *Dev. Biol. 112:* 230-240.

MEZQUITA, C. (1985). Chromatin proteins and chromatin structure in spermatogenesis. In *Chromosomal Proteins and Gene Expression* (Eds. G.R. Reeck, G.H. Goodwin and P. Puigdomenech). Plenum Press, New York, pp. 315-332.

MEZQUITA, C. and TENG, C.S. (1977a). Changes in nuclear composition and genomic activity during spermatogenesis in the maturing rooster testis. *Biochem. J. 164:* 99-111.

MEZQUITA, C. and TENG, C.S. (1977b). Changes in chromatin structure during spermatogenesis in maturing rooster testis as demonstrated by the initiation pattern of ribonucleic acid synthesis *in vitro*. *Biochem. J. 170:* 203-210.

OLIVA, R. and MEZQUITA, C. (1982). Histone H4 hyperacetylation and rapid turnover of its acetyl groups in transcriptionally inactive rooster testis spermatids. *Nucleic Acids Res. 10:* 8049-8059.

OLIVA, R. and MEZQUITA, C. (1986). Marked differences in the ability of distinct protamines to disassemble nucleosomal core particles in vitro. *Biochemistry 25:* 6508-6511.

OLIVA, R., BAZETT-JONES, D., MEZQUITA, C. and DIXON, G.H. (1987). Factors affecting nucleosome disassembly by protamines *in vitro*: Histone hyperacetylation and chromatin structure, time dependence and the size of the sperm nuclear proteins. *J. Biol. Chem. 262:* 17016-17025.

PANYIM, S. and CHALKLEY, R. (1969). High resolution acrylamide gel electrophoresis of histones. *Arch. Biochem. Biophys. 130:* 337-346.

PIÑA, B., MARTINEZ, P. and SUAU, P. (1987). Changes in H1 complement in differentiating rat-brain cortical neurons. *Eur. J. Biochem. 164*: 71-76.

RAPOPORT, S.M. (1986). Induction of reticulocytosis. In *The Reticulocyte*. C.R.C. Press, Boca Raton, Florida, pp. 91-102.

ROCA, J. and MEZQUITA, C. (1989). DNA topoisomerase II activity in nonreplicating, transcriptionally inactive, chicken late spermatids. *EMBO J. 8:* 1855-1860.

SANWAL, M., MUEL, A.S., CHANDIN, E., COURTOIS, Y. and COUNIS, M.F. (1986). Chromatin condensation and terminal differentiation process in embryonic chicken lens *in vivo* and *in vitro*. *Exp. Cell Res. 167:* 429-439.

SEYEDIN, S.M. and KISLER, W.S. (1980). Isolation and characterization of rat testis H1t: an H1 histone variant associated with spermatogenesis. *J. Biol. Chem. 255:* 5949-5954.

SHANNON, M.F. and WELLS, J.R.E. (1987). Characterization of the six chicken histone H1 proteins and alignment with their respective genes. *J. Biol. Chem. 262:* 9664-9668.

SMITH, B.J., COOK, Y., JOHNS, E.W. and WEIS, R.A. (1981). Absence of H1° from quiescent chicken cells. *FEBS Lett. 135:* 77-80.

TAL, M., SILBERSTEIN, A. and NUSSER, E. (1985). Why does coomasie brilliant blue R interact differently with different proteins? *J. Biol. Chem. 260:* 9976-9980.

TENG, N.N.H., PIATIGORSKY, J. and INGRAM, V.M. (1974). Histones of chick embryonic lens nuclei. *Dev. Biol. 41:* 72-76.

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

WINTER, E., LEVY, D. and GORDON, J.S. (1985a). Changes in the H1 histone complement during myogenesis. I. Establishment by differential coupling of H1-especies synthesis to DNA replication. *J. Cell Biol. 101:* 167-174.

WINTER, E., PALATNIK, C.M., WILLIAMS, D.L., COLES, L.S., WELLS, J.R.E. and GORDON, J.S. (1985b). Changes in the H1 histone complement during myogenesis. II. Regulation by differential coupling of H1 variant mRNA accumulation to DNA replication. *J. Cell Biol. 101:* 175-181.

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