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HISTONE-INDUCED CONDENSATION OF RAT TESTIS CHROMATIN: TESTIS-SPECIFIC H1t VERSUS SOMATIC H1 VARIANTS

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Due the likely role of H1 histone variants in inducing the formation of folded DNA filaments with different stabilities, the condensing capacity of the testis-specific H1t versus the somatic variants was tested. Circular dichroism analyses of rat testis H1-depleted oligonucleosomes (5-2kbp) revealed that H1t, which appears in germ cells during the meiotic prophase of mammalian spermatogenesis, exerts the lowest condensing effect as compared to the other variants. The distribution of H1 subtypes among different chromatin fractions was also investigated and gave evidence that H1t is more abundant in chromatin regions which are more sensitive to DNAase I digestion. © 1994 Academic Press, Inc.

In higher organisms the heterogeneity of the H1 histone family is such that at least five different H1 somatic subtypes have been reported (1,2). The relative amount of these genic variants (H1a, H1b, H1c, H1d, H1e), differ in various tissues and species and also depend on the development stages of the organism (1, 3).

In testis germ cells of a number of different organisms, during spermatogenesis, the usual somatic H1 variants are at least in part replaced by a testis-specific subtype, H1t (4). In particular, the H1t gene is reportedly first transcribed in pachytenic spermatocytes (5-6).

A specific role for the different H1 variants has not yet been established, but the presence of a large number of subtypes, as well as their possible post-synthetic modifications (7-8), are of the highest importance to explain the numerous structural and functional roles played by H1 histone in chromatin (9-11).

With regard to the structural role of the different H1 histones, previous analyses of both dinucleosomes (12) and linear and super-helical DNA (13), demonstrated that H1 somatic variants

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differ among themselves in their ability to condense DNA.

In order to address the question of functional differences between somatic and tissue-specific H1 variants, we have analyzed the condensing capacity of H1t versus somatic subtypes on rat testis oligonucleosomes.

Circular dichroism (CD) spectra regarding the condensing effect exerted by H1 somatic variants and H1t on H1-depleted oligonucleosomes as well as the different distribution of H1t in soluble and insoluble chromatin fractions, show that all somatic variants are more powerful than the testis subtype in inducing chromatin condensation.

MATERIALS AND METHODS

All chemicals and enzymes were of the highest purity available and were purchased from SIGMA and MERCK.

Nuclei were isolated from adult rat testis according to the procedure described by Utakoji et al. (14), except for the replacement of Ca^{2+} and Mg^{2+} by 0.15mM spermine, 0.75mM spermidine and 1mM EDTA/EGTA. Isolated nuclei were digested with DNAase I (EC 3.1.21.1; 41U/mg DNA), in the presence of 0.66mM $MnCl_2$ for 2 min at 30°C. Digested nuclei were suspended in 15mM Tris-HCl buffer, pH 7.5/1mM EDTA/1mM PMSF (lysis buffer) and lysed by incubation for 1-2 hours at 0°C with intermittent gentle agitation by passage through a Pasteur pipette (15). Lysed nuclei were centrifuged for 20 min at 9,000 rpm. The supernatant, soluble at low salt concentration, is referred to as "soluble chromatin"; the pellet represents the bulk of chromatin.

Experimental procedures were carried out at 4°C, chromatin-bound proteinases being irreversibly inhibited by the presence of 1mM phenylmethylsulfonyl fluoride in all steps except for nuclei digestion and lysis in which leupeptin 10µg/ml, chimostatin 10µg/ml, antipain 5µg/ml and pepstatin 5µg/ml were added.

Native oligonucleosomes (10-25 nucleosomes each) were obtained from the digested nuclei by separation on a 5%-35% (w/v) discontinuous sucrose gradient for 14 hours at 25.000rpm. When required, they were stripped of histone H1 by exposure to 0.6M NaCl and subsequent centrifugation for 14 hours through a 5% (w/v) sucrose cushion in the presence of 1mM EDTA and 0.6M NaCl (16). Reconstitution experiments were performed in 5mM Tris-HCl buffer, pH 7.5/60mM NaCl.

H1 histone variants were purified by reverse-phase HPLC (Beckman model 420) according to Quesada et al. (7) and analysed by gel electrophoresis in 15% (w/v) polyacrylamide in the presence of 0.1% SDS. Quantitative determination of proteins made use of a commercial adaptation (Pierce) of Bradford's method (17), using bovine serum albumin as standard.

The size of oligonucleosomal DNA was estimated by gel electrophoresis in 1% (w/v) agarose containing 0.1% SDS and the DNA content was determined on the basis of the absorbance at 260 nm (1.0 A_{260} = 50µg/ml DNA).

Circular dichroism (CD) spectra were recorded with a Jasco-500 A spectropolarimeter equipped with a DP-500 N data processor, using 2 mm light-path cells, at a temperature of 10 (±1)°C. The molar ellipticity, Θ, expressed as degrees x cm^2 x $dmol^{-1}$, was normalized to the number of DNA base pairs.

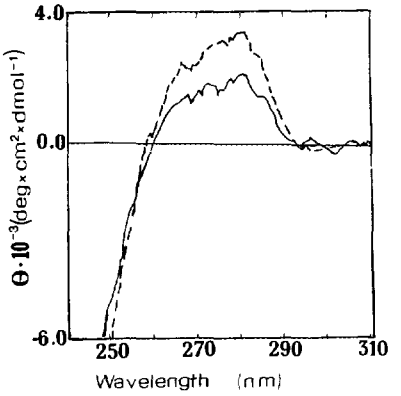

Fig.1. CD spectra of native (——) and H1-depleted (---) oligonucleosomes.
90µg DNA was used.

RESULTS

Oligonucleosomes, containing around 10-25 nucleosomes each, were used for circular dichroism experiments. H1-depleted oligonucleosomes, if compared to the native ones (Fig.1), exhibited, as a consequence of chromatin decondensation, an increased positive ellipticity of the nucleic acid chromophores around 280nm as well as a blue shift of their cross-over point.

H1-depleted oligonucleosomes were used to investigate the ability of H1t versus H1a, H1d-e, H1c somatic variants to induce condensation of rat testis chromatin.

The rat testis H1 subtypes were easily separated by reverse phase HPLC (Fig.2A) and their purity checked by SDS-PAGE (Fig.2B) and by amino acid composition analyses (data not shown).

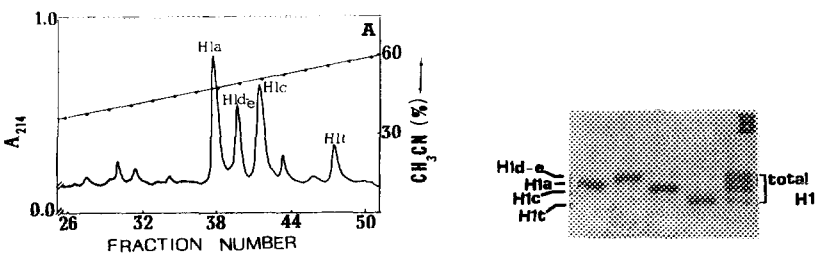

Fig.2. Separation of rat testis H1 subtypes by reverse phase HPLC (A) and their analysis by SDS/PAGE (B).
The 5% perchloric acid-soluble proteins from rat testis nuclei (800µg) were loaded onto a Nucleosil C_4 column (cm 0.5x24). Elution was carried out according to Quesada et al. (7), except that a 20%-60% solvent B linear gradient was applied. Flow-rate was 1ml/min; 1ml fractions were collected. (B) SDS/PAGE analysis of column fractions corresponding to H1 variants (3µg each).

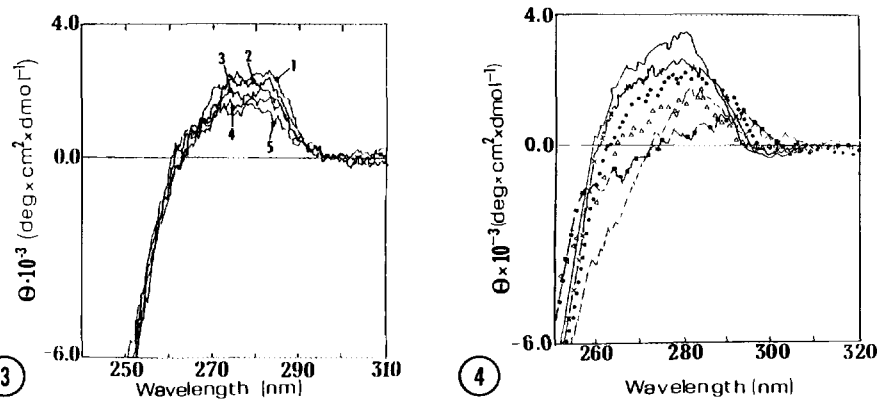

Fig.3. CD spectra of rat testis stripped oligonucleosomes before (1) and after (2, 3, 4) reconstitution with increasing amounts of H1a subtype.
DNA/H1 ratio ranged between 1:0.05 and 1:0.15 (w/w). (5) native oligonucleosomes.

Fig.4. Reconstitution of rat testis H1-depleted oligonucleosomes in the presence of different H1 variants.
CD spectra of reconstituted chromatin were determined at a constant DNA/H1 ratio 1:0.2 (w/w). (——) H1-depleted chromatin; (-x-x-) +H1t; (-▪-▪-▪-) +H1a; (---)+H1d-e; (....) +H1c; (△△)native chromatin.

When increasing amounts of purified H1a were added to H1-depleted oligonucleosomes, each addition was paralleled by a decrease of positive ellipticity at 283nm and by a red-shift of the cross-over point toward the spectrum values of native oligonucleosomes (Fig.3). The same dose-dependence was observed upon addition of the other variants (data not shown).

Reconstitution experiments where the H1 variant/DNA ratio was 0.2:1 (w/w, Fig.4 and Table I) showed that the degree of CD spectrum distortion of H1-depleted oligonucleosomes induced by the H1 variants tested varies greatly and follows the order H1a H1d-e

Table I- Ellipticity values and crossover points of H1 subtypes-reconstituted chromatin

	Intact nucleosomes[a]	H1-depleted nucleosomes[a]	H1[b] reconstituted nucl.			
			H1a	H1d-e	H1c	H1t
Crossover point (nm)	263	259	276	273	262.4	261.2
Θ_{283} [c]	1588	2973	1157	1516	2046	2561
$\%\Theta_{283}$ [d]	—	100	45	60	76	87

[a] 90µg/ml DNA.
[b] 1:0.2 (w/w) DNA/H1 ratio.
[c] Ellipticity in deg x cm^2 x dmole of nucleotide; values from the experiment of Fig.4.
[d] Mean values of three different determinations.

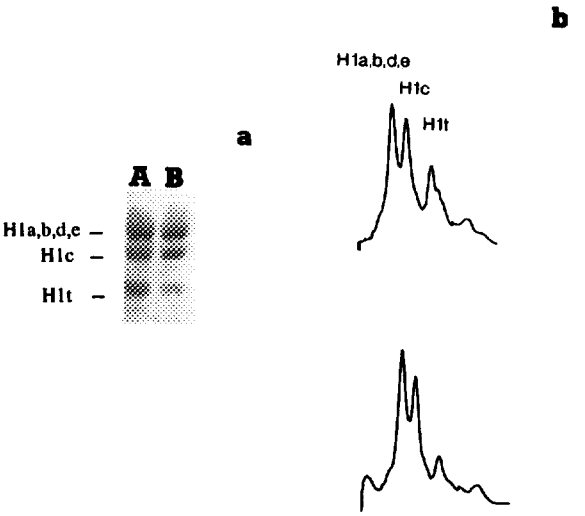

Fig.5. SDS/PAGE of the 20% TCA precipitate from soluble (A) and insoluble (B) chromatin fractions.
(a) Electrophoretic pattern of H1 variants.
A: 10μg protein corresponding to 79μg DNA.
B: 10μg protein corresponding to 44μg DNA.
(b) Densitometric profiles of (a). Scanning was performed in a Cellomatic densitometer (mod. CGA 2).

H1c H1t. These data indicate that H1t does not affect to a great extent the CD spectrum of stripped oligonucleosomal DNA.

To understand the role played by this specific variant in rat testis chromatin structure, we examined its quantitative distribution in chromatin fractions (soluble and insoluble), prepared by mild digestion of nuclei with DNAase I.

SDS PAGE analysis and the corresponding densitometric profile (Fig.5) show that the levels of H1t variant are higher in the soluble fraction, more sensitive to DNAase I digestion, than in the insoluble one (bulk chromatin). This result was corroborated by reverse-phase HPLC quantitative analyses of the H1 variants associated with both chromatin fractions, H1t accounting for 23% of the total H1 content of the soluble fraction but 15% in the bulk insoluble fraction (data not shown).

DISCUSSION

All genic H1 variants possess three domain structures: an N-terminal tail, a globular region and a C-terminal tail, respectively constituted of about 40, 80 and 100 amino acid residues. As for the nature of the differences among H1 somatic variants, peptide maps and subsequent amino acid sequence studies have revealed that, in terms of sequence conservation, the highly

cationic C-terminal tail shows less sequence similarities than the globular region which conserved a 98% identity (1).

It can be obviously expected that differences in the amino acid sequence can result in structural modifications with potential functional significance. H1 histone variants show, in particular, distinct differences in their sizes (1), in their mode of folding the three structural domains (1) and in their ability to undergo post-translational modifications (7,8).

In chromatin structure, histone H1 has a dual involvement in the coiling of DNA fiber. In nucleosome organization, the globular domain of H1 histone, through its ability to bind the DNA filament wrapped around the histone "core", seals the entrance and exit sites of DNA in the nucleosome, while the C-terminal tail domain interacts with the internucleosomal linker DNA. Moreover, the formation of higher order chromatin structures appears to require the presence of histone H1, which would thus play a key role in the coiling of the 10 nm nucleosomal fiber into the 30nm solenoidal filament.

Singer and Singer (18) have shown that at least part of the globular region of H1 (residues 72 to 106), which is identical in all somatic H1 variants, has a higher affinity for supercoiled than for relaxed DNA, and therefore is expecially important in the condensation of the former.

Evidence that H1 somatic variants differ in their ability to induce DNA condensation comes from CD, viscosity and sedimentation (1, 11) data, as well as from studies of the quantitative distribution of H1 variants between soluble and insoluble chromatin fractions (15). Liao & Cole (12) have proposed that in condensing dinucleosomal DNA, H1a plays a preferential role as compared to either H1d-e or H1c. On the other hand, Lennox (19) have hypothised a model in which the H1a and H1c variants, on the basis of their metabolic and evolutionary instability, are localized outside the solenoidal structure and therefore do not affect condensation.

The apparent contradiction between these two points of view can be solved if the ellipticity variation observed in the CD spectra is explained in terms of a fairly generic distortion of the DNA spectrum due to the binding of different H1 subtypes rather than by a well-defined chromatin condensation. This consideration is supported by the fact that, in our experiments, the different H1 variants produce the same effect on H1-depleted oligonucleosomes and on dinucleosomes (data not shown).

Our CD data show that H1a is more effective in modifying the DNA spectrum than H1d-e, H1c and, at last, H1t. The DNAase I sensitive chromatin fraction from rat testis is, on the other hand, enriched in the germinal H1t variant, as compared to the insensitive one.

The analysis of the first 108 residues of rat and boar H1t histone, compared to a consensus sequence characteristic of the standard somatic variants (20), shows that both forms of H1t histone share many substitutions within the H1 globular region, the portion of the molecule which is, as previously mentioned,virtually invariant among the common somatic variants (18). These substitutions within the globular domain of H1t contribute significantly to making this germinal subtype antigenically distinct from the other somatic histone variants (21). The variation observed in the amino acid sequence of the globular region of H1t, although occurring in different positions with respect to those involved in its binding to the "core" particle, might modulate the globular domain structure and, as a consequence, its preferential interaction with "core" supercoiled DNA, without excluding (also for this variant) the important role played by the C-terminal tails in favoring the higher levels of chromatin organization (9).

In conclusion,the high levels of H1t during the pachytene phase could suggest, for this H1 variant, a role in keeping chromatin in a decondensed state, thus leaving it "open" for the nuclear events, including genetic recombination, occurring in this spermatogenetic stage.

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REFERENCES

1. Cole,K.D. (1987) Int.J.Peptide Protein Res. 30, 433-449.
2. Davie,J.R. & Del Cuve,G.P. (1991) Biochem.J. 280, 491-497.
3. Lennox,R.W. & Cohen,L.H. (1988) Biochem Cell Biol. 66, 636-649.
4. Meistrich,M.L., Bucci,L.R., Trostle-Weige,P.K. & Brock,W.A. (1985) Developm. Biol. 112, 230-240.
5. Cole,K.D., Kandala,J.C. & Kistler,W.S. (1986) J.Biol.Chem. 261, 7178-7183.
6. Kremer,E.J. & Kistler,W.S. (1991) Exp.Cell Res. 197, 330-332.
7. Quesada,P., Farina,B. & Jones,R. (1989) Biochim.Biophys.Acta 1007, 167-175.

8. Wu, R.S., Panusz,H.T., Hatch,C.L. & Bonner,W.M.(1991) CRC Crit.Rev.Biochem. 20, 201-263.
9. Hill,C.S., Packman,L.C. & Thomas,J.O. (1990) EMBO J. 9, 805-813.
10. Clark,D.J. & Thomas, J.O. (1988) Eur.J.Biochem. 178, 225-233.
11. Zlatanova,J. & Yaneva,J. (1991) DNA & Cell Biol, 10, 239-248.
12. Liao,L.W. & Cole, K.D. (1981) J.Biol.Chem. 256, 10124-10128.
13. Welch,S.L. & Cole,K.D. (1980) J.Biol.Chem. 255, 4516-4518.
14. Utakoji,T., Muramatsu,M. & Sugano, H. (1968) Exp.Cell Res. 53, 366-368.
15. Huang,H.C. & Cole,K.D. (1984) J.Biol.Chem. 259, 14237-14242.
16. D'Erme,M., Santoro,R., Allegra,P., Reale,A., Marenzi,S, Strom,R. & Caiafa,P. (1993) Biochim.Biophys.Acta 1173, 209-216.
17. Bradford, M.M. (1976) Anal.Biochem. 72, 248-254.
18. Singer,D.S. & Singer,M.F. (1976) Nucl.Ac.Res. 3, 2531-2547.
19. Lennox,R.W. (1984) J.Biol.Chem. 259, 669-672.
20. Cole,K.D., York,R.G. & Kistler,W.S. (1984) J.Biol.Chem. 259, 13695-13702.
21. Markose,E.R. & Rao,M.R.S. (1989) Exp.Cell Res. 182, 279-283.