Quantitative changes of high mobility group non-histone chromosomal proteins HMG1 and HMG2 during rooster spermatogenesis

Manel Chiva and Cristóbal Mezquita*

Departamento de Fisiología, Facultad de Medicina, Universidad de Barcelona, Casanova, 143, Barcelona-36, Spain

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The quantitative changes of a group of non-histone chromosomal proteins identified by its solubility, electrophoretic mobility and amino acid analysis as the high mobility group proteins HMG1 and HMG2, were studied throughout rooster spermatogenesis. The ratio HMG1/HMG2 remained constant (0.66 ± 0.04) during the transition from dividing meiotic and premeiotic cells to nondividing spermatids and from transcriptionally active cells (spermatogonia, spermatocytes and early spermatids) to transcriptionally inactive late spermatids. The ratios HMG1/nucleosomal histone and HMG2/nucleosomal histone increased markedly at the end of spermiogenesis during the transition from nucleohistone to nucleoprotamine when nucleosomes are being disassembled. The high mobility group chromosomal proteins HMG1 and HMG2 were not detectable in the nuclei of rooster spermatozoa.

Non-histone protein *HMG1* *HMG2* *Chromatin* *Spermatogenesis*

1. INTRODUCTION

The high mobility group non-histone chromosomal proteins HMG1 and 2 are a very well characterized class of nuclear proteins of unknown function [1–3]. From the estimated amount of HMG proteins/cell nucleus, 10^6 molecules [4], a structural role of these proteins in chromatin has been proposed [5].

This study examines the correlation between quantitative changes in HMG1 and 2 and the structural and functional changes that chromatin undergoes throughout rooster spermatogenesis [6]. Spermatogonia and preleptotene spermatocytes are active in nuclear replication and transcription. Nondividing early spermatids are still active in nuclear transcription while late spermatids and spermatozoa are inactive. The chromatin of spermatids undergoing differentiation to spermatozoa (spermiogenesis) becomes relaxed, exposing binding sites on DNA, when nucleosomes are disassembled in late spermatids during the transition from nucleohistone to nucleoprotamine [7].

In accordance with the proposed structural role of the high mobility group non-histone proteins, our results show an enrichment of nucleohistone in HMG1 and 2 concomitant with the dramatic changes that chromatin structure undergoes during the disassembly of nucleosomes at the end of spermiogenesis.

2. EXPERIMENTAL

2.1. *Isolation and separation of rooster testis cell nuclei*

Hubbard White Mountain roosters (25–50 weeks old) were used throughout this study. Nuclei were isolated from fresh rooster testis and separated by sedimentation at unit gravity by the procedure previously described [6] except for the medium used: 3 mM $MgCl_2$, 25 mM KCl, 1 mM PMSF (pH 6.0).

* To whom correspondence should be addressed

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Fig.1. Electrophoretic analysis of HMG proteins isolated from rooster testis cell nuclei. (a) Electrophoretic mobility of marker proteins in SDS/polyacrylamide gel: (1) phosphorylase *b*; (2) bovine serum albumin; (3) ovalbumin; (4) carbonic anhydrase; (5) soybean trypsin inhibitor; and (6) α-lactalbumin. (b) Electrophoretic mobilities of 0.74 N perchloric acid-soluble nuclear proteins in SDS–polyacrylamide gels. (c) Electrophoretic pattern in acetic acid/urea/polyacrylamide gels of 0.35 M NaCl-soluble, 2% trichloroacetic acid-soluble nuclear proteins.

2.2. *Preparation and analysis of HMG proteins*

Purified nuclei were treated with 20 mM EDTA, 10 mM Tris–HCl (pH 7.5), 1 mM PMSF and perchloric acid was added to a final concentration of 0.74 N. After overnight extraction, perchloric acid-soluble proteins were precipitated with trichloroacetic acid (30%, w/v). No residual H1 or HMG1 and 2 proteins were extracted with 0.2 N H_2SO_4 after perchloric acid treatment of nuclei.

Alternatively, rooster testis cell nuclei were extracted with 0.35 M NaCl and the low mobility group non-histone proteins was precipitated by 2% trichloroacetic acid and the high mobility group proteins was recovered from the supernatant by acetone precipitation [8].

The HMG proteins were analyzed electrophoretically on 15% acetic acid–urea–polyacrylamide slab gels as in [9] or on exponential polyacrylamide–SDS gels (10–16%) as in [10]. The gels were stained with amido black and scanned with a 2410 Gilford linear transport scanner.

For amino acid analyses the high mobility group proteins was obtained by preparative SDS–polyacrylamide gel electrophoresis (3 mm slab gels). The gel was stained with Coomassie blue and the putative bands were excised, extracted and the dye removed as in [11]. Amino acid analyses were carried out using a Beckman 119 C amino acid analyzer after hydrolysis of the samples in 6 N HCl at 110°C for 24 h. No corrections were made for hydrolytic losses.

3. RESULTS

3.1. *Characterization of the HMG proteins obtained from rooster testis cell nuclei*

The electrophoretic pattern in SDS gels of 0.74 N perchloric acid-soluble proteins obtained from rooster testis cell nuclei is shown in fig.1b.

Table 1

Amino acid composition (%mol) of isolated HMG1 and 2 proteins from rooster testis cell nuclei and nuclei of chicken erythrocyte

Amino acids	HMG1 testis	HMG1 erythrocyte	HMG2 testis	HMG2 erythrocyte
Asp	10.7	11.5	9.9	12.9
Thr	3.2	2.7	3.9	2.5
Ser	6.1	5.8	6.2	5.7
Glu	14.8	18.1	16.7	15.3
Pro	9.7	5.1	10.3	6.1
Gly	9.4	5.9	9.3	7.2
Ala	12.8	8.7	12.8	10.0
Val	2.6	3.5	2.5	3.5
Met	0.7	0.5	1.2	0.4
Ile	1.0	1.8	1.5	1.7
Leu	2.2	2.7	2.7	2.3
Tyr	1.4	2.2	1.2	2.5
Phe	1.6	4.3	1.3	4.9
His	0.9	1.7	1.0	0.9
Lys	18.5	18.2	16.7	17.3
Arg	4.1	4.0	4.3	4.2

The amino acid composition of chicken erythrocyte HMG1 and 2 is from [12]

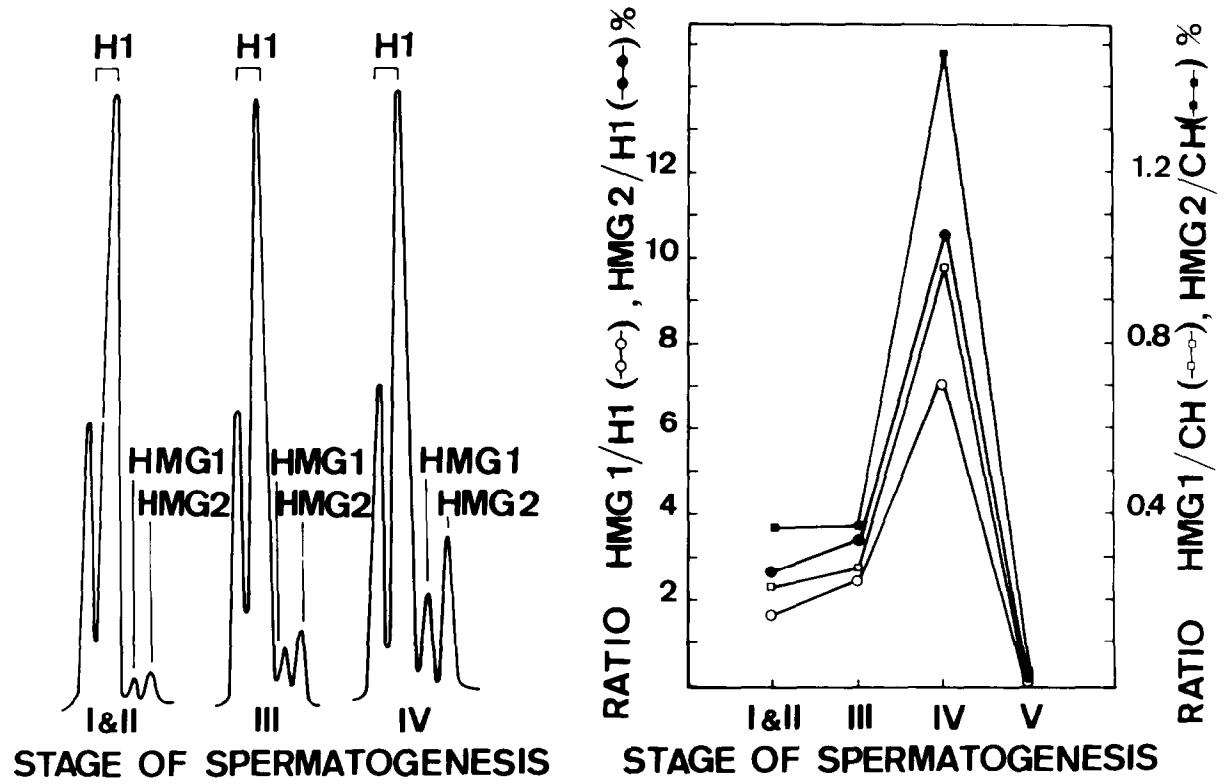

Fig.2. Changes in the relative proportion between HMG1 and 2 and nucleosomal histones during rooster spermatogenesis. Nuclei at different stages of rooster spermatogenesis were separated by sedimentation at unit gravity. HMG proteins and histone H1 were extracted from the different nuclei with 0.74 N perchloric acid and electrophoresed in SDS–polyacrylamide gels. The percentages plotted on the graph were determined from the densitometer scans showed in the figure and from the scans of SDS-gels containing 0.2 M H_2SO_4 nuclear-soluble proteins: stage I, tetraploid primary spermatocytes; stage II, small primary spermatocytes, secondary spermatocytes and spermatogonia; stage III, early spermatids; stage IV, late spermatids and testicular spermatozoa; stage V, spermatozoa from the vas deferens.

The apparent M_r-values of the main bands with higher mobility than histone H1 were estimated to be: 29000 (HMG1); 27000 (HMG2); and 17400 (HMG17).

The electrophoretic pattern in acetic acid–urea gels of 0.35 M NaCl-soluble, 2% trichloroacetic acid-soluble proteins obtained from rooster testis cell nuclei is shown in fig.1c. The electrophoretic mobilities of the main bands in relation to histone H1 were: 0.78 (HMG1), 0.85 (HMG2) and 1.91 (HMG17) in good agreement with the mobilities measured for the calf thymus HMG proteins.

The amino acid analysis of the high mobility group non-histone proteins HMG1 and 2 extracted from rooster testis cell nuclei and separated by preparative electrophoresis in SDS gels are shown in table 1. The amino acid composition of HMG1 and 2 isolated from rooster testis cell nuclei was similar to the composition of HMG1 and 2 from chicken erythrocyte nuclei [12].

3.2. *Changes in nuclear content of the high mobility group chromosomal proteins HMG1 and 2 throughout rooster spermatogenesis*

Rooster testis cell nuclei at different stages of spermatogenesis were separated by sedimentation at unit gravity and HMG proteins were extracted from each fraction with 0.74 N perchloric acid, as in section 2.

Four different fractions of rooster testis cell nuclei were obtained by sedimentation at unit gravity [6]: tetraploid primary spermatocytes

(stage I); small primary spermatocytes, secondary spermatocytes and spermatogonia (stage II); round and elongating spermatids (stage III); elongated spermatids and testicular spermatozoa (stage IV). An additional fraction was obtained by isolation of nuclei from spermatozoa of the vas deferens (stage V).

The ratio HMG1/HMG2 remained constant (0.66 ± 0.04) throughout the different stages of spermatogenesis. The relative proportions of these proteins did not change during the transition from dividing meiotic and premeiotic cells (stages I,II) to non-dividing spermatids (stage III) and from transcriptionally active nuclei (stages I–III) to transcriptionally inactive late spermatid nuclei (stage IV).

The ratios HMG1/histone H1, HMG2/histone H1, HMG1/core histones and HMG2/core histones increase markedly in late spermatids when nucleosomes are disassembled and histones are being replaced by protamine galline (fig.2). The HMG1 and 2 were not detectable in nuclei of spermatozoa obtained from the vas deferens.

4. DISCUSSION

A comparison of the nuclear content of the high mobility group chromosomal proteins HMG1 and 2 at different stages of rooster spermatogenesis showed that the ratio HMG1/HMG2 remained constant during the transition from dividing to non-dividing cells and from transcriptionally active to transcriptionally inactive cells. Changes in the relative amounts of HMG1 and 2 have been described during the differentiation of chicken skeletal muscle, when dramatic changes in the proliferation rates occur [13]. In different rat organs the loss of proliferative activity is also associated with a depletion of HMG2, whereas the level of HMG1 remains unchanged [14]. However, in agreement with our results, a comparable enrichment of both HMG1 and 2 proteins in rapidly proliferating rat cells has been described [15], and similar levels of these proteins in four calf tissues with widely differing proliferative activity have also been reported [16]. Methodological differences and the existence of various pools of HMG proteins may account for the disparate results obtained.

The ratio between high mobility group proteins HMG1 and 2 and nucleosomal histones increased markedly at the end of rooster spermiogenesis when nucleosomes are disassembled and histones are being replaced by protamine galline. The enrichment of nucleohistone in HMG1 and 2 in rooster late spermatids could play some role in the nucleohistone–nucleoprotamine transition or could simply reflect that histone removal takes place prior to the replacement of HMG1 and 2.

We have previously reported marked changes in the structure of chromatin in rooster late spermatids during the nucleohistone nucleoprotamine transition:

(1) Exposition of binding sites on DNA [7];
(2) Hyperacetylation of histone H4 and rapid turnover of its acetyl groups [17];
(3) Increase in the ratio uH2A/nucleosomal core histones [18].

The high mobility group proteins HMG1 and 2 might contribute to the conformational change of chromatin in late spermatids. In other systems, HMG1 and 2 can be released from nuclei by both micrococcal and DNase I nucleases and they are associated with highly acetylated domains of chromatin [5,19].

Acetylation of histone H4 is not detectable in late spermatids and the high mobility group proteins are not present at the end of spermiogenesis in species which retain histones in the sperm nucleus [20]. This observation is consistent with the hypothesis of a role for histone H4 hyperacetylation and for HMG1 and 2 during the nucleohistone–nucleoprotamine transition. A remarkable feature of the sequence of HMG1 and 2 is the presence of an unbroken run of glutamic acid and aspartic residues in the carboxyl half of the molecules [2,3]. Polyglutamic acid is capable of facilitating the assembly of core histones and DNA into nucleosomes [21]. We may speculate that through interaction of the highly acidic C-terminal domain of HMG1 and 2 with the very basic regions of histones [22] the high mobility group proteins might contribute to the nucleosome disassembly at the end of spermiogenesis. Further studies will be necessary to prove this possibility.

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REFERENCES

[1] Nicolas, R.H. and Goodwin, G.H. (1982) in: The HMG Chromosomal Proteins (Johns, E.W. ed) pp.41–68, Academic Press, London, New York.
[2] Walker, J.M. (1982) in: The HMG Chromosomal Proteins (Johns, E.W. ed) pp.69–87, Academic Press, London, New York.
[3] Bradbury, E.M. (1982) in: The HMG Chromosomal Proteins (Johns, E.W. ed) pp.89–110, Academic Press, London, New York.
[4] Goodwin, G.H., Walker, J.M. and Johns, E.W. (1978) in: The Cell Nucleus (Busch, H. ed) vol.4, pp.181–209, Academic Press, London, New York.
[5] Goodwin, G.H. and Mathew, C.G.P. (1982) in: The HMG Chromosomal Proteins (Johns, E.W. ed) pp.193–221. Academic Press, London, New York.
[6] Mezquita, C. and Teng, C.S. (1977) Biochem. J. 164, 99–111.
[7] Mezquita, C. and Teng, C.S. (1977) Biochem. J. 170, 203–210.
[8] Goodwin, G.H. and Johns, E.W. (1977) in: Methods in Cell Biology (Stein, G. et al. eds) vol.14, pp.257–267, Academic Press, London, New York.
[9] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346.
[10] O'Farrell, P.H. and O'Farrell, P.Z. (1977) in: Methods in Cell Biology (Stein, G. et al. eds) vol.14, pp.407–420, Academic Press, London, New York.
[11] Bernabeu, C., Sanchez-Madrid, F. and Amils, R. (1980) Eur. J. Biochem. 109, 285–290.
[12] Rabbani, A., Goodwin, G.H. and Johns, E.W. (1978) Biochem. Biophys. Res. Commun. 81, 351–358.
[13] Gordon, J.S., Randall, K. and Rosenfeld, B.I. (1981) Arch. Biochem. Biophys. 211, 709–721.
[14] Seyedin, S.M. and Kistler, W.S. (1979) J. Biol. Chem. 254, 11264–11271.
[15] Shastri, K., Isackson, P.J., Fishback, J.L., Land, M.D. and Reeck, G.R. (1982) Nucleic Acids Res. 10, 5059–5072.
[16] Rabbani, A., Goodwin, G.H. and Johns, E.W. (1978) Biochem. J. 173, 497–505.
[17] Oliva, R. and Mezquita, C. (1982) Nucleic Acids Res. 10, 8049–8059.
[18] Agell, N., Chiva, M. and Mezquita, C. (1983) FEBS Lett. 155, 209–212.
[19] Dixon, G.H. (1982) in: The HMG Chromosomal Proteins (Johns, E.W. ed) pp.149–192, Academic Press, London, New York.
[20] Kennedy, B.P. and Davies, P.L. (1980) J. Biol. Chem. 255, 2533–2539.
[21] Stein, A., Whitlock, J.P. and Bina, M. (1979) Proc. Natl. Acad. Sci. USA 76, 5000–5004.
[22] Reeck, G.R., Isackson, P.J. and Teller, D.C. (1982) Nature 300, 76–78.