Hypomethylation of DNA in meiotic and postmeiotic rooster testis cells

Nativitat Rocamora and Cristóbal Mezquita*

Department of Physiology, Faculty of Medicine, University of Barcelona, Casanova 143, 08036 Barcelona, Spain

Received 17 August 1984; revised version received 14 September 1984

To study whether changes in methylation of DNA are related to the structural and functional changes that chromatin undergoes throughout rooster spermatogenenis, we analyzed, by high-performance liquid chromatography, the 5-methylcytosine content of DNA purified from rooster testis cell nuclei at successive stages of the cell differentiation process. The DNA of meiotic and postmeiotic cells appears partially undermethylated, containing approximately 30% less methylcytosines than the DNA obtained from premeiotic and somatic cells.

DNA methylation *Spermatogenesis* *Meiosis* *Recombination*

1. INTRODUCTION

In mammals and birds, approximately 50–70% of the dinucleotides 5′-CpG of DNA are modified by enzymatic transfer of the methyl group of *S*-adenosyl-methionine to the cytosine residue. Other cytosines are rarely methylated [1–5]. A number of observations have suggested an inverse relationship between gene expression and gene methylation [6]. Other results indicate a direct correlation between gene expression and the extent of methylation [7]. An important area of current study involves the elucidation of the role of hypomethylation in cell transformation and cell differentiation. Substantial hypomethylation was found in genes of cancer cells compared with their normal counterparts [8]. Hypomethylation in cancer cells may be widespread, as the genes studied are localized in three different chromosomes [9]. Hypomethylation of oncogenes in primary human cancer has also been detected [10]. Hypomethylation can suppress the expression of class I antigens and thus lead to malignant transformation [7]. In addition, chemical carcinogens inhibit DNA methylation in vitro providing a mechanism for alteration of the state of cell differentiation [11–13].

Changes in the methylcytosine content of DNA during cell differentiation in several models average less than 10% [14]. However, the sperm DNA of several species contains only 50% of the methylcytosines found in somatic cells [15]. Chromatin undergoes dramatic changes in composition, structure and function during spermatogenesis [16–20], and offers an excellent model to investigate the role of DNA methylation in the structural and functional changes of chromatin throughout the differentiation process. Partially deficient methylation of cytosine stimulates genetic recombination of bacteriophage lambda [21]. It has been suggested [15] that hypomethylated DNA might be involved in pairing of chromosomes and recombination events which normally take place at meiosis. Here we show that the undermethylated pattern of sperm DNA is already present in meiotic and postmeiotic rooster testis cells.

2. MATERIALS AND METHODS

Hubbard White Mountain roosters (25–30 weeks old) and sexually immature chickens (8 weeks old) were used throughout this study. Nuclei were

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

isolated from fresh rooster testes or liver in citric acid and separated by sedimentation at unit gravity as in [16]. DNA was prepared from the isolated nuclei as in [23]. DNA samples were quantitatively hydrolyzed with DNase I, nuclease P1 and bacterial alkaline phosphatase [24]. The resulting deoxyribonucleosides were directly separated by reversed-phase high-performance liquid chromatography (RP-HPLC) using a modification of the method in [24], as indicated in fig.1.

3. RESULTS

We have determined the extent to which cytosine residues in the DNA are methylated at different stages of rooster spermatogenesis. A typical chromatographic analysis illustrating the separation of a hydrolysate of rooster testis DNA is shown in fig.1. The percentage of dG + dC content

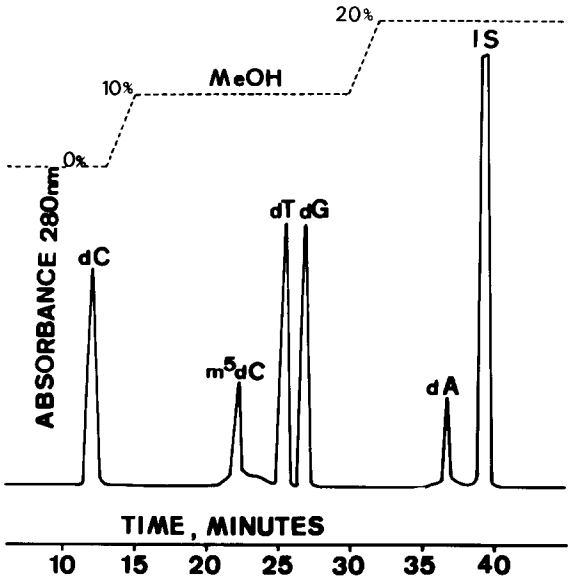

Fig.1. Reversed-phase high-performance liquid chromatographic determination of deoxyribonucleosides of rooster testis DNA. The separation was performed on one μBondapak C_{18} 300×4 mm column. Complete separation of the deoxyribonucleosides was achieved in 37 min at 15°C using a methanol gradient (– – –). A flow of 1.0 ml/min was maintained for 13 min and then increased to 1.5 ml/min. Elution times: deoxycytidine (dC), 12.02 min; 5-methyldeoxycytidine (m^5dC), 22.31 min; deoxythymidine (dT), 25.50 min; deoxyguanosine (dG), 26.85 min; and deoxyadenosine (dA), 36.76 min. IS, internal standard.

(40.59 ± 0.54) of the DNA isolated from rooster testis cell nuclei at successive stages of spermatogenesis agrees with published values of chicken DNA [25].

The various stages of differentiating rooster testis cell nuclei based on their sedimentation velocity [16] were designated as: DN (diploid nuclei, s = 1.7 mm/h), TN (tetraploid meotic nuclei, s = 2 mm/h), HN_1 (haploid nuclei of round spermatids, s = 0.8 mm/h), and NH_2 (haploid nuclei of elongated spermatids, s = 0.5 mm/h).

The methylcytosine content of DNA was maximal in the fraction of diploid nuclei (DN) containing nuclei of premeiotic gonial cells (fig.2) and also in diploid nuclei, with the same sedimentation velocity, obtained from immature testis enriched in spermatogonia (table 1). Similar levels of methylcytosine were detected in DNA from somatic nuclei of chicken liver (table 1). The amount of methylcytosine decreased by approximately 30% in DNA obtained from meiotic and postmeiotic nuclei (fig.2, table 1). The undermethylated pat-

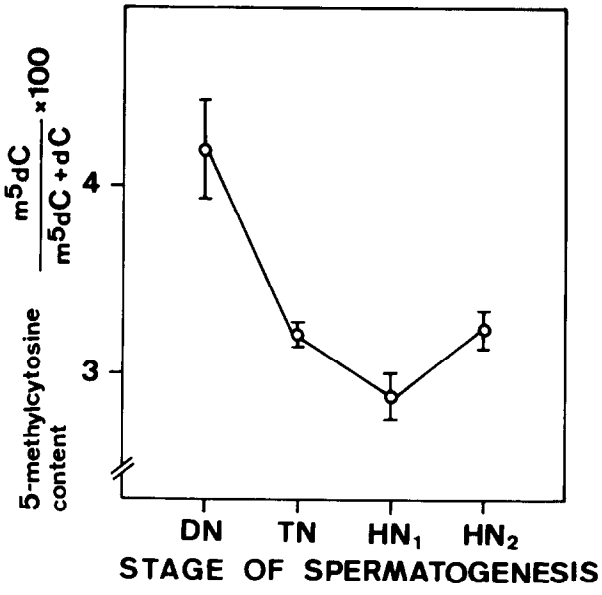

Fig.2. Mean methylcytosine levels in DNA obtained from rooster testis cell nuclei at successive stages of rooster spermatogenesis. DN, diploid nuclei; TN, tetraploid meiotic nuclei; HN_1, haploid nuclei of round spermatids; and NH_2, haploid nuclei of elongated spermatids.

Table 1

Methylcytosine content of DNA obtained from rooster germinal cells and liver

	$\frac{m^5C}{m^5C + C} \times 100$
Spermatozoa from the vas deferens	2.97 ± 0.09
Mature testis	3.43 ± 0.21
Immature testis	4.37 ± 0.17
Liver	4.05 ± 0.08

Analysis was by HPLC at the deoxynucleoside level. Determinations were made on at least 3 different batches of nuclei

tern of chicken sperm DNA was thus already present in meiotic and postmeiotic cells.

4. DISCUSSION

The experiments described here show that rooster sperm DNA contains approximately 30% less methylcytosines than DNA obtained from liver, immature testis or a fraction containing premeiotic rooster testis cell nuclei. The results also show that the undermethylated pattern of sperm DNA is already established in meiotic and postmeiotic cells.

DNA isolated from sperms of different species is undermethylated in relation to the 5-methylcytosine content of DNA isolated from the corresponding somatic tissues [15]. The methylcytosine content of different avian tissues has been reported [26]. These data are similar to the values we found in the liver and immature testis of chicken. Unfortunately, the published results did not detail the characteristics of the avian germinal tissue used as a source of DNA.

An important question to be solved is which DNA sequences are undermethylated in meiotic and postmeiotic cells. Certain chicken genes, such as ribosomal RNA genes or the 5′-end of the α(2) I collagen gene are hypomethylated in expressing tissues and also in genetically inactive sperm DNA [27]. Other chicken genes such as ovalbumin, conalbumin, α- and β-globin are undermethylated in expressing tissues and heavily methylated in sperm [28–30]. Meiosis does not appear to be a critical event involved in the establishment of methylation patterns of unique sequence genes [22].

The major cause of the undermethylation of bovine sperm DNA (2.5% of cytosines are methylated in sperm compared with 5.4% in calf thymus DNA) may be the presence of methyl-deficient satellite DNA. In somatic cells satellite DNA is highly methylated [15]. The ratio of methylcytosine content of the highly repeated human *Eco*RI family of DNA sequences from brain and sperm was 2.0:1.0 [31]. At an *Hha*I site in this repeated family, sperm DNA was 5–10-fold less methylated than somatic DNAs [31]. Undermethylation of mouse satellite DNA, examined by digestion with the restriction enzymes *Msp*I and *Hpa*II, has been detected in meiotic and postmeiotic cells as well as in spermatozoa [32,33]. Undermethylation of germ cell satellite DNA must occur very early in the germ cell lineage, because it is already present in immature mouse testis [32,33].

It has been suggested that satellite DNA could be involved in specifying interchromosomal recognition during pairing, or could affect the frequency of crossing-over or the distribution of chiasmata [34]. Partially deficient methylation of cytosine in DNA at $CC^{A}_{T}GC$ sites has been shown to stimulate genetic recombination of bacteriophage lambda [21]. The methylcytosine content of certain DNA sequences might control the extent of the recombination reactions that occur in meiotic and somatic cells.

If major changes in the methylcytosine content of highly repetitive DNA sequences are responsible for the undermethylated pattern observed in meiotic and postmeiotic cells, and these sequences are localized in constitutive heterochromatin, undermethylation might cause structural changes in these particular domains of chromatin during the differentiation of the germinal cell line. A drastic reduction in the number of constitutive heterochromatin blocks per cell nucleus has been detected during the transition from diploid rooster spermatogonia (20.9 heterochromatin blocks) to haploid late spermatids (3.2 heterochromatin blocks) [35].

Further studies will show if undermethylation in germinal cells affects DNA domains implicated in chromosomal pairing and genetic recombination during meiosis [36,37] and if it is responsible for major changes in the organization of DNA throughout spermatogenesis.

ACKNOWLEDGEMENTS

We thank Dr Emili Gelpí for providing the necessary facilities for HPLC during part of the work, and Dr Montserrat Pagès for her advice and interest. The work was supported by a CAYCIT 5-0668 grant.

REFERENCES

[1] Wigler, M.H. (1981) Cell 24, 285–286.
[2] Boehm, T.L.J. and Drahovsky, D. (1983) J. Natl. Cancer Inst. 71, 429–433.
[3] Taylor, J.H. (1984) Cell Biol. Monographs, vol.11. Springer-Verlag, Vienna.
[4] Cooper, D.N. (1983) Hum. Genet. 64, 315–333.
[5] Bird, A.P. (1984) Nature 307, 503–504.
[6] Busslinger, M., Hurst, J. and Flavell, R.A. (1983) Cell 34, 197–206.
[7] Tanaka, K., Appella, E. and Jay, G. (1983) Cell 35, 457–465.
[8] Feimberg, A.P. and Vogelstein, B. (1983) Nature 301, 89–92.
[9] McKusik, U.A. (1982) Cytogenet. Cell Genet. 32, 1–343.
[10] Feimberg, A.P. and Volgelstein, B. (1983) Biochem. Biophys. Res. Commun. 111, 47–54.
[11] Salas, C.E., Pfohl-Leszkowicz, A., Lang, M. and Dirheimer, G. (1979) Nature 278, 71–72.
[12] Pfohl-Leszkowicz, A., Salas, C.E., Fuchs, R.P.P. and Dirheimer, G. (1981) Biochemistry 20, 3020–3024.
[13] Wilson, V.L. and Jones, P.A. (1983) Cell 32, 239–246.
[14] Razin, A. and Riggs, A.D. (1980) Science 210, 604–610.
[15] Adams, R.L.P., Burdon, R.H. and Fulton, J. (1983) Biochem. Biophys. Res. Commun. 113, 695–702.
[16] Mezquita, C. and Teng, C.S. (1977) Biochem. J. 164, 99–111.
[17] Mezquita, C. and Teng, C.S. (1977) Biochem. J. 170, 203–210.
[18] Oliva, R. and Mezquita, C. (1982) Nucleic Acids Res. 10, 8049–8059.
[19] Agell, N., Chiva, M. and Mezquita, C. (1983) FEBS Lett. 155, 209–212.
[20] Chiva, M. and Mezquita, C. (1983) FEBS Lett. 162, 324–328.
[21] Korba, B.E. and Hays, J.B. (1982) Cell 28, 531–541.
[22] Rahe, B., Erickson, R.P. and Quinto, M. (1983) Nucleic Acids Res. 11, 7947–7959.
[23] Marmur, J. (1961) J. Mol. Biol. 3, 208–218.
[24] Kuo, K.C., McCune, R.A. and Gehrke, C.W. (1980) Nucleic Acids Res. 8, 4753–4776.
[25] Elofsson, B. and Bernardi, G. (1983) Eur. J. Biochem. 130, 241–245.
[26] Vanyushin, B.F., Mazin, A.L., Vasilyev, U.K. and Belozerski, A.N. (1973) Biochim. Biophys. Acta 299, 397–403.
[27] Cooper, D.N., Taggart, M.H. and Bird, A.P. (1983) Nucleic Acids Res. 11, 647–658.
[28] Mandel, J.L. and Chambon, P. (1979) Nucleic Acids Res. 7, 2081–2013.
[29] Weintraub, H., Larsen, A., Groudine, M. (1981) Cell 24, 333–344.
[30] McGhee, J.D. and Ginder, J.D. (1979) Nature 280, 419–420.
[31] Gama-Sosa, M.A., Wang, R.Y.H., Kuo, K.C., Gehrke, C.W. and Ehrlich, M. (1983) Nucleic Acids Res. 11, 3087–3095.
[32] Ponzetto-Zimmerman, C. and Wolgemuth, D.J. (1984) Nucleic Acids Res. 12, 2807–2822.
[33] Sanford, J., Forrester, L., Chapman, V., Chandley, A. and Hastie, N. (1984) Nucleic Acids Res. 12, 2823–2836.
[34] Bostock, C. (1980) Trends Biochem. Sci. 5, 117–119.
[35] Dressler, B. and Schmid, M. (1976) Chromosoma 58, 387–391.
[36] Hotta, Y. and Stern, H. (1981) Cell 27, 309–319.
[37] Hotta, Y. and Stern, H. (1984) Chromosoma 89, 127–137.