Genetics and Molecular Biology, 27, 1, 33-38 (2004)

www.sbg.org.br

Research Article

Analysis of topological organization of chromatin during spermatogenesis in mouse testis

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Abstract

Eukaryotic chromatin is organized as radial DNA loops with periodical attachments to an underlying nucleoskeleton known as nuclear matrix. This higher order chromatin organization is revealed upon high salt extraction of cells. To understand the sequential change in the functional organization of chromatin during spermatogenesis, we have analysed the higher order organization of chromatin in different testicular cell types and the epididymal sperm of laboratory mouse. The expansion and contraction of the nucleoid DNA following 2 M NaCl extraction was measured in a fluorescence microscope using ethidium bromide (2.5-200 μg/mL) as an intercalating dye to induce DNA positive supercoils. While the halo size varied among cell types (pachytene DNA most extended, round spermatid least), 5 μg/mL ethidium bromide (EtBr) removed maximum negative supercoils in all the cell types. At higher EtBr concentrations, maximum positive supercoiling occured in pachytene DNA loops. Consistent with this, the pachytene looped domains were maximally sensitive to DNase I, while the elongated spermatids and sperms were highly resistant. Our data suggest that pachytene DNA is in the most open chromatin conformation of all testicular cell types, while round spermatids show the most compact conformation in terms of EtBr intercalation.

Key words: chromatin, DNase I, nucleoid, spermatogenesis.

Received: June 13, 2003; Accepted: October 24, 2003.

Introduction

The 'ordered arrangement' of chromatin in the interphase nucleus is maintained by its attachment to the nuclear membrane (Comings, 1968; Comings and Okada, 1970). The 30 nm nucleofilament is further compacted into supercoiled looped DNA domains of 50-100 kb length through attachment to the nuclear matrix (Hancock, 1982; Pienta and Coffey, 1984; VanderWaal *et al*., 2002, Sheval *et al*., 2002). The supercoiled loops can be relaxed and visualized as DNA 'halo' around nuclear matrix in high salt buffer in the presence of low ethidium bromide (EtBr) concentrations (Benyajati and Worcel, 1976; Vogelstein *et al*., 1980).

During mammalian spermatogenesis, cells display differential expression of many histone variants in different stages (Trostle-Weige *et al*., 1982). Starting from the occurrence of the testis-specific and testis-enriched histones in spermatogenic cells, there is nearly complete replacement of histones by protamines and other basic proteins during spermiogenesis. It is likely that in response to these variations organizational modifications of chromatin take place in meiotic and post-meiotic cells. The most significant modification is observed during sperm maturation, during which the nucleosomal organization is dismantled in favour of a highly condensed, inert DNA-protein complex, stabilized by disulphide linkages (-S-S-crosslinks) (Ammer and Henschen, 1987).

Topological accommodation of DNA during such extreme chromatin modifications during spermatogenesis has been addressed in various ways. Similar to the somatic nuclei (Cook *et al*., 1976; Robinson *et al*., 1983; Pienta and Coffey, 1984; Nelson *et al*., 1986, VanderWaal *et al*., 2002), sperm DNA also occurs in a specific manner in relation to the sperm nuclear matrix (Balhorn, 1982; Risley *et al*., 1986; Ward *et al*., 1989; Ward and Coffey, 1990; Ward and Coffey, 1991), but its organization varies depending upon the proteins bound to it. While sperm nuclei of the histone-containing *Rana catasbeiana* contain negatively supercoiled DNA, the protamine-containing sperm DNA of *Xenopus laevis* and *Bufo fowleri* is not supercoiled (Risley *et al*., 1986). Likewise, in the human sperm nuclei, nearly 85% of the chromatin is organized as 'nucleoprotamine' and about 15% as 'nucleohistone' (Tanphaichitr *et al*., 1978; Gatewood *et al*., 1987). The DNA is slightly nega-

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tively supercoiled in about 15% of the supercoils of DNA per turn in the solenoid rather than nucleosomal configuration (Ward, 1993). Earlier studies on the chromatin organization in testis are mostly confined to sperm cells. The present study was undertaken with the objective of understanding the sequential changes from transcriptionally active spermatogonials and pachytenes to the transcriptionally inactive sperm cells during the course of terminal differentiation through spermatogenesis.

Materials and Methods

The white laboratory mice (*Mus musculus*, Parkes strain) bred and reared in our laboratory were used for the study.

Methods

Isolation of nuclei and nucleoid preparation

Isolation of nuclei and the preparation of nucleoids were carried out according to Buongiorno-Nardelli *et al*., 1982, and Risley *et al*., 1986, with slight modifications. Briefly, a cell suspension from adult mice testes was prepared by mincing the testes in cold (4 °C) PBS. The cells were pelleted at 3000 rpm for 10 min in a Sorvall SS-34 rotor and resuspended in NKETS buffer (10 mM NaCl, 40 mM KCl, 2 mM EDTA pH 8.0, 0.1 mM Spermine, 0.25 mM Spermidine, 15 mM Tris.HCl pH7.4, and 1mM PMSF), then Triton X-100 was added to a final concentration of 0.5%. The suspension was vortexed gently and then mixed rapidly with 1 vol. 80% glycerol in NKETS buffer (-20 °C). Nuclei were pelleted by centrifugation at 5000 rpm for 20 min at 0 °C in a Sorvall SS-34 rotor, resuspended in 40% glycerol in NKETS buffer (1-5x10^6/mL) and maintained at -20 °C prior to nucleoid preparation. The nuclei were pelleted and resuspended in high salt buffer (2.0 M NaCl, 0.2 mM $MgCl_2$, 10 mM Tris.HCl pH 7.5) in NKETS containing 2.5-200 µg/mL of ethidium bromide (EtBr) (1x10^6nuclei/mL). After 10 min incubation on ice, a drop of the nuclear suspension was placed on a clean glass slide, covered with cover slip and sealed with DPX. The slides were examined in a Leitz MPV-3 fluorescence microscope under UV-illumination. To avoid EtBr quenching, the observations and measurements of the nucleoids were done immediately after mounting the slides. The cell types were identified under phase contrast. Nucleoid diameter and halo radii were measured before photography (photography of fluorescent cells requires long exposures of 1-2 min, reducing the sharpness of the boundaries) for over 50 nuclei for each treatment to every cell type, using an ocular micrometer.

DNase I treatment of the nucleoid DNA

DNase I treatment of nucleiod DNA was done by adding 5 U/mL of DNase I (Sigma) to the nucleoid preparations and incubating for either 5 min (brief) or 30 min (prolonged) at 37 °C. The reaction was terminated by chilling on ice and adding 10 mM EDTA to final concentration. Fluorescence microscopy and nucleoid measurements were done as described before.

Results

Study of DNA topology in the nucleoids is one of the most effective tools to gain information on chromatin organization in a cell. At certain concentration of EtBr, the DNA loses all its negative supercoils and is relaxed and extended (equivalence point), giving an appearance of a 'halo' (Figure 1 e, f, h, i). Higher concentrations of EtBr introduce positive supercoils, resulting in recontraction of the DNA loops (Tables 1, 2).

Effects of ethidium bromide (EtBr) concentrations on nucleoid DNA of testicular cell types

Nucleoids were prepared in high salt (2.0 M NaCl) buffer containing different EtBr concentrations (2.5-200 µg/mL). The general shape of the nucleus from all the cell types was retained, regardless of the superhelical configuration of the DNA (Figure 1).

All the testicular cell types, viz., spermatogonials, pachytenes, round spermatids, elongated spermatids and testicular sperm, showed a biphasic change (condensed-relaxed-condensed) in their nucleoid as well as halo size in an EtBr concentration-dependent manner (2.5-200 µg/mL) (Tables 1, 2). Maximum halo size was obtained at a concentration of 5.0 µg/mL EtBr, above which halo size reduction took place. However, complete halo size reduction was not achieved even at 200 µg/mL EtBr concentration. Unlike the testicular cells, the epididymal sperm nucleiods were relaxed and expanded only at 2.5 µg/mL EtBr, and gradually condensed at increased EtBr concentrations (Tables 1, 2). At 5 µg/mL of EtBr, the nucleoid diameter increased to variable degrees in different cell types (Figure 1), viz., spermatogonial (153.2% of the isolated nuclei), pachytene (170.3%), round spermatids (61.3%), and elongated spermatids and testicular sperm (194.7%). In case of epididymal sperm, maximum increase in the nucleoid diameter (213%) occurred at 2.5 µg/mL EtBr. Of all the cell types, the pachytene nucleoid had the maximum length of loop domains (an average of 7.8 µm loop, *i.e.*, 15.6 µm of DNA domain). Although a halo was not detected in the nucleoids of round spermatids following 2.0 M NaCl extraction, a biphasic change in the diameter of nucleoids was observed as a function of EtBr concentrations (Table 1). When the round spermatids were subjected to a higher concentration of NaCl (2.4 M) for the nucleoid preparation, it resulted in much enlarged nucleoids (12.0 µm), with an average halo of 3.3 µm, an increase of 287.1% in nucleoid diameter as compared to isolated nuclei (Tables 1, 2; Figure 1i).

Table 1 - Nucleoid diameters (in micrometers) at different Ethidium bromide concentrations [Number of nucleoids].

Cell type	Nuclear diameter (control)		Ethidium bromide concentrations (µg/mL) in high salt buffer (HSB) containing 2.0 M NaCl											
			2.5 µg/mL		5 µg/mL		10 µg/mL		25 µg/mL		100 µg/mL		200 µg/mL	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Spermatogonial	7.7	0.8	10.5	1.2	19.5	2.8	16	1.6	14.8	1.3	14.2	1.5	11.9	1.2
	[62]		[53]		[54]		[54]		[52]		[52]		[51]	
Pachytene	10.8	1	16.4	2	29.2	4.1	24.5	2.5	21.3	2.2	20.1	1.8	17	2
	[55]		[51]		[56]		[51]		[52]		[50]		[52]	
Round spermatid	3.1	0.7	3.9	0.5	5	1.3	4.3	0.6	4.2	0.6	4	0.8	3.2	0.8
	[57]		[55]		[58]		[54]		[58]		[60]		[53]	
Round spermatid (in HSB containing 2.4 M NaCl)			-		12 [56]	1.2	-		-		-		-	
*Elongated spermatid & testicular sperm	1.9	0.7	3	0.6	5.6	1.4	4.7	1	4.5	1.2	3.7	0.9	3.2	0.7
	[56]		[57]		[52]		[54]		[61]		[59]		[59]	
*Epididymal sperm	1.5	0.3	4.7	0.7	4	0.7	3.3	0.5	2.9	0.4	2.5	0.4	2.3	0.5
	[56]		[56]		[56]		[56]		[56]		[56]		[56]	

*Nucleoids were measured at the region where the width was maximum.
S.D. = Standard Deviation.

Table 2 - Halo radii (in micrometers) at different Ethidium bromide concentrations [Number of nucleoids].

Cell type	Ethidium bromide concentrations (µg/mL) in high salt buffer (HSB) containing 2.0 M NaCl											
	2.5 µg/mL		5 µg/mL		10 µg/mL		25 µg/mL		100 µg/mL		200 µg/mL	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Spermatogonial	1.1	0.5	5.1	1.2	3.9	0.6	3.2	0.5	2.9	0.6	1.9	0.6
	[53]		[54]		[54]		[52]		[52]		[51]	
Pachytene	1.5	0.6	7.8	1.6	5.9	0.7	4.2	0.8	3.6	0.7	2.5	0.7
	[51]		[56]		[51]		[52]		[50]		[52]	
Round spermatid	-		-		-		-		-		-	
Round spermatid (in HSB containing 2.4 M NaCl)	-		3.3 [56]	0.4	-		-		-		-	
*Elongated spermatid & testicular sperm	0.3	0.3	1.4	0.4	0.9	0.4	0.8	0.3	0.4	0.3	0.3	0.3
	[57]		[52]		[54]		[61]		[59]		[59]	
*Epididymal sperm	1.5	0.3	1.2	0.3	0.9	0.2	0.7	0.2	0.5	0.7	0.4	0.2
	[56]		[56]		[56]		[56]		[56]		[56]	

*Halo were measured at the region where the width was maximum.
S.D. = Standard Deviation.

DNase I sensitivity of loop domains in nucleoids of different spermatogenic cell types

Since the chromatin sensitivity to DNase I depends upon its functional organization, we have investigated the sensitivity of DNA loops to brief (5 units/mL for 5 min) and prolonged (5 units/mL for 30 min) DNase I treatments at different stages of spermatogenesis. The loop domains of different cell types showed differential response to DNase I treatment. The maximum effect of DNase I was seen on pachytene nucleoids. The elongated spermatids and sperm nucleoids were minimally affected within 5 min (Table 3). While there was 18.8% loss in nucleoid diameter and 32.1% loss in halo radius of pachytenes, the elongated spermatid and sperm nucleoids remained stable, with apparently no loss of DNA (Table 3). After prolonged DNase I treatment (5 U/mL for 30 min), the loss in nucleoid diameter and halo radius was comparable among all the cell types (Table 3).

Discussion

Our findings reveal continuous changes in chromatin organization during spermatogenesis. The differences in nucleoid and halo sizes provide a measure of differential DNA topology in different cell types (Cook *et al*., 1976; Vogelstein *et al*., 1980; Risley *et al*., 1986). The biphasic change in the nucleoid diameters of testicular cell types as a function of increasing concentrations of EtBr establishes

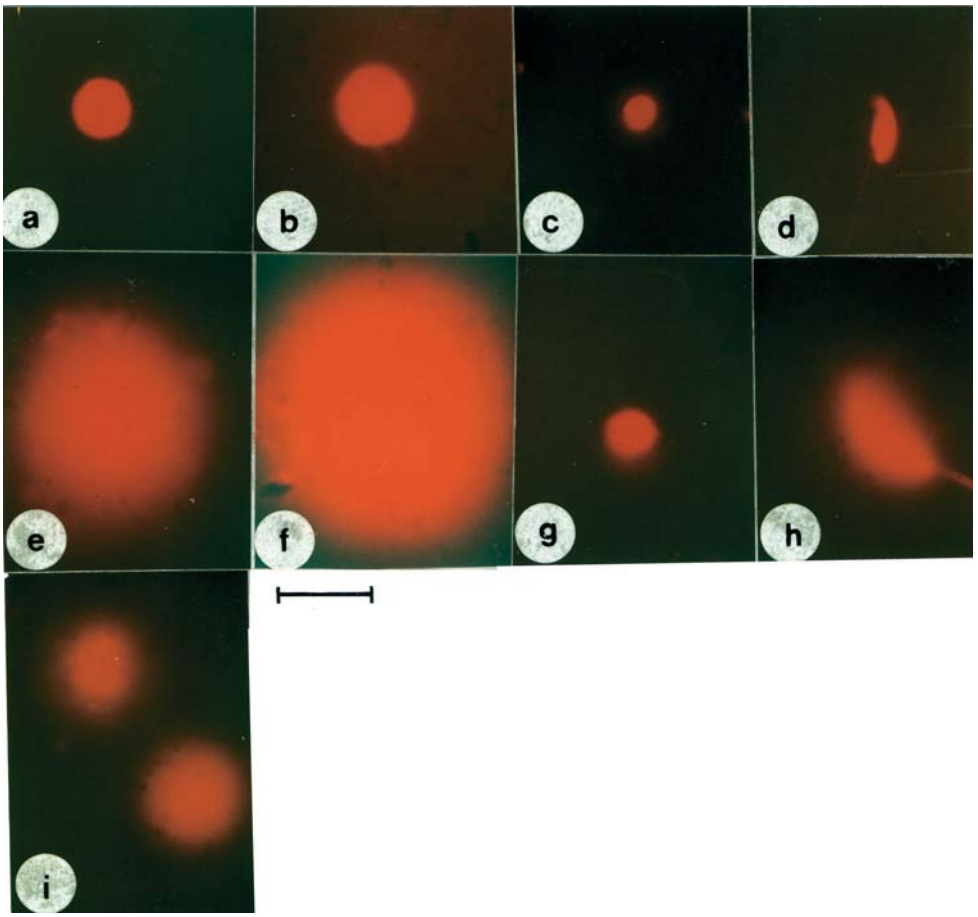

Figure 1 - Fluorescence photomicrographs of EtBr stained (5 µg/mL) mice spermatogonial (a, e), pachytene (b, f), round spermatid (c, g, i), and elongated spermatid/testicular sperm (d, h) nuclei (a, b, c, d) and nucleoids following 2.0 M NaCl extraction (e, f, g, h) and 2.4 M NaCl extraction (i). Note the absence of detectable halo in round spermatid nucleoid following 2.0 M NaCl extraction and a distinct halo following 2.4 M NaCl extraction. Bar = 10 µ m.

Table 3 - Nucleoid diameters and Halo radii (in micrometers) following DNase I treatment [Number of nucleoids].

Cell type	Without DNase I (EtBr = 5 µg/mL)				5 U/mL DNase I, 5 min (EtBr = 5 µg/mL)				5 U/mL DNase I, 30 min (EtBr = 5 µg/mL)			
	Nucleoid diameter		Halo radius		Nucleoid diameter		Halo radius		Nucleoid diameter		Halo radius	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Spermatogonial	19.5	2.8	5.1	1.2	18.5	1.8	4.3	1.6	12.4	1.7	1.6	0.7
	[54]		[54]		[56]		[56]		[55]		[55]	
Pachytene	29.2	4.1	7.8	1.6	23.7	2.6	5.3	1.8	18.4	2.3	2.4	1.7
	[56]		[56]		[52]		[52]		[53]		[53]	
Round spermatid	5	1.3	-		4.1	0.7	-		3.7	0.7	-	
	[52]		[52]		[60]		[60]		[53]		[53]	
*Elongated spermatid & testicular sperm	5.6	1.4	1.4	0.4	5.8	1.3	1.4	0.4	3.8	0.8	0.5	0.3
	[52]		[52]		[60]		[53]		[53]			
*Epididymal sperm	4	0.7	1.2	0.3	4	0.5	1.2	0.3	2.6	0.4	0.6	0.2
	[56]		[56]		[56]		[56]		[56]		[56]	

*Nucleoids/Halo were measured at the region where the width was maximum.
S.D. = Standard Deviation.

that, like in most other cell types studied, the DNA in the mouse testis cell types (Vogelstein *et al*., 1980; Pienta and Coffey, 1984; Risley *et al*., 1986) is organized into negatively supercoiled topological domains. However, the lack of similar biphasic modulation in the mature sperm derived from epididymis supports the loss of nucleosomal organization in mature sperm. A smaller spermatogonial DNA loop (at 5 µg/mL EtBr) than that of pachytenes indicates

more attachment sites at the matrix than the pachytene DNA. Since both DNA replication (Pardoll *et al*., 1980; Vogelstein *et al*., 1980; Buongiorno-Nardelli *et al*., 1982; Pienta and Coffey, 1984; Nelson *et al*., 1986; Haaf and Schmid, 1991) and transcription (Robinson *et al*., 1983; Hentzen *et al*., 1984; Mirkovitch *et al*., 1984; Small *et al*., 1985; Gasser and Laemmli, 1987; Steif *et al*., 1989) in the nuclei occur on the nuclear matrix, smaller than the pachytene loop domains in the gonials may be indicative of the active replication and transcription that goes on in the latter. Earlier experiments with DNase I sensitivity of the meiotic chromosomes (Rao *et al*., 1983; Chandley and McBeath, 1987; Raman *et al*., 1988) and those reported here suggest that among testicular cells the pachytene chromatin in its native form is the most accessible to the nucleolytic enzymes, and thus accessible to the requirement of recombination. We contend that the larger loop domain of pachytene nucleoids also facilitates its "openness" for accessibility to the recombinational machinery, which would need larger lengths of 'matrix-free' chromatin.

One of the striking observations is the absence of a detectable halo in the round spermatids at all the EtBr concentrations following 2.0 M NaCl and its appearance following 2.4 M NaCl extractions. The lack of halo indicates a chromatin organization in round spermatids that is remarkably different from other testicular cell types, even elongating spermatids. It was difficult to unequivocally distinguish between the elongating/elongated spermatids and testicular sperm in the EtBr-stained nucleoids. Therefore, these cell types were scored together. The occurrence of typical biphasic change in the nucleoid and the dimensions of halo after 2.0 M NaCl extraction imply that topologically negative supercoiling of DNA persists, even at higher degrees of spermatid differentiation. A curious aspect of this finding is that, though elongating spermatid (and testicular sperm) is a later and expectedly more condensed state of chromatin than round spermatid, it is the latter which is more resistant to the salt extraction. It seems unlikely that the apparent lack of loop domains is due to the large number of attachment points on the nuclear matrix. The spermatogenic calendar of the mouse shows that, while transition proteins (TPs) are transcribed in the round spermatids, the protamines (P1, P2) appear only in the elongating spermatids (Hecht, 1990). It is possible that, in the process of transition, the removal of histones by the protamines may relieve the constraints in the DNA, as it progresses from round spermatid to the elongating type. The retention of (-)ve supercoiled state and nucleosome structure in the elongating spermatid and testicular sperm suggests that the residual presence of histones is enough to generate the nucleosomal organization.

Since one of the possible reasons of DNase I sensitivity is the local displacement of histones, forming a nucleosome-free region of chromatin, it is appropriate to assay the sensitivity of histone-depleted and EtBr-relaxed chromatin. In the present investigation, nucleoids were exposed for 5 and 30 min to 5 units of DNase I. The results were more pronounced after the brief treatment. That the digestion does not lead to complete collapse of the halo even after the prolonged treatment suggests that, even in the histone-depleted condition, DNase I does not cleave randomly on the loop: the region close to the matrix may be less sensitive than the one distant from it in a gradient. The results in the elongated spermatids and sperm are to some extent surprising, because, in spite of the removal of the basic proteins and dismantling of the nucleosome structure in the sperm, chromatin of these cell types was almost inaccessible to the enzyme, although the prolonged exposure did digest the nucleoid to some extent. These results thus bring into consideration the role of other groups of non-histone structural DNA-binding proteins (consistent with Sheval *et al*., 2002), for instance high mobility group (HMG) proteins, in the organization of chromatin. It is evident that HMGs and other DNA-binding proteins have a role in the functional organization of chromatin, but it would need more information on their distribution and levels during different stages of chromatin for a meaningful comparison to be made.

Acknowledgements

Financial support for the work was provided by UGC, New Delhi, to GN and by CSIR, New Delhi, to RR. The authors are thankful to Dr. A.S. Balajee, Department of Radiation Oncology, Columbia University, New York, for his critical comments and suggestions.

References

Ammer H and Henschen A (1987) The major protamine from stallion sperm isolation and amino acid sequuence. Biol Chem Hoppe-Seyler 368:1619-1626.

Balhorn RA (1982) A model for the structure of chromatin in mammalian sperm. J Cell Biol 93:298-305.

Benyajati C and Worcel A (1976) Isolation, characterization and structure of the folded interphase genome of *Drosophila melanogaster*. Cell 9:393-407.

Buongiorno-Nardelli M, Micheli G, Carri MT and Marilley M (1982) A relationship between replicon size and supercoiled looped domains in the eukaryotic genome. Nature 298:100-102.

Chandley AC and McBeath S (1987) DNase I hypersensitive sites along the XY bivalent at meiosis in man include the XpYp pairing region. Cytogenet Cell Genet 44:22-31.

Comings D and Okada TA (1970) Association of chromatin fibers with the annuli of the nuclear membrane. Exp Cell Res 62:293-302.

Comings DE (1968) The rationale for an ordered arrangement of chromatin in the interphase nucleus. The Am J Hum Genet 20:440-460.

Cook PR, Brazell JA and Jost E (1976) Characterization of nuclear structuure containing superhelical DNA. J Cell Sci 22:303-324.

Gasser SM and Laemmli UK (1987) A glimpse at chromosomal order. Trends Genet 3:16-22.

Gatewood JM, Cook GR, Balhorn R, Bradbury EM and Schmid CW (1987) Sequence-specific packaging of DNA in human sperm chromatin. Science 236:962-964.

Haaf T and Schmid M (1991) Chromosome topology in mammalian interphase nuclei. Exp Cell Res 192:325-332.

Hancock R (1982) Topological organisation of interphase DNA: the nuclear matrix and other skeletal structures. Biol Cell 46:105-122.

Hecht NB (1990) DNA methylation and demethylation events during meiotic prophase in mouse testis. Mol Cell Biol 10:1828-1834.

Hentzen PC, Rho JH and Bekhor I (1984) Nuclear matrix DNA from chicken erythrocytes contains beta-globin gene sequences. Proc Natl Acad Sci, USA 81:304-307.

Mirkovitch J, Mirault M-E and Laemmli UK (1984) Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. Cell 39:223-232.

Nelson WG, Pienta KJ, Barrack ER and Coffey DS (1986) The role of the nuclear matrix in the organization and function of DNA. Ann Rev Biophys Chem 15:457-475.

Pardoll DM, Vogelstein B and Coffey DS (1980) A fixed site of DNA replication in eukaryotic cells. Cell 19:527-536.

Pienta KJ and Coffey DS (1984) A structural analysis of the role of the nuclear matrix and DNA loops in the organization of the nucleus and chromosome. J Cell Sci Suppl 1:123-135.

Raman R, Singh AP and Nanda I (1988) DNase I nick translation *in situ* on meiotic chromosomes of the mouse, *Mus musculus*. J Cell Sci 90:629-633.

Rao BJ and Rao MRS (1987) DNase I site mapping and micrococcal nuclease digestion of pachytene chromatin reveal novel structural features. J Biol Chem 262:4472-4476.

Risley MS, Einherber S and Bumcrot DA (1986) Changes in DNA topology during spermatogenesis. Chromosoma 94:217-227.

Robinson SI, Small D, Idzerda R, McKnight GS and Vogelstein B (1983) The association of transcriptionally active genes with the nuclear matrix of the chicken oviduct. Nucl Acid Res 11:5113-5130.

Sheval EV, Prusov AN, Kireev II, Fais D and Polyakov VYu (2002) Organization of higher-level chromatin structures (chromomere, chromonema and chromatin block) examined using visible light-induced chromatin photo-stabilization. Cell Biol International 26:579-591.

Small D, Nelkin B and Vogelstein B (1985) The association of transcribed genes with the nuclear matrix of *Drosophila* cells during heat shock. Nucl Acid Res 13:2413-2431.

Steif A, Winter DM, Stratling WH and Sippel AE (1989) A nuclear DNA attachment element mediates elevated and position-independent gene activity. Nature 341:343-345.

Tanphaichitr N, Sobhon P, Taluppeth N and Chalermisarachai P (1978) Basic nuclear proteins in testicular cells and ejaculated spermatozoa in man. Exp Cell Res 117:347-356.

Trostle-Weige PK, Meistrich ML, Brock WA, Nishioka K and Bremer JW (1982) Isolation and characterization of TH2A, a germ cell-specific variant of histone 2A in rat testis. J Biol Chem 237:5560-5567.

VanderWaal RP, Spitz DR, Griffith CL, Higashikubo R and Roti JLR (2002) Evidence that protein disulphide isomerase (PDI) is involved in DNA-nuclear matrix anchoring. J Cell Biochem 85:689-702.

Vogelstein B, Pordoll DM and Coffey DS (1980) Supercoiled loops and eukaryotic DNA replication. Cell 22:79-85.

Ward WS, Partin AW and Coffey DS (1989) DNA loop domains in mammalian spermatozoa. Chromosoma 98:153-159.

Ward WS and Coffey DS (1990) Specific organization of genes in relation to the sperm nuclear matrix. Biochem Biophys Res Commun 173:20-25.

Ward WS and Coffey DS (1991) DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod 44:569-574.

Ward WS (1993) Deoxyriboncleic acid loop domain tertiary structure in mammalian spermatozoa. Biol Reprod 48:1193-1201.

Editor: André Luiz Paranhos Perondini