HMGB4, a Novel Member of the HMGB Family, Is Preferentially Expressed in the Mouse Testis and Localizes to the Basal Pole of Elongating Spermatids

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ABSTRACT

We identified HMG4, a novel member of the HMGB family lacking the acidic tail typically found in this family. HMG4 is strongly and preferentially expressed in the adult mouse testis and weakly in the brain, but not in many other tissues. HMG4 associates with chromatin, and in transfection assays, in contrast to HMGB1, it acts as a potent transcriptional repressor. During spermatogenesis, HMG4 is present in the euchromatin of late pachytene spermatocytes and haploid round spermatids, whereas stronger expression is observed during the elongation phase, where it localizes to the basal pole of the nucleus in a manner mutually exclusive with H1FNT (H1T2) localized at the apical pole. HMG4 basal localization is lost in H1FNT-mutant spermatids, showing that H1FNT provides a positional cue for organizing chromatin domains within the nucleus. These results show that HMG4 and H1FNT specify distinct chromatin domains at the apical and basal poles of the elongating spermatid nucleus.

cytoskeleton, gene regulation, haploid cells, HMG2, H1FNT, polarity, spermatid, spermatogenesis, testis

INTRODUCTION

Mammalian spermatogenesis involves the differentiation of spermatogonia stem cells into spermatocytes and then, through two successive meiotic divisions, into haploid round spermatids. During spermiogenesis, the haploid round spermatids undergo an elongation phase that sculpts them into mature spermatozoa. This entails a major biochemical and morphological restructuring of the germ cell in which the majority of the somatic histones are replaced, first by transition proteins, then protamines packing the DNA into the sperm cell nucleus.

In round spermatids the DNA is organized in a histone-containing chromatin structure that comprises core histone variants, such as HIST1H2BA (also known as testis histone 2B [TH2B]), H2AL1 (H2A-like 1), H2AL2 (H2A-like 2), or H2BL1 (H2B-like 1), or linker histone variants, like HIST1H1T (also known as H1T) and H1FNT (also known as H1T2) [1–3]. (Note that the linker histone variants currently do not have official nomenclature.) Additional observations suggest that the DNA is not homogeneously packed in the spermatozoa and that positional cues exist within the elongating spermatid nucleus. For example, H1FNT localizes to the apical pole of round and elongating spermatids, revealing a polarity in chromatin organization [4]. Similarly, the core histone variants H2AL1 and H2AL2 localize specifically to the heterochromatin, where they heterodimerize preferentially with HIST1H2A, and telomeres were shown to be enriched in somatic-type core histones and HIST1H2B (H2B) variants [5–9].

In addition to the above, HMG2 (high-mobility group box 2) is a chromatin-associated protein preferentially expressed in male germ cells [10]. HMG2 is a member of the high-mobility group (HMG) proteins related to HMG1 and HMG3. These proteins are characterized by the presence of two HMG boxes, 80-amino acid domains composed of three alpha-helices that fold into a wedge-shaped domain that binds the minor groove of DNA with little or no sequence specificity, followed by a highly acidic C-terminal tail [11–14]. HMG proteins act as architectural factors capable of organizing dynamic active chromatin structures and by bending DNA [15, 16]. They can interact directly with nucleosomes, transcription factors, and nucleosome-remodeling machines to promote the global establishment of active and, more rarely, inactive chromatin domains (see, for example [17–20], reviewed in Agresti and Bianchi [16]).

Three members of the family have been characterized. In the adult, HMG1 is expressed everywhere except in neurons, whereas HMG2 is expressed in testis and HMG3 preferentially in lymphoid organs [16]. The generation of knockout mice for these proteins has shown that their functions are partially redundant: Hmg1−/− mice die immediately after birth, whereas Hmg2−/− mice are viable, but male mice have significantly reduced fertility, which is due to germ cell degeneration and immotile spermatozoa [10]. In addition to its role in chromatin, HMG1 has been shown to exert an endocrine inflammatory function upon release from necrotic cells [21]. Hmg3−/− mice are viable but erythrocytemic [22, 23].

In this study we describe HMG4, a novel member of the HMGB family, characterized by the absence of the acidic tail that is strongly and preferentially expressed in male germ cells, where it localizes to the basal pole of the elongating spermatid...
nucleus. Together, our results show that HMGB4 is a novel chromatin-associated protein that, together with H1FNT, specifies distinct positional domains within the elongating spermatid nucleus.

MATERIALS AND METHODS

RT-PCR and Cloning of HMGB4

The RT-PCR on RNA from different organs was performed using standard protocols. Primers used to amplify Hmgb4 were: forward: 5'-TCTGCATCTCGATCCTGAG-3', reverse: 5'-TTATTGTTTCAAACTGGTCTTGGC-3'.

A full-length cDNA encoding HMGB4 was amplified by RT-PCR from murine adult testis RNA using oligonucleotide primers located at the 5' and 3' ends as deduced from the analysis of the mouse expressed sequence tag (EST) public databases. Primers contained NotI and BamHI restriction sites. The 746-bp fragment was digested with NotI and BamHI and cloned in pXJ41 expression vector or in a modified pXJ41 expression vector containing the FLAG epitope linearized with the same enzymes. Transfection of HeLa and GC-1 cells and preparation of transfected cell extracts were as previously described [24].

Immunoblotting and Immunohistochemistry

Mouse seminiferous tube segments at defined stages were isolated using the transillumination-assisted microdissection method [25, 26]. Immunohistochemistry was performed on staged squash preparations of microdissected tubules from wild-type C57BL/6 mice, or H1fn1-/-/ mice, as previously described [4, 27]. All mice were maintained in the Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) mouse facility in accordance with the French national animal ethics regulations.

Antibodies against TBP and CBX5 (also known as HP1α H2AL1, and H1FNT) have been described previously [4, 28, 29], and monoclonal (4E1) and polyclonal antibodies were generated against the HMGB4 peptide, indicated in Figure 1, and coupled to ovalbumin.

Extract Preparation

Total extracts were prepared from frozen mouse testis ground in a mortar in the presence of liquid nitrogen. The powdered material obtained was resuspended in Laemmli buffer and heated at 95°C for 15 min before loading on an SDS-PAGE gel. Epididymal sperm was collected and resuspended directly in Laemmli buffer. Organ extracts were prepared by several cycles of the freeze-thaw in buffer A (50 mM Tris-HCl [pH 7.9], 10% glycerol, 1 mM
dithiothreitol [DTT], and 0.1% Nonidet P-40) plus 500 mM KCl, followed by incubation for 30 min on ice and microfugation for 10 min at 4°C.

Preparation of Testis Cell Chromatin Fibers and Hydroxyapatite Powder Fractionation

Nuclei of testis cells were prepared by dissociation of two testes in a potter with 2 ml lysis buffer (0.34 M saccharose, 60 mM KCl, 15 mM NaCl, 15 mM Tris-HCl [pH 7.4], 0.65 mM spermidine, 2 mM EDTA, 0.5 mM EGTA, 0.03% Triton X-100, 0.1 mM DTT, and 0.1 mM PMSF), centrifugation (10 min at 1500 rpm), washing in 10 ml lysis buffer, resuspension in 1 ml digestion buffer (lysis buffer without Triton X-100, EDTA, and EGTA), and filtering on 100-μm filters. Nuclei were resuspended in 300 μl digestion buffer and submitted to S7 MNase (Sigma) digestion (5 units per 2 × 10⁶ nuclei, 10 min, 37°C). Reaction was stopped by 5 mM EDTA (final concentration). After centrifugation (10 min at 2000 rpm), a first supernatant (SN1) containing oligonucleosomes was recovered. Longer fibers were extracted from the pelleted nuclei by resuspension in 300 μl of 0.1 mM EDTA, incubation for 1 h on ice, and centrifugation for 10 min at 10000 rpm (SN2). DNA analyses were performed on 10 μl of SN1 and SN2 by treatment with proteinase K followed by electrophoresis on a 1% agarose gel.

A total of 20 mg hydroxyapatite powder (Bio-Rad) was added to SN1 and SN2. After a 5 min incubation on ice and washes in 0.1 M potassium phosphate, pH 6.7, containing 0.15 M NaCl, the chromatin-associated proteins were eluted with phosphate buffer, containing 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 2 M NaCl.

Transfection Assays

GC-1 and COS1 cells were transfected with the 17m5-TATA-chloramphenicol transferase (CAT) reporter (2.5 μg), the cytomegalovirus-based β-galactosidase expression vector as internal control, the GAL4-VP16 activator plasmid (250 ng), and increasing concentrations (1, 2, and 5 μg) of expression vectors for F-HMGB4 or HMGB1 (kind gifts from M. Bianchi, Universita Vita Salute San Raffaele, Milano, Italy). Cells were transfected with JetPEI (Polyplus) and harvested 48 h after transfection, lysed, and assayed for CAT activity with a CAT ELISA kit (Roche). The CAT activity was normalized against the protein concentration of the extract (see Figure 6D) or the measured β-galactosidase levels (data not shown). All transfections were carried out in triplicate. For Western blotting, the transfected cells were resuspended in buffer A plus 250 mM KCl (200 μl per 10-cm dish) and subjected to three cycles of freeze-thaw, followed by incubation for 30 min on ice and microfugation at
2500 rpm for 10 min at 4°C. The supernatant is the cytoplasmic fraction. The pelleted nuclei were resuspended in buffer A plus 800 mM KCl (100 μl per 10-cm dish), and the supernatant corresponding to the nucleoplasmic fraction was recovered after three cycles of freeze-thaw and microfugation at 13,000 rpm for 10 min at 4°C. The residual chromatin pellet was recovered in SDS-PAGE loading buffer (100 μl per 10-cm dish).

RESULTS

HMGB4, a Novel Member of the HMGB Family, Preferentially Expressed in Testis

While performing a proteomic analysis of proteins coimmunoprecipitated with the testis-specific factor GTF2A1L (also known as ALF) [30], we identified several peptides belonging to a novel protein containing an HMG box (subsequent immunoblotting revealed that HMGB4 does not coprecipitate specifically with GTF2A1L, but is rather a nonspecific contaminant; data not shown). Nevertheless, given the testis-specific expression of this protein (see below), we decided to further investigate it.

Using primers based on mouse ESTs in the public databases, a full-length cDNA encoding this HMG protein was amplified from murine adult testis RNA by RT-PCR. Public databases show that the mouse gene is located on chromosome 4. It is an intronless gene transcribed as a unique 746-bp transcript encoding a protein of 181 residues characterized by two HMG boxes, A and B, typically present in various members of the HMGB family (Fig. 1).

Sequence comparison of HMGB4 from rat, mouse, and human with the sequences of HMGB1 and HMGB2 from different vertebrates shows that the similarity within the family is highest in the HMG boxes. However, even within the HMG domains there are several regions where residues that are highly conserved in the HMGB1 and two proteins are divergent in the HMGB4 subfamily. More importantly, a striking distinctive feature of HMGB4 is the lack of the acidic tail, typically present in the HMGB family (Fig. 1). Extensive reiterative BLAST analysis revealed orthologs of HMGB4 in other mammals but not in other vertebrates or nonvertebrates. HMGB4 is therefore a mammalian-specific protein characterized by a divergent HMG sequence and lack of an acidic tail.

To analyze HMGB4 expression, RNA from several adult murine tissues was analyzed by RT-PCR, showing that it is strongly expressed in testis, whereas only weak expression is seen in brain and kidney, and no expression was shown in other organs tested (Fig. 2A). We generated a monoclonal antibody against a synthetic peptide (underlined in Fig. 1) specific for HMGB4. This antibody recognized a 20-kDa protein in extracts from mouse testis and from HeLa cells transfected with an HMGB4 expression vector (Fig. 2B, see below, and data not shown). Despite the fact that Hmgb4 mRNA could be detected in brain and kidney, no significant expression of the HMGB4 protein could be detected in these tissues or in several other tested tissues (Fig. 2B). Thus, HMGB4 shows tissue-specific expression distinct from that of the other HMGB proteins, which are widely expressed, such as HMGB1, or restricted to testis and lymphoid organs, like HMGB2 and HMGB3, respectively.

To determine whether HMGB4 is associated with chromatin, we prepared micrococcal nuclease (MNase)-digested chromatin from testis. The nuclei were pelleted by centrifugation, and a first fraction (A) containing soluble nuclear proteins, mononucleosomes, and short-length oligonucleosomes was collected. Longer chromatin fibers then were extracted from the pelleted nuclei and recovered after centrifugation in a second fraction, B (Fig. 2C). HMGB4 is

FIG. 3. Developmental expression of HMGB4 in male germ cells. A) The stage of each microdissected seminiferous tubule segment is indicated to the left of each subpanel. Immunostaining with the anti-HMGB4 antibody is shown on the left, Hoechst-stained DNA is shown in the center column, and the merged image appears in the right column. Representative examples of cell types are indicated. P, Pachytene spermatocytes; RS, haploid round spermatids; ES, elongating spermatids; EES, early elongating spermatids. Original magnifications ×63 (stages VI–VII, XII, and I) and ×100 (stages VIII–IX). The white arrowheads indicate the expression of HMGB4 at the basal pole of the nucleus in early elongating spermatids. B) Detailed views of differentiating germ cells. Panels from top to bottom show late-stage pachytene spermatocytes and a round spermatid (circled), round spermatids, early elongating spermatids, and elongate spermatids. Note the exclusion of HMGB4 from the dense Hoechst-stained heterochromatin in the pachytene spermatocytes and the chromocenter (CC) in the round spermatid nuclei. Original magnification ×100.
found in both fractions A and B, unlike HIST1H1T and H1FNT, which are mainly associated with the longer chromatin fibers (Fig. 2D).

Nucleosomes and chromatin fibers from fractions A and B then were pulled down on hydroxyapatite, and the associated proteins were eluted with increasing salt concentration. A significant proportion of HMGB4 is readily released from chromatin by the MNase digestion, because most of the HMGB4 present in fraction A was not retained on hydroxyapatite (Fig. 2E, lanes 1 and 2) or eluted at low salt concentration (Fig. 2E, lanes 3 and 4). The HMGB4 present in fraction B was eluted between 0.3 and 0.4 M NaCl (Fig. 2E, lanes 3 and 4). Almost all the H1FNT or HIST1H1T was present in fraction B, and H1FNT eluted between 0.4 and 0.6 M NaCl and HIST1H1T (H18) at 0.6 M NaCl, as expected for a linker histone (Fig. 2E, lanes 4 and 5). These results show that HMGB4 binds chromatin with a lower affinity compared with the linker histone HIST1H1T. Moreover, H1FNT also appears to interact less strongly with the chromatin when compared with the more canonical HIST1H1T.

Developmental and Stage-Specific Expression of HMGB4 in Male Germ Cells

Spermatogenesis occurs in synchronized waves within the seminiferous epithelia. The cycle is divided into 12 stages in mouse, where each stage comprises a mix of cells in given developmental steps. During the first wave of spermatogenesis, germ cells are synchronized in their development, with different cell types appearing progressively at different postnatal times. To study the expression of murine HMGB4 in developing male germ cells, we first analyzed total extracts from 1- to 4-wk postnatal testis by Western blot analysis (Fig. 2F). HMGB4 can already be observed at 2 wk postpartum and persists in adult testis (Fig. 2, F and G) but, unlike H2AL1, it is not present in mature epididymal sperm (Fig. 2G). The timing of appearance of HMGB4 expression correlates with the appearance of pachytene spermatocytes and increases with that of haploid round spermatids in developing testis.

For a more refined analysis we used the anti-HMGB4 monoclonal antibody to study the expression and intracellular localization of HMGB4 during mouse spermatogenesis by immunohistochemistry. Segments of mouse seminiferous tubules corresponding to each developmental stage were isolated by transillumination-assisted microdissection to make squash preparations and subjected to immunostaining.

No expression of HMGB4 in spermatogonia, preleptotene, leptotene, and zygotene spermatocytes was observed, but it was present in the nuclei of Sertoli cells (data not shown). In germ cells, weak HMGB4 expression was first observed at stage V in the euchromatin of pachytene spermatocytes, then increased in pachytene cells until stage XI (Fig. 3, A and B, and data not shown). During spermiogenesis, HMGB4 was localized in the euchromatin of step 1 haploid round spermatids, but it was excluded from the heterochromatin of a step 2–5 round spermatids, then increased in pachytene cells until stage XI (Fig. 3, A and B, and data not shown). Its expression decreased somewhat in the nuclei of step 2–6 round spermatids (Fig. 3, A and B, and data not shown), but it increased during the elongation phase in the nuclei of step 8 early elongating spermatids up to step 13 elongate spermatids (Fig. 3, A and B, and data not shown). The specificity of these signals was verified in immunostaining experiments in the presence of an excess of epitope peptide, which almost completely eliminated the nuclear signals, leaving only nonspecific cytoplasmic staining (Supplemental Fig. 1, available online at www.biolreprod.org).

Strikingly, as round spermatids began to elongate, HMGB4 remained at the location of the round spermatid nucleus but did not spread to the elongating region (Fig. 3, A and B, and data not shown). During the elongation phase, HMGB4 was progressively localized to the basally polar region of the nucleus, and in later-stage elongate spermatids it was strongly expressed at the basal pole. HMGB4 localization in elongating spermatids is affected by the loss of H1FNT.

The above results are reminiscent of those obtained with H1FNT, which also has a polar localization, but at the apical pole of the round and elongating spermatid. Indeed, double
staining of elongate spermatids with antibodies against HMGB4 and H1FNT confirm that these two proteins localize to opposite poles of the nucleus (Fig. 5A). We have shown previously that H1FNT plays an important role in the process of chromatin condensation, because its loss results in defective spermatid elongation and diminished male fertility [4]. We next asked whether loss of H1FNT affects HMGB4 localization in elongating spermatids of H1fnt−/− mice. Staged squash sections of wild-type or H1fnt−/− seminiferous tubules were prepared by transillumination-assisted microdissection and subjected to immunostaining for HMGB4. At the time of step 8–11 elongating spermatids, localization of HMGB4 was similar to the wild type (data not shown); at later steps, two abnormalities were observed. In around half of the spermatids, HMGB4 expression was strongly reduced. In addition, in most of the others HMGB4 was mislocalized and no longer limited to the basal pole of the nucleus, but rather was found throughout the nucleus or as a ring around the nuclear membrane (Fig. 5B). These results indicate that loss of H1FNT and the subsequent disorganization of chromatin topology affect HMGB4 localization.

We have shown previously that loss of HMGB2 can affect H1FNT localization [31]. We therefore investigated the effect of loss of HMGB2 on HMGB4 expression using sections from Hmgb2−/− animals. Although no significant effect on HMGB4 was seen in round spermatids (data not shown), both H1FNT and HMGB4 were affected in elongating spermatids. Most elongating spermatids showed normal localization of each protein, but in the degenerate spermatids the normal localization was lost, resulting in a more homogenous staining, and in some cases colocalization of the two (Fig. 5C).

**Distinct Functional Properties of HMGB1 and HMGB4**

We next investigated some of the functional properties of HMGB4. A vector expressing recombinant F-HMGB4 was transiently transfected in HeLa, COS-1, and the immortalized spermatogonial GC-1 cells and the recombinant protein
detected by immunofluorescence with anti-HMGB4 and anti-FLAG antibodies or immunoblot (Fig. 6A and B). In all cases, a clear nuclear staining was observed (Fig. 6A and data not shown). Extracts were prepared from the nucleoplasmic and chromatin fractions of nuclei from GC-1 cells transfected with recombinant F-HMGB4. Little HMGB4 could be extracted in the nucleoplasmic fraction (Fig. 6C, lane 4), whereas the vast majority of the protein was present in the chromatin fraction (Fig. 6C, lane 3). As a control, the same extracts were also probed for the presence of HIST2H3C (also known as H3) and CBX5 (Fig. 6C), showing that HMGB4, HIST2H3C, and CBX5 are extracted in a similar manner. Therefore, transfected F-HMGB4, like the endogenous murine HMGB4, associates with chromatin.

We tested the effect of F-HMGB4 expression on transcriptional activation in GC-1 cells (Fig. 6D). We chose to use the potent GAL-VP16 activator and with a cognate reporter plasmid, a system that has been used widely to assess coactivator and repressor effects. The 17m5-TATA-CAT reporter plasmid, with five GAL4-binding sites in the promoter upstream of the Cat gene, was transiently transfected with a vector expressing the GAL4-VP16 activator and increasing concentrations of HMGB1 or F-HMGB4. Expression of GAL4-VP16 alone led to a potent stimulation of CAT activity (Fig. 6D, columns 1 and 2). Overexpression of HMGB1 had no significant effect on basal promoter activity (Fig. 6D, column 3), but expression of HMGB4 led to repression (Fig. 6D, column 4). In the presence of GAL4-VP16, expression of increasing amounts of HMGB1 had no significant effect on reporter activity (Fig. 6D, columns 5–7). In contrast, expression of F-HMGB4 led to a potent dose-dependent repression of GAL4-VP16 activity. Similar results were observed in COS-1 cells (data not shown). Therefore, HMGB1 and HMGB4 have different functional properties; HMGB1 has no significant effect, whereas HMGB4 is a potent transcriptional repressor.

**DISCUSSION**

**HMGB4, a Novel and Atypical Member of the HMGB Family**

In this paper we characterize HMGB4, a novel member of the HMGB family, and show that it is preferentially expressed in elongating spermatids, where it localizes to the basal pole. HMGB4 is characterized by the lack of an acidic tail and the presence of distinctive amino acids within the structured domain. Unlike the other HMGB proteins, HMGB4 is encoded by an intronless gene, suggesting that it is derived from a retrotransposed mRNA. HMGB4 seems specific to mammals, indicating that the retrotranscription event occurred early in the mammalian group. Several examples have been described where retrotransposed copies of X-linked genes have been relocated to autosomes to evade meiotic sex chromosome inactivation [32]. HMGB4 has therefore perhaps evolved to compensate meiotic inactivation of HMGB3 that is located on the X chromosome.

Biochemical fractionation experiments show that HMGB4 associates with chromatin in testsis and in transfected cells. A comparison with H1FNT and HIST1H1T shows that HMGB4 has a lower affinity for chromatin than H1FNT, which itself binds less avidly than HIST1H1T. Previous reports have shown that loss of the acidic tail, which is thought to bind to the first HMG box and impede its interaction with DNA, leads to increased DNA binding and blocks nucleosome sliding [19, 33, 34]. Despite the lack of the acidic tail, HMGB4 shows low affinity for chromatin, but nevertheless acts as a transcriptional repressor. Thus, although HMGB4 has chromatin-binding properties distinct from those of linker histones, it has similar functional properties in terms of transcriptional repression.
previous studies have suggested that HMG-type proteins competitively counteract the effects of linker histone H1, HIST1H1B, HIST1H1B and HMBG proteins bind competitively to nucleosomes, and although HMBG proteins have been shown generally to promote transcription by facilitating binding of transcription factors through DNA bending and/or direct interactions and enhancing nucleosome sliding and remodeling, HIST1H1B has generally the opposite effect by condensing chromatin [14, 35, 36]. therefore, our observations show that HMBG4 is an atypical HMBG-type protein.

HMBG4 is strongly expressed at the basal pole of the elongating spermatid nucleus, suggesting that at this stage it may act to repress transcription in this domain of the nucleus. Although this speculation is inferred from the observed properties in transfection experiments, it is evident that a full understanding of the role of HMBG4 in male germ cell differentiation will require generation of Hmgb4 knockout mice.

Specific Chromatin Domains in Elongating Spermatids

The striking feature of HMBG4 is that it localizes specifically to the basal pole of the elongating spermatid nucleus. Investigation of HMBG4 localization in H1FNT-null spermatids suggests that H1FNT may restrict HMBG4 localization, indicating its key role in organizing polar chromatin domains within the nucleus. The diminished HMBG4 expression also seen upon loss of H1FNT may be due to changes in HMBG4 transcription; however, at these late stages transcription is almost silenced. HMBG4 disappears from wild-type cells around step 14, suggesting that it is normally degraded at this time. A more probable explanation, therefore, is that loss of proper chromatin organization or other perturbations brought about by H1FNT inactivation affects the stability of HMBG4 such that it is prematurely degraded.

Our observations indicate a precise spatial topology of the DNA in two domains defined by the mutually exclusive presence of H1FNT or HMBG4. In addition, it has been shown previously that pericentric regions are associated with the novel H2A1L and H2A2L variants as well as HIST1H2BA [37]. It is striking that each of these domains is characterized by the presence of germ cell-specific or preferentially expressed variants. We have shown previously that H1FNT can be mislocalized within the nucleus by the alterations in chromatin structure that occur upon loss of TERF2 (TRF2) and HMBG2 [31]. Together, our observations therefore demonstrate the existence of a complex set of positional cues within the spermatid nucleus, suggesting that chromatin topology is important for organizing DNA condensation in a specific manner.

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