Attenuated chromatin compartmentalization in meiosis and its maturation in sperm development

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Germ cells manifest a unique gene expression program and regain totipotency in the zygote. Here, we perform Hi-C analysis to examine 3D chromatin organization in male germ cells during spermatogenesis. We show that the highly compartmentalized 3D chromatin organization characteristic of interphase nuclei is attenuated in meiotic prophase. Meiotic prophase is predominated by short-range intrachromosomal interactions that represent a condensed form akin to that of mitotic chromosomes. However, unlike mitotic chromosomes, meiotic chromosomes display weak genomic compartmentalization, weak topologically associating domains, and localized point interactions in prophase. In postmeiotic round spermatids, genomic compartmentalization increases and gives rise to the strong compartmentalization seen in mature sperm. The X chromosome lacks domain organization during meiotic sex-chromosome inactivation. We propose that male meiosis occurs amid global reprogramming of 3D chromatin organization and that strengthening of chromatin compartmentalization takes place in spermiogenesis to prepare the next generation of life.

The hallmark of germline development is meiosis, when the germ cell genome goes through recombination to facilitate genetic diversity in offspring.1 In late spermatogenesis, germ cells undergo cellular reconstruction and global chromatin remodeling that ultimately gives rise to functional sperm.2 Beginning in meiosis, global transcription networks are altered by the activation of germline-specific genes, and this process continues in postmeiotic spermatids3–5. As a result, the testis has the most diverse and complex transcriptomes of all organs.6 However, it remains unknown how the spatiotemporal organization of germ cell chromatin facilitates vital gene expression programs and sets the epigenetic state for the next generation of life.

By performing Hi-C on representative stages of male germ cells from mice, we found that meiosis occurs amid the attenuated compartmentalization of 3D chromatin organization; this compartmentalization is strengthened in haploid spermatids, giving rise to highly compartmentalized 3D chromatin organization in mature sperm. Our study suggests that the attenuated compartmentalization of 3D chromatin organization in male meiosis underlies complex germ-line transcriptomes and preparation for the next generation of life.

Results

The 3D chromatin organization of meiotic spermatocytes and postmeiotic spermatids. To determine the 3D chromatin organization of germ cells in late spermatogenesis, we isolated representative cell types from C57BL/6J mice and performed Hi-C experiments. Our analyses focused on pachytene spermatocytes, which are in the midst of meiotic prophase and the synopsis of homologous chromosomes, and round spermatids, which are haploid cells resulting from the second meiotic division (Fig. 1a). These two stages are accompanied by high levels of gene expression,7, last about one week each during spermatogenic differentiation, and represent a majority of testicular germ cells. We confirmed the high purity of isolated cells (Supplementary Fig. 1 and Supplementary Dataset 1), which was consistent with our previous studies.8–11. In addition to our processed Hi-C datasets (Supplementary Dataset 2 and Methods), we reanalyzed a published dataset for mature sperm.1 To provide a reference point for the analyses of spermatogenesis datasets, we also reanalyzed a mouse embryonic stem cell (ESC) dataset.14 A recent study demonstrated that sperm has highly compartmentalized 3D chromatin organization that resembles the 3D chromatin organization of ESCs.15 Although it was not possible using these data to distinguish homologous chromosomes or sister chromatids characteristic of pachytene spermatocytes, we were able to evaluate the global features of 3D chromatin organization in each dataset.

First, we compared general features of pachytene spermatocyte genome organization with those of sperm and ESCs. Interaction maps of pachytene spermatocytes revealed atypical forms of higher-order chromatin organization in comparison to previous studies of interphase nuclei, sperm, and ESCs.14,15 Consistent with the synopsis of homologous chromosomes and chromosome condensation, we detected an abundance of ‘near’ intrachromosomal interactions (strong interactions along the diagonal of the PS panel in Fig. 1b) relative to sperm and ESCs. In this respect, pachytene spermatocyte chromosomes bore resemblance to somatic mitotic chromosomes.
(prometaphase mitosis human foreskin fibroblasts)\(^{16}\) as well as the chromosomes of oocytes arrested in metaphase of meiosis II (MII oocytes)\(^2\) (Supplementary Fig. 2a).

Next, we examined the intrachromosomal contact probability \(P(s)\) for pairs of genomic loci stratified by genomic distance \(s\), which may be indicative of the general polymer state of chromatin\(^{17,18}\). In sperm and interphase chromosomes, intrachromosomal contact probability has been reported to follow a power law of \(P(s) \sim s^{-\alpha}\), which could be consistent with a fractal globule state\(^{17,18}\). In contrast, the chromosomes in pachytene spermatocytes displayed a power law decay of \(P(s) \sim s^{-0.60}\) at distances up to 3 Mb (Fig. 1c), followed by a steep drop in contact probability at larger distances. Of note, the chromatin in pachytene spermatocytes resembles that of mitotic chromosomes, with round spermatid chromatin presenting an intermediate state toward mature sperm.

To investigate the dynamics of chromatin organization after meiosis, we studied Hi-C interaction maps of round spermatids. Round spermatid data were found to reflect an intermediate state between pachytene spermatocytes and mature sperm (Fig. 1c), with more long-range interactions than pachytene spermatocytes but less than sperm. Following round spermatids, mature sperm evinced highly compartmentalized 3D chromatin (Fig. 1b,e). These results suggest that the large-scale structure of meiotic chromosomes in pachytene spermatocytes resembles that of mitotic chromosomes, with round spermatid chromatin presenting an intermediate state toward mature sperm.

**Fig. 1** Dynamic 3D chromatin organization in late spermatogenesis. a, Schematic of stages of late spermatogenesis analyzed in this study. PS, pachytene spermatocyte; RS, round spermatid. b, Heat maps showing normalized Hi-C interaction frequencies (128-kb bins, chromosome 2) in PS, RS, sperm, and ESCs. c,d, Hi-C intrachromosomal interaction frequency probabilities \(P(s)\) stratified by genomic distance \(s\) for each cell type shown (100-kb bins, all chromosomes). MII oocyte, metaphase meiosis II oocyte; HFF1-mitosis, synchronized prometaphase mitosis human foreskin fibroblasts. The blue shadow indicates intrachromosomal interactions up to 3 Mb, and the gray shadow indicates intrachromosomal interactions at and beyond 3 Mb. Scaling coefficients are shown. e, log\(_2\) ratio comparisons of the Hi-C interaction frequencies (128-kb bins, chromosome 2) for successive cell types. Details and metrics for Hi-C datasets are presented in Supplementary Dataset 2.
states of chromatin, A and B, in which each state preferentially interacts with other loci of the same state. Genomic compartments have been shown to be strongly associated with biological features such as chromatin epigenetic state (A, active/euchromatin; B, inactive/heterochromatin) and gene expression (A, expressed; B, silenced). In order to accentuate genomic compartments, we normalized the intrachromosomal interaction maps by genomic distance and then calculated Pearson correlation matrices. In contrast to somatic mitotic chromosomes and MII oocytes, which do not interact with other loci of the same state. Genomic compartments that show evidence of genomic compartments similar to those found in interphase, are present but attenuated in male meiosis. These features are maintained and strengthened in the transition from meiotic to postmeiotic germ cells, despite an overall dramatically different nuclear organization.

Interchromosomal interactions in late spermatogenesis. Next, we asked whether our Hi-C data recapitulate key features of chromosome organization during meiosis and in spermatids. In pachytene spermatocytes, homologous chromosomes have undergone synapsis, whereas chromosomes that are nonhomologous are separated from each other. Consistent with the separation of nonhomologous chromosomes during prophase, a low proportion of interchromosomal interactions in pachytene spermatocytes relative to sperm and ESCs was observed (Supplementary Fig. 5 and Supplementary Dataset 2). In order to accentuate interchromosomal interaction signals, we scaled the interaction matrix of each interchromosomal pair into a square matrix and calculated an average interchromosomal interaction frequency matrix over all such chromosome pairs (Methods). We found frequent interchromosomal interactions between the acrocentric ends of chromosomes (telomeres proximal to centromeres) during meiosis as well as the interchromosomal association of non-centromeric ends (telomeres distal to centromeres) during meiosis (Fig. 3a). These features may be due to the anchoring of telomeres to the nuclear membrane during meiosis (Fig. 3b). Studies utilizing microscopy demonstrated that, during meiotic prophase, both chromosome ends attach to the nuclear membrane and, in particular, the acrocentric ends tend to associate because of the frequent association of pericentromeric heterochromatin. Our Hi-C results are consistent with these microscopic observations.

This association of pericentromeric heterochromatin culminates in a single chromocenter in round spermatids, and this chromocenter persists into sperm (Fig. 1a). Consistent with this feature, we found that the acrocentric ends of chromosomes tended to associate in round spermatids and, to a lesser extent, in sperm (Fig. 3a and
Increased genomic compartment strength, we confirmed that interchromosomal interactions are present between chromosomes regardless of the stages of spermatogenesis and in ESCs (Fig. 3d). And as with intrachromosomal interactions in round spermatids, we observed an increased proportion of interchromosomal interactions in round spermatids relative to pachytene spermatocytes (Fig. 3e). By measuring genomic compartment strength, we confirmed that interchromosomal genomic compartments are also present in pachytene spermatocytes (Fig. 3f). These results are surprising, because they suggest that interactions associated with chromatin state are present between chromosomes in spite of their condensed form. Together, our data suggest that the observed interchromosomal interactions reflect key features of nuclear organization in spermatogenesis and that A compartments tend to self-associate between different chromosomes regardless of the stages of spermatogenesis (Fig. 3b,c).

Model shown in Fig. 3c; direct interactions between centromeres were not observed due to technical limitations related to sequencing highly repetitive regions of DNA). These general features were also observable, to a lesser degree, on an individual chromosome pair basis (for example, chromosomes 2 and 4 in Fig. 3d), as were interchromosomal interactions of genomic compartments (Fig. 3d). Intriguingly, we observed the strongest interchromosomal interactions as those of genomic A compartments, which are gene-rich and abundant in active histone modifications (Fig. 2c), and which persist throughout spermatogenesis and in ESCs (Fig. 3d). And as with intrachromosomal interactions in round spermatids, we observed an increased proportion of interchromosomal interactions in round spermatids relative to pachytene spermatocytes (Fig. 3e). By measuring genomic compartment strength, we confirmed that interchromosomal genomic compartments are also present in pachytene spermatocytes (Fig. 3f). These results are surprising, because they suggest that interactions associated with chromatin state are present between chromosomes in spite of their condensed form. Together, our data suggest that the observed interchromosomal interactions reflect key features of nuclear organization in spermatogenesis and that A compartments tend to self-associate between different chromosomes regardless of the stages of spermatogenesis (Fig. 3b,c).

Attenuated topologically associating domains in meiosis. In addition to genomic compartments, chromatin is spatially organized into regions of preferential interactions termed topologically associating domains (TADs)\textsuperscript{14,15}. TADs have been implicated in the regulation of gene expression\textsuperscript{15,27–29}, and meiosis and subsequent stages are notable for their active transcriptomes, which are among the most complex and diverse known\textsuperscript{16}. Thus, we identified TAD boundaries in late spermatogenesis with the software package HiCExplorer\textsuperscript{10,11} (Methods, Fig. 4a, and Supplementary Dataset 4). In pachytene spermatocytes and round spermatids, we observed weak, large TADs, ~1.1 Mb in average length (Fig. 4b, Supplementary Fig. 6, and Supplementary Dataset 4). While many TAD boundaries were unique to each germ cell dataset (or shared between two of the three datasets), a subset of boundaries persisted from pachytene spermatocytes through to sperm, ~28% of 2,233, and sperm (~14% of 4,541). As late spermatogenesis progressed, the large TADs of pachytene spermatocytes and round spermatids underwent consolidation, forming an abundance of stronger, smaller TADs in sperm, ~0.56 Mb in average length (Fig. 4b, Supplementary Fig. 6, and Supplementary Dataset 4).

Consistent with these observations, the levels of distance-normalized chromatin interactions around sites of sperm TAD boundaries (±2 Mb) are higher in pachytene spermatocytes and round spermatids relative to those in sperm and ESCs (Fig. 4d: the baseline is above 1.0 in the PS and RS datasets). We further
confirmed these data with 2D analyses of interaction frequencies in relative positions from start and end sites of sperm TAD boundaries (Fig. 4e). We detected weak structural features in pachytene spermatocytes and round spermatids (20-kb bins, chromosome 5, 118–138 Mb) showing dynamics of local interactions and TADs in PS, RS, sperm, and ESCs. Horizontal solid bars, TADs as delimited by the software package HiCExplorer (Methods); dashed transparent bars, sperm TAD start and stop boundaries. c, Numbers of intersections of TAD boundaries (n) between datasets. Vertical bars, overlap between TAD boundaries in the datasets below, which are further specified by solid black circles; black lines connecting the black circles indicate overlaps between multiple datasets. The intersections were plotted using the Intervene and UpSetR packages (Methods). d, Average observed/expected interaction frequencies at sperm TAD boundaries ± 2 Mb for all cell types (20-kb bins, chromosome 2). e, Schematic for interpretation of 2D matrix visualizations of observed/expected interaction frequencies at sperm TAD start and stop boundaries. f, 2D matrix visualizations of log, observed/expected interaction frequencies at sperm TAD start and stop boundaries ± 0.5 Mb for all cell types (20-kb bins, all chromosomes). In order to highlight their weak interaction patterns, PS and RS interactions are plotted on a different color scale. Genomic location information for TAD boundaries and results from the evaluation of TAD boundary intersections are presented in Supplementary Dataset 4.

Notably, in pachytene spermatocytes and round spermatids, the presence of common A and B compartments, along with large and weak TADs, evokes a chromosomal resemblance to paternal alleles in preimplantation development, which are in the midst of remodeling of 3D chromatin organization. Such features are distinct from those in somatic mitotic chromosomes and MII oocytes, which lack A and B compartments and TADs (20-kb bins, chromosome 2) (Supplementary Fig. 2b), thereby illuminating a chromatin configuration unique to meiotic chromosomes. This 3D chromatin status persists from meiotic prophase into round spermatids, which evince interphase-like nuclei after two rounds of reductional meiotic divisions. Through two successive rounds of meiotic divisions, chromosomes are condensed in two accompanying rounds of metaphase, during which 3D chromatin organization is presumed to have
Pairwise point interactions and active transcription during meiosis. Localized pairwise point interactions have been previously identified in high-resolution Hi-C interphase maps and have been associated with the activation of transcription and with gene-regulatory elements. These point interactions are thought to arise from the clustering of regulatory elements and genes through chromatin looping mechanisms. Chromosomes in meiotic prophase are distinct from those in mitotic M phase due to ongoing robust transcription. Thus, we examined the data for signs of such point interactions, both visually and computationally. We found that point interactions are clearly apparent in the data (Fig. 5a) and, using the interactions, both visually and computationally. We found that point interactions comprise a higher-order specificity of transcriptomes in pachytene spermatocytes and round spermatids that relates to the formation of TADs in sperm? To seek an answer, we analyzed the deposition of H3K27ac, H3K4me3, and H3K27me3 at the anchors versus other regions of the genome (Supplementary Fig. 8c). It is interesting to note that the loci of point interactions appear to persist into round spermatids (Supplementary Dataset 5). Next, we asked whether these point interactions are associated with specific genomic functions. We analyzed RNA-seq signals and the deposition of active histone modifications H3K27ac and H3K4me3, as well as silent histone modification H3K27me3, at the anchor sites of point interactions (Supplementary Dataset 5). We found that, on average, anchors are enriched in H3K27ac and H3K4me3 ChIP-seq signals, as well as RNA-seq signal (Fig. 5b). Likewise, relative read enrichments for the histone and RNA-seq datasets were higher at anchors versus other regions of the genome (Supplementary Fig. 8a,b), and correlation calculations demonstrated a weak positive correlation between the anchors and the ChIP- and RNA-seq datasets (Supplementary Fig. 8c). It is interesting to note that the loci of point interactions appear to persist into round spermatids (Supplementary Fig. 8d); this is consistent with the overall similarity of transcriptomes in pachytene spermatocytes and round spermatids after the mitosis-to-meiosis transition of spermatogenesis. Our data suggest that point interactions comprise a higher-order form of chromatin organization associated with active histone modifications and gene activation in late spermatogenesis.

Epigenetic mechanisms associated with 3D chromatin organization in the germline. This led us to an intriguing question: How does the higher-order organization of pachytene spermatocytes and round spermatids relate to the formation of TADs in sperm? To seek an answer, we analyzed the deposition of H3K27ac, H3K4me3, and H3K27me3 at sites of sperm TAD boundaries (Supplementary Dataset 4) across the four datasets. Surprisingly, we observed the strong enrichment of H3K27ac, H3K4me3, and H3K27me3 at the sites of sperm TAD boundaries in pachytene spermatocytes and round spermatids (Fig. 5c), indicating that sperm TAD boundaries are delineated with epigenetic marks as early as the pachytene spermatocyte stage. The enrichment of histone modifications at sperm TAD boundaries was also present in ESCs (Fig. 5d). These results raise the possibility that epigenetic mechanisms, which persist through meiotic divisions, determine 3D chromatin organization in the germline. In the germline, bivalent genomic domains that retain both H3K4me3 and H3K27me3 on gene regulatory elements are postulated to be responsible for epigenetic inheritance across generations. Therefore, such mechanisms could serve as persistent memories through meiotic divisions. In support of this notion, from pachytene spermatocytes through to sperm, H3K27me3 is enriched in A compartments, which are also enriched with H3K4me3 (Fig. 2c) and Supplementary Fig. 4).

3D chromatin organization of the XY body during meiosis and postmeiotic sex chromatin in round spermatids. Next, we shifted our attention from autosomal chromatin organization to chromatin organization of the X chromosome. In pachytene spermatocytes, the sex chromosomes undergo a phenomenon known as meiotic sex chromosome inactivation (MSCI) and form a transcriptionally silent compartment termed the XY body (also known as the sex body). MSCI begins when DNA damage signaling recognizes the unsynapsed status of the hemizygous X and Y chromosomes, resulting in their transcriptional repression. Consistent with the distinct regulation of the sex chromosomes in the XY body, interaction maps of pachytene spermatocytes revealed that X evinces chromatin organization that is highly— but not entirely—homogeneous (Fig. 6a–c; Supplementary Fig. 9), without chromatin compartments (Fig. 6a–c; Supplementary Fig. 9b). In this respect, the pachytene spermatocyte X closely resembles the MI oocyte X (Supplementary Fig. 9a,b). Interestingly, the chromatin features of the inactive X chromosome in meiosis are distinct from those of the inactive X chromosome in female X chromosome inactivation. In female X-chromosome inactivation, X is folded into two ‘megadomains’ that are established via a step-wise mechanism. However, the silent male X in meiosis does not have apparent megadomains (Fig. 6a). This could be explained by the mechanistic difference between male and female inactive X chromosomes: the male inactive X chromosome is established via DNA damage signaling, whereas the female inactive X is established through the action of non-coding Xist RNA.

The distinct regulation of the sex chromosomes persists into postmeiotic round spermatids, where either X or Y is organized into a silent compartment, termed postmeiotic sex chromatin, in the center of the nucleus (Fig. 1a). We observed the beginnings of higher-order chromatin organization in round spermatids in the form of distinct interactions greater than 10 Mb (Fig. 6b); however, as in pachytene spermatocytes, round spermatids lacked chromatin compartmentalization in postmeiotic sex chromatin (Fig. 6c,d). In support of these results, by measuring genomic compartment strength, we confirmed that intrachromosomal genomic compartments are infrequent on the X chromosome in pachytene spermatocytes and round spermatids (Fig. 6c). In round spermatids, a small number of sex-linked genes escape postmeiotic silencing to spur differentiation into sperm. However, these escape genes did not demonstrate apparent features of 3D chromatin organization in round spermatids (Supplementary Fig. 9c). By the time germ cells progressed to sperm, higher-order features of chromatin organization, such as compartments, were observable (Fig. 6a–d and Supplementary Fig. 9c). The overall features of the sperm X resemble those of the active X in ESCs, especially with respect to multidomain organization (Fig. 6a), whereas compartments are distinct between the sperm X and the active X in male ESCs, which is of maternal origin (Fig. 6c,d). Together, our data implicate 3D chromatin organization in the distinct regulation of the sex chromosomes in germ cell development.

Discussion
In this study, we revealed a form of 3D chromatin organization predominated by local, weakly preferential chromatin interactions in meiotic prophase that expand to distal, although still weakly preferential, interactions in postmeiotic spermatids. These features present intriguing clues to understand the structure of meiotic chromosomes in prophase. Through microscopy of mammalian cells, chromatin loop array structures in meiotic prophase were reported to be similar to those of their mitotic counterparts, which is an earlier Hi-C study modeled as compressed arrays of consecutive loops. However, to date, 3D structural information is lacking in mammalian meiotic prophase. In yeast, chromosome conformation capture (3C), the pioneering method that underlies Hi-C, provided keen structural insights into meiotic chromosomes and, recent yeast Hi-C studies demonstrated that meiotic chromosomes are comprised of dense, dynamic arrays of chromatin loops with variable sizes. However,
in comparison with those of other eukaryotes, yeast chromosomes do not display some features of higher-order chromatin organization, such as A/B compartments, thereby precluding direct comparisons between yeast and mammalian meiotic chromosomes.

Here, we show that mammalian meiotic chromosomes evince atypical features of higher-order chromatin organization: In comparison with somatic interphase chromosomes, the chromosomes of pachytene spermatocytes feature relatively attenuated structural features. One interpretation may account for this: because our Hi-C data for pachytene spermatocytes represent the average structural features from ~25 million nuclei (each nucleus with two complements of the diploid genome), loop positions may be random in the cell population, and loop sizes may be variable.

Nonetheless, in comparison with mitotic chromosomes, pachytene spermatocyte chromosomes displayed a steeper decrease in intrachromosomal contact probability (Fig. 1d), in addition to genomic compartmentalization (Fig. 2) and the presence of TADs (Fig. 4). It is intriguing to consider structural influences acting on the chromosomes of pachytene spermatocytes. Mechanistically, the structural features of meiotic chromosomes may be shaped by the meiosis-specific effects of cohesins and CTCF, which define 3D structural organization in interphase nuclei. During meiosis, chromosome axes are ubiquitously loaded with meiosis-specific cohesins, and CTCF has an isoform unique to meiosis and subsequent stages, BORIS. Thus, these factors may function in mechanisms that randomize loop positions.

Fig. 5 | Pairwise point interactions and sperm TADs are delineated with epigenetic marks. 

- **a**, Hi-C interaction heat maps (20-kb bins, chromosome 2, 48–55 Mb) of pachytene spermatocytes (PS) showing the dynamics of local interactions of active gene loci together with RNA-seq data and ChIP-seq data for H3K27ac, H3K4me3, and H3K27me3. Points, RPKM. Solid bars, TADs called with the software package HiCExplorer (Methods). Green and gray highlights, arrows, and dashed circles indicate localized pairwise point interactions and related features of interest. 

- **b**, RNA-seq data (top) and ChIP-seq data for H3K27ac, H3K4me3, and H3K27me3 (bottom) to examine enrichment at the center of pachytene spermatocyte point interaction anchors ±1 Mb (20-kb bins, all chromosomes). Point interactions were called with the software package cLoops (Methods). 

- **c, d**, ChIP-seq data for H3K27ac, H3K4me3, and H3K27me3 to examine enrichment at sperm TAD start and stop boundaries along with domain interior and exterior (±20 kb) portions (20-kb bins, all autosomes), in PS, RS, sperm, and ESCs. Genomic location information for pairwise point interactions are presented in Supplementary Dataset 5.
After our analyses of meiotic chromosomes, the observations of patterns found in mitotic and interphase chromosomes raise interesting questions, for example, what physical genome structures are consistent with both the randomized loop arrays proposed for mitotic chromosomes and the consistent structural features typically found in interphase chromosomes? How is genomic organization functional in spite of its attenuated form? And how do condensed chromosomes form interchromosomal interactions between active chromatin loci (Fig. 3)?

Although the features of chromatin loop arrays—random and/or structured—may be shared between autosomes and sex chromosomes during meiosis, we determined additional and unique structural features related to the inactive sex chromosomes in the male germline. Phase separation, a process by which membrane-less organelles form and behave as liquid droplets, has been proposed as a mechanism for the formation of heterochromatin. Furthermore, a phase separation mechanism was postulated for the stepwise establishment of the inactive X in females. Given the highly homogeneous and isolated 3D chromatin organization of the inactive X in late spermatogenesis, the XY body and postmeiotic sex chromatin may represent droplet-like structures that are self-associating and spatially segregated via phase separation mechanisms. Furthermore, phase separation mechanisms also underlie sites of active transcription. Therefore, it is tempting to speculate that phase separation mechanisms may underlie interchromosomal association mediated through A compartments.

In 1984, Robin Holiday proposed, in an article entitled “The biological significance of meiosis,” that a potential function of meiosis is the reprogramming of gametes to prepare for the next generation. In accordance with this hypothesis, we propose that attenuated compartmentalization of 3D chromatin organization in meiosis is itself a form of reprogramming for 3D chromatin organization that facilitates spermatogenic gene expression. Moreover, the attenuated compartmentalization of 3D chromatin organization corroborates the reorganization of various chromatin features in the mitosis-to-meiosis transition of the male germline. In summary, our results reveal that the attenuated compartmentalization of germline chromatin is associated with unique and diverse
transcriptomes, and that the maturation of germline chromatin to highly compartmentalized 3D chromatin organization in sperm prepares the next generation of life.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at [https://doi.org/10.1038/s41594-019-0189-y](https://doi.org/10.1038/s41594-019-0189-y).

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**Author contributions**

The manuscript was written by K.G.A., N.K., and S.H.N., with critical feedback from all other authors, and K.G.A and S.H.N. designed the Hi-C experiments. K.G.A and S.M. performed the Hi-C experiments. K.G.A., A.S., H.K., A.B., N.K., and S.H.N. designed and interpreted the computational analyses. K.G.A., A.S., H.K., and N.K. performed the computational analyses. N.K and S.H.N jointly supervised this work.

**Competing interests**

A.B. is a cofounder of Datirium, LLC.

**Additional information**

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Pachytene spermatocytes and round spermatids were isolated from adult testes through sedimentation velocity at unit gravity as described previously. At least 12 independent mice, at 90 to 120 days of age, were used for each isolation of germ cells. Purity was confirmed via fluorescence wide-field microscopy. All images were acquired with an ECLIPSE Ti-E microscope (Nikon) and Zyla 5.5 sCMOS camera (Andor Technology). Tissue was processed, mounted TIRF oil immersion lenses (Nikon), numerical aperture 1.40. Germ cells were identified by staining with 0.2 μg/ml Hoechst 33342. In keeping with previous studies from the Namekawa lab, 63% purity of ≥91% for pachytene spermatocytes and ≥94% for round spermatids was confirmed for each experiment (Supplementary Fig. 1 and Supplementary Dataset 1). To quantify the purity of isolated germ cells, images of cell fractions from sedimentation velocity at unit gravity, their details having been blinded, were fed into a partially automated Fiji/ImageJ 1.52 processing pipeline. Using a Gaussian filter, the images were smoothed before the application of a pixel intensity maximum filter to identify local maxima, which were automatically scored as cells. Then, each processed image was checked manually to correct for false positives and false negatives. Unprocessed versions of the blinded images were manually checked for heterogeneous cell contamination. Percent contamination was calculated for given sets of images as follows: number of heterogenous cells

number of non-heterogenous cells + number of heterogenous cells

a normalization factor to ensure a probability distribution. Next, we estimated the power-law decay coefficients of interaction frequency versus genomic distance, we used a maximum likelihood approach. Specifically, we assumed the probability of interaction between loci i and j is given by

\[ P(i, j) = \alpha \cdot \langle |i - j| \rangle^{\gamma} \]

where \( \gamma \) is a normalization factor to ensure a probability distribution; we assumed a Hi-C experiment can be described as multinomial sampling from the above probability distribution. Therefore, the likelihood of observing Hi-C

\[ X \]

is

\[ P(X|\alpha, \gamma) = \prod_i \left( \frac{1}{N_i} \right) \prod_j \left( \frac{1}{N_j} \right) \]

where

\[ N_i \]

is the number of reads mapping to region i. To estimate the best value for \( \gamma \), we computed

\[ \alpha = \frac{\sum X_{ij} \cdot \langle |i - j| \rangle^{-\gamma}}{\sum X_{ij}} \]

we then used SciPy LM-BFGS quasi-Newton optimization to find the value that maximizes the likelihood. Since some cases, the data adhere to regimes with different powers; we selected an appropriate range of genomic distances that were used to estimate \( \alpha \) as follows: pachytene spermatocytes, 0.5–3 Mb; MII oocytes, 0.5–3 Mb; HFF1-mitosis, 0.5–7 Mb.

**Hi-C: A/B compartment analyses.** Hi-C matrices at 100- or 128-kb resolution were imported to HiCEXplorer (version 2.1.3 cooler_correction_patch) for use with the applications hicTransform and hicPlotMatrix. hicTransform was called to convert interaction frequency matrices to distance-normalized matrices (that is, matrices taken from dividing observed interactions by expected interactions as described), then to subsequently generate Pearson correlation coefficient matrices. hicPlotMatrix was used to visualize the Pearson correlation coefficient matrices.

To call genomic compartments, the HiCEXplorer application hicPCA was employed to perform principal component analysis (PCA) on Pearson correlation coefficient matrices at 100- or 128-kb resolution (Supplementary Dataset 3). The largest eigenvalue (EV1) represented the genomic compartment profile, which is consistent with previous reports. For information, consecutive eigenvectors were evaluated; eigenvectors beyond EV1 represented profiles distinct from genomic compartments (data not shown). Per convention, genomic compartments were assigned one of two designations, ‘A’ (active/euchromatic compartments) and ‘B’ (inactive/heterochromatic compartments), based on associated biological features, including gene density, mRNA transcription, and markers of chromatin state such as histone post-translational modifications. In comparison to B compartments, A compartments were denoted by higher gene densities (data not shown), increased enrichment for mRNA transcription, and increased enrichment of H3K27ac and H3K4me3, post-translational modifications conventionally associated with euchromatin and active transcription.

**Hi-C: Evaluation of genomic compartment strength.** To evaluate the level of genomic compartmentalization between datasets, a new approach that calculates the ‘genomic compartment strength’ was developed due to the limitations of PCA for this task. This approach quantifies the genomic compartment signal by examining the level of interaction in regions belonging to the same compartment versus regions belonging to different compartments. The definition of compartments is given by an eigenvector as explained in the preceding section; the choice of this vector is independent from the rest of the analysis. Then, we selected the highest 25% and lowest 25% of values, which indicate, respectively, strong A and strong B loci. Next, a Hi-C interaction matrix was distance-normalized by taking the log2 of observed interactions, the resulting normalized matrix was referred to as a “LOE” matrix. Then, genomic compartment strength was defined as

\[ \text{mean(AA LOE interactions)} + \text{mean(BB LOE interactions)} - 2\text{mean(AB LOE interactions)} \]

Thus, if AA and BB interactions were stronger than AB interactions, a positive value was expected; if AA and BB interactions were equivalent to AB interactions, then a value close to zero was expected.

**Hi-C: Genomic compartment strength analysis in a controlled setting.** In order to test whether genomic compartment strength analysis accurately quantifies changes in compartment strength, different portions of the sperm Hi-C matrix...
S (which has the highest GC strength) were mixed with a matrix M that does not have any genomic compartments. Matrix M was constructed by taking the pachytene spermatocyte interaction frequency map and converting it to an expected interaction map such that the interaction probability at distance x is the average interaction probability of all loci within that distance. It was verified that M has a genomic compartment strength of approximately zero. To obtain a mixed matrix with fraction f reads from the sperm matrix S, we sampled \( \frac{x}{\text{sum}(S)} \) reads multinomially according to the probabilities given by matrix S, and combined these with \( (1-f) \times \text{sum}(S) \) reads sampled multinomially by the probabilities of matrix M. We created mixed matrices at controlled levels, where \( f \in [0.0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9,1.0] \), and genomic compartment strength was calculated for each such matrix, both for cis and trans interactions. To avoid eigenvector distortion resulting from weak compartment signals, all mixed samples used the eigenvector extracted from S. To account for randomness introduced by sampling, we repeated our analysis on 10 times.

An example call for the evaluation of TAD boundaries: S intervene upset -t SBS_bed_30kb SBS_bed_30kb

**Hi-C: Evaluation of TAD boundary intersections.**

To compute 2D line plots depicting interaction tendencies 0 to 2 Mb from TAD boundaries (Fig. 4d and Supplementary Fig. 7a), distance-normalized submatrices (observed interactions/expected interactions), derived from Hi-C interaction frequency matrices at 20-kb resolution, were called with the application hicAggregateContacts. In brief, submatrices centered at the intersections of TAD boundary start and end regions were selected, and all interactions from 0 to 2 Mb of the boundaries were considered. These intersections were split over 100 bins from the center of the submatrix in the horizontal and vertical directions. Then, the submatrices were pooled by computing their averages at all positions. The interaction frequencies of the averaged submatrices were normalized by dividing individual values by the total interaction frequencies in the submatrix. Then, the diagonal, from top left to bottom right, of the pooled, averaged submatrix was output as a 2D line plot. hicAggregateContacts was called with the following arguments: --avgType mean --transform obs/exp --range 20000:2020000 --numberOfBins 100 --plotType 2d --diagnosticHeatmapFile $file_.bedpe -o Soutputhic_s2-eps 10000,15000,20000,25000 -minPts 10,20,30,40,50 -hic. We identified 12,990 significant point interactions. However, manual assessment of the results indicated that a majority of these could either not be confirmed visually or were not found in both replicates. We therefore reduced the set into a high-confidence set of 1,985 point interactions (Supplementary Dataset 5), which better matched visual assessment and was more consistent between replicates. To reduce the set, we selected only significant point interactions with a binomial p value \( p < 1 \times 10^{-6}\). We found that this filtered set of point interactions was also consistent with an independent peak-calling method based on signal-processing filters (data not shown).

**RNA-seq and ChIP-seq: Sourcing, alignment, processing, and visualization.**

RNA-seq and ChIP-seq datasets were obtained from published work: for RNA-seq, pachytene spermatocytes and round spermatids (PMID 25703348, GEO GSE53860); sperm and embryonic stem cells (PMID 22242016, DDBJ DRA000484); for ChIP-seq of H3K27ac, pachytene spermatocytes and round spermatids (PMID 29462142, GEO GSE107398); sperm (PMID 28178516, GEO GSE9230); and embryonic stem cells (PMID 22673441, GEO GSE89902); for ChIP-seq of H3K4me3, pachytene spermatocytes and round spermatids (PMID 25703348, GEO GSE89902); sperm (PMID 28178516, GEO GSE9230) and embryonic stem cells (PMID 22673441, GEO GSE89902); and sperm (PMID 28178516, GEO GSE9230) and embryonic stem cells (PMID 25303531, GEO GSE57912).
RNA- and ChIP-seq datasets were processed and visualized through one of two pipelines: (1) the BioWardrobe Experiment Management Platform®, which indexes and normalizes .bam files, makes use of NCBI RefSeq annotation to categorize reads from .bam files, and then, for viewing, uploads the data to the UCSC genome browser; and (2) the deepTools® (version 3.1.0) application bamCoverage, which was used to normalize indexed .bam files and bin resulting values in 50-bp windows; then, the output of bamCoverage was imported to HiCExplorer (version 2.1.3 cooler_correction_patch) for visualization via the applications hicPlotMatrix and hicPlotTADs.

RNA- and ChIP-seq enrichment with respect to TAD boundaries, genomic compartments, and point interaction anchor centers. Using sorted .bam files and the software package ngsplot (version 2.6.3), we calculated the enrichment of RNA-seq data and/or ChIP-seq data for histone post-translational modifications with respect to EV1 lists (Supplementary Dataset 3), TAD boundary lists (Supplementary Dataset 4), and lists of point interaction anchor centers (Supplementary Dataset 5). An example command line call for point interaction anchor centers: $ ngs.plot.r -G mm10 -R bed -C $n foramergen_file -O Soutout_file -SE 0 -FL 400 -L 1000000.

RNA- and ChIP-seq read enrichment at pairwise point interaction centers. Using bedGL files output by cLoops (version 0.9), we generated a bed file containing genomic location information for individual pairwise point interaction anchors on pachytene spermatocyte chromosomes (Supplementary Dataset 5). Using the program bedTools (version 2.27.1) and its application intersector, we also generated a bed file for all genomic regions excluding point interaction anchors. Then, we calculated read enrichment for ChIP- and RNA-seq data at anchor or anchor-excluded (that is, “other”) genomic regions using bedTools intersect, for example, $ bedTools intersect -c -a $bed_file_for_regions -b $sorted_bam_file_for_RNA_seq -o $output_file reads_enrichment_at_regions.txt. Resulting regional read enrichment was normalized for sequencing depth using a counts per million (CPM) calculation, that is, uniquely mapped reads were scaled by the total number of sequenced reads multiplied by 1,000,000: regional read enrichment X (real number of sequenced reads X 1,000,000). To test for statistical differences between read enrichment at “anchor” versus “other” regions, we performed Wilcoxon rank sum tests with Bonferroni post corrections.

RNA- and ChIP-seq enrichment correlation with pairwise point interactions. The pairwise point interaction anchor bed file (Supplementary Dataset 5) was converted to a .bam file for all bedTools (version 2.27.1) application multim BamSummary to stratify the anchor, RNA-, and ChIP-seq .bam files in 40-kb bins and calculate the genome coverage for each bin. Resulting coverage matrices were processed with the deepTools application plotCorrelation in order to compute the Pearson and Spearman correlation coefficients, and to visualize the correlation calculation results as hierarchically clustered heat maps.

Figure preparation. Plots were generated with, alone or in combination, Excel (2013, Microsoft), the R software package ggplot2 (version 3.1.0)®, and the various plotting programs employed by the other software packages used in this study. Illustrator (CS6, Adobe) was used for composing figures.

Code availability. Source code for software used in this study, with documentation, examples, and additional information, is available at the following URLs:

- https://github.com/dekkerlab/cMapping
- https://github.com/mirnylab/cooler
- http://bowtie-bio.sourceforge.net/bowtie2
- https://github.com/deeptools/HiCExplorer
- https://github.com/asnetch/intervene
- https://github.com/hms-dbmi/UpSetR
- https://github.com/YaqiangCao/cLoops
- https://github.com/alexdoxin/STAR
- http://bowtie-bio.sourceforge.net/
- https://github.com/taoliu/MACS
- https://github.com/deeptools/deepTools
- https://github.com/aarg5x/bedtools2
- https://github.com/shenlab-sinais/ngsplot
- https://github.com/tidyverse/ggplot2

Other code used in this study, including code for compartment strength analysis and code for average trans interaction analysis, is available at https://github.com/KaplanLab/Spermatogenesis. A version of HiCExplorer that includes the cooler_correction_patch branch is forked at https://github.com/Namekawalab/HiCExplorer. Any further code is available upon request. Information for the BioWardrobe Experiment Management Platform®, which is commercial software, is available at https://biowardrobe.com and https://github.com/Barski-lab/biowardrobe.

Statistical analysis. No statistical methods were used to predetermine sample sizes. No data were excluded from analyses. The experiments were not randomized and, except where noted, investigators were not blinded to allocation during experiments and outcome assessment.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All Hi-C sequencing data used in this study, including processed files for published datasets, have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) under the accession number GSE119905. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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☐  A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐  The statistical test(s) used AND whether they are one- or two-sided

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection  Data were collected with GNU Wget from the following web databases: NCBI Gene Expression Omnibus (GEO), EMBL-EBI ArrayExpress, and NIG DNA Data Bank of Japan (DDBJ).

Data analysis  We used the following software and code for data analyses: cMapping, cooler, bowtie2, HiCEXplorer, Intervene, UpSetR, cLoops, STAR, bowtie, MACS, deepTools, bedtools, ngs.plot.r, ggplot2. The commercial software BioWardrobe Experiment Management Platform was also used. Other code used for data analysis, including code for compartment strength analysis and code for average trans interaction analysis, is available at https://github.com/KaplanLab/Spermatogenesis. A version of HiCEXplorer that includes the cooler_correction_patch branch is forked at https://github.com/NamekawaLab/HiCEXplorer.

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- A description of any restrictions on data availability

All Hi-C sequencing data used in this study, including processed files for published datasets, have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) under the accession number GSE119805. All other data are available upon request.
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All studies must disclose on these points even when the disclosure is negative.

**Sample size**
No statistical methods were used to predetermine sample sizes.

**Data exclusions**
No data were excluded from analyses.

**Replication**
We confirmed consistent results between two independent biological replicates for pachytene spermatocyte and round spermatid Hi-C experiments based on comparisons between replicate Hi-C matrix heatmaps, consistency between independent replicates under genomic compartment strength and point interaction calling experimental conditions, and pairwise correlations between replicate matrices.

**Randomization**
The experiments were not randomized.

**Blinding**
Except for measurements of germ cell purity for Hi-C library preparation, the investigators were not not blinded during allocation and outcome assessment. Measurements of germ cell purity were blinded to allocation before processing as described in the Supplementary Methods.

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| Mammals                           | ChIP-seq |
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Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**
Wild-type C57BL/6J were used for Hi-C analyses of germ cells; at least 12 independent mice, at 90 to 120 days of age, were used for each isolation of germ cells.

**Wild animals**
Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

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For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

**Ethics oversight**
All experimental work was approved by the Institutional Animal Care and Use Committee, protocol no. IACUC2015-0032.

Note that full information on the approval of the study protocol must also be provided in the manuscript.
ChIP-seq

Data deposition

☑ Confirm that both raw and final processed data have been deposited in a public database such as GEO.
☑ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

RNA- and ChIP-seq data were not generated for this study. Instead, RNA- and ChIP-seq datasets used in Hi-C analyses were obtained from published work: for RNA-seq, pachytene spermatocytes and round spermatids (PMID 25703348, GEO GSE55060), sperm and embryonic stem cells (ESCs; PMID 22242016, DDBJ DRA0040484); for ChIP-seq of H3K27ac, pachytene spermatocytes and round spermatids (PMID 29462142, GEO GSE107398), sperm (PMID 28178516, GEO GSE79230), and ESCs (PMID 22763441, GEO GSE29218); for ChIP-seq of H3K4me3, pachytene spermatocytes and round spermatids (PMID 25703348, GEO GSE89502), sperm (PMID 28178516, GEO GSE79230), and ESCs (PMID 22763441, GEO GSE29218); for ChIP-seq of H3K27me3, pachytene spermatocytes and round spermatids (PMID 25703348, GEO GSE89502), sperm (PMID 28178516, GEO GSE79230), and ESCs (PMID 25303531, GEO GSE57912).

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Provide a link to an anonymized genome browser session for "initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

For RNA-seq analyses, .fastq files were aligned to the Mus musculus mm10 reference genome via Spliced Transcripts Alignment to a Reference (STAR; version 2.4.2a), and only unique alignments were allowed with a maximum of two errors per alignment. For ChIP-seq analyses, .fastq files were aligned to the Mus musculus mm10 reference genome with Bowtie (version 1.1.1), and only unique alignments were allowed with a maximum of one error per alignment. To estimate fragment sizes, and to find islands of enrichment, MACS2 (version 2.1.0.20140616) was used with an FDR q value threshold of <0.2.

RNA- and ChIP-seq datasets were processed and visualized through one of two pipelines: (1) the BioWardrobe Experiment Management Platform, which indexes and normalizes .bam files, makes use of NCBI RefSeq annotation to categorize reads from .bam files, and then, for viewing, uploads the data to the UCSC genome browser; and (2) the deepTools (version 3.1.0) application bamCoverage, which was used to normalize indexed .bam files and bin resulting values in 50-bp windows; then, the output of bamCoverage was imported to HiCEXplorer (version 2.1.3 cooler_c,correction,patch) for visualization via the applications hicPlotMatrix and hicPlotTADs.