Dynamic reorganization of the genome shapes the recombination landscape in meiotic prophase

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In meiotic prophase, chromosomes are organized into compacted loop arrays to promote homolog pairing and recombination. Here, we probe the architecture of the mouse spermatocyte genome in early and late meiotic prophase using chromosome conformation capture (Hi-C). Our data support the established loop array model of meiotic chromosomes, and infer loops averaging 0.8–1.0 megabase pairs (Mb) in early prophase and extending to 1.5–2.0 Mb in late prophase as chromosomes compact and homologs undergo synapsis. Topologically associating domains (TADs) are lost in meiotic prophase, suggesting that assembly of the meiotic chromosome axis alters the activity of chromosome-associated cohesin complexes. While TADs are lost, physically separated A and B compartments are maintained in meiotic prophase. Moreover, meiotic DNA breaks and interhomolog crossovers preferentially form in the gene-dense A compartment, revealing a role for chromatin organization in meiotic recombination. Finally, direct detection of interhomolog contacts genome-wide reveals the structural basis for homolog alignment and juxtaposition by the synaptonemal complex.

In the specialized meiotic cell division program, homologs must identify one another, pair along their length and physically link to ensure their accurate segregation in the meiosis I division. Interhomolog links are formed by homologous recombination, in which DNA double-strand breaks (DSBs) are first introduced along each chromosome and are then repaired using the homolog as a template. A subset of DSBs are repaired as interhomolog crossovers, reciprocal exchanges of genetic material that drive eukaryotic evolution by shuffling alleles along chromosomes in each generation, and also constitute specific physical links between each pair of homologs. Failure to form interhomolog crossovers can cause chromosome mis-segregation in the meiosis I division. In humans, aneuploidy resulting from meiotic chromosome mis-segregation is a major cause of miscarriage and the source of developmental disorders including Down syndrome.

To promote the formation of accurate interhomolog crossovers, chromosomes undergo dramatic morphological changes during meiotic prophase. In leptotene (Latin for ‘thin threads’), chromosomes become individualized and compacted as linear loop arrays around the proteinaceous chromosome axis. The axis comprises cohesin complexes with meiosis-specific subunits plus filamentous axis ‘core’ proteins that, together, aid chromosome compaction and serve as a platform for recombination. Later, in zygonema (paired threads), telomeres cluster on the nuclear envelope and form a distinctive ‘bouquet’ arrangement, and homologs begin to undergo synapsis. Synapsis, mediated by assembly of the synaptonemal complex between paired chromosome axes, is completed in pachynema (thick threads) along with further linear compaction of chromosomes. Meiotic recombination occurs alongside these morphological changes, with DSBs introduced in leptotene, and interhomolog recombination driving pairing and synopsis of homologs in zygonema and pachynema. Finally, the synaptonemal complex is disassembled in diplonema (two threads), followed by further compaction and homolog segregation in meiosis I.

In mice, meiotic prophase occurs over the course of ~10 days, during which time the chromosomes are also highly transcriptionally active. Overall transcription levels are low in early prophase, then massively increase in mid-pachynema to support sperm development. Thus, meiotic prophase chromosomes must achieve a balance between two seemingly conflicting needs: first, overall compaction and organization around the meiotic chromosome axis to support homolog pairing and synopsis; and second, high-level transcription at many loci. This balance between compaction and transcriptional activity contrasts with mitosis, where transcription is largely shut down as chromosomes become tightly packed in mitotic prophase.

While recent technological advances have driven a fundamental rethinking of the forces driving mammalian chromosome organization in interphase and mitosis, the organization of the meiotic genome and how it relates to somatic cell genome organization is largely unknown. Here, we performed Hi-C on synchronized mouse spermatocytes in both early and late meiotic prophase, revealing how chromosomes are reorganized to meet the needs of this unique developmental stage. We find that meiotic chromosomes show an almost complete loss of long-range contacts as they are reorganized around the meiotic chromosome axis. We show that TADs, a key organizational feature of interphase chromosomes, are lost as cohesin complexes become integrated into the meiotic chromosome axis, with DNA breaks and interhomolog crossovers preferentially forming at A compartments. Meiotic DNA double-strand breaks (DSBs) are then repaired by homologous recombination to form long-range loops.

Articles
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a high-polymorphism density hybrid allows us to define the physical parameters of homolog pairing by the synaptonemal complex as cells progress from zygonema to pachynema. Finally, we show that chromosome compartments are maintained in meiotic prophase, and that both DSBs and crossovers show a strong bias toward the gene-dense A compartment, revealing a key role for chromatin state in meiotic recombination.

Results

Hi-C analysis of mouse spermatogenesis. While Hi-C methods have recently enabled an unprecedented exploration of eukaryotic genome structure and regulation, analysis of mammalian meiotic prophase by Hi-C has been limited by an inability to isolate pure populations of meiotic prophase cells. To overcome this challenge, we developed methods to purify large numbers of highly synchronized mouse spermatocytes (Supplementary Fig. 1a–c) and performed Hi-C in both early prophase (zygonema) and late prophase (late pachynema/diplonema) (Fig. 1a and Supplementary Table 1). To capture interhomolog contact durations during recombination and synthesis, we isolated spermatocytes from C57BL6/J (B6) × Mus musculus castaneus (CAST/EiJ; CAST) F1 hybrid mice, which possess 0.83% overall single-nucleotide polymorphism (SNP) density between haplotypes. We performed Hi-C using 100-base paired-end sequencing reads, theoretically allowing us to unambiguously assign B6 versus CAST haplotype for over half of individual reads, and over a quarter of paired-end reads. We generated 351 million Hi-C contacts for zygonema from two independent samples, and 487 million contacts for pachynema from three independent samples (Supplementary Fig. 1e–g and Supplementary Table 2). The resulting Hi-C contact maps from the two prophase stages were visually distinct yet maps from biological replicates showed high reproducibility (Supplementary Fig. 2), demonstrating the robustness of our synchronization and purification method. We could assign 3.3% of zygonema read pairs (11.7 million) and 3.6% of pachynema read pairs (17.7 million) as unambiguous interhomolog contacts (Methods and Supplementary Table 2). As a control, we used a recent Hi-C dataset from unsynchronized cultured mouse embryonic stem cells (hereafter termed interphase) Overall, our data provide an unprecedented picture of dynamic genome reorganization in mammalian meiotic prophase.

Meiotic prophase chromosomes maintain compartment structure but lose topologically associating domains. The eukaryotic genome is organized in all developmental and cell cycle stages to achieve the particular needs of each cell. In interphase, chromosomes occupy individual ‘territories’ in the nucleus and also show multiple levels of internal organization. Dynamic DNA binding, loop extrusion modulated by chromosome-bound CCCTC-binding factor (CTCF), and dissociation from DNA by cohesin complexes give rise to megabase-sized TADs with high local interaction propensity. Interphase chromosomes are also arranged into ‘compartments’, with the gene-dense and transcriptionally active A compartment physically separated from the gene-poor, heterochromatic B compartment. In contrast to TADs, compartments are not formed through dynamic loop extrusion and are not dependent on cohesin, rather, they probably form through the tendency of heterochromatin to self-associate through a phase separation-like mechanism.

In meiotic prophase, we observe an almost complete loss of very long-range contacts (over ~5–10 Mb), consistent with the known organization of meiotic chromosomes as linear arrays of loops anchored to the meiotic chromosome axis (Fig. 1b). We also observe X-shaped interchromosomal contact patterns consistent with the alignment of chromosomes into the prophase bouquet, which are particularly strong in zygonema but also detectable in pachynema (Supplementary Fig. 3a,b). Despite the reorganization of chromosomes into loop arrays, we find that meiotic prophase chromosomes maintain strong A/B compartment identity, observable in Hi-C contact maps as a checkerboard pattern near the diagonal axis (Fig. 1b). A/B compartments are also clearly visible in chromosome-wide Pearson correlation matrices (Fig. 1c), and are remarkably consistent with interphase compartments (Fig. 1d and Supplementary Fig. 4a–c). Thus, despite the reorganization of chromosomes into loop arrays in meiotic prophase, the fundamental organization of chromatin into A/B compartments is maintained.

We next examined TADs, which are visible on Hi-C contact maps as squares with high contact propensity, often with strong corner signals that result from looping interactions between TAD boundaries. We find that, in meiosis, TADs are mostly lost despite the continued presence of cohesin on chromosomes (Fig. 2a)–(c). A few loci show evidence of looping interactions between TAD boundaries (Fig. 2b), but most show a complete loss of both the square and corner TAD signals. These data suggest that if cohesin-constrained loops are present in meiotic chromosomes, as ample cytological and electron microscopy data suggest, the locations of these loops most probably vary from cell to cell. This may arise from a reduction in the influence of CTCF on loop positioning, or from modulation of cohesin activity following association with the filamentous chromosome axis core proteins. We propose that association with the chromosome axis reduces the dynamics of chromosome association and dissociation by cohesin, leading to the formation of a stable loop array. Our data do not reveal whether cohesin-mediated loop extrusion activity is reduced following axis association, though the increase in average loop size as cells progress from zygonema to pachynema (see below) suggests that loop extrusion continues through prophase (Fig. 2c). Our data, indicating a lack of reproducible loop positions in meiosis, contrast with recent Hi-C analyses of Saccharomyces cerevisiae meiosis, which showed strong looping interactions between cohesin binding sites across the genome in pachynema (X). While binding sites for S. cerevisiae meiotic cohesin complexes are highly reproducible, probably leading to these strong looping signals, to date there is no evidence for reproducible cohesin binding along chromosomes in mouse spermatocytes.

Formation of transcription hubs on meiotic chromosomes. While we observe a near-complete loss of TAD signal in meiotic chromosomes, a large fraction of the genome shows looping or clustering interactions at the 1–10 Mb scale, which are present in zygonema but very pronounced in pachynema (Fig. 3a,b) and Supplementary Fig. 5). When we overlaid Hi-C contact maps with RNA polymerase II-bound loci in both prophase stages, we found that the clustered loci correspond to loci undergoing active transcription in both meiotic stages (Fig. 3a). Some clusters also correspond to highly transcribed clusters of Piwi-interacting RNAs (piRNAs), short RNAs with specialized roles in transposon silencing and sperm development (Fig. 3b). These data suggest that transcribed loci self-associate or condense within the meiotic chromosome structure to form clusters or hubs (Fig. 3c). While previous studies have shown that transcription machinery can localize to transcription factories and form phase-separated condensates within the nucleus, the strong interactions evident in our Hi-C contact maps suggest that meiotic prophase chromosomes are particularly susceptible to these influences. Supporting the idea of transcription hub formation in meiotic prophase, several previous studies have shown that RNA polymerase II (refs. ) and nascent RNA transcripts form highly punctate localization patterns in mouse and human spermatocytes.

Global organization of meiotic chromosomes. To characterize the global organization of meiotic chromosomes, we next analyzed genome-wide Hi-C contact probability (P) as a function of genomic distance (s). We find that for genomic distances less than...
~5 Mb, contact probability \( P(s) \) follows a power-law scaling proportional to \( s^{-0.5} \), dramatically different from the typical scaling of interphase chromosomes (between \( s^{-1} \) and \( s^{-1.5} \)\(^{-16,43} \)). The \( P(s) \sim s^{-0.5} \) scaling we observe in meiosis is similar to previous findings on mitotic chromosomes, which are organized as helical arrays of loops by cohesin-related condensin complexes\(^{13,14,44} \). Meiotic chromosomes are also morphologically similar to early mitotic prophase chromosomes, being individualized and compacted but much longer than mitotic prometaphase or metaphase chromosomes\(^{45} \). In agreement with this idea, the \( P(s) \) curves of meiotic prophase cells are most similar to those of chromosomes in early mitotic prophase, which have lost detectable TADs and are organized as linear arrays of loops but have not yet formed the highly compacted helical arrays characteristic of metaphase chromosomes\(^{44} \). While contact probability in mitotic prophase chromosomes drops sharply beyond ~2 Mb (ref. \(^{46} \)), meiotic chromosomes retain a \( P(s) \sim s^{-0.5} \) scaling relationship up to ~5 Mb (Fig. 4a). Chromosomes in pachynema show high contact probability at slightly longer distances than in zygonema, suggesting that cohesin-constrained loops may continue to extend through zygonema until final stabilization of the loop array in pachynema. This model agrees with previous reports of axis compaction as cells progress from zygonema to pachynema, and the more general inverse relationship between loop size and axis length in mutants of both meiosis-specific cohesin subunits (for example, Smc1\(^{15} \)) and chromosome axis core proteins (SYCP3)\(^{46-48} \). To estimate average loop length genome-wide, we examined plots of the slope, or derivative, of the \( P(s) \) function, maxima in which have been shown to correlate with average loop lengths inferred from polymer simulations\(^{49} \). This analysis suggests that average loop lengths are 0.8–1 Mb in zygonema,
and extend to 1.5–2 Mb in pachynema (Fig. 4a, lower panel). To estimate average loop density along chromosomes, we measured the total length of the synapsed chromosome axis in B6 × CAST pachynema spermatocytes at 215 ± 33 μm (Supplementary Fig. 1d). If the entire 2.8 Gb (haploid) genome is contained within loops averaging 1.5 Mb in length, this suggests an average loop density of ~10 loops per micron of chromosome axis in pachynema.

**Hi-C captures homolog pairing in meiotic prophase.** Meiotic prophase is the only developmental stage in mammals where homologous chromosomes are physically associated along their length. The 0.83% SNP density between B6 and CAST haplotypes in our F1 hybrid mice allowed us to assign 3.3% of zygonema read pairs (11.7 million) and 3.6% of pachynema read pairs (17.7 million) as unambiguous interhomolog contacts, enabling analysis of interhomolog contacts genome-wide (Methods and Supplementary Table 2). Hi-C contact maps constructed using only interhomolog contacts showed a strong diagonal signal in all intra-chromosomal maps, clearly indicating that homologs are aligned along their length (Fig. 4c,d and Supplementary Fig. 7). This general relationship was true in both zygonema and pachynema, despite the fact that chromosomes are only partially synapsed in the former. Preferential association within A/B compartments, visible as a checkerboard pattern in the interhomolog Hi-C maps, was also evident along the entire lengths of most chromosomes (Fig. 4c,d and Supplementary Fig. 7a,b). This finding supports a model in which the chromatin loops of paired homologs are extensively interdigitated (Fig. 4b), allowing preferential self-association of the A and B compartments between these chromosomes. In agreement with this idea, we also observe evidence of transcription-mediated interactions between homologs (Supplementary Fig. 7c,d).

We next plotted contact probability versus genomic distance specifically for interhomolog contacts (Fig. 4e). The interhomolog P(s) function shows a significantly shallower slope than the intra-homolog
The $P(s)$ function, with a power-law scaling roughly proportional to $s^{-0.18}$ (Fig. 4e). When considering the structure of a synapsed homolog pair, we envision that two factors may contribute to this shallower slope. First, synapsed homologs are aligned and juxtaposed arrays of chromatin loops, whose bases are held apart by the synaptonemal complex but which can probably interdigitate extensively (Fig. 4b). The effect of this loop interdigitation can be modeled mathematically as a convolution of two $P(s) = s^{-0.5}$ functions, which results in a power-law scaling function proportional to $P(s) = s^{-0.2}$ (Supplementary Fig. 8). Second, chromosomes are unlikely to be held in perfect juxtaposition by the synaptonemal complex. Local variation in packing density due to differences in loop size and positioning, plus variations in axis structure, probably give rise to small displacements of aligned homologs relative to one another.

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**Fig. 3** | Transcription-mediated interaction hubs in meiotic chromosomes. 

**a**, High-resolution view of a region of chromosome 9 in interphase, zygonema and pachynema. Shown in green are RNA polymerase II peaks detected at 10 dpp (zygonema) or 16 dpp (pachynema). Shown in blue are piRNA clusters transcribed at 12.5 days postpartum (dpp), during pachynema of the first wave of spermatogenesis (piRNA clusters measured at 14.5 dpp, later in pachynema, were nearly identical), and shown in green are RNA polymerase II peaks detected at 10 dpp (zygonema of the first wave of spermatogenesis) or 16 dpp (pachynema). 

**b**, High-resolution view of a region of chromosome 7 in interphase, zygonema and pachynema. Shown in blue are piRNA clusters transcribed at 12.5 dpp (zygonema of the first wave of spermatogenesis) or 16 dpp (pachynema). 

**c**, Left: model for assembly of transcription-mediated interaction hubs. In the absence of dynamic cohesin complexes disrupting chromatin–chromatin interactions, highly transcribed loci (green) will condense through cooperative self-interaction into interaction hubs. Right: schematic of Hi-C contact maps resulting from assembly of interaction hubs. Highly transcribed regions show depletion of short-range contacts with non-transcribed regions, and increased interactions with highly transcribed regions up to several megabase pairs away. See Supplementary Fig. 6 for additional examples of transcription-mediated interaction hubs.
Meiotic recombination frequency is strongly correlated with compartment structure. Spo11-catalyzed DSBs, which initiate meiotic recombination, occur preferentially in hotspots whose locations are dictated by a combination of chromatin structure and protein factors, and in yeast correlate with regions of high guanine-cytosine (GC) content. In most mammals, hotspot locations are controlled by PRDM9, a histone methyltransferase that generates trimethylated histone H3 lysine 4 and 36 (H3K4me3, H3K36me3) marks in chromatin near its binding sites. PRDM9 has been shown to direct recombination away from functional elements like promoters at the fine scale, but control of DSB formation at larger scales is not well understood in mammals. Previous reports that the recombinase RAD51 preferentially localizes to R-band (A compartment) chromatin in meiotic prophase, and also that meiotic chiasma appear more frequently in R bands in mouse spermatocytes, have hinted that compartment identity may play a role in mammalian meiotic recombination.
More recently, PRDM9 was shown to be involved in meiotic DSB formation more effectively in euchromatin than in heterochromatin or lamin-associated regions, suggesting that chromatin accessibility may directly affect meiotic recombination rates through differential PRDM9 binding. Finally, genome-wide maps of meiotic DSBs have shown a bias toward nucleosome-depleted regions flanked by H3K4me3 and H3K36me3 nucleosomes in euchromatin.

To further explore the connection between chromosome compartments and meiotic recombination, we overlaid the chromosome compartment structure with a previously reported map of meiotic DSB hotspots in B6 × CAST F1 hybrids. We found that both hotspot density (Fig. 5a,b and Supplementary Fig. 9a) and relative intensity (Fig. 5c and Supplementary Fig. 9e) are significantly higher in the A compartment compared to the B compartment. The A compartment is also enriched in both PRDM9-bound sites (Supplementary Fig. 9b) and H3K4me3 peaks (Supplementary Fig. 9c) in B6 × CAST spermatocytes. Finally, a set of ~800 crossovers between B6 and CAST chromosomes in the multi-species Collaborative Cross also show a strong bias toward the A compartment (Supplementary Fig. 9d). Overall, these data indicate that meiotic recombination landscape, while controlled at the fine scale by the location of PRDM9 binding sites, is strongly correlated at the megabase scale with compartment identity and chromatin state.

Isolation and silencing of the X chromosome in pachynema. In mammalian meiosis, chromosomes that fail to pair and synapse are subject to a pathway termed meiotic silencing of unsynapsed chromatin, in which these regions obtain repressive chromatin marks and are transcriptionally silenced. In male mice, the X and Y chromosomes pair, synapse, and form crossovers in a ~1 Mb pseudo-autosomal region, but the majority of these chromosomes remain unpaired. As spermatocytes enter pachynema, the unsynapsed regions of the X and Y are silenced by meiotic silencing of unsynapsed chromatin, also termed meiotic sex chromosome inactivation, and become isolated from other chromosomes as they are packaged into the sex body or XY body.

Our Hi-C contact maps clearly illustrate the reorganization of the X chromosome in pachynema. In zygonema, the X chromosome behaves equivalently to autosomes, showing strong X-shaped interchromosomal interaction patterns (Supplementary Fig. 3c) and maintaining compartment structure while losing visible TADs (Fig. 6a,b). While these features are maintained through pachynema on autosomes, the X chromosome shows dramatic changes. First, it becomes strongly isolated from all autosomes in pachynema, completely losing the X-shaped interchromosomal contact pattern observed in zygonema (Supplementary Fig. 3c). Second, the X chromosome's compartment structure is completely lost in pachynema (Fig. 6b) and Supplementary Fig. 4d). Third, consistent with the idea that the looping or clustering interactions we observe on autosomes are linked to transcription, we observe a near-complete loss of this clustering on the X chromosome as it becomes transcriptionally silenced in pachynema (Fig. 6b).

While these data reveal significant reorganization of the X chromosome in pachynema, its underlying structure as a linear array of loops appears mostly unaffected. We plotted P(s) for the X chromosome in both zygonema and pachynema, and found that while it shows a subtly different contact probability curve in pachynema compared to autosomes, the overall shape and slope of the curve is largely unchanged from zygonema (Supplementary Fig. 6b,i). Thus, the pachynema X chromosome can be considered as representing a ‘basal state’ of meiotic chromosome organization in which the axis-associated loop structure is unperturbed by either transcription-mediated clustering of loci or A/B compartment structure. In agreement with the idea that meiotic chromosome axis-associated chromatin loop locations are mostly stochastic, we observe no evidence of reproducibly located loops along the pachynema X chromosome in our Hi-C contact maps (Fig. 6a,b).

Discussion

In meiotic prophase, although chromosomes are highly organized by the meiotic chromosome axis and synaptonemal complex to promote homolog recognition and recombination, these chromosomes must also be transcriptionally active to support later stages...
of spermatogenesis. Here, we use Hi-C to directly visualize chromosome reorganization in meiotic prophase, revealing the physical parameters of chromosome organization by the meiotic chromosome axis, and of homolog juxtaposition by the synaptonemal complex. We find that meiotic chromosomes lose TADs, retain strong A/B compartment structure and form transcription hubs through clustering of highly transcribed loci. These changes can be explained by a model in which association of cohesin complexes with the meiotic chromosome axis stabilizes their association with chromatin, reduces the influence of CTCF on the positioning of

Fig. 6 | X chromosome organization in pachynema. a, Hi-C contact maps for the X chromosome in interphase, zygonema and pachynema. b, Close-up view of a region of chromosome X showing transcription-mediated clustering of loci in zygonema, which is largely lost in pachynema. Shown in green are RNA polymerase II binding peaks at 10 dpp (zygonema) or 16 dpp (pachynema). c, Pearson correlation matrices for the X chromosome in interphase, zygonema and pachynema. See Supplementary Fig. 4d for Eigenvector analysis of the X chromosome.
cohesin-constrained loops and may also affect loop extrusion rate or processivity. As cohesin complexes coalesce on the chromosome axis, they mediate the assembly of a stable array of loops, and our data suggest that loop length continues to increase as the axis undergoes linear compaction through leptotene/zygonema and into pachynema.

The loss of TADs, retention of A/B compartment structure and formation of transcription hubs are all strongly reminiscent of recent reports on the effects of cohesin depletion in somatic cells. Cohesin depletion causes an almost immediate loss of TADs in aggregate Hi-C data, strengthening and fragmentation of the A/B compartment structure, and the formation of multivalent enhancer clusters, or superenhancer hubs\(^8,9,13\). The similarity of meiotic chromosomes to chromosomes that have lost cohesin supports our model in which cohesin dynamics are strongly suppressed as they are repurposed for assembly of a stable chromatin loop array. The loops themselves, which exceed 1 Mb in length in pachynema, are essentially free of dynamic cohesin complexes which would otherwise counteract the tendency of both heterochromatin and transcription machinery to self-associate.

The overall structure of meiotic chromosomes as linearly compacted loop arrays organized by cohesin complexes and other axis components is highly conserved throughout eukaryotes\(^9\). When we compare our data from \textit{M. musculus} spermatocytes to two recent Hi-C analyses of \textit{S. cerevisiae} meiotic chromosomes\(^9,13\), we find some clear differences but overall a striking level of agreement. The most obvious difference is that while Hi-C contact maps of \textit{S. cerevisiae} chromosomes show strong evidence of looping between known cohesin binding sites, \textit{M. musculus} chromosomes show little evidence for reproducible loop locations across the cell population. This difference is probably due to the known preferential binding of \textit{S. cerevisiae} meiotic cohesin complexes near the 3' ends of open reading frames, and particularly between convergent gene pairs;\(^10,10\) there is so far no evidence for preferred cohesin binding sites in mouse meiocytes. Another major difference between \textit{S. cerevisiae} and \textit{M. musculus} is the length of chromatin loops: our data suggest that average chromatin loops in \textit{M. musculus} chromosomes extend from 0.8–1 Mb in zygonema to 1.5–2 Mb in pachynema, while Hi-C and polymer simulations of \textit{S. cerevisiae} chromosomes indicate an average loop length of \~26kb in this organism. Loop lengths are shorter, \~20kb, in the absence of interhomolog synapsis (zip1Δ), in agreement with our finding that loops extend as chromosomes undergo synapsis during the zygonema–pachynema transition. The extremely short loops in \textit{S. cerevisiae} meiotic chromosomes, combined with the preferential cohesin binding sites and the transient nature of pachynema in \textit{S. cerevisiae}, probably precludes any clustering of transcribed loci as we observe in \textit{M. musculus} spermatocytes. While loop lengths are \~50-fold different between \textit{S. cerevisiae} and \textit{M. musculus}, the density of loops along the chromosome axis is remarkably similar in the two organisms. With a total pachytene axis length of \~36μm in \textit{S. cerevisiae} (based on measurements in ref. \(^9\)), and 65% of the genome packaged into 26kb loops as estimated by Schalbetter et al.\(^13\), we estimate a loop density of \~8.5 per micron of axis, very close to our estimate of ten loops per micron in \textit{M. musculus}. This finding agrees with previous proposals that while loop lengths vary widely between eukaryotes, scaling roughly with overall genome size, the architecture of the chromosome axis and its looping structure are highly conserved\(^9\).

Thus, while the details of meiotic chromosome structure vary among organisms, the fundamental architecture of the chromosome axis-constrained loop array is extremely consistent.

Previous studies have indicated strong morphological similarities between chromosomes in meiotic prophase and very early mitotic prophase\(^26\), in which chromosomes are individualized and partially compacted but have not yet become the highly compacted helical loop arrays found in mitotic prometaphase and metaphase\(^13,14\). Whereas mitotic prophase is a transient state characterized by the replacement of cohesins with condensins and eviction of transcriptional machinery, meiotic prophase is a highly stable state mediated by meiosis-specific cohesin complexes and characterized by high transcriptional activity. Nonetheless, in keeping with chromosomes' overall morphological similarity, we find that global genome organization in these two states is similar, with \(P(s)\) curves showing power-law scaling proportional to \(s^{-0.5}\). Meiotic chromosomes retain a \(P(s)\sim s^{-0.5}\) scaling relationship over longer distances than mitotic prophase chromosomes (5 versus 2 Mb, ref. \(^9\)), consistent with significantly longer loops in meiotic prophase (1.5 Mb in pachynema compared to 60–80 kb in mitotic prophase\(^8\)).

The high transcriptional activity of meiotic chromosomes impacts chromosome organization in several ways. First, meiotic chromosomes retain physically separated A and B compartments, in contrast to mitotic chromosomes which lose compartment separation entirely\(^8,9\). Second, we observe strong clustering of highly transcribed loci, suggesting that these loci tend to self-associate or condense during the extended meiotic prophase. This condensation is probably a consequence of the loss of dynamic cohesin complexes on extended chromatin loops, which would otherwise act to dissociate these clusters much as they counteract the self-association of A and B compartments in somatic cells\(^6,10\). Due to meiotic chromosome organization as linear loop arrays, clustering of transcribed loci can only occur locally, within a 5–10 Mb range, but apparently can occur between loci on homologs due to interdigitation of the paired loop arrays.

The maintenance of A/B compartments and high-level transcription in meiotic prophase shows that the genome retains many chromatin-structure features of interphase chromosomes, despite reorganization by the meiotic chromosome axis and in stark contrast to chromosomes entering mitosis. Chromatin structure in turn strongly affects the overall distribution of meiotic DSBs and eventual interhomolog crossovers, strongly biasing recombination toward the gene-dense A compartment over the more heterochromatinic B compartment. This effect is probably due to differential chromatin accessibility, with easier access of PRDM9 to its binding sites and the generation of H3K4me3 marks in the A compartment\(^7\).

Newly developed methods for the synchronization and purification of mouse spermatocytes, and their analysis by Hi-C, can provide a new window into the organization and function of meiotic chromosomes. These advances will be critical to increasing our understanding of the roles of structural proteins, including cohesins and chromosome axis components, the interplay between chromosome organization and transcription, and homolog interactions during recombination and synopsis.

**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/s41594-019-0187-0](https://doi.org/10.1038/s41594-019-0187-0).

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Author contributions
K.D.C., F.C. and B.R. conceived and planned the study. R.K. performed spermatocyte isolation and characterization. L.P. adapted and implemented the Hi-C data analysis pipeline and performed Hi-C data analysis. S.C. and R.H. prepared sequencing libraries and performed initial Hi-C data analysis. S.R., Y.Q. and R.R. provided valuable input for data processing and analysis. K.D.C. and F.C. wrote the manuscript with input from B.R. and all other authors.

Competing interests
The authors declare no competing interests.

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Methods

Mouse husbandry and spermatocyte isolation. We mated female C57BL/6J and male CAST/EiJ mice (obtained from The Jackson Laboratory). We treated male F1 neonates with the retinoic acid inhibitor WIN18,446 at 2 days postpartum to block spermatogonial differentiation, then injected retinoic acid at 9 days postpartum as previously described \(^\text{1}\) (Supplementary Fig. 1a). Treated animals were allowed to recover for 25–47 days to enable isolation of synchronized cell populations in zygenome and pachynema of waves 2–5, with harvesting times calculated as previously described \(^\text{2}\) (Supplementary Table 1). Spermatocytes from synchronized testes were isolated and stained with Hoechst 33342 to allow isolation of cells with 4C DNA content (prophase I) by flow cytometry \(^\text{3}\). Compared to unsynchronized cells, isolated from synchronized testes showed a significantly different cell profile with only a few densely populated 4C regions (Supplementary Fig. 1b). Purity and prophase stage were determined by chromosome spreads of sorted cells, stained with antibodies to SCP3 (c 74569, mouse monoclonal clone D-1 from Santa Cruz) and H3K4me3 (a gift from Dr. Mary Ann Handel) (Supplementary Fig. 1c). Final cell numbers and purity for each sample are noted in Supplementary Table 1.

Hi-C library preparation and sequencing. Hi-C experiments were performed largely as previously described \(^\text{4}\). Briefly, between 300 × 10^6 and 800 × 10^6 cells (Supplementary Table 1) were crosslinked with 2% formaldehyde for 10 min at room temperature; the reaction was quenched using 200 mM glycine for 5 min at room temperature, 15 min on ice, then samples were frozen in liquid nitrogen. Nuclei were isolated and directly applied for digestion using the four-base cutter restriction enzyme MboI (NEB) at 37 °C for 37 min. The single-strand overhang was filled with HotHoechst 33342 to allow isolation of cells with 14-DATP (Life Technologies) using Klenow DNA polymerase (New England Biolabs). In contrast to traditional Hi-C, ligation was performed when the nuclear membrane was still intact (in situ protocol). DNA was ligated for 4 h at 16 °C using T4 ligase (New England Biolabs). Protein was degraded by proteinase K (New England Biolabs) at 55 °C for 30 min. Crosslinking was reversed with the addition of 500 mM NaCl and incubation overnight. DNA was purified by ethanol precipitation, sonicated to 300–700 bp fragments and size-selected using SPIRi magnetic beads as described \(^\text{5}\). Biotinylated DNA was selected with Dynabeads MyOne T1 Streptavidin beads (Life Technologies). Sequencing libraries were prepared on beads, checked using an Agilent Bioanalyzer 2100 and quantified using a QuantiBead (Life Technologies). Libraries were sequenced on an Illumina Hiseq 4000 with 100 cycles of paired-end reads.

Hi-C data analysis and bioinformatics. Hi-C data pre-processing and analysis was performed largely as previously described \(^\text{6}\), with modifications for assignment of haplotype of each read. We aligned each read to the mm10 genome assembly using BWA-MEM \(^\text{7}\) with default parameters, except for the clipping penalty (-l flag) being set to 13. Next, WASHI \(^\text{8}\) was adapted to identify reads containing one or more SNPs and then the read was realigned after flipping each allele to the value in the CAST genome. For SNP identification, we used data from the Wellcome Sanger Institute Mouse Genomes Project \(^\text{9}\), accession code ESR076381. Dividing 226,138,14 SNPs by a total genome length of 2,725,521,370 (one copy of each chromosome including X and Y) gives 0.83% SNP density, or one SNP in 120 bp sequencing data have been deposited in the NCBI Gene Expression Omnibus database under accession number GSE122622: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE122622. All previously published data used in our analysis are available at the links below: GSM1908921: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1908921; GSM1954839: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1954839; ESR076381: ftp://ftp-mouse.sanger.ac.uk/current_snps/strain_specific_vcfs/CAST_EiJ.mgp.v5.snps.dbSNP142.vcf.gz; GSE101406: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE101406; GSM1083638: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1083638; SSRR7720230 (processed): http://www.slimrnagroup.uni-munich.de/piRNAClusterDB.html; SSRR772029/GSM1096583 (raw data): https://www.ncbi.nlm.nih.gov/sra?人生/sra/SRR248863; SSRR7720230/GSM1096584 (raw data): https://www.ncbi.nlm.nih.gov/sra?人生/sra/SRR248864

Contact probability calculation. Contact probability versus genomic distance \((P(s))\) curves were calculated as previously described \(^\text{10}\). Briefly, we divided all genomic separations into logarithmically sized bins, starting at 10 kb and increasing by a factor of 1.1 per bin. We first calculated the number of possible Hi-C contacts in each dataset that fall into each bin. We next calculated the number of possible Hi-C contacts at each distance across the genome or within an individual chromosome, using a fragment size of 250 bp to approximate the ~ 256 bp size of MboI-generated restriction fragments. Dividing contact number by potential contacts in each bin yielded contact probability \(P(s)\), which we then normalized by setting the value of \(P(s)\) at a distance of 1000 kb to 1. Due to their distinctive organization in meiotic prophase, the X and Y chromosomes were considered separately in this analysis.

Data availability

All custom scripts and code are available at Github (https://github.com/lucaspatel/nsmb; mousehic) or from the authors (K.D.C) upon request. All sequencing data have been deposited in the NCBI Gene Expression Omnibus database under accession number GSE122622: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE122622. All previously published data used in our analysis are available at the links below: GSM1908921: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1908921; GSM1954839: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1954839; ESR076381: ftp://ftp-mouse.sanger.ac.uk/current_snps/strain_specific_vcfs/CAST_EiJ.mgp.v5.snps.dbSNP142.vcf.gz; GSE101406: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE101406; GSM1083638: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1083638; SSRR7720230 (processed): http://www.slimrnagroup.uni-munich.de/piRNAClusterDB.html; SSRR772029/GSM1096583 (raw data): https://www.ncbi.nlm.nih.gov/sra?人生/sra/SRR248863; SSRR7720230/GSM1096584 (raw data): https://www.ncbi.nlm.nih.gov/sra?人生/sra/SRR248864

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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Sequencing data were collected on an Illumina HiSeq 4000 running HiSeq Control Software version 3.3.76

Data analysis

Sequencing data were analyzed using BWA-MEM (http://bio-bwa.sourceforge.net) and WASP (https://github.com/bmvdgeijn/WASP), then Hi-C contact maps were calculated using Juicer (https://github.com/theaidenlab/juicer). Data was visualized with JuiceBox (https://www.aidenlab.org/juicebox/) and Integrated Genomics Viewer (http://software.broadinstitute.org/software/igv/). Compartm
ent analysis was performed with regioneR (http://bioconductor.org/packages/release/bioc/html/regioneR.html). Custom scripts used in this study have been uploaded to Github (https://github.com/lucaspatel/nsmb_mousehic).

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Life sciences study design

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Sample size

For Hi-C analysis, we obtained the maximum feasible number of cells from each mouse testis and sequenced the resulting libraries to the maximum extent possible, given library complexity calculated from pilot experiments. While the "resolution" of a Hi-C contact map is not well-defined and depends on a number of variables, a recent study (Rao et al (2014) Cell 159(7): 1665-1680) calculated resolution as the bin size at which at least 80% of bins contain at least 1000 Hi-C contacts. By this criterion, our combined zygonema and pachynema maps can both be interpreted at a resolution of 10 kb.

Data exclusions

Read pairs where one or both reads showed SNPs matching both B6 and CAST haplotypes (0.5-0.6% of each dataset) were excluded from downstream analysis.

Replication

For zygonema Hi-C data, two separate biological replicates were independently processed and sequenced, then separate Hi-C maps were calculated and visually compared before combination. For pachynema Hi-C data, three separate biological replicates were independently processed and sequenced, then separate Hi-C maps were calculated and visually compared before combination.

Randomization

We did not perform comparisons between different populations or individuals, therefore randomization does not apply to this study.

Blinding

We did not perform comparisons between different populations or individuals, therefore blinding does not apply to this study.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study
☐ Unique biological materials
☐ Antibodies
☐ Eukaryotic cell lines
☐ Palaeontology
☐ Animals and other organisms
☐ Human research participants

Methods

n/a Involved in the study
☐ ChIP-seq
☐ Flow cytometry
☐ MRI-based neuroimaging

Antibodies

Antibodies used

The SYCP3 antibody (clone D-1) is a mouse monoclonal IgG1 (kappa light chain) from Santa Cruz (sc-74569). The H1t antibody is a hamster polyclonal antibody from the laboratory of Mary Ann Handel.

Validation

These antibodies have been used extensively by the field, published in at least 50 papers, and are widely believed to be specific. Each has been shown by immunoblotting and immunofluorescence to be testis and spermatocyte cell-stage specific for expression and localization. Additionally, each antibody has been validated by staining spermatocyte spreads from either Sycp3-/- or H1t-/- animals, respectively.
Animals and other organisms

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

| Laboratory animals | All mice (Mus musculus) used were male C57BL6/J x CastEiJ F1 hybrids of the ages described in the text. |
|--------------------|-----------------------------------------------------------------------------------------------------|
| Wild animals       | N/A                                                                                           |
| Field-collected samples | N/A                                                                                       |

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Decapsulated testes were incubated with shaking (500 RPM) in 15ml Gey’s Balanced Salt Solution (GBSS) supplemented with 0.5mg/ml collagenase for 15 minutes at 33°C. The separated seminiferous tubules were washed in 15 ml GBSS and digested with 15ml GBSS/0.5mg/ml trypsin/20 to 50 μl of 0.4mg/ml DNase I for 15 minutes. The amount of DNase I was dependent upon the size of the testis. Then, 750μl of newborn calf serum (NCS) was added and cells were mechanically separated using a transfer pipette for 3 minutes. Individualized cells were filtered through a 70μm cell strainer and spun at 2,000 RPM for 3 minutes. After supernatant removal, the pellet was re-suspended in 25μl of 0.4mg/ml DNase I by tapping and washed once with 10ml GBSS/10μl 0.4mg/ml DNase I/500μl NCS for 3 minutes at 2,000 RPM. Cell pellet was re-suspended in 6ml GBSS/2% NCS/12μl 0.4mg/ml DNase I and stained with 5μg/ml of Hoechst 33342 (2.5μg/μl in DMSO, stored at 4ºC in light-protected tubes) for 45 minutes at 33°C at 500 RPM. Finally, 0.2μg/ml of propidium iodide (PI) was added. Cells were filtered through 70μm cell strainer and placed under BD Aria or Fusion flow cytometer machines for sorting.

Instrument

Becton Dickinson FACSAria™Fusion SORP and FACSAria™Fusion

Software

Becton Dickinson FACSDivaTM software

Cell population abundance

Shown in Table S1

Gating strategy

Cells were initially separated by side scatter (y-axis) vs. forward scatter (x-axis) plot to disregard doublets followed by side scatter (y-axis) vs. PI(x-axis) plot to gate only live cells. Spermatocytes were separated by blue fluorescence (y-axis) vs. red fluorescence (x-axis) emitted by Hoechst 33342. Cells emitting the highest blue fluorescence were identified as 4C spermatocytes in meiotic prophase I. Synchronized 4C spermatocytes were found in densely compacted 4C populations and gated for sorting into 2ml GBSS containing 5% NCS.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.