Principles of meiotic chromosome assembly revealed in *S. cerevisiae*

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During meiotic prophase, chromosomes organise into a series of chromatin loops emanating from a proteinaceous axis, but the mechanisms of assembly remain unclear. Here we use *Saccharomyces cerevisiae* to explore how this elaborate three-dimensional chromosome organisation is linked to genomic sequence. As cells enter meiosis, we observe that strong cohesin-dependent grid-like Hi-C interaction patterns emerge, reminiscent of mammalian interphase organisation, but with distinct regulation. Meiotic patterns agree with simulations of loop extrusion with growth limited by barriers, in which a heterogeneous population of expanding loops develop along the chromosome. Importantly, CTCF, the factor that imposes similar features in mammalian interphase, is absent in *S. cerevisiae*, suggesting alternative mechanisms of barrier formation. While grid-like interactions emerge independently of meiotic chromosome synapsis, synapsis itself generates additional compaction that matures differentially according to telomere proximity and chromosome size. Collectively, our results elucidate fundamental principles of chromosome assembly and demonstrate the essential role of cohesin within this evolutionarily conserved process.

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uring meiosis, eukaryotic chromosomes are broken, repaired and paired with their homologues followed by two rounds of segregation—a series of events accompanied by dynamic structural changes of the chromosomes (Fig. 1a, top). Most prominent is the paired arrangement of pachytene chromosomes into a dense array of chromatid loops emanating from proteinaceous axes linked by a central core, the synaptonemal complex (SC), which is highly conserved across eukaryotes. In S. cerevisiae, structural components include the meiotic cohesin kleisin subunit, Rec8, the transverse filament, Zip1, the axial/lateral elements, Hop1 and Red1, and the pro-DSB factors Rec114-Mei4-Mer2 (RMM). Rec8 is a major component of the meiotic axis—its absence disturbs the localisation patterns of Red1 and Hop1, with no axial or central elements detected by electron microscopy (EM). In the absence of Hop1 or Zip1, unsynapsed axial elements are formed. Much of our understanding of meiotic chromosome structure has been deduced from a combination of EM, immunofluorescence microscopy and the genome-wide patterns of protein localisation determined by ChIP. However, clarifying the link between key meiotic protein complexes, chromosome conformation and genomic sequence is of great interest.

Chromosome conformation capture (3C) techniques generate maps of pairwise contact frequencies that are snapshots of chromosome organisation. 3C methods were originally applied to assay chromosome conformation in S. cerevisiae, including during meiosis. Now they are widely used across a range of organisms and cellular contexts to link 3D organisation directly with genomic sequence, revealing important roles of the Structural Maintenance of Chromosomes (SMCs) cohesin and condensin in genomic organisation, where they likely mediate chromosome compaction via the process of loop extrusion.

Recent studies have utilised Hi-C to investigate meiotic chromosome structure in mammals and S. cerevisiae. Consistent

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**Fig. 1** Chromosome conformation during yeast meiosis. **a** Upper panel: cartoon of chromosome morphology during the stages analysed in the meiotic time course. The Rabl-structure observed in G1 is characterised by centromere clustering (in blue), meiotic axis proteins are represented in green, with the fully assembled synaptonemal complex (SC) represented in light green. Lower panels: cells were collected during meiosis at indicated timepoints and analysed by FACS. At 0 h the cells are in G1. Representative Hi-C contact maps of chromosomes 6, 11 and 18 plotted at 5 kb resolution. Centromeres, telomeres and arm fold-back at the centromere are indicated by blue, red and grey arrows, respectively, and axial compaction by the width of the main diagonal relative to the fixed-width black clamp. For interactive HiGlass views see: http://higlass.pollard.gladstone.org/app/?config=ZiwKpjzQpePCXXyvuYGeQ. **b** Meiotic entry assessed by FACS; at 4 h, the majority of cells show a 4C peak indicating completion of DNA replication. **c** Meiotic progression was monitored by quantification of nuclear divisions determined by DAPI staining. Around 4 h, cells start to undergo meiotic divisions I and II. The majority of cells undergo meiotic divisions between 4 h and 8 h, indicating the degree of heterogeneity within the cell population. Source data are provided as a Source Data file.

**d** Upper panels: Average trans centromere–centromere contact maps. Lower panels: trans telomere–telomere contact maps. Right: ratio of cis to total contact frequency. **e** Intra-arm contact probability versus genomic distance, P(s), indicating the emergence (left) and disappearance (right) of chromosome arm compaction during meiosis. Shaded area bounded above and below by the two ndt80Δ 8 h replicates. **f** Meiosis was induced in ndt80Δ cells for 8 h and meiotic entry was checked by monitoring DNA replication by FACS. **g** ndt80Δ cells were grown for 8 h in sporulation media and analysed by Hi-C. Log2 ratio of ndt80Δ cells 8 h over G1 (right). Centromeres and telomeres are indicated by blue and red arrows, respectively, and axial compaction by a black clamp.

**h** Left: contact probability of individual chromosome arms stratified by length. Right: contact probability stratified by the distance from the telomere.
with compaction by a loop array, pachytene chromosome structure displays increased short-range cis interactions and a shoulder in contact frequency versus distance curves\textsuperscript{17–20}. Mouse and monkey pachytene chromosomes additionally display a loss of the topological domains (TADs) characteristic of mammalian interphase\textsuperscript{17–19}. In \textit{S. cerevisiae}, Hi–C with a synthetically re-designed chromosome found low levels of interhomologue contacts, and increased insulation at Rec8 sites\textsuperscript{20}. However, it remains to be determined whether cohesin is required for the formation of meioteic chromosome structure, as measured by Hi–C, and what mechanisms organise meiotic chromosomes.

Here we employ yeast meiosis as a model system to elucidate mechanisms of chromosome assembly, and define the role of key meiotic chromosome components, including cohesin and the SC. We show that meiotic chromosome compaction is accompanied by the emergence of punctate grid-like interactions. These interactions are dependent on Rec8 and their underlying DNA loci are preferred Rec8 association sites. Our data agrees with polymer simulations of loop extrusion with barriers, which suggest a remarkable heterogeneity in loop size and location from cell-to-cell. We further show that the synaptonemal complex modulates compaction differentially along chromosome arms.

**Results**

**Chromosome compaction emerges and subsides in meiosis.**

Starting with a synchronised G1 population we analysed time-points encompassing DNA replication, meiotic prophase and both meiotic divisions (Fig. 1a–c, Supplementary Fig. 1a–c). In G1, we detect strong centromere clustering (Fig. 1a, d) and folding back of the arms at the centromeres (Fig. 1a, Supplementary Fig. 2), characteristic of a Rabl conformation\textsuperscript{12,21}. During meiosis, centromere clustering is transiently dissolved (3–5 h, Fig. 1a, d, Supplementary Fig. 1a); this coincides with a global decrease in inter-chromosomal contact frequency at mid-prophase, reflecting chromosome individualisation. Subtelomeric clustering also decreases during meiotic prophase (Fig. 1a, d, Supplementary Fig. 1a, Supplementary Fig. 3). Our wild-type timecourse displayed no evidence of a telomere bouquet, likely due to its transience, which has been measured by microscopy\textsuperscript{22}.

Entering meiosis, contact frequency versus distance, $P(s)$, curves display a shoulder, consistent with the linear compaction of chromosome arms increasing due to cis-loop formation (2–4 h, Fig. 1e, Supplementary Fig. 1d, e.g. as defined\textsuperscript{23}; for review\textsuperscript{16}). This change in $P(s)$ is reminiscent of the SMC-dependent changes observed via Hi–C during mitosis across species\textsuperscript{24–28}. Compaction coincides with meiotic prophase I and the formation of the SC at pachytene, and is lost at later stages (Fig. 1e, Supplementary Fig. 1d).

To study meiotic chromosome compaction in more detail, and to eliminate cell-to-cell heterogeneity (Fig. 1b, c), we enriched for pachytene cells in subsequent experiments by inactivating Ndt80, a transcription factor required for exit from meiotic prophase\textsuperscript{29}. \textit{ndt80} cells entered meiosis synchronously, assessed by microscopy\textsuperscript{30}. Our data agrees with polymer simulations of loop extrusion with barriers, which suggest a remarkable heterogeneity in loop size and location from cell-to-cell. We further show that the synaptonemal complex modulates compaction differentially along chromosome arms.

**Rec8-dependent punctate interactions emerge in meiosis.**

Zooming in to consider within-arm organisation revealed punctate grid-like Hi–C interactions between pairs of loci during prophase (Fig. 2a), particularly prominent in \textit{ndt80} cells (Fig. 2a, b). Indeed, the focal meiotic patterns we observe resemble peaks between CTCF sites\textsuperscript{31} rather than TADs,\textsuperscript{32,33} detected in mammalian interphase Hi–C maps, and likely arise from a heterogeneous mixture of ‘transitive’ interactions and ‘skipping’ of peak bases (Fig. 2c).

Genomic regions underlying the punctate Hi–C interactions display a remarkable visual (Fig. 2a, b), and quantitative (Fig. 2d–g), correspondence with previously characterised sites of high Rec8 occupancy\textsuperscript{30}. A reciprocal analysis of calling Hi–C peaks and assaying the frequency of Rec8 sites around peak anchors confirmed this correspondence (Supplementary Fig. 4a). At pachytene, Rec8 sites display elevated cis/total contact frequencies (Fig. 2d), enriched contact frequency (Fig. 2e, f), and evidence of insulation (Fig. 2g)—features that correlate with Rec8 occupancy measured by ChIP (Fig. 2a, lower) consistent with recent observations\textsuperscript{29}. In wild-type cells, Rec8–Rec8 interactions became visible in early prophase (2 h), peaked at mid-prophase (4 h), and were especially prominent in the homogenous \textit{ndt80} cell population (Fig. 2a, b, f, Supplementary Fig. 4b, c). Importantly, Rec8–Rec8 enrichments are strongest between adjacent sites, decrease between non-adjacent sites with increasing genomic separation, and are absent in trans (Supplementary Fig. 4b, c). As for enrichments between CTCF sites in mammalian interphase\textsuperscript{34}, these observations argue that a cis-acting process generates such focal interactions in meiosis.

Rec8 is a central component of the meiotic chromosome axis\textsuperscript{4}. \textit{rec8Δ} mutants fail to assemble chromosome axes as detected by EM, and exhibit delayed and inefficient chromosome segregation producing few viable spores\textsuperscript{4}. Assaying a \textit{rec8Δ} mutant in the \textit{ndt80} background enabled us to determine that Rec8 is absolutely required for the emergence of the grid-like Hi–C patterns present in meiosis (Fig. 2a, b). Moreover, \textit{rec8Δ} cells completely lose the shoulder in $P(s)$, indicative of a dramatic loss of arm compaction (Fig. 2b, Supplementary Fig. 4d), similar to that caused by deletion of SMCs in diverse contexts\textsuperscript{24,26,28,35–38}. Instead of assembling an axis of loops, \textit{rec8Δ} cells appear to be caught in a state with highly clustered telomeres (Supplementary Fig. 3, Supplementary Fig. 4e), consistent with previous observations by microscopy\textsuperscript{39,40}. Moreover, in \textit{rec8Δ} cells cis contact frequency is reduced (Fig. 2d, Supplementary Fig. 2c), similar to G1 cells, and cis/totals no longer correlates with Rec8 occupancy. Instead, \textit{rec8Δ} cis/totals displays a decreasing trend along chromosome arms (Supplementary Fig. 4e), likely due to persistent telomere clustering (Supplementary Fig. 3a). Importantly, because focal interactions in wild-type cells are detected as early as cells start progressing through S phase (Fig. 2a, 2 hours), the lack of such interactions in \textit{ndt80}–arrested \textit{rec8Δ} cells, which have completed DNA replication (Supplementary Fig. 2d), cannot be explained by partial arrest prior to a pachytene-like stage.

**Meiotic chromosomes modelled by loop extrusion with barriers.**

To test how compaction and grid-like interaction patterns could jointly emerge in meiosis, we developed polymer simulations (Fig. 3a, Methods) similar to those used to successfully describe the assembly of TADs in mammalian interphase chromosomes\textsuperscript{16}. Importantly, these simulations employ the cis-acting process of loop extrusion, where extruders form progressively larger chromatin loops, unless impeded by adjacent extruders or...
barrier elements (Fig. 3a). Extrusion dynamics are controlled by parameters dictating the processivity (average loop size) and separation (number of active extruders), as well as the strength of barriers (Methods). Because the accumulation of Rec8 at ChIP-seq sites is indicative of barriers to extrusion, we positioned bi-directional barriers at Rec8 sites.

To find loop extrusion dynamics in agreement with experimental data, we computed the goodness-of-fit between experimental ndt80Δ Hi–C maps and simulated Hi–C maps generated for a wide range of parameter combinations (Fig. 3, Methods). Models with excellent fits were identified in which ~64% of the genome is covered by extruded loops (Fig. 3b, c) - a far denser array than present in S. cerevisiae mitosis, but still less compact than human mitotic cells. Even though extrusion can generate compaction independently of barriers (Fig. 3d, iii), an intermediate barrier strength is essential to match the grid-like patterns observed.
Fig. 2 Emergence of a Rec8-dependent grid of punctate interactions in meiosis. a Hi–C contact maps of chromosome 11 for the indicated genotypes at 2 kb resolution, showing near-diagonal interactions. Lower panels: log2(insulation); cis/total ratio, Rec8 ChIP-seq. Insulation and cis/total calculated from ndt80Δ maps. Green circles: positions of Rec8 sites. Genome-wide cis/total (Spearman’s R = 0.62, P < 1e-10) and insulation (R = -0.23, P < 1e-10, insulation window = 20 kb) profiles are correlated with Rec8 occupancy. Colour scale as in Fig. 2b. b Zoom-in of chromosome 11 (0–200 kb) for wt-4h, ndt80Δ and rec8Δ. Contact probability versus genomic distance, P(s), for G1 (ndt80Δ-0h) and ndt80Δ and rec8Δ. Data are the average (n = 2) except for wt-4h. While faint locus-specific patterns exist in rec8Δ, there is no global enrichment at Rec8 sites (see f and g). Rec8 peak sites called from ChIP-seq data are indicated in green. Interactive view: http://higlass.pollard.gladstone.org/app/?config=Twrh61jGT4s5xotaguTI1g. Comparison to published ndt80Δ–arrested Hi-C data: http://higlass.pollard.gladstone.org/app/?config=NKoclicPJRTuah4ZrQPPm_Q. c Simplified illustration of how a grid of peaks on a Hi–C map can emerge between Rec8 sites either by transitive contacts between adjacent loops, or by loops that skip over adjacent sites. Experimentally observed grids extend much further than separation = 2 (Supplementary Fig. 4c). d Cis/total ratios for Rec8 (green) and nonRec8 (grey) sites for indicated datasets, showing an elevated cis/total frequency (0.85 versus 0.77) at Rec8 sites in ndt80Δ. e Contact probability versus genomic distance, P(s), between Rec8-Rec8 sites (green), Rec8-nonRec8 sites (light green) and nonRec8-nonRec8 sites (grey). Note elevated pairwise contact frequency (2-fold at 20 kb) at Rec8 sites in ndt80Δ. f Log2 ratio of contact frequency between adjacent Rec8 sites (separation = 1) compared to average cis interactions. g Log2 ratio of contact frequency centred at Rec8 sites compared to average cis interactions, showing mild insulation at Rec8 sites in ndt80Δ. These distinctions (d–g) are similar in wild-type pachytene (4h) yet absent in G1 (ndt80Δ-0h) or in rec8Δ

experimentally (Fig. 3d, i). Despite the simplifying assumptions, simulated chromosomes displayed many features observed experimentally: (i) chromosomes fold into a loose polymer brush, with a Rec8-rich core (Fig. 4a, Supplementary Fig. 5a, b); (ii) a grid-like interaction pattern naturally emerges in simulated Hi-C maps (Fig. 3d); (iii) importantly, because loop extrusion is a cis-acting process, pairs of Rec8 sites at increasing separations naturally have lower contact frequency (Fig. 3e).

Simulations also highlight the stochasticity of loop positions in the best-fitting models, with most barriers (73%) unoccupied by an extruder, and extruders paused with barrier elements on both sides only a minority of the time (15%) (Fig. 4b–d). Because of this, the majority (65%) of extruded loops cross over Rec8 sites, consistent with an average loop size roughly twice the average distance between Rec8 ChIP peaks (26 kb versus 12 kb, Fig. 4e), and remarkably consistent with estimates made using EM (~20 kb)41. Genome-wide simulations for these best-fitting parameters show that the majority of chromosomes display similar goodness-of-fit with meiotic Hi-C data as on chr13 (Supplementary Fig. 6). Most strikingly—despite the prominence of Rec8-dependent grid-like features in the experimental data (Fig. 2a, b)—our simulations indicate that Rec8 sites are not always occupied by extruding cohesins and thus are present at the meiotic chromosome core in only a subset of cells, as inferred previously43. Notably, when loop extrusion operates independently on each chromatid, as in our simulations, the positions and sizes of loops are naturally heterogeneous, even between sister chromatids (Fig. 4d). Such heterogeneity agrees with a recent microscopy study in C. elegans which argues for asymmetric chromatin loops on sister chromatids in meiosis44.

The range of loop extrusion parameters we explored encompassed the situation where Rec8 sites always halt extrusion and cis-loops are formed between each consecutive Rec8 site. However, simulations with these parameters have quantitatively poor fits with experimental maps (Fig. 3d–e, ii): the bend in P(s) comes too early to recapitulate experimental P(s), and Rec8-Rec8 contacts are much too strong. The poor fit of such ‘direct-bridging’ simulations underscores the conclusion that only a fraction of Rec8 sites are occupied in a given cell, and argues that cohesin-dependent cis-loops must link regions that are not primary Rec8 binding sites in order to provide compaction without making Rec8-Rec8 enrichments overly strong. As expected, certain loop extrusion parameter sets give rise to TAD-like patterns. However, simulations with TAD-like patterns show poor quantitative agreement with experimental ndt80Δ data (Fig. 3d–e, iv), arguing that the patterns we observe in meiosis are better described as grids-of-peaks rather than a segmentation into TADs, and underscoring how a single process, loop extrusion limited by barriers, can give rise to multiple distinct 3D contact patterns.

A crucial prediction of our loop extrusion simulations is that depletion of extruders in meiosis would lead to both decompaction (Supplementary Fig. 5a–c) and loss of the grid-like pattern of Hi–C interactions. When we repeated our fitting procedure for rec8Δ, the best fits were for simulations with either no, or very few, extruded loops (Supplementary Fig. 5e). The lack of compaction in these simulations is consistent with previous EM showing decompacted chromatids in rec8Δ4. Such joint consistency between Hi–C and imaging data further supports loop extrusion as a mechanism underlying assembly of the cohesin-rich core and contributing to chromosomal compaction in meiosis. Our simulations also open the possibility that overly shortened axes observed upon Wapl45,46 and Pds52–5727 deformation may reflect heightened extruder processivity48 upon which shortened SCs are assembled, and predict that such perturbations would cause a rightward shift in the P(δ) shoulder measured via Hi-C (Supplementary Fig. 5c).

The synaptosomal complex modulates chromosome compaction. To investigate how homologue synopsis affects chromosome conformation, we assayed pachytene cells in the absence of Zip1, the transverse filament of the SC, and Hop1, an axial element required for Zip1 loading (Fig. 5a, b). Despite unsynapsed axial elements forming, hop1Δ and zip1Δ mutants proceed through both meiotic nuclear divisions—with a partial delay in prophase I in zip1Δ cells—generating spores with low viability49–51. Thus, to aid direct comparisons we again combined the use of the ndt80Δ allele to prevent exit from prophase. Both zip1Δ and hop1Δ mutants display punctate Hi–C interactions (Fig. 5b, Supplementary Fig. 4b, c), and displayed compaction relative to G1 or rec8Δ, but with the P(δ) shoulder shifted left relative to ndt80Δ (Fig. 5c). Attempts to model the known zip1Δ and hop1Δ defects in chromosome synopsis simply by removing interhomologue crosslinks from best-fitting ndt80Δ simulations did not recapitulate the P(δ) shift observed experimentally (Supplementary Fig. 5f). Instead, best-fitting simulations had shifts towards slightly lower processivity and larger separation (Fig. 5d), consistent with less axial compaction relative to the ndt80Δ control (Fig. 5c). Interestingly, subtelomeric regions no longer displayed a distinct P(δ) in zip1Δ and hop1Δ (Fig. 5e), suggesting that chromosome compaction at chromosome termini is regulated differentially.

Discussion

Our analysis of meiotic chromosome organisation via Hi–C reconciles the function and localisation of factors thought to shape meiotic chromosomes with their 3D organisation (Fig. 6a), revealing the emergence of a punctate grid of interactions
Modelling meiotic chromosome compaction. 

In simulations, yeast Chr13 was represented as a polymer fibre confined to the nucleus subject to additional meiosis-specific constraints. These include extruded loops, sister crosslinks and homologue crosslinks (Methods). Barriers to extruded loops were placed at Rec8 sites. We imposed inter-sister and inter-homologue crosslinks at sites of extruded loop bases in order to approximate the paired arrangement of homologues at pachytene. For each set of extruded loop parameters (processivity, separation and barrier strength), conformations were collected and used to generate simulated contact maps. Roughly, processivity dictates the size of an extruded loop unimpeded by collisions, separation controls the number of active extruders on the chromosome, and barrier strength controls the probability that an extruder gets paused when attempting to step past a barrier. Goodness-of-fit was then evaluated using the combined average fold discrepancy between $P(s)$ curves for Rec8-Rec8, Rec8-non and non-non bin pairs at 2 kb resolution. Note that a value of 1 indicates perfect agreement between simulations and experimental data.

**b** Goodness-of-fit for indicated barrier strengths over coarse grids of processivity and separation demonstrate that intermediate barrier strengths are required to agree with experimental ndt80Δ Hi-C maps. 

**c** Goodness-of-fit for a fine grid of processivity versus separation at barrier strength 0.95 (and for 0.90; Supplementary Fig. 5d). Best-fitting models had separation ~32 kb and processivity ~76 kb, corresponding to ~64% coverage of the genome by extruded loops of average length 26 kb.

**d** From left to right: contact maps for chromosome 13 for experimental data, and simulations with (i) best-fitting parameters, (ii) relatively stable loops between neighbouring Rec8 sites, (iii) no barriers, (iv) square TAD-like patterns of enriched contacts. Positions for each of these parameter sets indicated in **b, c, e** $P(s)$ split by Rec8-Rec8, Rec8-non and non-non, as in Fig. 2e.

**Fig. 3** Modelling meiotic chromosome compaction.

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concomitant with initial stages of meiotic chromosome compaction. Crucially, we formally demonstrate the link between preferential positioning of meiotic cohesin along the genome\textsuperscript{11,30} and the inference that these loci come into close proximity based on the localisation of Rec8 to the chromosomal axes\textsuperscript{4}. In the context of recent characterisations of mammalian meiosis via Hi–C\textsuperscript{17–19}, our results highlight similarities and differences across species. In all cases, meiotic contact frequency versus distance curves display a prominent shoulder consistent with cis-loop formation. Only \textit{S. cerevisiae}, however, display punctate grid-like patterns of Hi–C enrichment all along chromosomal arms. This argues that the positioning of underlying loops may be much more stochastic from cell-to-cell in mammalian meiosis. Additionally, the plaid-like patterns observed in mammalian meiosis, yet not observed in yeast, suggest that additional mechanisms, beyond loop extrusion, are at play in mammalian meiosis.

Remarkably, the punctate cohesin-dependent interactions in yeast meiosis emerge despite the absence of CTCF in this organism; this challenges previous models where focal Hi–C peaks are strictly dependent on CTCF\textsuperscript{31,37,52}, and indicates that alternative mechanisms of loop positioning must exist. Transcription constitutes a promising candidate for a mechanism of loop positioning that does not rely on CTCF\textsuperscript{53–55}. Indeed, previous studies highlight the correspondence between cohesin positioning and convergent transcription in both yeast meiosis\textsuperscript{11} and mitosis\textsuperscript{56,57}. Moreover, whilst much less prominent than in meiosis, we find that locus-specific folding is evident in new high-resolution Hi–C maps of mitotic cells (Fig. 6b, c). Finally, in
agreement with a role for transcription in loop positioning, we observe an enrichment of convergently oriented TSSs around both meiotic and mitotic peak anchors (Fig. 6d, e).

However, whether or not it is transcription per se or the binding of large protein complexes-like RNA polymerase that influences loop positioning is unclear. We favour the view that transcription-associated machinery acts as a barrier to cohesin-dependent loop extrusion (Fig. 6a), rather than as a motive force as previously proposed [11,8,39], consistent with transcription-independent compaction by cohesin in mammalian interphase [60] and direct observation of extrusion by the related SMC condensin in vitro [61]. Indeed, the fact that chromosome compaction is interrupted at centromeres in both meiosis (Fig. 1a, g) and mitosis [5,4,6] supports the concept that large protein complexes—like the kinetochore or RNA polymerase—act as potent barriers to loop extrusion.

The reason for why loops are more prominent and strictly positioned in meiosis compared to mitosis is intriguing. However, our observations enable us to rule out the axial element, Hop1, the SC lateral element, Zip1 and the process of homologous recombination mediated by Spo11, Sae2 and Dmc1 (unpub. obs.) as important for the generation of such patterns. The axial element Red1, however, localises to chromosome axes prior to Hop1 in budding yeast [6], and the additional observation that Rec10 (ScRed1) binds to cohesion in fission yeast meiotic prophase [64] make Red1 a possible candidate for regulating loop extrusion dynamics. The strong meiotic Hi-C patterns are also reminiscent of the grid-like Hi-C patterns observed in interphase mammalian cells upon depletion of the cohesin unloader, Wap1 [37,60], wherein “vermicelli”-like chromatids arise with a cohesin-rich backbone [65]—emphasising the influence of cohesin dynamics on loop extrusion, and suggesting that differential cohesin regulation might underpin the differences between meiosis and mitosis.

Exploring our Hi-C data via polymer simulations enabled us to reveal a nuanced picture of meiotic chromosome assembly: loops are, on average, larger than the inter-Rec8 peak distance, and more than half of the loop bases are not associated with preferred sites of Rec8 binding. It is likely that loop sizes and positions vary widely from one cell to another, making classifications of genomic regions as ‘axis’ or ‘loop’ a great oversimplification. Our simulations also illustrate how a single mechanism—loop extrusion—can give rise to divergent Hi-C patterns, either TADs or grids-of-peaks, relevant in different cellular contexts. Indeed, simulations allow us to quantitatively test mechanisms of chromosome folding in addition to reporting the patterns observed in Hi-C maps. Looking more broadly, the agreement between our simulations and experimental data furthers the case for loop extrusion as a general mechanism [29,28,34,66–69] that is flexibly employed and regulated in interphase, mitosis and meiosis.

Finally, our results also reveal how the interplay between the synopsis components, Hop1 and Zip1, influences chromosome
Fig. 6 Underlying mechanisms of chromosome conformation in meiosis and mitosis. a Pathway of meiotic chromosome compaction: Rec8-dependent loop formation leads to initial chromosome arm compaction and emergence of a grid-like pattern of Hi-C interactions that jointly agrees with a mechanism of loop extrusion including barrier elements. We suggest that transcription could impose such barriers. Hop1 and Zip1 are dispensable for this step, but are required for synopsis, where additional compaction occurs differentially along chromosome arms. b Hi-C contact maps of chromosome 11 for meiotic (ndt80Δ, pachytene - top) and mitotic (wild type, nocodazole arrest - bottom) plotted at 2 kb bin resolution. c Zoom-in into contact maps on chromosome 11 (0–200 kb) of ndt80Δ (top) and mitotic (bottom). Arrowheads indicate sites of visually prominent focal interactions. d, e Frequency of TSSs by orientation around meiotic (d) and mitotic (e) Hi-C peak anchors, in 500 bp bins smoothed with a sliding window of three bins to emphasise the enrichment patterns (Methods). N = number of peaks analysed.

Morphology. That Hop1 and Zip1 are both required to increase chromosome compaction at pachytene likely points at their joint role in promoting synopsis, and supports the view that synopsis itself modulates axial compaction (Fig. 6a). While mouse spermatocytes defective for SC formation also show changes in chromosome compaction, developmental arrest of these mutants in a zygotene-like state makes it difficult to interpret the contribution of the SC to this phenotype. Our data suggests that the SC does have an impact on chromosome compaction because even though zip1Δ cells are partially defective in exiting prophase I, hop1Δ cells are not. Interestingly, whilst Zip1 binds largely uniformly along the arms of pachytene chromosomes, subtelomeres and short chromosomes display an increase in short-range contacts and an earlier shoulder in P(s), consistent with smaller loops or less compression of spacers between loops in these regions, and therefore less axial compaction. Because such differences correlate with disproportionate retention of Hop1 in these regions and diminished efficiency of synopsis, it is possible that Hop1 impedes the pathway whereby Zip1 imposes additional compaction upon synopsis. Nevertheless, it is unclear whether Zip1 mediates this effect by modifying loop extrusion dynamics, or via a distinct process of axial compression, as has been argued for higher eukaryote mitotic chromosome compaction. Given the influence that chromosome structure has over so many aspects of meiosis, teasing apart these mechanisms is of great future interest.

Methods

Yeast strains and cell culture growth. Strains used in this study were derived from SK1 and are listed in Supplementary Table 1. Key genes of interest are summarised in Supplementary Table 2.

Monitoring DNA replication and nuclear divisions. Cells were fixed in 70% EtOH, digested with 1 mg/ml RNase (10 mM Tris-HCl pH 8.0, 15 mM NaCl, 10 mM EDTA pH 8.0) for 2 h at 37 °C, 800 rpm in Thermomixer (Eppendorf) and subsequently treated with 1 mg/ml Proteinase K in 50 mM Tris-HCl pH 8.0 at 50 °C, 800 rpm (as above) for 30 min for analysis by FACS. Cells were then washed in 50 mM Tris-HCl pH 8.0 and stained in the same buffer with 1 μM Sytox green or 1 μg/ml Propidium Iodide (PI) overnight in the fridge. Samples were processed on an Accuri C6. Collected FACS profiles were plotted with R using the library hwglabr2 (https://github.com/hochwagenlab/hwglabr2), applying the following gates: For Sytox green (gate = c (200000, 3000000)) and for PI (gate = c (800, 10000)). Fixed cells were also used for quantification of nuclear divisions by spreading onto a microscope slide, mounting with Fluoroshield containing DAPI followed by analysis with a Zeiss Scope.A1 microscope.
**Hi-C library preparation.** The Hi-C protocol used was amended from 25% to 5-fold reduction in all materials and volumes. Briefly, for meiotic samples, S. cerevisiae cells were synchronized in Gei-0 at 30 °C –16 h in 30 ml YPA (1% Yeast extract, 2% Peptone, 1% K-acetate) to OD600 of ~4, harvested, washed and resuspended in prewarmed sporulation medium (2% K-acetate with 0.2x nutritional supplements adenine, histidine, leucine, tryptophan and uracil) before fixing for 5 ml aliquots (20–30 ODs) of relevant timepoints with formaldehyde at 3% final concentration for 20 min at 30 °C, in an orbital shaker at 280 rpm, then quenched by incubating with a final concentration of 0.35 M Glycine (2x the volume of Formaldehyde added) for an additional 5 min. Cells were washed with water, split into two samples (for two libraries) and stored at 80 °C ready for library preparation.

For meiotic samples, S. cerevisiae diploid cells were grown in YPD (1% Yeast extract, 2% Peptone, 2% Glucose) to exponential phase, 10 µg/ml of nocodazole were added and 100 ml of cells (50–80 OD, sufficient for 1 Hi-C library) were fixed and stored (as described above). Cells were thawed, washed in spheroplasting buffer (SB, 1 M Sorbitol, 50 mM Tris pH 7.5) and digested with 100 µg/ml 100 T Zymolyase in SB containing 1% beta-Mercaptoethanol for 15–20 min at 35 °C. Cells were suspended in restriction enzyme buffer (NER1), chromatin was solubilised by adding SDS to 0.1% and incubating at 10 °C for 10 min. Excess SDS was quenched by addition of Triton X100 to 1%, and chromatin was incubated with 2.07 μl of DpnII overnight at 37 °C. DNA ends were filled in with nucleotide triphosphates using 100 μl of Klenow fragment DNA polymerase I at 37 °C for 2 h, followed by addition of DpnII to 0.5% and incubation at 65 °C for 20 min to inactivate Klenow and further solubilise the chromatin. The sample volume was diluted 15-fold, crosslinked DNA ends ligated at 16 °C for 8 h using 0.024 μl of T4 DNA ligase, and crosslinks reversed by overnight incubation at 65 °C in the presence of proteinase K. DNA was purified by phenol/chloroform:isoamyl alcohol precipitation and precipitated with ethanol, dissolved in TE and passed through an Amicon 30 kDa column. DNA was further purified by phenol: chloroform/isoamyl alcohol extraction and precipitated again before treating with RNase A at 37 °C for 1 h. Biotin was removed from unligated ends by incubation with T4 DNA polymerase and low abundance of dNTPs (0.05 mM) at 20 °C for 4 h and at 37 °C for 4 h. DNA was still fragmented using a Covaris M220 (Duty factor 200, 200 cycles/burst, 350 ± 20 °C), purified with Qiagen MinElute columns and DNA ends were repaired using T4 DNA polymerase, T4 Polynucleotidase Kinase and Klenow fragment DNA polymerase I. A 2.5 x molar excess of Klenow and A-tailed biotinylated isolated fragments of 100–250 bp using a Blue Pippin (Sage). Biotinylated fragments were enriched using streptavidin magnetic beads (Clontrex Bioo Scientific) barcode adapters were ligated while the DNA was on the beads. Resulting libraries were minimally amplified by PCR and sequenced with paired-end 42 bp reads on a NextSeq500 (Illumina; Brigham Genomics).

**Hi-C data processing and analysis.** Hi-C sparse matrices were generated at varying spatial resolutions using the Hi-C-pro pipeline73, using a customised S288c genome view by dividing the genome into 2032 bins (Δ80 kb), each bin being 0.2x nutritional supplements adenine, histidine, leucine, tryptophan and uracil) and 4200 bins (Δ800 kb). In wt-0h and wt-4h, chr1 was excluded from downstream analysis as few informative bins remained after this distance, was calculated for each pair of bins-pairs where one bin was closer to a centromere than telomere along that arm. Contacts were recorded between any two monomers in a given conformation at steady-state, generated simulated chromosome 13 Hi-C maps, of the full system, which includes intra- and inter-sister, and interhomologue contacts. Because experimental Hi-C maps show homologues (13 pairs-of-pairs). For simulations with sister crosslinks, these were added (following Goloborodko et al.84) when extruded loop bases were present at cognate positions ±30 monomers (~20 kb) on both chromatids (distance = 20 nm); homologous crosslinks were added similarly when sister crosslinks were present on both chromatids (distance = 100 nm); centromeres and telomeres were always paired, and both presented impermeable (strength ~1) boundaries to extruders. To avoid introducing pseudo-knots, if extruded loops were nested only the outer cohens were considered as possible bases for sister crosslinks, sister crosslinks were only allowed between the same side of loop bases (i.e. left-to-left arm or right-to-right arm), and sister crosslinks were only added between bases at the reciprocal monomer distance.

To calculate simulated Hi-C maps, contacts were recorded from conformations of the full system, which includes intra- and inter-sister, and interhomologue contacts. Because experimental Hi-C here does not distinguish either sisters or homologues, contacts were then aggregated into one simulated map. For each model and parameter set we investigated, we collected ensemble of conformations at steady-state, generated simulated chromosome 13 Hi-C maps, and compared their features and P(s) with those from experimental Hi-C maps. Contacts were recorded between any two monomers in a given conformation separated by <60 nm. Each simulated chromosome 13 map represented an average over 40 independent simulations, and for each model and parameter set we investigated, we collected ensemble of conformations at steady-state, generated simulated chromosome 13 Hi-C maps, and compared their features and P(s) with those from experimental Hi-C maps.
Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Raw sequence reads are accessible via the SRA repository GSE127940. Hi-C matrices publicly viewable via the interactive HiGlass viewer1, hosted athttp://higlass.pollard.gladstone.org. All other relevant data supporting the key findings of this study are available within the article and its Supplementary Information files or from the corresponding authors upon reasonable request. Data monitoring the cell culture informations the cell culture communicated. Additional data are available within the article and its Supplementary Information.

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Author contributions

SAS and MJN planned the study, performed wet-lab work and data analysis. GF and KSP developed polymer simulations and performed data analysis. SAS, GF, JB, KSP and MJN discussed results and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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