Condensin-mediated remodeling of the mitotic chromatin landscape in fission yeast

Yasutaka Kakui, Adam Rabinowitz, David J Barry & Frank Uhlmann

The eukaryotic genome consists of DNA molecules far longer than the cells that contain them. They reach their greatest compaction during chromosome condensation in mitosis. This process is aided by condensin, a structural maintenance of chromosomes (SMC) family member. The spatial organization of mitotic chromosomes and how condensin shapes chromatin architecture are not yet fully understood. Here we use chromosome conformation capture (Hi-C) to study mitotic chromosome condensation in the fission yeast Schizosaccharomyces pombe. This showed that the interphase landscape characterized by small chromatin domains is replaced by fewer but larger domains in mitosis. Condensin achieves this by setting up longer-range, intrachromosomal DNA interactions, which compact and individualize chromosomes. At the same time, local chromatin contacts are constrained by condensin, with profound implications for local chromatin function during mitosis. Our results highlight condensin as a major determinant that changes the chromatin landscape as cells prepare their genomes for cell division.

Mitotic chromosomes are one of the most recognizable structures in eukaryotic cells. While their microscopic description goes back to the nineteenth century, knowledge of their molecular architecture remains scarce. Mitotic chromosome condensation is promoted by the chromosomal condensin complex, a ring-shaped multisubunit protein assembly that topologically entraps DNA and that is thought to establish linkages between more than one DNA. How condensin promotes chromosome condensation remains a topic of intense current interest. Chromosome conformation capture is a powerful tool to visualize patterns of chromosomal DNA contacts, yielding insight into how the DNA is arranged inside chromosomes. This technique identified large-scale homogenous folding of the chromatin chain within human mitotic chromosomes. How local chromatin behavior changes as a result of mitotic chromosome compaction and how the condensin complex contributes to this process remain important open questions.

To understand the changes of mitotic chromatin organization at high resolution, we applied a high-throughput chromosome conformation capture variant, Hi-C, to fission yeast. The small size of the fission yeast genome, distributed among three chromosomes, and its use of a fundamentally conserved chromosome condensation machinery make this organism an attractive choice. We compared chromosomal DNA contact maps between asynchronously growing cells, >90% of which are in the G2 stage of interphase, and cells arrested in mitosis by transcriptional repression of the Slp1Cdc20 activator of the anaphase-promoting complex (Supplementary Fig. 1a). In our Hi-C analysis, mapped paired-end reads indicative of a DNA interaction were assigned to the nearest restriction site used for DNA fragmentation (DpnII), and these sites were then grouped into 2-kb bins for further analysis (Supplementary Fig. 2a). This resulted in high-resolution DNA interaction frequency maps of the fission yeast genome (Fig. 1a). High reproducibility of the interaction maps under these and each of the following biological conditions was confirmed by clustering of the Euclidean distance between the directionality plots of three independent repeats of each experiment (Supplementary Fig. 2b,c).

In addition to the expected richness of local DNA interactions along the three fission yeast chromosomes, seen in dark colors along the diagonal of the interphase contact map, prominent inter-centromeric and inter-telomeric interactions between chromosomes were apparent (Fig. 1a, lower left triangle; compare to Supplementary Fig. 3 for an annotated map). This is expected from the Rabl orientation of fission yeast chromosomes in interphase. In mitosis, interactions appeared to spread to greater distances from the diagonal, seen by the broadening of the dark region (Fig. 1a, upper right triangle). In contrast, interactions between chromosomes and between the two arms of each chromosome were reduced, as indicated by the lighter orange color of these regions. Contact probability distribution plots confirmed a quantitative enrichment of interactions within chromosome arms in mitosis (Fig. 1b), at the expense of interactions between arms and between chromosomes. This provides a molecular correlate of the cytologically observed chromosome individualization and chromosome arm stiffening in mitosis (Supplementary Fig. 1a). The changes in contact probabilities between interphase and mitosis were seen more clearly on Hi-C difference maps, obtained by simple subtraction of the normalized contact probabilities (Fig. 2a, upper right triangle). This confirmed an increased frequency of interactions within chromosome arms in mitosis, as well as the accompanying reduction in inter-arm and inter-chromosomal interactions. To elucidate

1Chromosome Segregation Laboratory, The Francis Crick Institute, London, UK. 2Bioinformatics Core, The Francis Crick Institute, London, UK. 3Advanced Light Microscopy Facility, The Francis Crick Institute, London, UK. Correspondence should be addressed to F.U. (frank.uhlmann@crick.ac.uk).

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the contribution of condensin to the altered contact probabilities in mitosis, we established a conditional shutoff allele for the condensin subunit Cut14Smc2 by combining promoter repression with an auxin-inducible degron (cut14SO). The efficiency of Cut14 shutoff was confirmed by loss of mitotic chromosome compaction, measured by the distance between two fluorescent marks on chromosome I (Supplementary Fig. 1c–e). A Hi-C difference map between cells in interphase and condensin-depleted mitotic cells showed hardly any changes in contact probabilities (Fig. 2a, lower left triangle). Contact probability distribution plots confirmed that the previously seen changes in mitosis, namely increased intra-arm interactions, as well as reduced inter-chromosomal and inter-arm interactions, all depended on condensin (Fig. 2b; compare to Fig. 1b). Similar results were obtained after depletion of the condensin subunit Cnd3CAP-G or following inhibition of Aurora B kinase, a positive regulator of mitotic condensation, although Cnd3CAP-G was depleted less efficiently than Cut14Smc2 and Aurora B inhibition only partly blocked chromosome condensation (Supplementary Figs. 1b, f and 4a–c). A Hi-C difference map of mitotic cells containing or depleted of Cut14Smc2 further illustrated that condensin is responsible for most of the observed contact probability changes in mitosis (Fig. 2c).

Hi-C differences indicate relative, but not necessarily absolute, changes of contact probability values. We therefore used 3C followed by quantitative real-time PCR (3C–qPCR) to calibrate our Hi-C difference maps. We chose inter-centromeric interactions as a benchmark, which are among the strongest inter-chromosomal contacts and are reliably detectable. 3C–qPCR showed that the interaction frequency between centromeres dropped by just over twofold in mitosis, consistent with the almost twofold reduction in inter-centromeric interactions seen in the Hi-C difference maps (Fig. 2d). Both the Hi-C difference map and 3C–qPCR suggested that inter-centromeric contacts were retained in mitotic cells lacking condensin (Fig. 2d).

To confirm this by an independent technique, we microscopically visualized centromere positioning in wild-type and cut14SO cells arrested in mitosis. Centromeres were separated and distributed along the short metaphase spindle in wild-type cells, as previously described (Fig. 2e,f). Centromeres remained clustered close to the spindle poles in Cut14-depleted cells, often displaying stretched signals. This pattern is consistent with persistent inter-centromere contacts and reminiscent of mitotic defects that have previously been reported in the absence of condensin. Thus, the contact frequency changes reported in our Hi-C difference maps are a good reflection of absolute changes that occur during mitotic chromosome condensation.

We next focused our analysis on the most frequent DNA contacts, those within chromosome arms, as these are the most likely drivers of mitotic chromosome architecture changes. Figure 3a shows a magnified view of the Hi-C difference map along the right arm of chromosome II, demonstrating increased longer-range interactions within the arm. Unexpectedly, we also noticed a marked reduction in short-range contacts, seen as a blue line along the base. Both changes
Figure 3 Condensin replaces local contacts with longer-range interactions in mitosis. (a,b) Hi-C difference maps of the chromosome II right arm comparing interphase and mitosis in wild-type (a) and cut14SO (b) cells. (c) Median contact probabilities in interphase and mitosis as a function of distance along the chromosome II right arm are shown in wild-type (top) and cut14SO (bottom) cells. (d) 4C-like plot of Hi-C contact probability from a viewpoint on the chromosome II right arm. The positions of 3C–qPCR primer-binding sites are shown (dotted and solid lines). (e) Interaction changes between interphase and mitosis determined by 3C–qPCR (mean ± s.e.m. of three biological repeats) are compared to 4C-like contact probability changes based on the Hi-C data (dots). Lines represent smoothed 4C-like contact probability changes. (f) Box plots of the distribution of contact frequencies within all chromosome arms under the indicated conditions: I, interphase; M, mitosis. Box plots show the median and 25th and 75th percentiles; whiskers indicate the 95% confidence interval. Outliers are also shown. (g) Moving intra-arm median interacting distances along chromosome II (solid lines) are shown together with shaded areas representing the 25th and 75th percentiles.

The reduction of megabase-scale contacts is reminiscent of reduced longest-range interactions as human chromosomes condense, a probable reflection of chromosome-arm stiffening as mitotic chromosomes gain shape.

The chromosome volume decreases during mitotic condensation, resulting in chromatin compaction. A decrease in local chromatin contacts within mitotic chromosomes came therefore as a surprise. To further investigate this, we plotted interaction frequencies from one selected viewpoint in the Hi-C data to obtain a 4C-like plot.

Figure 3d shows such a plot from a viewpoint close to the middle of the chromosome II right arm. We selected seven distances from this viewpoint and compared contact frequency in interphase and mitosis by 3C–qPCR (Fig. 3e). This provided quantitative confirmation of the 4C-like plot. Intra-arm contacts closer than 90 kb were reduced up to twofold in mitosis, while longer-range interactions increased to a similar extent, consistent with the changes seen in the Hi-C difference map. Both effects again depended on condensin. Thus, an unanticipated consequence of chromosome condensation is a reduction in local chromatin contacts.

4C-like analysis also allowed us to address whether chromosomes condense homogeneously or whether compaction varies along chromosome arms. We used a sliding viewpoint and recorded the contact distributions at each position along chromosome II. The median length of all interactions was around 50 kb in interphase, which increased to around 150 kb in mitosis (Fig. 3f). Plotting the sliding median along chromosome II showed somewhat longer interactions in the middle of both the left and right chromosome arms and shorter

Figure 4 Condensin-dependent chromatin domain expansion in mitosis. (a) Normalized Hi-C maps are shown with domain boundaries along a section of the chromosome I left arm under the indicated conditions. For domain visualization, a bin size of 5-kb was used. Black triangles indicate domain boundaries. (b) Density plots of domain size distributions. (c) Distribution of normalized contact probabilities of 5-kb bins containing or not containing a condensin-binding site. A Wilcoxon Mann–Whitney test was used to test the null hypothesis that contact probabilities between condensin-binding and non-binding sites are the same. (d) Contact probability changes between interphase and mitosis are plotted as a function of distance, separated into bins containing or not containing a condensin-binding site.
interactions toward the centromeres and chromosome ends (Fig. 3g). This trend was not an artifact of the iteratively corrected normalization used to generate our Hi-C maps and was similarly seen when using an alternative normalization strategy, as well as in the raw read counts (Supplementary Fig. 5). This implies that fission yeast chromosomes are slightly more compact in the middle of their arms than in centromere-proximal regions or chromosome ends.

Chromosomes are thought to consist of a series of topologically associating domains (TADs)9,24–26, within which chromatin interactions are enriched. We analyzed TADs along fission yeast chromosomes in interphase and mitosis by plotting the directionality of interactions along the chromosome (Supplementary Fig. 2b), from which we determined TAD boundary positions (Fig. 4a). These boundaries correlated with local minima of insulation scores, thus validating the boundary assignments (Supplementary Fig. 2d,e). This analysis confirmed the existence of numerous TADs in interphase with a median size of 267 kb. The median TAD size nearly doubled in mitosis to 481 kb, dependent on condensin as well as reduced local chromatin motility.

We started to address how condensin promotes longer-range interactions by performing ChIP to determine condensin-binding sites. We divided the chromosome into 5-kb bins that were categorized into those that did or did not contain a condensin-enriched site (Supplementary Table 1). The contact probability plot of these 5-kb bins showed that condensin-binding sites have a small but significantly greater probability of engaging in intra-arm interactions, as compared to non-binding sites (Fig. 4c). This suggests that condensin acts by engaging in contacts between its binding sites. Next, we analyzed whether condensin-binding sites show a preference for longer-range DNA interactions. The mitotic interaction frequency change of condensin-binding sites as a function of genomic distance confirmed that these sites engage in mitotic longer-range interactions in a condensin-dependent manner. However, sites not binding condensin showed a qualitatively similar behavior (Fig. 4d). Therefore, condensin-binding sites are preferential targets for intra-arm interactions that facilitate contacts between neighboring DNA sequences. While site-specific looping between condensin-binding sites has been seen by ChIA-PET27, our Hi-C approach finds that intra-arm interactions are widely spread. Condensin binding to chromosomes with limited sequence preference could be a reason for this.

To further clarify the role of condensin-binding sites in chromatin organization, we analyzed their relationship with chromatin boundary positions. Most interphase and mitotic boundaries contained a condensin-binding site (Supplementary Fig. 8), consistent with a role for condensin in domain formation and their fusion in mitosis.

An unexpected consequence of mitotic chromosome condensation was the reduction in local chromatin contacts. To address whether this is due to restricted chromatin motility in mitosis, we monitored local movement of two loci on chromosomes I and II, visualized using LacO repeats bound by LacI-GFP and TetO repeats bound by TetR-tdTomato, respectively, by high-speed confocal microscopy. Kymographs of the Lac-GFP movements on chromosome I showed greater motility in interphase than mitosis (Fig. 5a). To quantify this difference, we tracked the GFP locus at 20-ms intervals and derived mean square displacement plots. This confirmed that chromatin motility is constrained in mitosis (Fig. 5b). The same was observed on chromosome II (Supplementary Fig. 9). Constrained mitotic chromatin motility depended on condensin. Motility markedly increased following condensin depletion, exceeding even that observed in interphase (Fig. 5b). We could not directly measure condensin’s impact on interphase motility, as the condensin depletion that we achieve during mitotic arrest requires longer than cells usually spend in interphase.

Our study describes the conformational changes of eukaryotic chromatin during mitotic chromosome condensation. Condensin replaces predominantly short-range local contacts in interphase with longer-range interactions in mitosis. Our results do not distinguish whether condensin establishes longer-range interactions by extruding or expanding DNA loops, by stabilizing stochastic chromatin interactions, or both. In either scenario, a slower dissociation rate of condensin from chromosomes, which has been observed in mitosis, could promote longer-range interactions. It will be interesting to computationally study predictions from the two models and compare them to our high-resolution experimental data sets. A notable consequence of mitotic longer-range interactions is restricted chromatin motility, accompanied by reduced local contacts (Fig. 5c). Establishment of gene silencing in budding yeast requires passage through mitosis, while transcriptional reprogramming in mammalian nuclei is promoted by mitotic factors, in ways that are not fully understood. Future studies will explore whether reduced local chromatin motility facilitates either process. In line with the central role played by condensin during biochemically reconstituted chromosome assembly, our findings illustrate condensin’s principal contribution to chromatin reorganization during mitotic chromosome condensation.

URLS. ParticleTracker plugin for tracking chromatin motility, https://bitbucket.org/djbarry/particletracker; Gene Expression Omnibus, https://www.ncbi.nlm.nih.gov/geo/; computer code for analysis of Hi-C data at GitHub, https://github.com/adam-rabinowitz/Condensin-mediated-remodeling-of-the-mitotic-chromatin-landscape.
METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.K. and F.U. conceived the study, Y.K. performed the experiments, Y.K., A.R. and D.I.B. analyzed the data, and Y.K. and F.U. wrote the manuscript with input from A.R.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

S. pombe strains and culture. All the strains used in this study are listed in Supplementary Table 2. PCR-based gene targeting45 and standard genetic manipulation46 were used during strain construction. To obtain the cut14bio and cnd3 strains, the corresponding gene sequence for the C terminus was fused to the sequence-encoding the auxin-inducible degron module IAA17 (ref. 37), and the endogenous gene promoter was replaced by the nmt81 promoter48 in cells harboring Skp1–Tirf1 (ref. 16). For the Zz-Cnd3-GFP strain, a plasmid containing the cnd3 gene fused with GFP under the regulation of the endogenous cnd3 promoter was constructed by the module-based Golden Gate method for chromosomal integration plasmids in fission yeast49. The resultant plasmid was linearized by enzymatic digestion (FseI) and integrated at the Zz locus. To replace the endogenous slp1 promoter, a Kan’–nmt81 promoter–slp1 fragment was amplified from a previously described slp1 shuts off strain53 and integrated.

All strains were cultured in Edinburgh minimal medium (EMM) supplemented with l-glutamic acid as a nitrogen source. To shut off slp1 transcription and induce mitotic arrest, cells were cultured at 25 °C for 3 h after addition of 5 µg/ml thiamine. After this, 60–75% of cells were arrested in mitosis, displaying a short mitotic spindle. The efficiency of this arrest was unaffected by condensin depletion or Aurora B kinase inactivation. To deplete Cut14 or Cnd3, cells were cultured at 25 °C for 3 h with 5 µg/ml thiamine as well as 0.5 mM of the auxin 1-naphthaleneacetic acid (NAA). To inhibit Aurora B kinase (Ark1), ankt1–as3 cells48 were cultured with 5 µg/ml thiamine at 25 °C for 1.5 h and then with 5 µM 1NM-PP1 or the equivalent amount of DMSO as a control for a further 1.5 h.

Fluorescence microscopy. Cells were fixed with 70% ethanol and stained with 4′,6-diamidino-2-phenylindole (DAPI). Images were acquired as serial sections along the z axis on a DeltaVision microscope system (Applied Precision) and combined using the quick projection algorithm in SoftWoRx. To measure the distance between genomic loci marked with LacO repeats bound by LacI-GFP and TetO repeats bound by TetR-tdTomato, z stacks were merged and the distance was measured in 2D using Fiji57. To track chromatin motility, live-cell imaging was performed using an α Plan Apochromat 100×/1.46 NA oil objective (Carl Zeiss) in a temperature-controlled chamber. Images were captured on an Evolve S12 EMCCD camera (Photometrics), operated in streaming mode and mounted on an inverted microscope (AxioObserver.Z1, Carl Zeiss), the constituents of a custom-built spinning-disc confocal system (Intelligent Imaging Innovations). All hardware was controlled with SlideBook software (Intelligent Imaging Innovations). Images were collected at 20 Hz (TetR-tdTomato) or 50 Hz (LacI-GFP), following excitation with either a 488- or 568-nm laser. Fluorescent spot movement was automatically tracked, and MSD was calculated using the ParticleTracker plugin in Fiji (see URLs).

Immunoblotting. Cell extracts were prepared by glass bead cell breakage as detailed below. Protein levels were analyzed by SDS–PAGE followed by immunoblotting using antibody to AID (Cosmo Bio, CAC-APC004AM) or Tat1 (a mouse monoclonal antibody generated in house against α-tubulin) as primary antibody.

Hi-C and 3C sample preparation. The Hi-C experiment was performed as previously described, with modifications2. Approximately 7.5 × 10^9 fission yeast cells were fixed with 0.5% formaldehyde at 25 °C for 30 min. Excess formaldehyde was quenched with 0.125 M glycine at 25 °C for 15 min. Fixed cells were washed three times with wash buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100 and 1 mg/ml NaN3) and resuspended in FA buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100 and 0.1% sodium deoxycholate) containing Complete ULTRA protease inhibitor cocktail (Roche). Cells were ruptured using glass bead breakage in a Multi Beads Shocker (Yasu Kikai). Crude extract was centrifuged to retrieve the chromatin fraction at 20,800g at 4 °C for 15 min. Precipitated chromatin was washed once with DpnII restriction enzyme incubation buffer (New England BioLabs) and then resuspended in DpnII buffer and incubated at 65 °C for 10 min with 0.1% SDS. After addition of 1% Triton X-100, chromatin was digested overnight with DpnII (New England BioLabs) and RNase A (Sigma) at 37 °C. After heat inactivation of DpnII at 65 °C for 20 min, the digested chromatin was divided into three parts, one for 3C library preparation and the other two for Hi-C library preparation. For Hi-C library preparation, DpnII overhangs were filled in with biotin-14-dATP, dCTP, dGTP and dTTP using DNA polymerase I Klenow fragment (New England BioLabs) at 37 °C for 45 min. To capture proximity, chromatin was diluted to 8 ml in T4 DNA ligase buffer followed by incubation at 16 °C for 8 h with T4 DNA ligase (New England BioLabs). For 3C library preparation, digested chromatin was similarly ligated, but without the fill-in step. DNA was in both cases recovered by proteinase K digestion and reversal of cross-linking overnight at 65 °C, followed by phenol–chloroform extraction and isopropanol precipitation. Hi-C library preparation proceeded by removing biotin-14-dATP at unligated ends by incubation at 20 °C for 4 h in the presence of 300 U of T4 DNA polymerase (New England BioLabs). After inactivating T4 DNA polymerase by addition of EDTA, DNA was fragmented using a focused ultrasonicator (Covaris, S220) and purified on AMPure XP beads (Beckman Coulter). The ends of the fragmented DNA were repaired, and sequencing adaptors were ligated using NEBNext Ultra DNA Library Prep kit reagents and NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, New England BioLabs). Junction-containing biotylated DNA fragments were adsorbed to MyOne Streptavidin C1 Dynabeads (Thermo Fisher) at room temperature for 15 min. Bead-bound DNA was amplified using NEBNext High-Fidelity 2x PCR Master Mix with index primer sets. DNA fragments in the 200- to 700-bp range were purified by double selection on SPRI selection beads (Beckman Coulter), followed by sequencing on the Illumina HiSeq platform (100-bp paired-end reads).

3C–qPCR. 3C libraries were prepared as described above. The efficiency of proximal ligation was quantified using primer pairs corresponding to interactions of interest (listed in Supplementary Table 3). Real-time qPCR was performed with PowerUp SYBR Green Master Mix using a QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems). Ct values were normalized using internal controls (YK377–YK378 for inter-centromeric interactions, YK717–YK718 for intra-arm interactions), and ΔCt values comparing interphase and mitosis were then calculated. For quantification of inter-centromeric interactions, ΔCt values were converted to a linear scale to derive fold-change values. Two technical replicates were averaged in each of three biological repeats of the experiment. The median and range of values from the three repeat experiments are reported. To quantify intra-arm interactions, again two technical replicates were averaged in each of three biological repeats. The mean of ΔCt values between interphase and mitosis in the three biological repeats was plotted together with the standard error.

ChiP–seq sample preparation. Cells were fixed with 0.5% formaldehyde, and crude cell extracts were then prepared by glass bead breakage as described above. Extracts were sonicated using a Bioruptor (Diagenode). After removing debris by centrifugation, extracts were split into input and ChiP samples. The ChiP sample was incubated with antibody to HA (F-7; Santa Cruz Biotechnology, sc-7392) overnight at 4 °C. Precipitated DNA–protein complexes were washed twice with FA buffer, twice with FA buffer containing 500 mM NaCl, twice with ChiP wash buffer (10 mM Tris–HCl pH 8, 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate and 1 mM EDTA) and once with TE. DNA ends were repaired by incubation with Klenow enzyme (New England BioLabs) in the presence of dNTPs at 25 °C for 30 min. End-repaired DNA–protein complex was eluted with TE containing 1% SDS by incubating at 25 °C for 30 min. Eluted DNA–protein complexes were incubated with T4 DNA ligase and then incubated at 65 °C overnight, followed by incubation with RNase A at 37 °C for 60 min. DNA was recovered by proteinase K digestion and reversal of cross-linking at 55 °C for 2 h, followed by phenol–chloroform extraction and ethanol precipitation. Sequencing adaptors were ligated to the ends of purified DNA using the NEBNext Ultra DNA Library Prep kit and NEBNext Multiplex Oligos for Illumina. The adaptor-ligated DNA was amplified using NEBNext High-Fidelity 2x PCR Master Mix with index primer sets. Amplified fragments were purified by double selection on SPRI selection beads (Beckman Coulter), followed by sequencing on the Illumina HiSeq platform.

Hi-C data analysis. Identification of ligation events. Reads containing DpnII recognition motifs were trimmed to immediately after the first occurrence of the motif. If either read of the resultant read pair was shorter than 20 bp, the
pair was discarded. Each read of the read pair was aligned individually to the *S. pombe* genome (build ASM294v2.21) using the BWA-mem algorithm (version 0.7.12)\(^\text{42}\). Read pairs with unmapped reads or read alignments with a mapping quality of \(< 10\) were discarded. To remove reads derived from unligated genomic fragments, concordantly mapped read pairs within 2 kb of each other on the genome were also discarded. The sonicatation fragmentation step within the library preparation protocol should generate unique read pairs from the ligation of any two DpnII sites within the genome. Identically mapped read pairs were therefore assumed to be PCR duplicates. A single read from these putative duplicate reads was randomly selected, and the other reads were discarded. The remaining reads were extended to the nearest DpnII recognition site downstream of the read start. Read pairs where either read start site was \(> 1\) kb upstream of a DpnII site were presumed to be derived from unligated fragments and were discarded. Ligation between the remaining read pairs is presumed to have occurred between the downstream DpnII sites. The number of read counts at each step for each of our Hi-C libraries is summarized in Supplementary Table 4.

**Generation of interaction matrices.** Count matrices were generated for chromosomes I, II and III. The genome was divided into equally sized bins (2 kb or 5 kb). Where chromosome length was not an exact multiple of bin length, the maximum number of non-overlapping bins was generated and the most telomeric regions of the chromosome were not analyzed. Few reads, if any, are accurately assigned to these regions owing to their non-unique sequences. Thus, no information is lost, while the equally sized bins ease subsequent statistical analyses. The count matrix is filled by ascribing individual DpnII ligation events to the bins containing their respective DpnII sites. When the ligation event occurs at a bin boundary, the event is ascribed to the bin containing the mapped read. To examine subsets of interactions, for example, inter-chromosome, intra-arm or inter-arm ligation, the count matrices for these regions were simply extracted from the larger count matrix. **Normalization of interaction matrices.** The observed interaction frequency between two genomic regions is affected by many factors other than proximity. Normalization of the interaction matrices aims to remove these ‘non-proximity’ effects. Two techniques were used to normalize the matrices. The first technique, iterative correction\(^\text{43}\), does not attempt to model the source of these non-proximity factors. Instead, it operates on the principle that all genomic regions should be equally visible and partake in an equal number of interactions. The second technique, HiCNorm\(^\text{44}\), attempts to remove the impact of three non-proximity factors known to affect observed interactions—fragend frequency, GC content and mappability. The iteratively corrected normalized matrices were used for most of our analyses, except in Supplementary Figure 5a, where HiCNorm was used.

Prior to normalization, the intra-bin ligation were removed from the matrix by setting the diagonal of the count matrix to zero. Additionally, bins involved in fewer inter-bin interactions than a specified threshold were removed from the matrix to ensure optimal functioning of the normalization algorithm. A threshold of 500 interactions was used for the 2-kb bins (Supplementary Fig. 2a) and a threshold of 1,000 was used for the 5-kb bins. Matrices for the 2-kb bins were used for our analysis if not stated otherwise. The iterative correction algorithm was used to normalize the altered count matrices such that all rows and columns were set to sum to 1. The resultant matrix can therefore be considered to be a probability matrix where the value at point \((i,j)\) is the probability that region \(i\) is found to interact with region \(j\), and vice versa. HiCNorm (version 08.05.2012)\(^\text{44}\) was provided with the mappability scores, GC content and active fragend size of all the 2-kb bins. Mappability scores and GC content were calculated from all regions within a bin that were within 200 bp of a DpnII cut site. Active fragend size is the cumulative length of these regions. Mappability scores were calculated by aligning tiled 50-bp reads (10-bp window shift), extracted from the regions of interest, and identifying the proportion of reads with a mapping quality of 10 or greater as defined by BWA-mem (version 0.7.12)\(^\text{42}\). A floor value for mappability scores was set as 0.1.

**Interaction directionality and Euclidean clustering.** To calculate the interaction directionality for a given bin, the interaction probabilities between the bin and its 50 upstream and 50 downstream bins were extracted from the intra-arm normalized matrix. The upstream and downstream probabilities were then paired by their absolute distance from the bin of interest. The interaction directionality value is the mean value of the log\(_2\) ratio of each pair of upstream and downstream probabilities. A value greater than 0 (a positive log\(_2\) directionality value) indicates that the bin preferentially interacts with upstream regions, whereas a value smaller than 0 (a negative log\(_2\) directionality value) indicates that downstream interactions are favored. Where one or more of the paired probability values were equal to zero, the pair was excluded from the calculation. Where fewer than two paired distance probabilities were available, owing to zero values or the end of the chromosome, no interaction directionality was calculated. Samples were clustered by calculating the Euclidean distance between vectors of log\(_2\) interaction directionality values and performing complete linkage agglomerative hierarchical clustering on the resulting distance matrix.

**Histogram of contact probabilities.** Contact probabilities in whole-genome normalized matrices were categorized into inter-chromosomal, intra-arm and inter-arm interactions according to the position of the bins. The contact probabilities categorized above were used to plot histograms of count distribution in normalized contact probabilities using ggplot2 in R. Contact probabilities in intra-arm normalized matrices were further divided into condensin-binding and non-binding groups according to the presence or absence of ChIP peaks, respectively (see below for detection of ChIP peak positions). Hi-C bins with ChIP peaks were identified using ChiPpeakAnno in Bioconductor\(^\text{45}\). Contact probabilities categorized as above were used to plot histograms.

**Hi-C maps and Hi-C difference maps.** Normalized matrices were plotted using ggplot2 in R. To prepare Hi-C difference maps, differential contact probability matrices were produced by subtraction of a normalized matrix in condition 1 (for example, mitosis) from a normalized matrix in condition 2 (for example, interphase). Then, matrix differences were plotted using ggplot2 in R. Normalized Hi-C maps with domain boundaries were prepared as below. Normalized Hi-C matrices from 5-kb bins were plotted, and the positions of the identified domain boundaries were then plotted on top using ggplot2 in R.

**Calculation of fold change of inter-centromeric interactions.** Bins encompassing centromere sequences and the surrounding 10 kb were identified as centromeric bins. Fold change in inter-centromeric interaction probabilities was calculated after adding up the Hi-C contact probabilities within the centromeric bins from whole-genome normalized matrices. Three biological replicates of Hi-C samples were used to generate box plots for each condition.

**Contact probability as a function of distance.** All the contact probabilities in intra-arm normalized matrices were classified according to the distance between the two underlying bins. The median contact probability was calculated for each distance and then plotted against distance for each condition. To calculate probability–distance quartiles, all the interaction probabilities of a bin of interest were extracted from an intra-arm normalized matrix or from the raw count matrix in the case of Supplementary Figure 5b. The bin probabilities were then ordered by their absolute distance from the bin of interest, and the cumulative probability of the bins was calculated. The 25th, 50th and 75th percentiles are the distances at which the cumulative probability first equaled or exceeded 0.25, 0.50 and 0.75, respectively. 25th-, 50th- (median) and 75th-percentile distances for the bins along chromosome II were plotted for each condition using ggplot2 in R.

**4C-like plots.** A bin in which a primer for 3C–qPCR analysis annealed on the right arm of chromosome II was selected as a viewpoint to generate 4C-like plots. Hi-C contact probabilities between the viewpoint and all other bins within the right arm of chromosome II were then plotted as dots in each condition. Lines of smoothed 4C-like contact probabilities, calculated using the loess-smoothing method within ggplot2 in R, were also plotted.

**Determination of domain boundaries.** Domain boundaries were determined using either log\(_2\) directionality or the TopDom software package (version 0.0.2)\(^\text{46}\) from the intra-arm normalized matrices for 2-kb bins. The log\(_2\) directionality values were calculated as below. A 40-kb sliding window was used to traverse the chromosome arm. For each window position, the maximum log\(_2\) value (log\(_2\) max) within the 20-kb upstream half of the window and the minimum log\(_2\) value (log\(_2\) min) within the 20-kb downstream half of the window were identified. A log\(_2\)-shift value for the window was then calculated by subtracting log\(_2\) min from log\(_2\) max. A putative domain boundary was identified between the log\(_2\) min and log\(_2\) max values if log\(_2\)-shift was greater or equal to 0.5. Putative boundaries were then filtered by grouping overlapping boundary
domains and discarding all domains except the one with the highest log2 shift value. The domain boundary was then assigned to the midpoint between the log2 max and log2 min values for the remaining domain boundaries. To improve the reliability of domain boundary identification, only domain boundaries that were identified in the same location (± 5 kb) in each of three biological replicates were classed as true boundary locations. To plot average log2 directionality at the boundaries, log2 directionality values within 40-kb upstream and downstream regions were normalized. The normalized log2 directionality values were plotted using the geom_smooth function provided by ggplot2 in R. Alternatively, TopDom was used as follows. A window size of ten bins was used to identify boundary positions. Common boundaries that were identified in three biological replicates were used for further analyses. Domain size was calculated as the distance between neighboring boundary positions.

Insulation scores. Insulation scores were generated from the intra-arm normalized matrices for 2-kb bins. Scores were calculated by taking the mean interaction probability across the two bins on either side of the bins of interest. To determine insulation score around the boundaries determined by log2 directionality, insulation scores within 50 kb upstream and downstream were normalized by log2 (insulation score/mean insulation score in the selected region). Average normalized insulation scores were calculated for all the normalized insulation scores at every 10-kb insulation distance. The average normalized insulation scores as a function of insulation distance were plotted using ggplot2 in R.

ChIP–seq data analysis. Each read of the paired-end ChIP–seq library was trimmed to a length of 50 bases. Reads were aligned to the S. pombe genome (build ASM294v2.21) using the BWA-mem algorithm (version 0.7.12)42. ChIP peaks were identified from the alignments using version 2.1.0 of macs2 software with the input format set to BAMPE46. ChIP peaks are listed in Supplementary Table 1.

Code availability. The computer code used to generate the results reported in this manuscript has been deposited with GitHub (see URLs).

Data availability. The Hi-C and ChIP–seq data reported in this manuscript have been deposited with the Gene Expression Omnibus under accession GSE94478. A Life Science Reporting Summary is available.

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Experimental design

1. Sample size

Describe how sample size was determined.

This study uses the fission yeast Schizosaccharomyces pombe as a model. Synchronous cultures of several million cells formed the basis for the Hi-C analyses. When individual cells were analyzed to determine centromere clustering or the chromosome condensation status, we scored at least 100 cells to ensure robust and statistically significant comparisons.

2. Data exclusions

Describe any data exclusions.

No data was excluded, the results of all cells was always included in our analyses.

3. Replication

Describe whether the experimental findings were reliably reproduced.

We confirmed all results reported in this manuscript in three independently performed repeats of each experiment. In the case of the Hi-C analyses, the major tool in our study, the reproducibility among these three repeats is documented in Supplementary Figure 2c. In the case of the cytological analysis contained in Figure 2f, all three obtained datapoints, their mean and standard deviations are reported.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

n/a

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

n/a

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

| n/a | Confirmed |
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| ☐   | ✗         |
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| ☐   | ✗         |
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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
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- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted
- A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Describe the software used to analyze the data in this study. All the software used in our study has been deposited in GitHub and can be freely accessed using the link provided in the Online Methods section.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The Nature Methods guidance for providing algorithms and software for publication may be useful for any submission.

8. Materials and reagents

Materials and reagents

Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies were only used for the western blot contained in Supplementary Figure 2c. The details of these antibodies are contained in the Online Methods, including clone and catalogue number. The specificity of the anti-aid antibody is confirmed in our own study by depletion of the signal in response to auxin-induced degradation of the respectively tagged condensin subunits.

Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

- The widely used Leupold’s 972 isogenic fission yeast strain background was used in all experiments.

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12. Description of human research participants
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