Transcription factors mediate condensin recruitment and global chromosomal organization in fission yeast

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It is becoming clear that structural-maintenance-of-chromosomes (SMC) complexes such as condensin and cohesin are involved in three-dimensional genome organization, yet their exact roles in functional organization remain unclear. We used chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) to comprehensively identify genome-wide associations mediated by condensin and cohesin in fission yeast. We found that although cohesin and condensin often bind to the same loci, they direct different association networks and generate small and larger chromatin domains, respectively. Cohesin mediates associations between loci positioned within 100 kb of each other; condensin can drive longer-range associations. Moreover, condensin, but not cohesin, connects cell cycle–regulated genes bound by mitotic transcription factors. This study describes the different functions of condensin and cohesin in genome organization and how specific transcription factors function in condensin loading, cell cycle–dependent genome organization and mitotic chromosome organization to support faithful chromosome segregation.

The three-dimensional genome structure is coupled to important nuclear processes such as transcription, DNA replication and repair. To understand global genome organization, next-generation sequencing combined with chromosome conformation capture (Hi-C) has been applied to a variety of organisms, from bacteria to humans. These studies collectively show that genomes are highly ordered by a hierarchy of organizing events ranging from gene associations to topologically associating domain (TAD) formation. TADs have recently been identified as functional genome-organizing units related to transcriptional coregulation and replication timing. Condensin and cohesin are implicated in TAD organization, although their roles in domain formation remain enigmatic.

ChIA-PET is a genomic approach that combines chromatin immunoprecipitation (ChIP), proximity ligation and next-generation sequencing to identify the genome-wide associations mediated by particular proteins. This approach has been used to identify genomic associations involving estrogen receptor, CCCTC-binding factor (CTCF), cohesin, RNA polymerase II (Pol II) and histone H3 Lys4 methylation. Mechanistically, cohesin is recruited to CTCF binding sites through interaction with CTCF and mediates associations among genes and their regulatory elements. Another important SMC complex, condensin, is also involved in higher-order genome organization in yeast and other organisms. Condensin binds to Pol III–transcribed genes such as those encoding transfer RNA (tRNA) and 5S rRNA, to Pol II–transcribed housekeeping genes and to long terminal repeat (LTR) retrotransposons, and it clusters these dispersed genetic elements, often associating them with centromeres in fission yeast. It has been shown that cohesin and condensin frequently colocalize to several different genetic elements, including Pol III genes, gene promoters or enhancers and chromatin domain boundaries, in a variety of organisms. However, it remains largely unclear how these two complexes cooperate to organize a functional genome architecture. Using the ChIA-PET genomic approach, we show here how condensin and cohesin mediate domain organizations and genome-wide associations in fission yeast.

RESULTS
Specific cell cycle–regulated genes are bound by condensin
On the basis of ChIA-PET self-ligation reads, condensin (Cut14) and cohesin (Rad21) proteins were mapped to 485 and 475 significant binding sites, respectively, across the genome (Supplementary Fig. 1a and Supplementary Note). Cut14 and Rad21 were often colocalized and detected at dispersed repetitive elements including tRNA and 5S rRNA genes, LTR retrotransposons and centromeres (Fig. 1a and Supplementary Fig. 1b,c). They were also localized at highly expressed Pol II–transcribed genes (Fig. 1b). Transcription levels of Pol II genes appeared to be positively correlated with Cut14 and Rad21 enrichment. The peak of cohesin enrichment was located at the 3′ end of genes but not within gene bodies. The peak of condensin enrichment was also located at the 3′ end, although condensin was also detected to some extent at gene bodies. Previous studies have predicted that condensin and cohesin slide along genes. This result suggests that condensin and cohesin are recruited to highly expressed genes and translocated along gene bodies.

It has been shown that cohesin often localizes at intergenic regions between convergent genes. We observed that cohesin was enriched at convergent gene loci and was more enriched at convergent gene loci in the context of class 1 than in class 2, whereas condensin was only slightly enriched at convergent gene loci (Supplementary Fig. 1d).

Although condensin and cohesin frequently shared the same binding sites across the genome, we found that binding of condensin and cohesin showed a clear difference at some loci (for example, the eng1

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locus in Fig. 1c). Such condensin-specific sites often coincided with histone genes and a group of genes highly expressed during S and M-G1 phases (Fig. 1d). It is known that the expression of histone genes and of M-G1-phase genes is induced by the cell cycle–regulated transcription factors Ams2 and Ace2, respectively.45,46. Ams2 is a GATA-type transcription factor and responsible for cell cycle–dependent transcription of histone genes.46. Ace2 is known to control the cell cycle–dependent expression of genes required for septin ring formation and cell separation;47,48 we identified two potential Ace2 target genes, exg1 and SPAC343.20 (Supplementary Fig. 1e,f). Condensin, but not cohesin, was most enriched at the 3′ end of the Ace2 and Ams2 target genes, implying that condensin is recruited to gene promoters through its interaction with Ace2 and Ams2 and slides along gene bodies (Fig. 1e). Among the 18 Ace2 and Ams2 target genes, 18 (100%) are bound by condensin and 6 (33%) are bound by cohesin. We observed prominent condensin peaks at the Ace2 and Ams2 target genes, but negligible or no cohesin peaks were present (Fig. 1c,e).

Figure 1 Condensin binds to cell cycle–regulated Pol II genes. (a) Summary of Cut14 (Cut14-Pk) and Rad21 (Rad21-Myc) binding sites. Active genes are among the top 10% highest transcribed Pol II genes. (b) Highly transcribed Pol II genes are bound by condensin and cohesin. Pol II genes were classified into 20 groups on the basis of transcription level. Each group contains 162 Pol II genes. Average binding patterns of Cut14-Pk (top) and Rad21-Myc (bottom) for the respective gene groups were plotted. Gene sizes from transcriptional initiation to termination sites were normalized to the same length for all the genes investigated. (c) Distributions of Cut14-Pk (orange) and Rad21-Myc (blue) at the genomic region including the Ace2 target gene (eng1) and Pol III genes (tRNA and 5S rRNA). (d) Expression of Pol II genes bound by Rad21-Myc (left) or Cut14-Pk (right) during the cell cycle.45. Pol II genes were ordered from top to bottom on the basis of enrichment of Rad21-Myc or Cut14-Pk. Dotted lines indicate M and S phases, in which Ace2 and Ams2 target genes are upregulated, respectively. (e) Binding patterns of Cut14-Pk and Rad21-Myc at mid2 and hta2. Red bars indicate Ace2- and Ams2-binding motifs.
Different-sized domains formed by condensin and cohesin

To understand the general features of condensin- and cohesin-mediated genomic associations, we mapped ChIA-PET reads against the fission yeast genome, which was divided into 20 kb sections, and we found that both condensin- and cohesin-mediated associations were most enriched along the diagonal, indicating that both condensin and cohesin drive local associations to the highest degree (Fig. 2a). At a 20-kb resolution, we observed chromatin domains with associations mediated by condensin but not cohesin (Fig. 2a and Supplementary Fig. 2a). In contrast, at a resolution of 5 kb, we detected smaller chromatin domains with associations mediated by cohesin but not condensin (Fig. 2b). Cohesin-mediated associations were observed between flanking gene loci positioned less than 100 kb apart (Supplementary Fig. 2b). The frequency of condensin-mediated associations decreased as distances between two loci increased, but the decay curve was not as acute as for cohesin-dependent associations, and condensin-mediated associations were frequent between loci separated by as far as 1 Mb (Supplementary Fig. 2b). Note that condensin-mediated associations also occur beyond 1 Mb, as further described below. These associations are nonrandomly distributed across the genome, thereby showing the domain organizations mediated by condensin and cohesin.

We next estimated the positions of chromatin boundaries formed by condensin on the basis of a boundary index (Supplementary Note). Condensin-mediated association domains, referred to as condensin domains, were demarcated by boundaries (condensin boundaries) where condensin was generally highly enriched (Fig. 2c and Supplementary Fig. 2a). Similarly, cohesin domains were separated by boundaries where cohesin was enriched (Fig. 2d). The average sizes of cohesin and condensin domains were 84 kb (median 70 kb) and 349 kb (median 300 kb), respectively (Fig. 2e).

A previous Hi-C study indicated that cohesin mediates the formation of 100 kb chromatin domains across the fission yeast genome. Consistently with those results, we observed cohesin domains of similar size, and the positions of the boundaries estimated from the cohesin ChIA-PET heavily overlapped those determined from the Hi-C data (Fig. 2e and Supplementary Fig. 2c), although the cohesin ChIA-PET data showed clearer domains, probably owing to the purity of the data reflecting only cohesin-mediated associations. Cohesin boundaries were, in general, formed at cohesin binding sites, most typically at convergent gene loci (Fig. 2f and Supplementary Fig. 2d,e).

In contrast, condensin boundaries (where condensin was highly enriched) coincided with positions of Ace2 and Ams2 target genes (exg1, C343.20, adg2, adg1, cft4, exg1, hta1 and htb1), RNA-encoding genes, LTR retrotransposons and highly transcribed Pol II genes (Fig. 2f). The 7 condensin boundaries (out of 27 total) were formed at the Ace2 and Ams2 target gene loci, and only 11 loci consist of all 18 Ace2 and Ams2 target genes. The observation that boundaries are often formed at the Ace2 and Ams2 targets suggests that these transcription factors are involved in boundary organization. Together, these results indicate that the condensin and cohesin complexes establish chromatin domains that have distinct sizes and are demarcated by boundaries bound by these complexes. It is important to note that the distance decay in the Rad21 ChIA-PET data was clearly different from the decay in the Hi-C data, indicating that associations detected by ChIA-PET are not derived from randomly sampled in vivo associations (Supplementary Fig. 2f).

Condensin associates Ace2 and Ams2 target genes

To elucidate how condensin and cohesin build association networks across the genome, we focused on significant associations among association spots (FDR < 0.01; Supplementary Table 1 and Supplementary Note). The Mango algorithm predicts association spots and significance of the observed associations by considering the distance-dependent background association frequency. The association spots predicted by the Mango algorithm and the condensin binding sites estimated on the basis of self-ligation reads (Fig. 1a and Supplementary Fig. 1a) overlapped heavily, but association spots generally included relatively weak binding sites: every condensin and cohesin binding site, except for one condensin site, was included in association spots. There were 1,554 condensin- and 1,340 cohesin-association spots, and 905 and 805 significant associations between association spots were mediated by condensin and cohesin, respectively (Supplementary Table 1). Using this algorithm, we found that condensin mainly associated two loci positioned more than 100 kb apart (Fig. 3a). In contrast, cohesin-dependent associations were restricted to loci separated by less than 100 kb (Fig. 3b). Condensin- and cohesin-mediated associations appeared to depend upon distances between loci (Fig. 3c).

Regarding the association context, we found that condensin connected loci carrying LTR retrotransposons, Pol III genes or highly transcribed Pol II genes to a certain degree (Supplementary Fig. 3a). Condensin-mediated associations involving Ace2 and Ams2 target genes consistently occurred with much greater frequencies than associations estimated by the randomized simulation, suggesting that Ace2 and Ams2 targets serve as association hot spots (Supplementary Fig. 3a). Among cohesin binding sites, convergent loci preferentially associated together (Supplementary Fig. 3b).

We observed condensin-mediated long-range associations among boundaries and centromeres (Supplementary Fig. 3c). Relatively strong associations were often derived from condensin boundaries (Supplementary Fig. 3d). We previously showed that condensin binding sites are preferentially targeted to centromeres. The condensin ChIA-PET data also demonstrated that many condensin bindings sites dispersed across the genome were connected to centromeres and centromere-proximal regions (Supplementary Fig. 3c). In contrast, cohesin solely mediated short-range associations between cohesin boundaries present at 50- to 70-kb intervals (Supplementary Fig. 3e).

Because condensin favorably mediates associations of Ace2 and Ams2 target genes, we extracted only the associations involving those genes. This chromosome conformation capture on chip (4C)-like analysis again identified associations among the Ace2 and Ams2 targets and centromeres (Fig. 3d). These condensin-dependent associations were detected between two loci separated by 3–4 Mb, indicating that condensin can mediate long-range associations.

Mitotic associations of Ace2 and Ams2 targets via condensin

The transcription factors Ace2 and Ams2 are highly expressed during mitosis and upregulate their target genes during M–G1 and S phases, respectively. Therefore, we speculated that associations among Ace2 and Ams2 target genes and centromeres might also be cell cycle dependent. We first investigated Cut14 enrichment at Ace2 and Ams2 targets during the cell cycle and observed that condensin was most enriched at their target genes during mitosis and that the enrichment decreases as the cell cycle proceeds (Fig. 4a and Supplementary Fig. 4a). We next analyzed associations of Ace2 targets distributed across chromosome 1 (Fig. 4b) and found that Ace2 targets were best associated with each other during mitosis and to a lesser extent during S and early G2 phases, whereas the association between the Ace2 target (agn1) and the c110 negative control locus was continuously at background level during the cell cycle (Fig. 4c). Likewise, the Ams2 target histone gene hta1 associated with centromeres to the highest
Figure 2 Different sizes of chromatin domains are formed by condensin and cohesin. (a) Heat maps of associations mediated by Cut14 (Cut14-Pk) and Rad21 (Rad21-Myc) at 20-kb resolution. Association frequencies between 20-kb genomic sections were counted as number of reads assigned to respective combinations. ChIA-PET data from biological and technical replicas were combined. Arrows indicate chromatin domains. (b) Cut14-Pk- and Rad21-Myc-mediated genomic associations at 5-kb resolution. (c) Cut14-Pk-mediated associations between 20-kb genomic sections. Boundary indexes were calculated as described in Supplementary Note. Boundary index, Cut14-Pk binding score and gene annotations are shown below the association map. Pink lines indicate positions of predicted domain boundaries. (d) Comparison of Rad21-Myc ChIA-PET and Hi-C data. Association frequencies between genomic sections and boundary indexes were calculated for Hi-C and cohesin ChIA-PET data at 10-kb and 5-kb resolution, respectively, and shown with Rad21-Myc binding score and gene annotations. Filled circles represent potential boundaries formed at the class I convergent gene loci. The class II convergent loci at significant cohesin binding sites are also shown. (e) Distributions of domain sizes predicted from Cut14-Pk and Rad21-Myc ChIA-PET and Hi-C data. Boxes show center quartiles, and whiskers extend to the data point that is no more than 1.5x the interquartile range from the box (n = 36, 150 and 100 for Cut14-Pk and Rad21-Myc ChIA-PET and Hi-C data, respectively). (f) Breakdown charts of condensin and cohesin boundaries.
degree during mitosis (Supplementary Fig. 4b). As condensin-mediated association is a transient event, the association is scored as a frequency of nearby localization of gene loci. In addition, we observed that the associations of the Ace2 target (eng1) and Ams2 target genes (hht2 and hta1) with centromeres during mitosis were significantly impaired by the cut14-208 temperature-sensitive condensin mutation (P < 0.005, Mann–Whitney U-test), while relative positioning between the c110 negative control locus, which lacks condensin localization, and centromeres was not affected (Fig. 4d and Supplementary Fig. 4c). Similarly, the mitotic association between the Ace2 targets eng1 and agn1 was significantly disrupted by the cut14-208 mutation (P < 0.001, Mann–Whitney U-test), whereas positioning between the agn1 gene and c110 locus was not affected (Fig. 4d). Negative control data indicate that the disruption of gene associations by the condensin mutation did not result from indirect global effects.

Figure 3 Condensin and cohesin mediate different gene associations. (a,b) Significant associations mediated by Cut14 (Cut14-Pk; a) and Rad21 (Rad21-Myc; b). Significant associations were defined by Mango software (FDR < 0.01; Supplementary Note). Associations were categorized into two groups according to the distance (<100 kb and >100 kb) between two loci. Color intensities correlate with P values of respective associations. The graphs were drawn with Circos software. Centromeres are represented by yellow. (c) Frequencies of Cut14-Pk- and Rad21-Myc-mediated significant associations plotted against distance. Associations were divided into 100-kb bins. (d) 4C-like plots for Cut14-Pk ChIA-PET data. Ace2 and Ams2 target gene loci and cen1 were used as baits. The entire chromosome 1 was divided into nonoverlapping 20-kb windows, and read numbers indicating associations between the bait regions and 20-kb sections were plotted (top). Significant associations from the bait regions are also shown (bottom).
Moreover, condensin enrichment was reduced in the ace2 and am2 deletion mutants (ace2Δ and am2Δ) at the Ace2 and Ams2 targets but not at the cnt1 centromeric region of chromosome 1 (Fig. 4e and Supplementary Fig. 4d). It has been shown that condensin loading to fission yeast centromeres is compromised by the psc1 monopolin–kinetochore deletion (psc1Δ)52. We observed that the associations of Ace2 and Ams2 targets with centromeres were significantly impaired by the psc1Δ mutation, which did not affect relative positioning of the c110 control locus and centromeres, suggesting that centromeric condensin loaded by the monopolin is involved in the associations of Ace2 and Ams2 target genes with centromeres (Supplementary Fig. 4e). Together, these results suggest that Ace2 and Ams2 recruit condensin to their target genes most efficiently during mitosis when these transcription factors are highly expressed, and that condensin in turn mediates associations among Ace2 and Ams2 targets and centromeres.

Condensin ChIA-PET using mitotic cells

We used asynchronous culture to identify condensin-mediated gene associations (Fig. 3), and our microscopic investigation suggested that those associations are promoted during mitosis (Fig. 4c and Supplementary Fig. 4b). Therefore, we hypothesized that the asynchronous data reflect mainly mitotic associations. To test this hypothesis, we decided to conduct the condensin ChIA-PET analysis with mitotic and G2 cells. We observed that condensin localization patterns in mitosis and G2 cells were, in general, similar to those in asynchronous cells (Supplementary Fig. 5a); for example, condensin localization was consistently observed at tRNA and SS rRNA genes (Supplementary Fig. 5b). Condensin localization at the Ace2 and Ams2 targets mid2 and hta2 was observed in asynchronous and mitotic cells but not in G2 cells (Supplementary Fig. 5c). In this regard, the nuclear localization of condensin was more enhanced during mitosis than in interphase, whereas cohesin localized in the nucleus throughout the cell cycle53,54 (Supplementary Fig. 5d).

Comparing condensin domains between asynchronous and mitotic cells, we observed that positions of condensin boundaries in asynchronous and mitotic cells were very similar across the genome (Supplementary Fig. 5e,f). As in asynchronous cells, the distance decay in the condensin ChIA-PET data from mitotic cells was different from that in the cohesin data (Supplementary Fig. 5g); for example, cohesin-mediated associations were enhanced between flanking gene loci positioned less than 100 kb apart. Condensin ChIA-PET data were in general highly similar between asynchronous and mitotic cells (r = 0.962; Supplementary Fig. 5b). Moreover, significant condensin-mediated associations (FDR < 0.022; Supplementary Table 1) detected were mainly between two loci positioned more than 100 kb apart in both asynchronous and mitotic cells, whereas cohesin-dependent associations were predominantly restricted to loci separated by less than 100 kb (Supplementary Fig. 5i,j). Together, these results strongly suggest that condensin ChIA-PET data from asynchronous cells reflect mainly domain organization and gene associations during mitosis.

Ace2 binding sites form domain boundaries

We postulated that the Ace2 target genes eng1 and C343.20 form domain boundaries (Fig. 5a). To test this hypothesis, we deleted the two Ace2 binding motifs from eng1 and the entire C343.20 gene; these deletions are referred to as m1A and m2A, respectively (Fig. 5a). We observed that condensin enrichment around eng1 and C343.20 was clearly diminished in m1A and m2A cells, respectively, as well as in ace2Δ cells, suggesting that Ace2 recruits condensin to its target genes (Fig. 5b). Consistently, condensin-mediated associations of eng1 and C343.20 with centromeres were significantly disrupted by the m1Δm2Δ double deletions (P < 0.001, Mann–Whitney
Mitotic cells were prepared as described in the methods. Relative positioning of the c110 control locus and centromeres was not affected by the deletions (Fig. 5c).

We next asked whether eng1 and C343.20 function as domain boundaries. For this purpose, we investigated how the m1Δ and m2Δ mutations affect intra- and interdomain associations. m1Δ, but not m2Δ, significantly enhanced the interdomain association between the A and B loci bound by condensin (P < 0.001, Mann–Whitney U-test; Fig. 5d). The m2Δ mutation specifically increased the interdomain association between the C and D loci. Both m1Δ and m2Δ slightly reduced the intradomain association between the B and C loci. These observations, together with the condensin ChIA-PET data, strongly suggest that the two Ace2 binding sites serve as domain boundaries to prevent interdomain associations.

**Mitotic transcription factors recruit condensin**

As it was still not clear whether Ace2 and Ams2 have a direct role in recruiting of condensin to their targets, we performed coinmunoprecipitation (co-IP) experiments and found that Cut14 interacts with Ace2 and Ams2, and that these interactions remained even after DNase I treatment (Fig. 6a,b).

![Image of Figure 5](https://example.com/image5)

**Figure 5** Ace2 target sites form domain boundaries. (a) Deletion of the Ace2 binding sites. m1Δ removes the two Ace2 binding motifs from the eng1 gene promoter, and m2Δ deletes the C343.20 gene. Red lines indicate transcribed regions. (b) ChIP results showing enrichment of Cut14-Pk around the eng1 and C343.20 genes in WT, ace2Δ, m1Δ and m2Δ strains. Primer positions are indicated in a. ChIP enrichment was normalized against leu1. Experiments were independently repeated three times, and data are represented as mean ± s.d. (c) FISH-immunofluorescence (FISH-IF) data and representative images summarizing distances between the indicated gene loci and centromeres in WT and m1Δm2Δ strains. Cells were treated with hydroxyurea for cell cycle synchronization. Mitotic cells were defined by spindle staining (tubulin), and the distance between two FISH foci was measured in more than 50 mitotic cells and binned into one of the three categories (top right). The regions between these loci are consistently ~350 kb. Mitotic cells were prepared as described in Figure 4a (40 min after cell cycle release; top left). The distance between two FISH foci was measured in more than 100 cells and binned into one of the three categories.
We employed a system to test the hypothesis that tethering of Ace2 and Ams2 could result in the recruitment of condensin to a chromatin locus. Specifically, lacO repeats were integrated into the c887 locus, where no significant condensin binding peaks were observed in our previous ChIP-seq data. We also made strains expressing either Ace2–LacI–3Flag or Ams2–LacI–3Flag, in which Ace2 and Ams2 are fused to the Lac repressor (LacI) and three Flag epitopes. It has previously been shown that the condensin-loading process could be validated by the same approach. After expressing Ace2–LacI–3Flag or Ams2–LacI–3Flag, we first observed, using ChiP, that both were enriched at the lacO locus, indicating that these LacI-tagged proteins were successfully tethered to the lacO repeats (Fig. 6d). We then found that tethering of Ace2–LacI–3Flag and Ams2–LacI–3Flag resulted in condensin becoming enriched at the lacO locus, firmly establishing that Ace2 and Ams2 have an important role in recruiting of condensin to a gene locus (Fig. 6d). Additionally, our results suggest that the condensin loading by Ace2 and Ams2 unlikely involves the TATA box-binding protein (TBP), Tbp1 in fission yeast, and a mitotic transcription factor, Sep1 (Supplementary Fig. 6 and Supplementary Note). We also used the tethering system to examine the association of the lacO locus with centromeres by FISH analysis (Fig. 6e). We found that when Ace2–LacI–3Flag or Ams2–LacI–3Flag was expressed, the lacO locus was frequently located near centromeres and that the localization patterns in cells with and without expression of the LacI-3Flag-fused proteins were significantly different (P < 0.001, Mann–Whitney U-test; Fig. 6e). This colocalization of the lacO locus with centromeres by expression of Ace2–LacI–3Flag and Ams2–LacI–3Flag was not observed in the cut14-208 mutant, indicating that the Ace2- and Ams2-mediated associations of the locus with centromeres are dependent on condensin (Fig. 6f).

Mitotic defects caused by ace2Δ and boundary deletions

We next investigated the functions of Ace2-dependent gene associations in mitotic chromosome organization and segregation. Mitotic defects, including lagging chromosomes, were observed in ace2Δ and m1Δm2Δ cells but not in wild-type (WT) cells (Fig. 7a). The m1Δm2Δ double deletion removes the Ace2 binding sites eng1 and C343.20, which serve as strong association hot spots and as domain boundaries (Figs. 3d and 5). The mitotic defects in ace2Δ cells were suppressed by exogenous expression of Ace2 proteins (Fig. 7b). As Ams2 is involved in loading of centromeric histone H3 variant (CENP-A) to centromeres and thereby chromosome segregation, it is difficult to assess the mitotic roles of Ams2-dependent gene associations. In contrast, we observed that CENP-A loading and centromeric clustering were not affected by the ace2Δ mutation (Fig. 7c,d). During mitosis in ace2Δ cells, the centromeric associations of the Ace2 target genes agn1 and eng1 and the association between the Ace2 target genes were significantly disrupted (P < 0.001, Mann–Whitney U-test), whereas positioning between the c110 negative control locus and centromeres was not affected (Fig. 7e). In anaphase WT cells, the Ace2 target genes associated together, and centromeres associating with the Ace2 target gene locus were pulled by the spindle microtubules and the mitotic chromosomes were properly segregated (Fig. 7f). In contrast, in the ace2Δ mutant, the associations among the Ace2 target genes and centromeres were disrupted, but centromeres were still pulled by the microtubules, causing a defect in mitotic chromosome segregation (Fig. 7f). Re-examining the fidelity of chromosome segregation in the ace2Δ mutant using the Chr16 mini-chromosome system, we found that the frequency of mini-chromosome loss was roughly ten times more elevated in the ace2Δ mutant than in WT cells (Fig. 7g). Because the mini-chromosome does not consist of Ace2 target genes, the observed mini-chromosome loss in ace2Δ cells probably accompanies segregation defects of other chromosomes. Moreover, we observed modest but significant defects in mitotic chromosome segregation when the two Ace2 target sites were removed by the m1Δm2Δ deletion (Fig. 7h). These results suggest that associations among Ace2 target genes and centromeres and, potentially, domain organization contribute to the faithful segregation of mitotic chromosomes.

Using the tethering system, we examined local chromosomal compaction around the c887 lacO locus in mitotic cells (Fig. 7i). We observed that the two foci representing the c887 left and c887 lacO repeats were significantly different (P < 0.001, Mann–Whitney U-test; Fig. 7i). Although in WT cells, the two foci were co-localized, this localization pattern was disrupted in the ace2Δ mutant, indicating that the Ace2-dependent association of the lacO gene locus with centromeres contributed to compaction in mitotic cells.
right regions completely overlapped in approximately 50% of mitotic cells when Ace2–LacI–3Flag was expressed (Fig. 7i,j). In contrast, we observed primarily two separate foci when the LacI-fused proteins were not expressed, indicating that the local chromosome compaction around the lacO locus was significantly facilitated by the expression of Ace2–LacI–3Flag proteins ($P<0.001$, Mann–Whitney U-test; Fig. 7j). The local compaction mediated by Ace2–LacI–3Flag was diminished in the cut14-208 condensin mutant, indicating that this compaction process is mediated by condensin (Fig. 7k). Together, these results indicate that local chromosomal compaction during mitosis is mediated by condensin molecules, which are recruited by factors such as Ace2, thus participating in mitotic chromosome assembly. Because condensin binds to Ace2 and Ams2 target genes, we postulated that condensin might regulate the expression of those genes. To test this hypothesis, we investigated the effect of the cut14-208 condensin mutation on the expression of Ace2 and Ams2 targeting genes. Our results suggest that Ace2 and Ams2 proteins are stabilized in condensin mutant cells, thereby leading to higher expression of their target genes in the mutant (Supplementary Note and Supplementary Fig. 7).
DISCUSSION

In this study we found that both the condensin and the cohesin complexes frequently bind to the same loci including rRNA and 5S rRNA genes, LTR retrotransposons and highly expressed Pol II genes. Condensin—but not cohesin—additionally localizes at the cell cycle-regulated genes bound by the transcription factors Ace2 and Ams2. It has been shown by ChIP-seq approach that condensin is enriched at mitotically activated genes\(^{58,59}\). However, our results indicate that condensin is preferentially targeted to specific genes bound by these mitotic transcription factors. Moreover, we show that Ace2 and Ams2 interact with condensin and recruit it to chromatin (Fig. 6). As Ace2 and Ams2 bind only to their target genes, these transcription factors allow condensin to bind to specific genes, although other factors may interact with Ace2 and Ams2 to help recruit condensin to their targets.

Even though condensin and cohesin frequently bind to the same loci, they drive distinct genome-wide association networks. Cohesin-dependent associations are restricted mostly to loci positioned within 100 kb, whereas condensin can associate gene loci separated by more than 3–4 Mb apart, indicating that condensin can mediate long-range associations. Cohesin and condensin form chromatin domains of 50–100 and 300–500 kb, respectively, and domain boundaries are occupied by these complexes (Fig. 2).

TAD organization is observed among eukaryotic species and linked to transcriptional coregulation and replicating timing coordination\(^9\). Condensin has been implicated in TAD organization, and our work supports this finding by demonstrating that fission yeast condensin is involved in forming chromatin domains that are similar to the mammalian TADs in size, ranging from 100 kb to 1 Mb (refs. 14,17). We have observed that the mitotic domain organization is disrupted by the deletions of Ace2 binding sites, demonstrating that this mitotic transcription factor participates in the cell cycle–dependent arrangement of chromatin domains.

Using the LacI tethering system, we have found that condensin is recruited by Ace2 and mediates both the association of a gene locus with centromeres and local chromosomal compaction during mitosis. Condensin is targeted to centromeres through a mechanism involving kinetochore protein\(^52,60\). Condensin is known to interact with another condensin molecule to mediate associations between chromatin fibers\(^61,62\). Therefore, condensin molecules present at centromeres and Ace2 target genes can mediate their associations through condensin–condensin interactions.

It has also been shown that the condensin complex interacts with another condensin in the presence of DNA and introduces positive supercoiling into the chromatin loop\(^51,63\). We thus hypothesize that chromatin loops generated by associations among gene loci and centromeres are supercoiled by condensin, leading to local chromosomal compaction. This looped and supercoiled chromosome arrangement contributes to the efficient transmission of physical force at the kinetochore to the chromosomal arms, improving the fidelity of chromosome segregation. Therefore, when gene associations are disrupted by the ace2A mutation, Ace2 target genes no longer associate with centromeres, but centromeres still segregate without holding the Ace2 target genes, leading to chromosomal segregation defects (Fig. 7f). We propose that mitotic transcription factors interact with and recruit condensin to mitotic chromosomes and participate in the mitotic chromosome organization required for the fidelity of chromosome segregation.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Sequence Read Archive: data were deposited under accession number SRP061635.

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

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AUTHOR CONTRIBUTIONS

H.T. performed the bioinformatics analyses. O.I. performed the tethering assays. K.-D.K. performed ChIA-PET and other experiments. K.N. conceived and designed the study. All authors contributed to analyzing the data and writing the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

URLs. Mango software, https://github.com/dphanst/i/mango; Circos software, http://circos.ca/software/download/circos/.

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ONLINE METHODS

Strains and culture conditions. Cut14, Rad21, Ace2, Ams2, and Sep1 were tagged with Myc, Flag, or Pk at the C terminus using a PCR-based module method\textsuperscript{64,65}. Tagged proteins were expressed from their endogenous gene loci with their own promoters. Strain constructions were performed using conventional genetic crosses. The fission yeast (\textit{Schizosaccharomyces pombe}) strains were cultured in yeast-extract adenine (YEA) or Edinburgh minimal medium (EMM).

ChIA-PET. Chromatin was fixed with 3\% paraformaldehyde followed by further cross-linking with 10 mM dimethyl adipimidate (DMA). Cross-linked chromatin was sheared into 300–500 bp DNA fragments by Bioruptor (Diagenode). Cut14-Pk and Rad21-Myc proteins were immunoprecipitated using mouse monoclonal anti-Pk (SV5-Pk1, Serotec) or mouse monoclonal anti-Myc (9E10, Clontech), and protein-G-coupled Dynabeads (Life Technologies). Immunoprecipitated chromatin was subjected to the ChIA-PET procedure\textsuperscript{20}, ChIP DNA was split into an equal amount and separately ligated to either A or B linkers, and mixed ligation products were further subjected to proximity ligation. The ligation product was digested by EcoP15I and purified using streptavidin-coupled magnetic beads (Invitrogen). Illumina sequencing adaptors were added to DNA fragments, which were then subjected to PCR amplification (Phusion PCR Master Mix; New England BioLabs). This PCR was performed in a total of 32 separate tubes for each ChIA-PET library. Assembled PCR products were sequenced on the Illumina HiSeq 2000 platform to obtain 100-bp single end reads. ChIA-PET data analysis is described in the Supplementary Note.

Immunoprecipitation, FISH, tethering analysis, mini-chromosome assay and qRT-PCR. Methods for ChIP, FISH, IP, tethering analysis, mini-chromosome assay and qRT-PCR are described in the Supplementary Note.

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