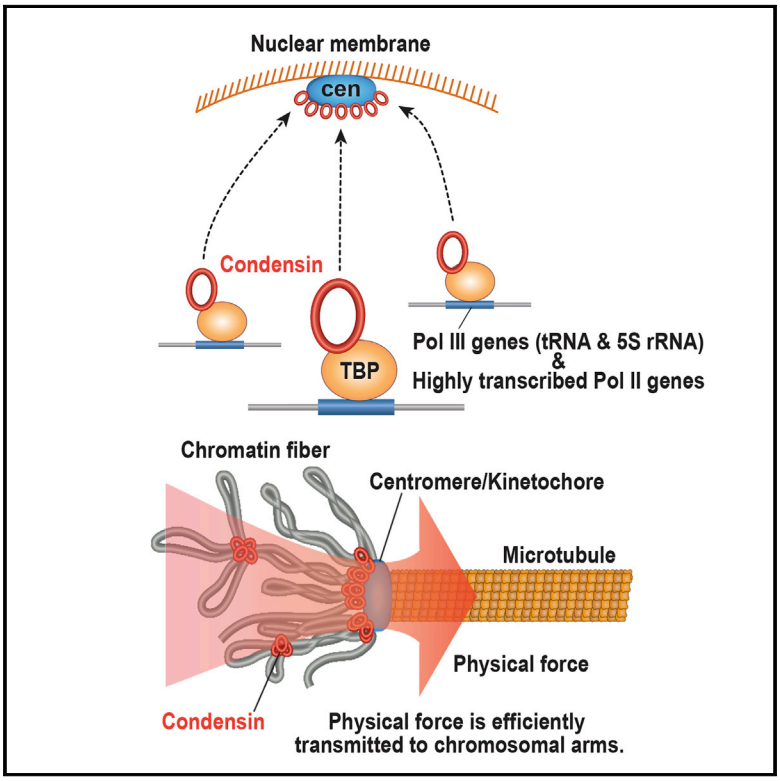
Article

Molecular Cell

Interaction between TBP and Condensin Drives the Organization and Faithful Segregation of Mitotic Chromosomes

Graphical Abstract



Authors

Osamu Iwasaki, Hideki Tanizawa, Kyoung-Dong Kim, ..., Emmanuel Skordalakes, Louise C. Showe, Ken-ichi Noma

Correspondence

noma@wistar.org

In Brief

Determinants of condensin localization across the genome are unclear. Iwasaki et al. show that the general transcription factor, TBP, recruits condensin to Pol III-transcribed genes and Pol II-transcribed housekeeping genes in S. pombe. This promotes their association with centromeres and facilitates faithful chromosome segregation during mitosis.

Highlights

- TATA box-binding protein, TBP, interacts with the kleisin condensin subunit
- TBP recruits condensin to Pol III genes and highly transcribed Pol II genes
- TBP-condensin interaction is essential for faithful chromosome segregation
- TBP plays a key role in chromosome/genome organization through condensin

Accession Numbers

GSE60273

Iwasaki et al., 2015, Molecular Cell 59, 755–767
September 3, 2015
http://dx.doi.org/10.1016/j.molcel.2015.07.007

Molecular Cell
Article

Interaction between TBP and Condensin Drives the Organization and Faithful Segregation of Mitotic Chromosomes

Osamu Iwasaki,[1] Hideki Tanizawa,[1] Kyoung-Dong Kim,[1] Yuhki Yokoyama,[1] Christopher J. Corcoran,[1] Atsunari Tanaka,[1] Emmanuel Skordalakes,[1] Louise C. Showe,[1] and Ken-ichi Noma[1,*]
[1]The Wistar Institute, Philadelphia, PA19104, USA
*Correspondence: noma@wistar.org
http://dx.doi.org/10.1016/j.molcel.2015.07.007

SUMMARY

Genome/chromosome organization is highly ordered and controls various nuclear events, although the molecular mechanisms underlying the functional organization remain largely unknown. Here, we show that the TATA box-binding protein (TBP) interacts with the Cnd2 kleisin subunit of condensin to mediate interphase and mitotic chromosomal organization in fission yeast. TBP recruits condensin onto RNA polymerase III-transcribed (Pol III) genes and highly transcribed Pol II genes; condensin in turn associates these genes with centromeres. Inhibition of the Cnd2-TBP interaction disrupts condensin localization across the genome and the proper assembly of mitotic chromosomes, leading to severe defects in chromosome segregation and eventually causing cellular lethality. We propose that the Cnd2-TBP interaction coordinates transcription with chromosomal architecture by linking dispersed gene loci with centromeres. This chromosome arrangement can contribute to the efficient transmission of physical force at the kinetochore to chromosomal arms, thereby supporting the fidelity of chromosome segregation.

INTRODUCTION

Protein complexes containing structural maintenance of chromosome (SMC) factors form ring-shaped structures and function as chromatin linkers (Hirano, 2006). The two best-known SMC complexes, namely condensin and cohesin, play major roles in mitotic chromosome assembly and in holding sister chromatids together, respectively (Nasmyth and Haering, 2005). Importantly, recent evidence suggests that the condensin and cohesin complexes are also involved in various nuclear processes through their genome-organizing activities (Wood et al., 2010). Although it is accepted that cohesin mediates genome-wide associations and activates gene expression by mediating interactions between enhancers and promoters, how condensin drives the functional organization of the genome has remained elusive (Kagey et al., 2010).

Recent studies have shown that mitotic chromosomes can largely be compacted in the absence of condensin in several organisms, although partial decondensation is observed (Hagstrom et al., 2002; Hudson et al., 2003; Steffensen et al., 2001). Importantly, the mitotic chromosome structure appears to be disorganized in the absence of condensin, and sister chromatids are entangled (Hagstrom et al., 2002; Hudson et al., 2003; Saka et al., 1994). Therefore, condensin mediates the establishment of an integral configuration of mitotic chromosomes, which is required for the fidelity of chromosome segregation, and also plays a limited role in chromosome compaction. Critical questions that remain are how the specific mitotic chromosome structure is assembled by the condensin complex and how the properly assembled architecture promotes correct chromosome segregation during mitosis.

Using fission yeast as a model system, we have studied condensin-mediated genome organizations and their roles in nuclear events and demonstrated that Pol III genes, such as tRNA and 5S rRNA genes dispersed across the chromosomal arms, associate with centromeres (Iwasaki et al., 2010; Tanaka et al., 2012). We now show that condensin is recruited to Pol III genes and highly transcribed Pol II genes through its interaction with TBP, thereby mediating associations between these genes and centromeres. Using *cnd2* condensin mutations that inhibit the Cnd2-TBP interaction, we show that failure of Cnd2 to bind to TBP disrupts condensin binding across the genome, impairs the proper assembly of mitotic chromosomes, and causes a severe defect in the segregation of genes, followed by cellular lethality. In summary, the Cnd2-TBP interaction identified by this study plays a pivotal role in 3D chromosome organization and ensures the fidelity of the segregation of actively transcribed genes.

RESULTS

Interaction between the Cnd2 Condensin Subunit and TBP

We previously found that condensin mediates clustering of Pol III genes at centromeres in fission yeast, although the molecular mechanism underlying this genome organization was not clear. Our hypothesis was that a component of the Pol III transcription

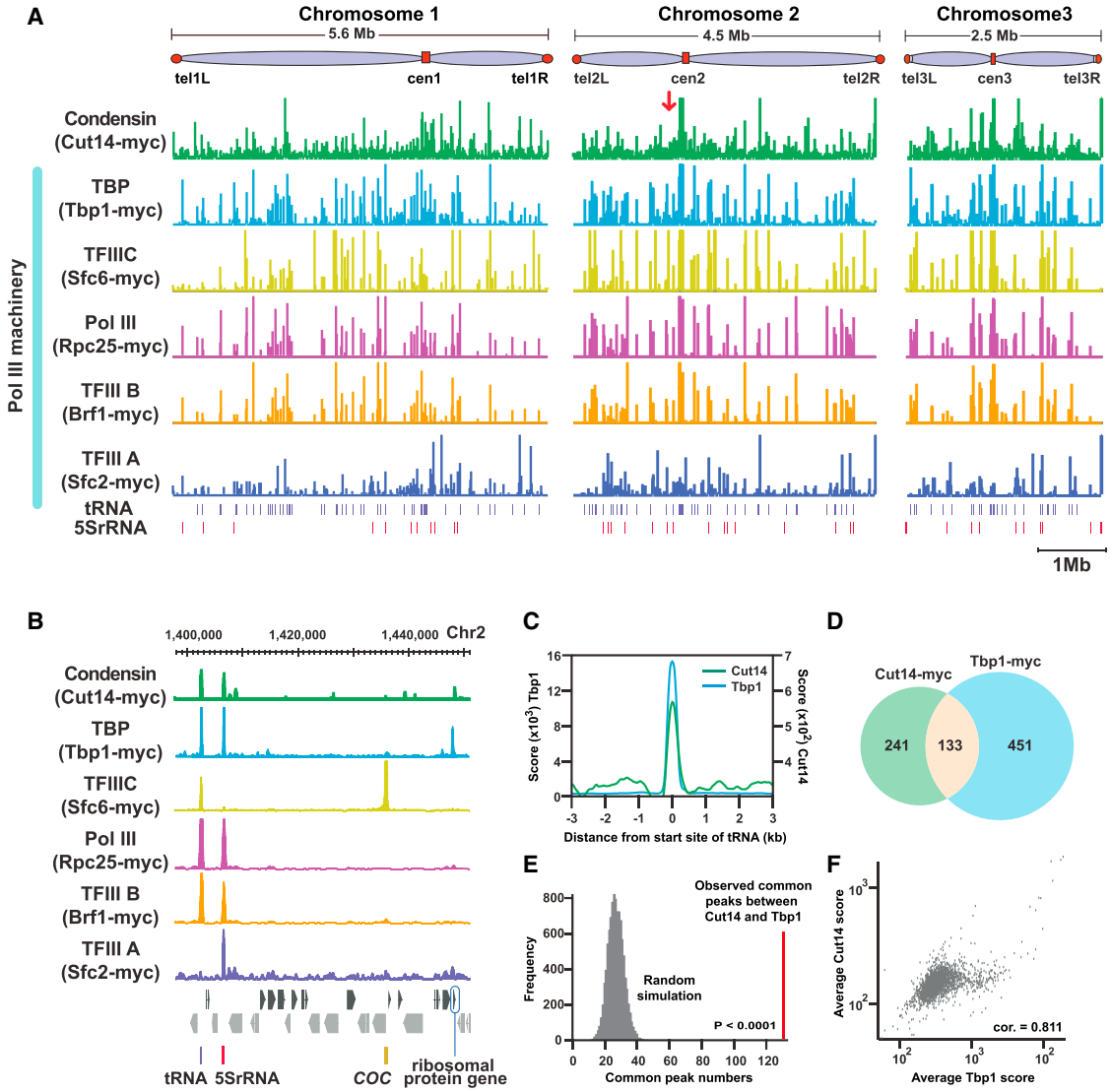


Figure 1. Co-localization of the Cut14 Condensin Subunit and Tbp1

(A) Chromosomal distribution profiles of Cut14 and the components of the Pol III transcription machinery were determined by a ChIP-seq approach.

(B) The genomic region indicated by the red arrow in (A) is magnified to exemplify the similar binding profiles of the Cut14 condensin subunit and Tbp1. The *COC* region, which was only bound by TFIIIC, had a negligible binding peak of Cut14 (Noma et al., 2006).

(C) Average binding patterns of Cut14 and Tbp1 proteins at tRNA genes.

(D) Venn diagram showing the overlap between binding sites of Cut14-myc and Tbp1-myc. Significant peaks located within same windows (300 bp) were counted as co-localization.

(E) Significance of co-localization between Cut14 and Tbp1. Cut14 and Tbp1 binding peaks were assigned to 1 kb windows, and common peaks were counted. The same number of 1 kb windows as Cut14 binding peaks were randomly selected from the entire genome, and any overlap with Tbp1 peaks was counted as co-localization. This random sampling was repeated 10,000 times and the background distribution of co-localization was used to calculate the p value (permutation test).

(F) The *S. pombe* genome was divided into non-overlapping windows (5 kb). Average scores of Cut14-myc and Tbp1-myc for every window were plotted.

machinery must recruit condensin to Pol III genes. If this is correct, then condensin and the Pol III machinery should co-localize across the fission yeast genome. To explore this possibility, we performed chromatin immunoprecipitation sequencing (ChIP-seq), a chromatin immunoprecipitation assay combined with next-generation sequencing, and examined genome-wide distributions of condensin and the Pol III transcription machinery (Figure 1A). Pol III transcription requires the transcription factor complexes (TFIIIA, TFIIIB, and TFIIIC) that direct the accurate positioning of Pol III on tRNA and 5S rRNA genes (Huang and Maraia, 2001). We observed that the Cut14 condensin subunit generally co-localized with the Pol III transcriptional machinery and that Cut14 binding peaks were frequently associated with peaks of the TATA box-binding protein (TBP), Tbp1, in fission

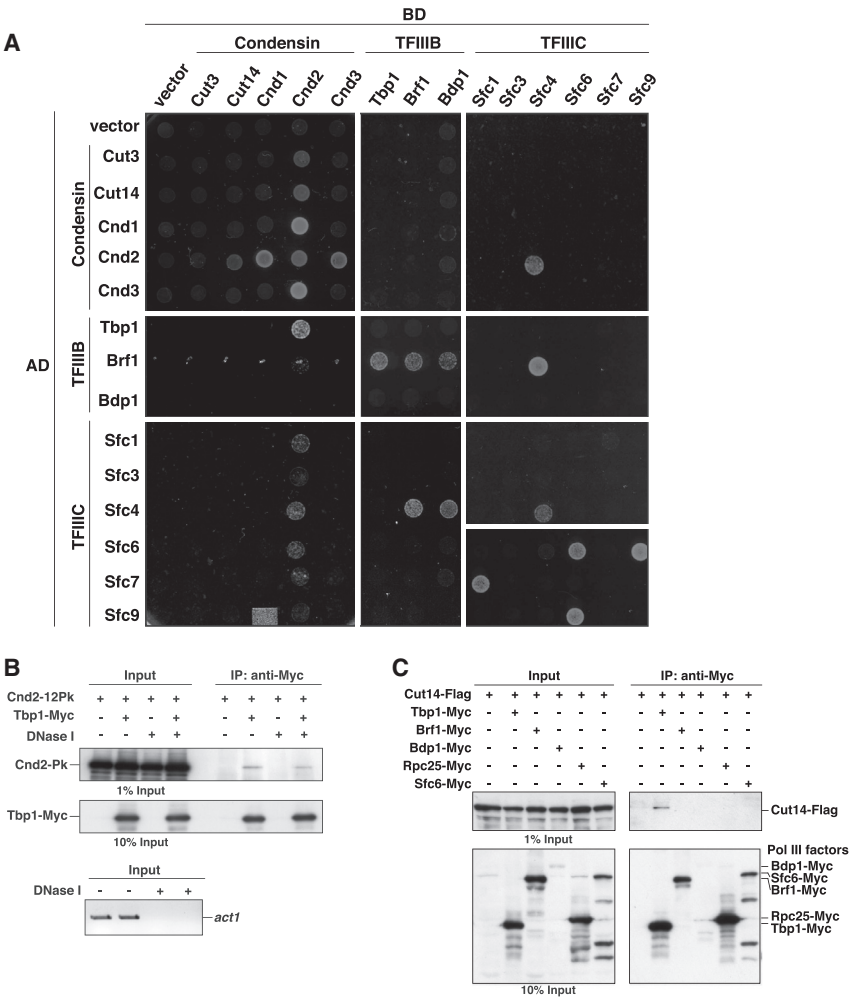


Figure 2. Interaction between the Cnd2 Condensin Subunit and Tbp1

(A) Two-hybrid analysis for the components of condensin, TFIIIB, and TFIIIC complexes. The square shadow positioned at the Cnd1-Sfc9 combination reflects a pattern of culture dish.

(B) CoIP result showing the interaction between the Cnd2 condensin subunit and Tbp1. CoIP assay was performed with and without DNase I treatment. DNA digestion was verified by a PCR-based assay to detect *act1*$^{+}$ gene region as a control (bottom).

(C) CoIP assay was performed to investigate the association between condensin (Cut14) and the components of the RNA Pol III transcription machinery, including TFIIIB subunits (Tbp1, Brf1, and Bdp1), RNA Pol III subunit (Rpc25), and TFIIIC subunit (Sfc6).

See also Figure S1.

yeast (Figure 1B). Binding peaks for Cut14 and Tbp1 at tRNA genes were located at the exact same position (Figure 1C). In addition, binding sites of Cut14 and Tbp1 throughout the genome overlapped in a highly significant manner (Figures 1D and 1E). Moreover, the ChIP enrichment values of Cut14 and Tbp1 showed a clear correlation across the entire genome (Pearson's r = 0.811; Figure 1F). These data suggest that Tbp1 might recruit condensin to Pol III genes.

To more closely investigate interactions between condensin and the Pol III transcription machinery, we performed yeast two-hybrid analyses and comprehensively examined interactions between all 14 proteins of the condensin, TFIIIB, and TFIIIC complexes (Figures 2A and S1A). Among the many interactions detected between the components of the respective complexes, we observed the interactions of the Cnd2 condensin subunit with Tbp1 and the Sfc4 TFIIIC subunit. Moreover, using a co-immunoprecipitation (coIP) assay, we could reproducibly show that Cnd2 interacts with Tbp1 and Sfc4 and that these interactions remained even after DNase I treatment (Figures 2B and S1B). We also performed coIP analysis to investigate the association between another condensin subunit, Cut14, and the various components of the Pol III transcription machinery; this analysis revealed that Cut14 associates with Tbp1, suggesting that the condensin complex associates with Tbp1 through the Cnd2-Tbp1 interaction (Figure 2C). This interaction was observed in G2 and mitotic cells, suggesting that Tbp1 and condensin associate constitutively during the cell cycle (Figure S1C). To examine whether Cnd2 directly binds to Tbp1, we investigated the interaction between Cnd2 and Tbp1 proteins that were overexpressed more than 100 times compared to the endogenous proteins (Basi et al., 1993). We found reciprocal interactions between these overexpressed proteins (Figure S1D). We also performed in vitro binding assays using recombinant Cnd2 and Tbp1 proteins purified form *E. coli* cells and further demonstrated that Cnd2 interacts with Tbp1 (Figure S1E). These data consistently suggest that Tbp1 directly interacts with the Cnd2 condensin subunit and may recruit condensin to Pol III genes.

Recruitment of Condensin via Tbp1

Since it was not clear whether Tbp1 alone is sufficient for the recruitment of condensin to Pol III genes, we developed a system to directly test the hypothesis that tethering of Tbp1 could result in the recruitment of condensin to a chromatin locus (Figure 3A; Yamazaki et al., 2012). Specifically, we integrated *lacO* repeats to the c887 non-Pol III gene locus, where no significant condensin binding peaks were observed in our previous ChIP-seq data (Kim et al., 2013; Tanaka et al., 2012). We also made strains expressing either Cut3 (condensin)-LacI-3Flag or Tbp1-LacI-3Flag (Figures 3A and S2A). After expressing Cut3-LacI-3Flag, we observed the ChIP enrichment of both the Cut3 and Cut14 condensin subunits at the *lacO* locus, suggesting that Cut14 can be recruited to the *lacO* locus as a part of the condensin complex through specific binding of Cut3-LacI-3Flag to the *lacO* repeats (Figure S2B). More importantly, we observed that tethering of Tbp1-LacI-3Flag resulted in Cut14

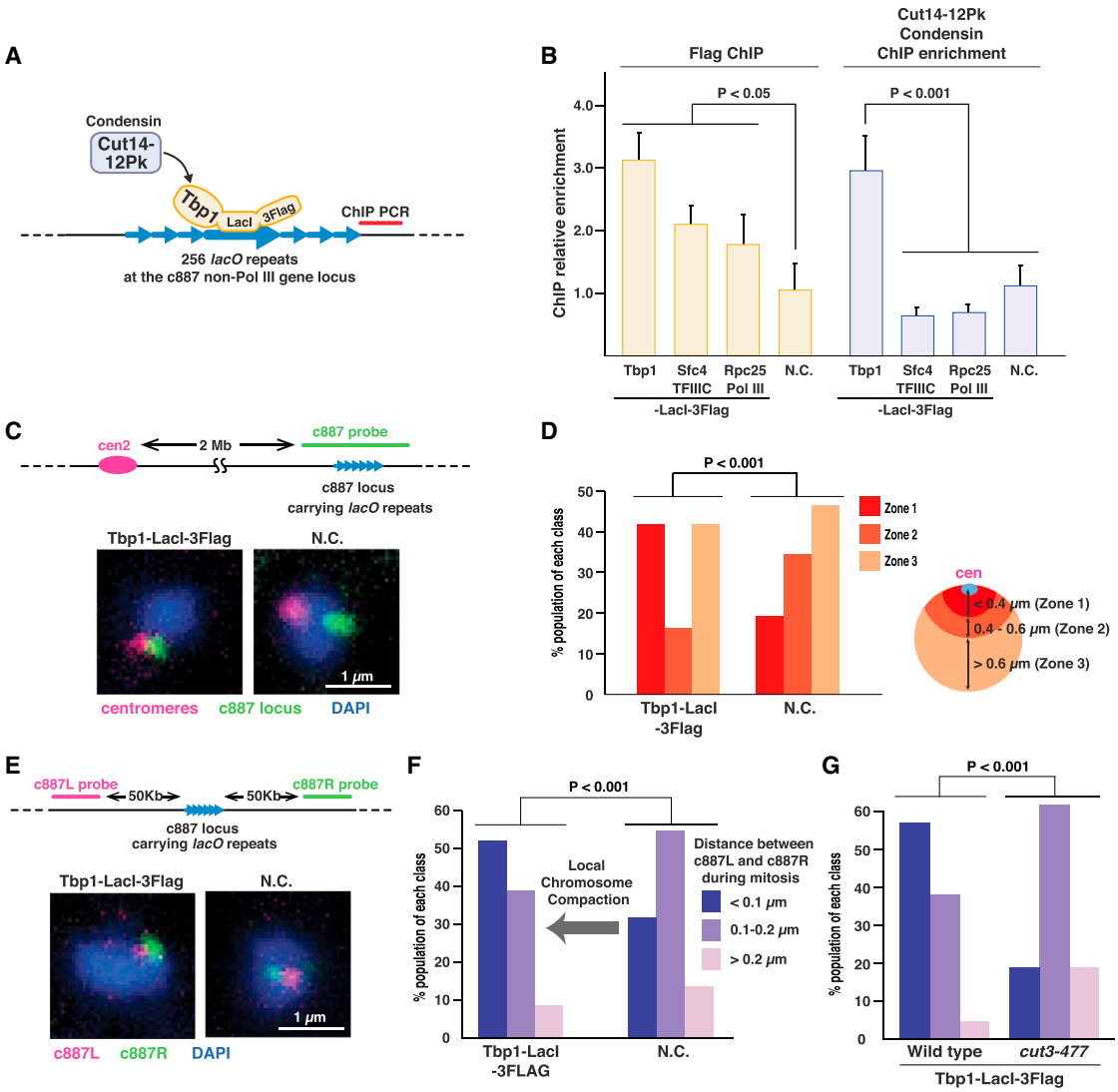


Figure 3. Tbp1 Recruits Condensin

(A) Tbp1 fused to LacI-3Flag is tethered to *lacO* repeats at the c887 non-Pol III gene locus.

(B) Tbp1 tethering is sufficient to recruit condensin to the *lacO* region. Cells expressing Tbp1, Sfc4, or Rpc25 fused to LacI-3Flag were subjected to ChIP experiments. ChIP analysis was performed as described in Figure S2B. Data are represented as mean ± SD.

(C) Representative FISH results showing the c887 *lacO* locus (green) and centromeres (red) in interphase cells expressing Tbp1-LacI-3Flag or carrying an empty vector (N.C.).

(D) The c887 *lacO* locus frequently localizes near centromeres in interphase cells expressing Tbp1-LacI-3Flag. The distance between the *lacO* locus and centromeres was measured in more than 100 cells, and FISH data were summarized as detailed in Figure S2D.

(E) FISH data showing the c887L (red) and c887R (green) regions in mitotic cells expressing Tbp1-LacI-3Flag or carrying an empty vector (N.C.). FISH analysis was accompanied by IF staining, which visualizes spindle microtubules. Mitotic cells were defined by spindle staining.

(F) Local chromosomal compaction around the *lacO* locus is promoted by expression of Tbp1-LacI-3Flag. FISH-IF analysis was performed as described in Figure S2F.

(G) Local chromosomal compaction driven by Tbp1-LacI-3Flag is diminished by the *cut3-477* condensin mutation. Wild-type or *cut3-477* mutant cells expressing Tbp1-LacI-3Flag were cultured at restrictive temperature (36°C) for 2 hr prior to FISH-IF analysis.

See also Figure S2.

loading to the *lacO* locus, firmly establishing that Tbp1 is sufficient to recruit condensin to chromatin (Figure 3B). In contrast, tethering of the Sfc4 TFIIIC and Rpc25 Pol III subunits fused to LacI-3Flag did not cause Cut14 localization at the *lacO* locus (Figure 3B).

Condensin Recruited by Tbp1 Mediates Centromeric Localization of a Gene Locus and Local Chromosomal Compaction during Mitosis

Using the tethering system, we also examined the association of the *lacO* locus with centromeres by fluorescent in situ

hybridization (FISH) analysis during interphase (Figures 3C and S2C). We found that when Tbp1-LacI or Cut3-LacI was expressed, the *lacO* locus was frequently located near centromeres and that the localization patterns in cells with and without expression of the LacI-fused proteins were significantly different ($p < 0.001$, Mann-Whitney U test), indicating that condensin recruited by Tbp1-LacI and Cut3-LacI can mediate the association between a genetic locus and centromeres (Figures 3D and S2D). Since centromeric association of a gene locus is a transient event during interphase, the association is scored as a frequency of localization of the locus near centromeres (Kim et al., 2013). In addition, we examined local chromosomal compaction around the c887 *lacO* locus in mitotic cells (Figures 3E and S2E). We observed that the two foci representing the c887L and c887R regions were completely overlapped in more than 50% of mitotic cells when Tbp1-LacI or Cut3-LacI was expressed (Figures 3F and S2F). In contrast, we primarily observed two separate foci without expression of the LacI-fused proteins, indicating that local chromosomal compaction was significantly facilitated by condensin molecules recruited by Tbp1-LacI or Cut3-LacI ($p < 0.001$, Mann-Whitney U test). We also observed that local compaction mediated by Tbp1-LacI was diminished in the *cut3-477* condensin mutant, suggesting that local chromosomal compaction induced by Tbp1-LacI is dependent on condensin activity (Figure 3G). Together, these results suggest that Tbp1-mediated recruitment of condensin can cause centromeric association of a gene locus and local chromosome compaction during mitosis (Figure S2G). Since condensin tethering via Tbp1-LacI and Cut3-LacI has the same effects on centromeric localization of the gene locus and local chromosomal compaction, we conclude that condensin localization is sufficient for driving these molecular processes.

The C-Terminal Domain of Cnd2 Is Required for Its Interaction with Tbp1

Thus far, our study supports the premise that the Cnd2-Tbp1 interaction mediates condensin localization at Pol III genes, thereby leading to associations between Pol III genes and centromeres. To further test this hypothesis, we sought specific *cnd2* mutations that might impair or abolish the interaction between Cnd2 and Tbp1. Toward this end, we carried out a genetic screen employing the yeast two-hybrid system (Figure S3A). By monitoring yeast two-hybrid phenotypes reflecting the Cnd2 interaction with TBP and Cnd3, we isolated specific *cnd2* gene mutations (*m1*, *m2*, and *m3*) from more than 2,000 independent colonies (Figure 4A). Two of the *cnd2* mutations, *cnd2-m1* and *m2*, abolished the Cnd2-Tbp1 interaction but did not affect the Cnd2-Cnd3 interaction, which plays a role in condensin complex formation (Figure 4B). In contrast, the *cnd2-m3* mutation abolished the Cnd2-Cnd3 interaction but did not drastically affect the Cnd2-Tbp1 interaction (Figure 4B). CoIP analysis to examine the interactions in fission yeast cells revealed that the Cnd2-Tbp1 interaction was impaired by the *cnd2-m1* and *m2* mutations, but not by the *m3* mutation (Figure 4C). On the other hand, the Cnd2-Cnd3 interaction was only disrupted by the *cnd2-m3* mutation (Figure 4D). Interestingly, the C-terminal domain of Cnd2 is eliminated by the *cnd2-m1* and *m2* mutations, as they contain nonsense mutations (Figure 4A). We thus created a C-terminal deletion mutation, *Δ176*, which deletes the last 176 aa residues of the C terminus but otherwise encodes wild-type Cnd2. The *cnd2-Δ176* mutation specifically compromised the Cnd2-Tbp1 interaction, but not the Cnd2 interaction with Cnd3 (Figures 4C and 4D). These results indicate that the 176 aa domain of the Cnd2 C terminus is important for its interaction with Tbp1 but is dispensable for the Cnd3 interaction.

The kleisin subunit interacts with all other condensin subunits to assemble the condensin complex (Figure 2A; Onn et al., 2007). Therefore, we next investigated the effect of the *cnd2* mutations on condensin complex formation (Figure 4E). After purification of Cnd2-Flag and its associating proteins, we observed interactions of Cnd2-m1, m2, and Δ176 with Cnd1 and Cnd3, but not with the Cut3 SMC subunit of condensin. We also detected the interaction of Cnd2-Δ176 with another SMC subunit, Cut14, although this interaction was weaker than the wild-type Cnd2-Cut14 interaction. This may be because the Cnd2-Cut14 interaction is strengthened by a Cnd2-Cut3 interaction. These results collectively indicate that the 176 aa domain of the Cnd2 C terminus mediates its interaction with Tbp1 and Cut3.

A Ten-Residue Domain of the Cnd2 C Terminus Is Critical for the Tbp1 Interaction

Because the *cnd2-Δ176* mutation has an inhibitory effect on the Cnd2-Tbp1 interaction, we predicted that this 176 aa domain of the Cnd2 C terminus harbors an interaction surface for Tbp1. To explore this further, we generated 18 *cnd2* mutants carrying a series of ten-residue deletions within the C-terminal domain (Figure S3B). Somewhat unexpectedly, our yeast two-hybrid data on these mutants indicated that they did not affect the Cnd2-Tbp1 interaction, while cells expressing the Cnd2-d7, d8, and d14 showed an auto-activation phenotype (Figure S3C). As this phenotype is known to interfere with the two-hybrid assay (Van Criekinge and Beyaert, 1999), we next assessed the interaction of these mutant proteins with Tbp1 using the co-immunoprecipitation assay. We observed that the *d14* mutation strongly affected the Cnd2-Tbp1 interaction compared to the *d7* and *d8* mutations (Figure S3D). Moreover, the Cnd2 interaction with the Cut3 SMC subunit was also severely impaired by the *d14* mutation (Figure S3E). These data suggest that the ten residues removed by the *d14* mutation play important roles in the Cnd2 interactions with both Tbp1 and Cut3.

The *cnd2* Point Mutation Inhibits the Cnd2-Tbp1 Interaction

The C-terminal domain of the kleisin subunit is evolutionarily conserved from bacteria to human and is predicted to fold into a specific protein structure, referred to as winged helix (Fennell-Fezzie et al., 2005). We noted that the d14 domain of the Cnd2 C terminus is located within a helix of the conserved winged-helix motif. Next, we generated three point mutations (*F701R*, *C703R*, and *L705R*) in the d14 domain (Figure 4F). CoIP analysis indicated that these point mutations consistently disrupt the Cnd2-Tbp1 interaction (Figure 4G). Importantly, only the *cnd2-C703R* mutation did not affect the condensin complex assembly, whereas the *F701R* and *L705R* mutations compromised the Cnd2-Cut3 interaction (Figure 4H). These results indicate that the Cnd2-Tbp1 interaction is specifically

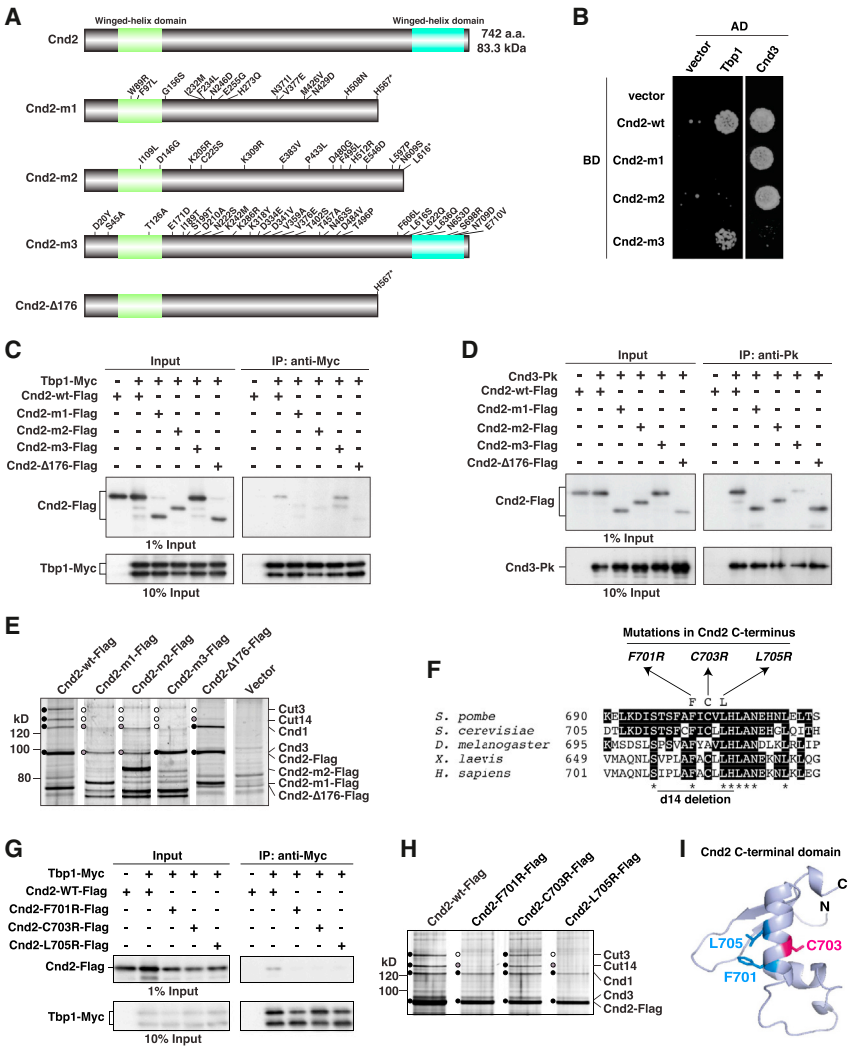


Figure 4. The *cnd2* Point Mutations Inhibit the Cnd2-Tbp1 Interaction

(A) Mapping of the *cnd2-m1*, *m2*, and *m3* mutations.

(B) Two-hybrid analysis to examine the interactions of mutant Cnd2 with Tbp1 and the Cnd3 condensin subunit.

(C) CoIP analysis for the interaction between mutant Cnd2 and Tbp1. Wild-type and mutant Cnd2 proteins were expressed from *ura4*/ars1/pUC19-based plasmids carrying its endogenous promoter.

(D) CoIP analysis for the interaction between mutant Cnd2 and the Cnd3 condensin subunit.

(E) Disruption of condensin complex formation by the *cnd2* mutations. Cnd2-Flag and its interacting proteins were affinity purified using anti-Flag M2 magnetic beads and visualized by silver staining. The analysis was carried out as described in Figure S3E.

(F) The three *cnd2* point mutations. An alignment of the kleisin condensin subunits from different species is shown. Amino acid residues identical to the fission yeast Cnd2 are boxed. Asterisks indicate the conserved residues among the species.

(G) CoIP analysis testing the interaction between mutant Cnd2 (F701R, C703R, and L705R) and Tbp1. The experiment was performed as described in (C).

(H) Effects of the *cnd2* point mutations on condensin complex formation were investigated as described in (E).

(I) Ribbon representation of the Cnd2 C-terminal domain (residues from 668 to 733). The C-terminal portion of Cnd2 has 27% sequence identity to the budding yeast Scc1 cohesin subunit, which comprises a winged-helix motif (PDB ID: 1W1W) (Haering et al., 2004). The model of Cnd2 was generated using PHYRE 2 (Kelley and Sternberg, 2009).

See also Figure S3.

inhibited by the *cnd2-C703R* mutation without affecting the formation of the condensin complex. The *C703R* mutation is therefore most useful for probing the function of the condensin-TBP interaction.

We modeled the protein structure of the Cnd2 C terminus and found that the F701 and L705 residues have their side chains on one side of the helix present within the winged-helix domain, while the side chain of the C703 residue faces the opposite side (Figure 4I). It has previously been shown that the cognate mutations (*F701R* and *L705R* in fission yeast) within the Scc1 kleisin subunit of the budding yeast cohesin complex abolish the interaction between Scc1 and Smc1 and that the helix within the winged-helix domain makes contact with the Smc1 head domain (Haering et al., 2004). Therefore, we speculate that one side of the helix within the winged-helix of the Cnd2 C terminus probably mediates the Cut3 interaction and the C703 residue on the other side is potentially involved in the Tbp1 interaction. It is also possible that the *cnd2-C703R* mutation affects the confirmation of the Cnd2 C terminus in such a way that it inhibits the Cnd2-Tbp1 interaction.

Cnd2-Tbp1 Interaction Is Essential for Faithful Chromosome Segregation

It is known that the condensin complex is essential for the viability of fission yeast cells (Saka et al., 1994; Sutani et al., 1999). We tested whether the expression of mutant Cnd2 can restore cell viability after endogenous Cnd2 proteins were depleted by the auxin-based degron system (Kanke et al., 2011; Nishimura et al., 2009). We found that expression of only wild-type Cnd2 restored cell viability, whereas mutant Cnd2 (F701R, C703R, and L705R) failed, suggesting that the Cnd2-Tbp1 interaction plays a vital role in condensin functions (Figure 5A). We also found that mutant Cnd2 was not efficiently loaded onto mitotic chromosomes compared to wild-type Cnd2, indicating that the Cnd2-Tbp1 interaction is required for the association of condensin molecules with mitotic chromosomes (Figure 5B).

Surprisingly, after 4 hr of Cnd2 depletion, expression of mutant Cnd2 proteins resulted in mitotic defects in more than 50% of the cells, whereas expression of wild-type Cnd2 did not affect mitotic chromosome segregation (Figure 5C). In wild-type anaphase cells, we observed that centromeres associating

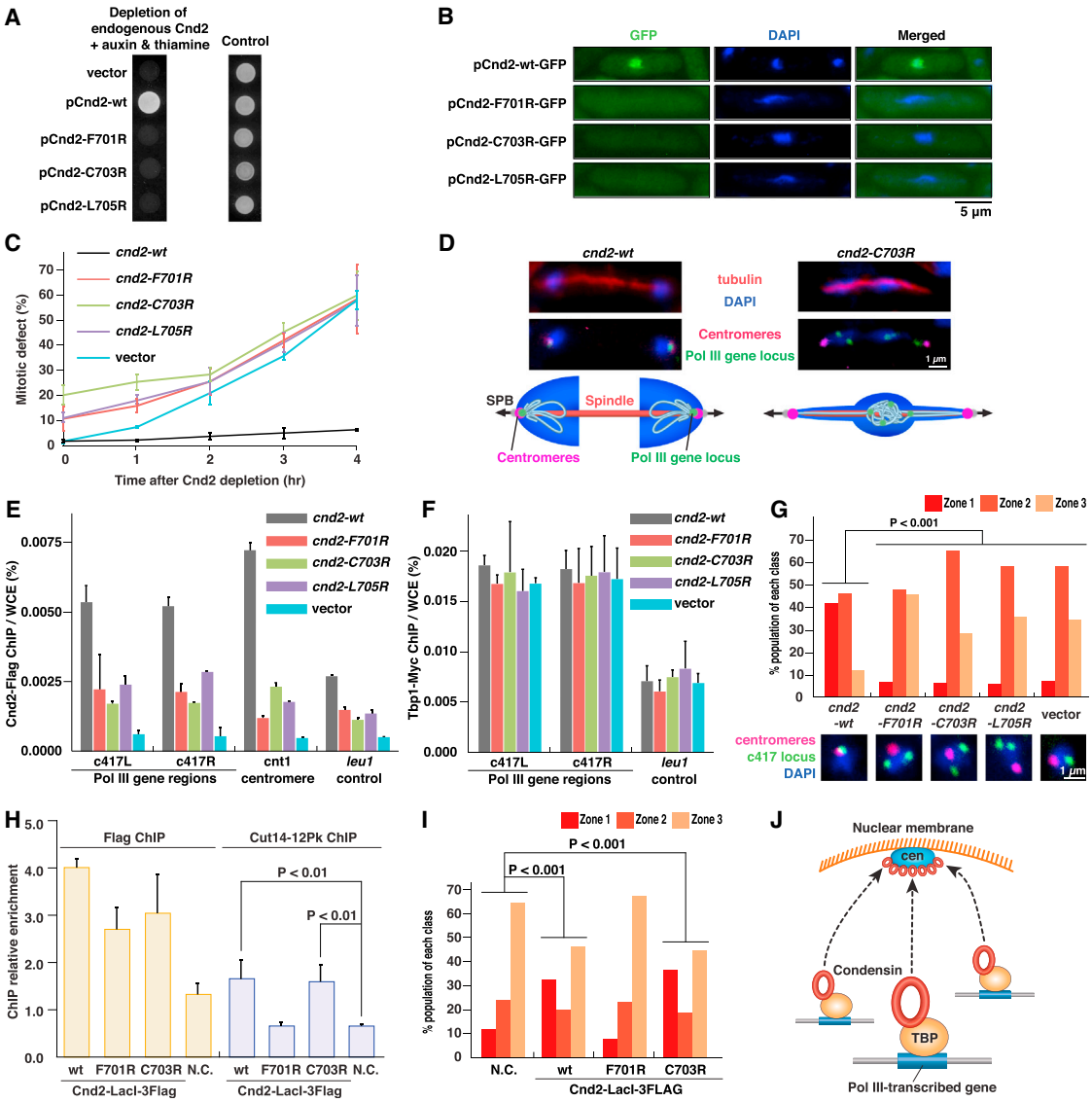


Figure 5. The *cnd2* Point Mutation Causes Chromosome Segregation Defects

(A) Expression of mutant Cnd2 does not suppress a growth defect caused by Cnd2 depletion. Endogenous Cnd2 proteins were depleted by adding auxin and thiamine into culture medium, and wild-type and mutant Cnd2 were expressed from plasmids. Logarithmically growing cells were plated onto EMM (control) and EMM + auxin + thiamine plates.

(B) Localization of Cnd2 proteins. Wild-type and mutant Cnd2 were visualized using GFP fusion proteins (green). GFP signals were detected in ethanol-fixed cells (Dovey and Russell, 2007).

(C) Mitotic defects are accumulated in cells expressing mutant Cnd2. Wild-type and mutant Cnd2 proteins were continuously expressed from plasmids, and endogenous Cnd2 proteins were depleted by the degron system. Cells were subjected to IF experiments to visualize tubulin and DAPI signals, and mitotic cells were defined by spindle staining. In fission yeast, mitotic defects are observed as stretched chromosomes (Saka et al., 1994). The percentage of mitotic defects is plotted against time.

(D) Expression of Cnd2-C703R causes a chromosomal segregation defect. Representative IF images show tubulin (red) and DAPI signals (blue) in anaphase cells (top). FISH images represent the c417 Pol III gene locus (green), centromeres (red), and DAPI signals (blue, middle). Intra-nuclear positioning of the Pol III gene locus and centromeres is depicted in the schematic diagrams (bottom).

(E) ChIP results showing enrichment of Cnd2-Flag proteins (WT, F701R, C703R, and L705R) at the indicated loci. Endogenous Cnd2 proteins were depleted for 2 hr, when most cells were in interphase. The same conditions were used for the experiments described in (F) and (G).

(F) ChIP data representing Tbp1 localization in cells expressing wild-type or mutant Cnd2.

(G) FISH results showing centromeric localization of the c417 Pol III gene locus in cells expressing wild-type or mutant Cnd2 proteins. Representative FISH images visualizing the Pol III gene locus (green) and centromeres (red) are shown at the bottom. FISH data were summarized as described in Figure S2D.

(H) Tethering of Cnd2-C703R to the c887 *lacO* locus triggers condensin localization at the locus. Cnd2 proteins (WT, C703R, and F701R) fused to LacI-3Flag were expressed from plasmids. ChIP analysis was performed as described in Figure S2B.

(legend continued on next page)

with the Pol III gene locus were pulled by the spindle microtubules and the mitotic chromosomes were properly segregated (left panels in Figure 5D). In contrast, in the *cnd2-C703R* mutant, we found that the Pol III gene locus was detached from centromeres, while centromeres were still pulled by the microtubules, causing a defect in mitotic chromosome segregation (right panels in Figure 5D). These results suggest that the Cnd2-Tbp1 interaction is required for a faithful segregation of mitotic chromosomes and thereby cell viability.

Cnd2-Tbp1 Interaction Mediates Condensin Loading onto Pol III Genes

Using the *cnd2* point mutations, we tested whether the Cnd2-Tbp1 interaction functions in the recruitment of condensin molecules onto Pol III genes. We found that the *cnd2* point mutations impaired Cnd2 localization at Pol III genes, but Tbp1 binding was not affected, suggesting that the Cnd2-Tbp1 interaction is required for condensin loading onto Pol III genes (Figures 5E and 5F). As a result, the condensin-mediated association between the Pol III gene locus and centromeres was significantly impaired by the *cnd2* mutations ($p < 0.001$, Mann-Whitney U test; Figure 5G).

Interestingly, Cnd2 localization at the centromere (cnt1) was reduced by the *cnd2* mutations, suggesting that the Cnd2-Tbp1 interaction is required for condensin localization not only at Pol III genes but also at centromeres (Figure 5E). To further examine the effect of the *cnd2* mutations on condensin distribution, we also performed ChIP-seq analysis and observed that Cnd2 peaks were associated with genomic regions, including Pol III genes such as tRNA and 5S rRNA genes, as well as centromeres (Figure S4A). Localization of mutant Cnd2 proteins was impaired at Pol III gene regions and the centromere 2 (Figures S4B and S4C). In addition to Pol III genes and centromeres, we found that enrichment of mutant Cnd2 proteins (F701R and C703R) was generally reduced throughout the genome compared to wild-type Cnd2 (Figures S4A and S4D). Expression levels of mutant Cnd2 proteins were comparable to that of the wild-type counterpart (Figure 4G).

We further characterized the *cnd2-F701R* and *C703R* mutations using the Cnd2-LacI tethering system. We found that tethering of Cnd2-C703R-LacI-3Flag to the *lacO* locus recruits condensin to the locus and mediates its centromeric association (Figures 5H and 5I). These results suggest that the *cnd2-C703R* mutation specifically inhibits condensin loading. Therefore, bypassing the Tbp1-dependent recruitment by the LacI-based tethering allows condensin to drive centromeric association of the *lacO* locus. In contrast, the mutant Cnd2-F701R-LacI-3Flag mediates neither condensin recruitment nor centromeric association, because the *F701R* mutation inhibits condensin complex formation (Figures 5H and 5I). Together, these results demonstrate that the Cnd2-Tbp1 interaction mediates condensin recruitment to Pol III genes and association of Pol III genes with centromeres (Figure 5J).

Highly Transcribed Pol II Genes Associate with Centromeres

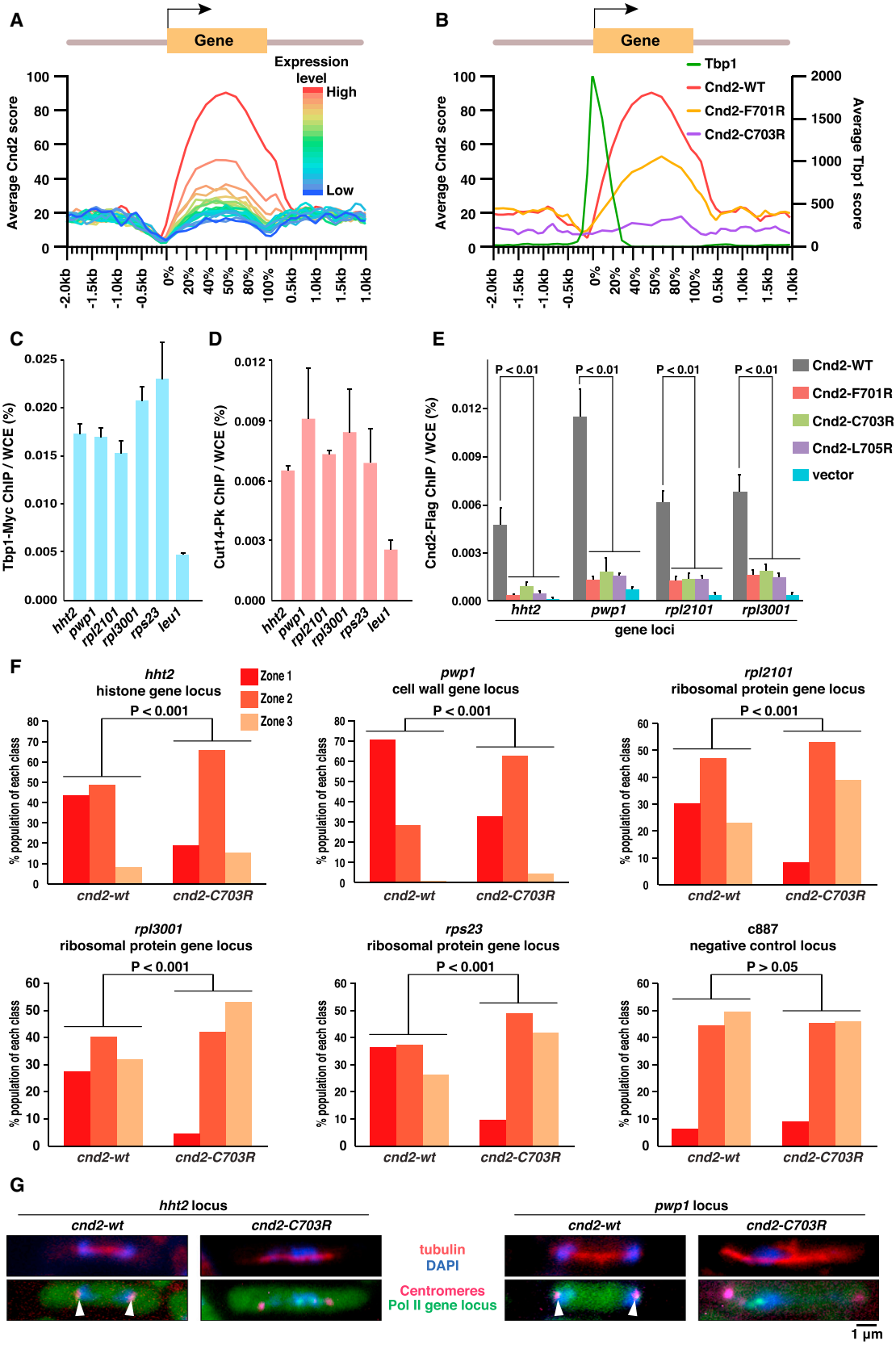
TBP is a general transcription factor essential for transcription driven by RNA polymerases I, II, and III (Hernandez, 1993). Among the Tbp1 binding sites located throughout the genome, we found that Pol III genes were those most stably bound by Tbp1 (Figure 1). This preferential localization of Tbp1 at Pol III genes might be reflective of the transcription frequency of these genes. To address this possibility, we compared Cnd2 localization and transcription levels of Pol II genes. We observed that Cnd2 enrichment was clearly correlated with transcription levels of genes and that condensin was most enriched at the most highly transcribed genes including many ribosomal protein genes (Figure 6A). Interestingly, we found that Cnd2 binding peaks were generally positioned downstream of the Tbp1 binding sites at gene promoters (Figure 6B). Since it has previously been predicted that condensin slides along chromatin in a transcription-dependent fashion (D'Ambrosio et al., 2008), it is likely that condensin molecules recruited by Tbp1 slide along genes. In addition, we performed a conventional ChIP assay and found that Tbp1 and Cut14 were co-localized at several highly transcribed Pol II genes, including histone and ribosomal protein genes (Figures 6C and 6D). We also found that the *cnd2* point mutations, which disrupt the Cnd2-Tbp1 interaction, impaired Cnd2 localization at the highly transcribed Pol II genes (Figures 6B and 6E). These results suggest that the Cnd2-Tbp1 interaction is required for condensin localization at the highly transcribed Pol II genes.

Moreover, the highly transcribed Pol II gene loci were frequently located near centromeres, and this centromeric localization was significantly compromised by the *cnd2-C703R* mutation ($p < 0.001$, Mann-Whitney U test; Figure 6F). In contrast, localization of the c887 control locus was not affected by the *cnd2* mutation ($p > 0.05$, Mann-Whitney U test; Figure 6F), indicating that disruption of the centromeric localization of the gene loci caused by the mutation does not result from indirect global effects. Moreover, we observed that the Pol II gene loci (*hht2* and *pwp1*) localized at centromeres in wild-type anaphase cells, whereas the gene loci dissociated from centromeres in the *cnd2-C703R* mutant (Figure 6G). These results collectively suggest that Cnd2-Tbp1 interaction is required for the distribution of condensin molecules at highly transcribed Pol II genes and their associations with centromeres.

The *tbp1* Mutations Impair Cnd2-Tbp1 Interaction and Condensin Loading

We next investigated which domain of Tbp1 interacts with the Cnd2 condensin subunit. To this end, we made 18 *tbp1*

(I) Centromeric localization of the c887 *lacO* locus in cells expressing Cnd2-C703R fused to LacI-3Flag. FISH images were analyzed as described in Figure S2D.
(J) Schematic model explaining how condensin-TBP interaction mediates associations between Pol III genes and centromeres.
In the experiments shown in (A)–(G), endogenous Cnd2 proteins were depleted by the degron system, and wild-type and mutant Cnd2 proteins were expressed from plasmids.
Data in (C), (E), (F), and (H) are represented as mean ± SD.
See also Figure S4.



(legend on next page)

mutations carrying a series of ten-residue deletions, which cover the conserved domain among TBP proteins in different species (Figure S5A; Hernandez, 1993). We performed a yeast two-hybrid assay to investigate the interaction between Cnd2 and mutant Tbp1 and found that several *tbp1* mutations (d6, d9, d10, d12, d15, and d17) affected the interaction in varying degrees, although *tbp1-d10* completely abolished the interaction (Figure S5B). Therefore, we then generated the *tbp1* point mutations in the Tbp1 d10 domain (Figure 7A). The yeast two-hybrid and coIP results consistently indicated that the *tbp1* point mutations impaired the Cnd2-Tbp1 interaction, suggesting that the Tbp1 d10 domain is important for the Cnd2 interaction (Figures 7B and 7C). Expression levels of mutant Tbp1 proteins were comparable to that of the wild-type counterpart.

TBP has been well studied in terms of its structure and interacting partners (Burley and Roeder, 1996). We generated a model of the 3D structure of fission yeast TBP with high accuracy using the known structures of proteins with more than 80% sequence identity (Figure 7D). The structure revealed the classic TATA box-binding folds and was highly similar to the budding yeast TBP (PDB: 4B0A). Interestingly, the d10 domain of Tbp1 does not completely overlap with the other domains required for TBP interactions with DNA, the Pol II transcription machinery (TFIIA, TFIIB, and TFIID), and the Pol III transcription machinery (TFIIIB), although the TBP domain interacting with the Brf1 TFIIIB subunit is adjacent to the d10 domain (Andel et al., 1999; Cormack and Struhl, 1993; Juo et al., 2003; Kim et al., 1993; Nikolov et al., 1995; Tan et al., 1996). It is possible that the condensin (Cnd2) interaction with Tbp1 might compete with the Brf1 interaction.

We further characterized the *tbp1* point mutations using the Tbp1-LacI tethering system. We found that tethering of mutant Tbp1-LacI-3Flag to the *lacO* locus impaired condensin loading to the locus and its centromeric association compared to wild-type Tbp1, suggesting that the Cnd2-Tbp1 interaction mediated by the Tbp1 d10 domain is required for condensin loading and centromeric association of a gene locus (Figures 7E and 7F).

The *tbp1-1* ts Mutation Is Not Suppressed by the *tbp1* Point Mutations

We performed PCR-based random mutagenesis for the *tbp1* gene and generated the *tbp1-1* mutant, which showed sensitivity to restrictive temperature (36°C) (Figures S5C and S5D). We tested whether expression of mutant Tbp1 suppresses temperature-sensitive (ts) growth of the *tbp1-1* mutant. We found that expression of only the wild-type Tbp1, but not the mutant proteins, suppressed the *tbp1-1* mutation (Figure S5D). We also observed that the *tbp1* point mutations (*D145V-K147I* and *D145I-F146R-K147I*) impaired Cnd2 localization at Pol III genes and the centromere 1 (Figure S5E). Moreover, the *tbp1* point mutations significantly compromised centromeric association of the c417 Pol III gene locus and mitotic chromosome compaction ($p < 0.01$, Mann-Whitney U test; Figures 7G and 7H). These results suggest that the Cnd2-Tbp1 interaction mediated by the Tbp1 d10 domain participates in condensin loading to endogenous target loci, their centromeric associations, and local chromosomal compaction. Based on observations that the *cnd2* and *tbp1* mutations that disrupt the Cnd2-Tbp1 interaction consistently compromise condensin recruitment (Figures 7E, S4, and S5E), we propose that this interaction has a role in the condensin loading process.

We detected the interaction between the hCAP-H kleisin subunit of the human condensin complex and hTBP (Figures S5F and S5G). We did not observe the interaction between hCAP-G and hTBP, which was previously identified and implicated in gene bookmarking (Figure S5F; Xing et al., 2008). Surprisingly, hCAP-H interacted with Tbp1 and the condensin subunits (Cnd1, Cnd2, and Cnd3), all of which are derived from fission yeast (Figure S5F). In addition, Cnd2 interacted with human TBP and hCAP-G (Figure S5F). These results suggest that the condensin-TBP interaction and the interactions required for condensin complex assembly are conserved between the fission yeast and human systems, and, importantly, those interactions are maintained even when the proteins are switched between the two evolutionarily distant organisms.

DISCUSSION

Results described here reveal that a general transcription factor, TBP, interacts with the Cnd2 condensin subunit. By employing the *cnd2* and *tbp1* gene mutations that inhibit the Cnd2-TBP interaction, we show that this protein interaction is required for condensin localization at dispersed loci including Pol III genes and highly transcribed Pol II genes. Moreover, using the LacI

Figure 6. Highly Transcribed Pol II Genes Are Bound by Condensin and Tbp1 and Associate with Centromeres

(A) Highly transcribed Pol II genes are bound by condensin. Pol II genes were classified into 20 groups based on their transcription levels. Each group contains 162 Pol II genes. Average binding patterns of Cnd2-Flag in the respective gene groups were plotted. Gene size from transcriptional initiation to termination sites was normalized to the same length for all the genes investigated.

(B) Average binding patterns of Tbp1-Myc and Cnd2-Flag (WT, F701R, and C703R) in the highest transcribed gene group shown in (A).

(C) ChIP results showing enrichment of Tbp1-Myc at the indicated Pol II gene loci. The *hht2*, *pwp1*, *rpl2101*, *rpl3001*, and *rps23* genes, except for *leu1*, are highly transcribed by Pol II.

(D) Cut14-Pk localization at the Pol II gene loci.

(E) ChIP enrichment of Cnd2-Flag proteins (WT, F701R, C703R, and L705R) at the *hht2*, *pwp1*, *rpl2101*, and *rpl3001* gene loci.

(F) FISH data representing centromeric localization of the Pol II gene loci in cells expressing wild-type Cnd2 or Cnd2-C703R proteins. FISH data were summarized as described in Figure S2D.

(G) Expression of Cnd2-C703R causes dissociation of the Pol II gene loci (*hht2* and *pwp1*) from centromeres in mitotic cells. FISH-IF analysis was performed as described in Figure 5D. Arrowheads indicate association of FISH foci corresponding to centromeres and the Pol II gene loci.

In the experiments shown in (A), (B), (E), (F), and (G), endogenous Cnd2 proteins were depleted by the degron system, and wild-type and mutant Cnd2 proteins were expressed from plasmids.

Data in (C)–(E) are represented as mean ± SD.

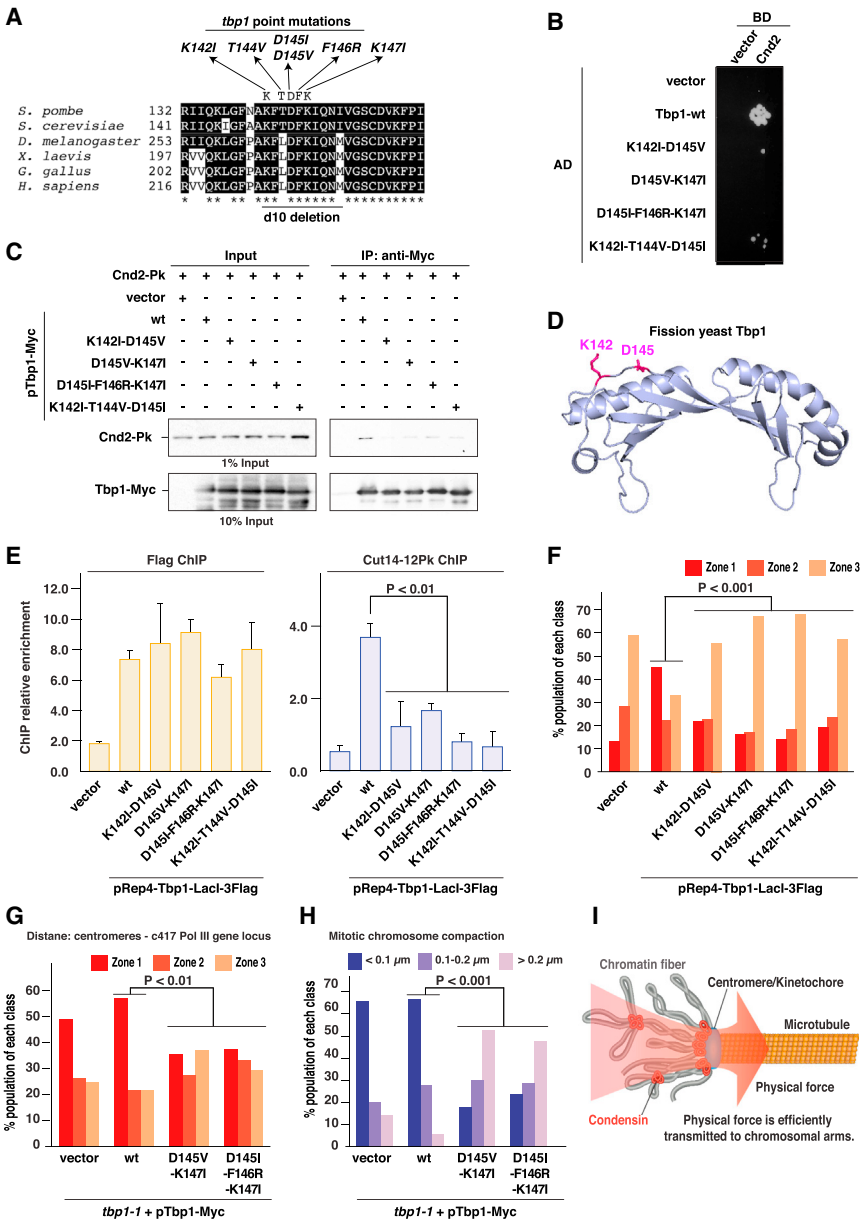


Figure 7. The *tbp1* Mutations Compromise the Cnd2-Tbp1 Interaction and Condensin Loading

(A) *tbp1* point mutations. An alignment of TBP proteins from different species is shown. Amino acid residues identical to fission yeast Tbp1 are boxed. Asterisks indicate conserved residues.

(B) Two-hybrid analysis to examine the interaction between Cnd2 and mutant Tbp1.

(C) CoIP analysis testing the interaction between Cnd2 and mutant Tbp1. Wild-type and mutant Tbp1 proteins were expressed from plasmids.

(D) Ribbon diagram of fission yeast Tbp1. Fission yeast Tbp1 has 87% sequence identity to the full-length budding yeast TBP, the structure of which is available in the RCSB database (PDB: 4B0A) (Anandapadamanaban et al., 2013). The model of the fission yeast Tbp1 was generated using PHYRE 2 (Kelley and Sternberg, 2009).

(E) The *tbp1* mutations affect condensin recruitment. Wild-type and mutant Tbp1 proteins fused to LacI-3Flag were expressed from plasmids. ChIP analysis was performed as described in Figure S2B. Data are represented as mean ± SD.

(F) Centromeric localization of the c887 *lacO* locus in cells expressing Tbp1 proteins (WT and point mutants) fused to LacI-3Flag. FISH data were summarized as described in Figure S2D.

(G) Centromeric association of the c417 Pol III gene locus in *tbp1-1* mutant cells expressing wild-type or mutant Tbp1 proteins. The *tbp1-1* ts mutant was cultured at restrictive temperature (36°C) for 6 hr.

(H) The Cnd2-Tbp1 interaction is required for mitotic chromosome compaction. The *tbp1-1* mutant expressing wild-type or mutant Tbp1 proteins was subjected to FISH-IF analysis visualizing the c417 Pol IIII gene locus, the c162 non-Pol III gene locus (120 kb distant form the c417 locus), and spindle microtubules. Mitotic cells were defined by spindle staining. The distance between the c417 and c162 loci was measured in more than 20 mitotic cells.

(I) A model to introduce how centromeric association of Pol III genes and highly transcribed Pol II genes facilitates effective chromosome segregation during mitosis (see Discussion for details).

See also Figure S5.

tethering system, we demonstrate that TBP recruits condensin onto chromatin. These observations indicate that TBP present at Pol III genes and highly transcribed Pol II genes loads condensin molecules onto these genes through the Cnd2-TBP interaction. It was previously predicted that TFIIIC recruits condensin to Pol III genes in budding yeast (D'Ambrosio et al., 2008; Haeussler et al., 2008). It is possible that TFIIIC cooperates with TBP to facilitate condensin recruitment to Pol III genes. We also find that inhibition of the Cnd2-TBP interaction impairs localization of the condensin complex at centromeres, although residual condensin is still detected at centromeres (Figure S4C). Since many Pol III genes are located at centromeres, TBP can recruit condensin to centromeres. It has also been shown that condensin is targeted to centromeres through a mechanism involving kinetochore proteins (Nakazawa et al., 2008; Tada et al., 2011). Therefore, the two mechanisms involving TBP and kinetochore proteins probably participate in the recruitment of condensin to centromeres.

Condensin is known to interact with another condensin molecule to mediate associations between chromatin fibers (Hirano et al., 2001; Yoshimura et al., 2002). Therefore, condensin molecules present at centromeres, Pol III genes, and highly transcribed Pol II genes can mediate associations between these genes and centromeres through condensin-condensin interactions (Figure 5J). Comparatively more condensin molecules localize at centromeres than other genomic loci, which might explain why Pol III and highly active Pol II genes frequently associate with centromeres (Nakazawa et al., 2008). We also find the

interaction between the kleisin subunit of the condensin complex and TBP in the human system. Moreover, condensin is known to mediate clustering of tRNA genes in the budding yeast nucleolus (Haeusler et al., 2008). These observations suggest that a similar genome-organizing mechanism is at least in part conserved in other eukaryotes and deserves additional study.

It has previously been hypothesized that condensin mediates the specific organization of mitotic chromosomes, which is critical for faithful chromosome segregation (Hudson et al., 2003). However, what the chromosomal architecture assembled by condensin is, and how the structure contributes to chromosomal segregation, was not known. Using the LacI tethering system, this study indicates that condensin recruited by TBP mediates both the association of a gene locus with centromeres and local chromosomal compaction during mitosis. We have previously shown that the centromeric association of Pol III genes is significantly increased during mitosis compared to interphase (Iwasaki et al., 2010). It is known that phosphorylation of the Cut3 condensin subunit by Cdc2 functions in accumulation of condensin molecules in the mitotic nucleus, while condensin localization patterns are similar during interphase and mitosis (Iwasaki et al., 2010; D'Ambrosio et al., 2008; Sutani et al., 1999). Therefore, condensin molecules enriched in the nucleus can facilitate the centromeric association of gene loci during mitosis. It has also been shown that the condensin complex interacts with another condensin in the presence of DNA and introduces positive supercoiling into chromatin loops (Hirano et al., 2001; Kimura et al., 1999). We thus hypothesize that the chromatin loop generated by the association between a specific locus and centromeres is supercoiled by condensin, leading to local chromosomal compaction (Figure S2G). Since several hundreds of Pol III genes and highly transcribed Pol II genes are bound by condensin, and they are dispersed across the fission yeast genome, many twisted chromatin loops are potentially generated by this mechanism. This chromosome arrangement contributes to the efficient transmission of physical force at the kinetochore to chromosomal arms, improving the fidelity of chromosome segregation (Figure 7I). Therefore, when the Cnd2-Tbp1 interaction is disrupted by *cnd2* mutations, Pol III genes and highly transcribed Pol II genes no longer associate with centromeres, and physical force at the kinetochore is diffused and inefficiently transmitted to chromosomal arm regions, resulting in chromosomal segregation defects and cellular lethality (Figure 5D). We propose that this genome-organizing mechanism contributes to an effective transmission of important genetic materials, such as Pol III genes and highly transcribed housekeeping genes, to daughter nuclei during mitosis.

EXPERIMENTAL PROCEDURES

Strain Construction and Culture Conditions

Cnd2, Tbp1, and Sfc4 proteins were tagged with Myc, Flag, Pk, or GFP at the C termini of their proteins using a PCR-based module method (Bähler et al., 1998). Strain constructions were performed using conventional genetic crosses. Yeast cells were cultured in Yeast-Extract Adenine (YEA) or Edinburgh Minimal Medium (EMM) media.

Tethering Analysis

The tethering experiment was conducted as previously described (Yamazaki et al., 2012). Tbp1, Cnd2, Cut3, Sfc4, and Rpc25 fused to LacI-3Flag at the C termini of their proteins were expressed from pREP4-based plasmids (Maundrell, 1993). Those genes are under control of the *nmt1* promoter, which is activated by the absence of thiamine in culture medium. The insertion of the 256 *lacO* tandem array at the c887 control locus was previously generated (Kim et al., 2013). The c887 control locus does not have condensin-binding sites, as determined by the ChIP-seq analysis (Tanaka et al., 2012).

Depletion of Cnd2 Proteins

Cnd2 proteins were efficiently depleted from fission yeast cells using an auxin-based degron system (Kanke et al., 2011; Nishimura et al., 2009). Skp1-atTIR1 fusion protein expressed from the *Padh15-skp1-AtTIR1-NLS-9Myc* locus mediates polyubiquitination of the Aid degron when it binds to auxin. The DNA fragment containing the *hphMX6-nmt81-cnd2*$^{+}$*-aid-kanMX6* was generated by a PCR-based approach and used for yeast transformation. Cnd2 fused to the Aid degron is expressed from the endogenous *cnd2* gene locus. Cells were cultured in EMM medium containing 15 μM thiamine and 0.5 mM auxin.

ACCESSION NUMBERS

The accession number for the data reported in this paper is GEO: GSE60273.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2015.07.007.

ACKNOWLEDGMENTS

We would like to thank F. Ishikawa and H. Masukata for experimental materials including fission yeast strains and plasmids; K. Gull for anti-tubulin TAT1 antibody; the Wistar Institute Genomics and Bioinformatics Facilities for high-throughput sequencing and genomic data analyses; the Imaging Facility for microscopic analysis; and the Proteomics Facility for mass spectrometry analysis. We also thank M. Murphy, P. Lieberman, and M. Showe for critically reading the manuscript and S. Shaffer for editorial assistance. This work was supported by the G. Harold & Leila Y. Mathers Charitable Foundation, the National Institutes of Health (NIH) grant CA010815, and the NIH Director's New Innovator Award Program (DP2-OD004348) to K.N.

Received: September 30, 2014
Revised: April 16, 2015
Accepted: July 1, 2015
Published: August 6, 2015

REFERENCES

Anandapadamanaban, M., Andresen, C., Helander, S., Ohyama, Y., Siponen, M.I., Lundström, P., Kokubo, T., Ikura, M., Moche, M., and Sunnerhagen, M. (2013). High-resolution structure of TBP with TAF1 reveals anchoring patterns in transcriptional regulation. Nat. Struct. Mol. Biol. *20*, 1008–1014.

Andel, F., 3rd, Ladurner, A.G., Inouye, C., Tjian, R., and Nogales, E. (1999). Three-dimensional structure of the human TFIID-IIA-IIB complex. Science *286*, 2153–2156.

Bähler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast *14*, 943–951.

Basi, G., Schmid, E., and Maundrell, K. (1993). TATA box mutations in the Schizosaccharomyces pombe nmt1 promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. Gene *123*, 131–136.

Burley, S.K., and Roeder, R.G. (1996). Biochemistry and structural biology of transcription factor IID (TFIID). Annu. Rev. Biochem. *65*, 769–799.

Cormack, B.P., and Struhl, K. (1993). Regional codon randomization: defining a TATA-binding protein surface required for RNA polymerase III transcription. Science *262*, 244–248.

D'Ambrosio, C., Schmidt, C.K., Katou, Y., Kelly, G., Itoh, T., Shirahige, K., and Uhlmann, F. (2008). Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. Genes Dev. *22*, 2215–2227.

Dovey, C.L., and Russell, P. (2007). Mms22 preserves genomic integrity during DNA replication in Schizosaccharomyces pombe. Genetics *177*, 47–61.

Fennell-Fezzie, R., Gradia, S.D., Akey, D., and Berger, J.M. (2005). The MukF subunit of Escherichia coli condensin: architecture and functional relationship to kleisins. EMBO J. *24*, 1921–1930.

Haering, C.H., Schoffnegger, D., Nishino, T., Helmhart, W., Nasmyth, K., and Löwe, J. (2004). Structure and stability of cohesin's Smc1-kleisin interaction. Mol. Cell *15*, 951–964.

Haeusler, R.A., Pratt-Hyatt, M., Good, P.D., Gipson, T.A., and Engelke, D.R. (2008). Clustering of yeast tRNA genes is mediated by specific association of condensin with tRNA gene transcription complexes. Genes Dev. *22*, 2204–2214.

Hagstrom, K.A., Holmes, V.F., Cozzarelli, N.R., and Meyer, B.J. (2002). C. elegans condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis. Genes Dev. *16*, 729–742.

Hernandez, N. (1993). TBP, a universal eukaryotic transcription factor? Genes Dev. *7* (7B), 1291–1308.

Hirano, T. (2006). At the heart of the chromosome: SMC proteins in action. Nat. Rev. Mol. Cell Biol. *7*, 311–322.

Hirano, M., Anderson, D.E., Erickson, H.P., and Hirano, T. (2001). Bimodal activation of SMC ATPase by intra- and inter-molecular interactions. EMBO J. *20*, 3238–3250.

Huang, Y., and Maraia, R.J. (2001). Comparison of the RNA polymerase III transcription machinery in Schizosaccharomyces pombe, Saccharomyces cerevisiae and human. Nucleic Acids Res. *29*, 2675–2690.

Hudson, D.F., Vagnarelli, P., Gassmann, R., and Earnshaw, W.C. (2003). Condensin is required for nonhistone protein assembly and structural integrity of vertebrate mitotic chromosomes. Dev. Cell *5*, 323–336.

Iwasaki, O., Tanaka, A., Tanizawa, H., Grewal, S.I., and Noma, K. (2010). Centromeric localization of dispersed Pol III genes in fission yeast. Mol. Biol. Cell *21*, 254–265.

Juo, Z.S., Kassavetis, G.A., Wang, J., Geiduschek, E.P., and Sigler, P.B. (2003). Crystal structure of a transcription factor IIIB core interface ternary complex. Nature *422*, 534–539.

Kagey, M.H., Newman, J.J., Bilodeau, S., Zhan, Y., Orlando, D.A., van Berkum, N.L., Ebmeier, C.C., Goossens, J., Rahl, P.B., Levine, S.S., et al. (2010). Mediator and cohesin connect gene expression and chromatin architecture. Nature *467*, 430–435.

Kanke, M., Nishimura, K., Kanemaki, M., Kakimoto, T., Takahashi, T.S., Nakagawa, T., and Masukata, H. (2011). Auxin-inducible protein depletion system in fission yeast. BMC Cell Biol. *12*, 8.

Kelley, L.A., and Sternberg, M.J. (2009). Protein structure prediction on the Web: a case study using the Phyre server. Nat. Protoc. *4*, 363–371.

Kim, Y., Geiger, J.H., Hahn, S., and Sigler, P.B. (1993). Crystal structure of a yeast TBP/TATA-box complex. Nature *365*, 512–520.

Kim, K.D., Tanizawa, H., Iwasaki, O., Corcoran, C.J., Capizzi, J.R., Hayden, J.E., and Noma, K. (2013). Centromeric motion facilitates the mobility of interphase genomic regions in fission yeast. J. Cell Sci. *126*, 5271–5283.

Kimura, K., Rybenkov, V.V., Crisona, N.J., Hirano, T., and Cozzarelli, N.R. (1999). 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. Cell *98*, 239–248.

Maundrell, K. (1993). Thiamine-repressible expression vectors pREP and pRIP for fission yeast. Gene *123*, 127–130.

Nakazawa, N., Nakamura, T., Kokubu, A., Ebe, M., Nagao, K., and Yanagida, M. (2008). Dissection of the essential steps for condensin accumulation at kinetochores and rDNAs during fission yeast mitosis. J. Cell Biol. *180*, 1115–1131.

Nasmyth, K., and Haering, C.H. (2005). The structure and function of SMC and kleisin complexes. Annu. Rev. Biochem. *74*, 595–648.

Nikolov, D.B., Chen, H., Halay, E.D., Usheva, A.A., Hisatake, K., Lee, D.K., Roeder, R.G., and Burley, S.K. (1995). Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature *377*, 119–128.

Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T., and Kanemaki, M. (2009). An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nat. Methods *6*, 917–922.

Noma, K., Cam, H.P., Maraia, R.J., and Grewal, S.I. (2006). A role for TFIIIC transcription factor complex in genome organization. Cell *125*, 859–872.

Onn, I., Aono, N., Hirano, M., and Hirano, T. (2007). Reconstitution and subunit geometry of human condensin complexes. EMBO J. *26*, 1024–1034.

Saka, Y., Sutani, T., Yamashita, Y., Saitoh, S., Takeuchi, M., Nakaseko, Y., and Yanagida, M. (1994). Fission yeast cut3 and cut14, members of a ubiquitous protein family, are required for chromosome condensation and segregation in mitosis. EMBO J. *13*, 4938–4952.

Steffensen, S., Coelho, P.A., Cobbe, N., Vass, S., Costa, M., Hassan, B., Prokopenko, S.N., Bellen, H., Heck, M.M., and Sunkel, C.E. (2001). A role for Drosophila SMC4 in the resolution of sister chromatids in mitosis. Curr. Biol. *11*, 295–307.

Sutani, T., Yuasa, T., Tomonaga, T., Dohmae, N., Takio, K., and Yanagida, M. (1999). Fission yeast condensin complex: essential roles of non-SMC subunits for condensation and Cdc2 phosphorylation of Cut3/SMC4. Genes Dev. *13*, 2271–2283.

Tada, K., Susumu, H., Sakuno, T., and Watanabe, Y. (2011). Condensin association with histone H2A shapes mitotic chromosomes. Nature *474*, 477–483.

Tan, S., Hunziker, Y., Sargent, D.F., and Richmond, T.J. (1996). Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature *381*, 127–151.

Tanaka, A., Tanizawa, H., Sriswasdi, S., Iwasaki, O., Chatterjee, A.G., Speicher, D.W., Levin, H.L., Noguchi, E., and Noma, K. (2012). Epigenetic regulation of condensin-mediated genome organization during the cell cycle and upon DNA damage through histone H3 lysine 56 acetylation. Mol. Cell *48*, 532–546.

Van Criekinge, W., and Beyaert, R. (1999). Yeast two-hybrid: State of the art. Biol. Proced. Online *2*, 1–38.

Wood, A.J., Severson, A.F., and Meyer, B.J. (2010). Condensin and cohesin complexity: the expanding repertoire of functions. Nat. Rev. Genet. *11*, 391–404.

Xing, H., Vanderford, N.L., and Sarge, K.D. (2008). The TBP-PP2A mitotic complex bookmarks genes by preventing condensin action. Nat. Cell Biol. *10*, 1318–1323.

Yamazaki, H., Tarumoto, Y., and Ishikawa, F. (2012). Tel1(ATM) and Rad3(ATR) phosphorylate the telomere protein Ccq1 to recruit telomerase and elongate telomeres in fission yeast. Genes Dev. *26*, 241–246.

Yoshimura, S.H., Hizume, K., Murakami, A., Sutani, T., Takeyasu, K., and Yanagida, M. (2002). Condensin architecture and interaction with DNA: regulatory non-SMC subunits bind to the head of SMC heterodimer. Curr. Biol. *12*, 508–513.