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Centromeric Localization of Dispersed Pol III Genes in Fission Yeast

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The eukaryotic genome is a complex three-dimensional entity residing in the nucleus. We present evidence that Pol III–transcribed genes such as *tRNA* and *5S rRNA* genes can localize to centromeres and contribute to a global genome organization. Furthermore, we find that ectopic insertion of Pol III genes into a non-Pol III gene locus results in the centromeric localization of the locus. We show that the centromeric localization of Pol III genes is mediated by condensin, which interacts with the Pol III transcription machinery, and that transcription levels of the Pol III genes are negatively correlated with the centromeric localization of Pol III genes. This centromeric localization of Pol III genes initially observed in interphase becomes prominent during mitosis, when chromosomes are condensed. Remarkably, defective mitotic chromosome condensation by a condensin mutation, *cut3-477*, which reduces the centromeric localization of Pol III genes, is suppressed by a mutation in the *sfc3* gene encoding the Pol III transcription factor TFIIIC subunit, *sfc3-1*. The *sfc3-1* mutation promotes the centromeric localization of Pol III genes. Our study suggests there are functional links between the process of the centromeric localization of dispersed Pol III genes, their transcription, and the assembly of condensed mitotic chromosomes.

INTRODUCTION

Large-scale DNA sequencing of a variety of organisms has led to the detailed annotation of genes and regulatory elements dispersed throughout their genomes. Eukaryotic genomes exist as complex three-dimensional structures in the nucleus. Understanding the functional relationships between intranuclear positioning of the genomic loci and the DNA regulatory activities including transcription and replication is an important problem in current genome biology (Misteli, 2007). It has been proposed that transcription of Pol II genes involves higher-order genome organization via "transcriptional factories," although clustering of Pol II genes is likely mediated by the nuclear speckles (SC-35 domains) containing numerous mRNA metabolic factors (Cook, 1999; Lamond and Spector, 2003; Chakalova *et al.*, 2005; Brown *et al.*, 2008; Lawrence and Clemson, 2008; Sutherland and Bickmore, 2009). Likewise, various DNA regulatory activities are known to impact the global genome structure in the nucleus (Misteli, 2007). However, the significance of higher-order genome structures in individual DNA regulatory processes and molecular mechanisms of the global genome organizations coupled to DNA regulatory processes remain unclear.

In eukaryotes, RNA polymerase (Pol) III transcribes the *tRNA* and *5S rRNA* genes as well as several small noncoding RNA genes (Willis, 1993; Roeder, 1996; Paule and White, 2000; Huang and Maraia, 2001). The Pol III transcription machinery includes several transcription factor complexes that direct the accurate positioning of Pol III on *tRNA* and *5S rRNA* genes (Paule and White, 2000; Geiduschek and Kassavetis, 2001). Transcription of the *tRNA* genes involves the initial recognition of A and B box promoter sequences located within the *tRNA* gene by the transcription factor TFIIIC. Binding of TFIIIC directs the transcription factor complex, TFIIIB, to bind upstream of the transcription start site, and TFIIIB in turn recruits Pol III to the *tRNA* gene. Once transcription is initiated, transcriptional elongation results in TFIIIC dissociation from the *tRNA* gene promoter, whereas TFIIIB stably binds to the DNA and directs multiple rounds of Pol III transcription. Transcription of *5S rRNA* genes requires an additional transcription factor, TFIIIA, which consists of only one subunit, Sfc2, in fission yeast (Schulman and Setzer, 2002). TFIIIA first recognizes the internal promoter sequences, and then recruits TFIIIC and TFIIIB, allowing TFIIIB to then recruit Pol III to *5S rRNA* promoter.

In budding yeast, it has been shown that dispersed *tRNA* genes cluster in the nucleolus, suggesting that Pol III transcription of these genes likely affects the global genome structure (Thompson *et al.*, 2003). However, it remains to be determined whether the nucleolar clustering of *tRNA* genes observed in budding yeast is a generally conserved mechanism, as its occurrence in other organisms has not been investigated. It has been shown that a *tRNA* gene situated between the heterochromatin and euchromatin domains functions as a barrier (also called chromatin boundary) to prevent the spread of heterochromatin (Oki and Kamakaka, 2005; Noma *et al.*, 2006; Scott *et al.*, 2006, 2007). In higher eukaryotes, short interspersed repeated DNA elements (SINEs) originate from Pol III genes and are transcribed by

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Abbreviation used: *COC*, chromosome-organizing clamp.

Pol III machinery (Deininger, 1989). Approximately 500,000 copies of the *Alu* elements consisting of Pol III promoters are dispersed in the human genome. Interestingly, *Alu* and another SINE element, *B2*, are also involved in forming chromatin boundaries (Willoughby *et al.*, 2000; Lunyak *et al.*, 2007). These findings, from yeast and mammals, suggest a general role for the Pol III genes and their transcription machinery in genome organization.

The fission yeast *Schizosaccharomyces pombe* offers an excellent model system to investigate the molecular mechanisms that organize the functional genome. Its genome is ~13.8 Mb, consisting of ~5000 genes located on three chromosomes, whose organization and composition are similar to those in higher eukaryotes (Wood *et al.*, 2002). For instance, the fission yeast genome contains large blocks of heterochromatin at centromeric and subtelomeric regions, both of which are located at the nuclear periphery (Funabiki *et al.*, 1993; Hall *et al.*, 2003; Cam *et al.*, 2005). Moreover, fission yeast centromeres range from 35 to 110 kb and consist of a central kinetochore surrounded by heterochromatic satellite repeats (Takahashi *et al.*, 1992). The centromeric architecture essential for chromosome segregation is similar between fission yeast and human (Yanagida, 2005). Interestingly, 52 of 174 fission yeast *tRNA* genes are located at centromeres (Takahashi *et al.*, 1991), and some centromeric *tRNA* genes have been shown to function as a heterochromatin barrier (Noma *et al.*, 2006; Scott *et al.*, 2006, 2007). The observation that many *tRNA* genes are located at centromeres, suggests that centromeric *tRNA* genes may have an uncharacterized role in centromere functions essential for chromosome segregation.

We have recently shown that TFIIIC participates in organizing the higher-order genome structure in fission yeast (Noma *et al.*, 2006). Our genome-wide ChIP-chip analysis revealed that more than 60 loci dispersed across the fission yeast genome contain bound TFIIIC without Pol III association. These loci were referred to as *COC* (chromosome-organizing clamps), based on the observation that in addition to being occupied by high concentrations of TFIIIC, they are tethered to the nuclear periphery. TFIIIC binding to specific DNA sequences is critical for boundary function demarcating chromosomal domains. However, whether and how Pol III genes dispersed across the fission yeast genome are involved in global genome organization remain unclear.

In this study, we utilize an integrated approach, combining microscopic and genetic analyses, to gain comprehensive insights into the higher-order genome organization by Pol III genes and their transcription machinery. Our analyses reveal a global chromosome organization by which dispersed *tRNA* and *5S rRNA* genes frequently localize in proximity to centromeres.

MATERIALS AND METHODS

Strain Construction and Culture Conditions

Sfc6 (TFIIIC subunit), Brf1 (TFIIIB), Rpc25 (RNA Pol III), and Sfc2 (TFIIIA) proteins were tagged with Myc, Flag, or TAP, at the C-termini of their proteins using a PCR-based module method (Bahler *et al.*, 1998). The *sfc3-1* mutation was created by a PCR-based method using oligos containing a single substitution of Glu for Gly at position 361 (G361E). The insertion of the two Pol III genes (*tRNA^{asn} and 5S rRNA*) at the c162 locus was generated using the DNA fragments constructed by PCR. The two Pol III genes are derived from the c417 locus. All other strain constructions were performed using conventional genetic crosses. Yeast cells were cultured in yeast-extract adenine (YEA) medium at 30°C.

Immunofluorescence

Immunofluorescence (IF) experiments were performed as described (Noma and Grewal, 2002). Fixed cells were incubated with primary antibodies, such as mouse anti-Myc (9E10, Clontech, Palo Alto, CA), mouse anti-Flag (M2, Sigma, St. Louis, MO), and rabbit anti-Myc (Novus Biologicals, Littleton, CO), at 1:4000 dilution. Rabbit anti-Swi6 (Abcam, Bedford, MA), mouse anti-Nop1 (Encor Biotechnology, Alachua, F), and mouse anti-tubulin TAT1 (Woods *et al.*, 1989) were used at 1:1000, 1:100, and 1:10 dilutions, respectively. Cells were subsequently incubated with secondary antibodies, such as Cy3-conjugated anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA), Alexa Flour 488 anti-rabbit IgG, and Alexa Flour 488 anti-mouse IgG (Molecular Probes, Eugene, OR), at 1:2000 dilution. Immunostained cells were analyzed by a Zeiss Axioimager Z1 fluorescence microscope with oil immersion objective lens (Plan Apochromat, 100×, NA 1.4, Zeiss, Thornwood, NY). Images were acquired at 0.2-μm intervals in the *z*-axis and deconvolved by Axiovision 4.6.3 software (Zeiss). More than 100 cells were analyzed for each experiment if not otherwise specified.

Fluorescent In Situ Hybridization

Fluorescent in situ hybridization (FISH) experiments were performed as described (Sadaie *et al.*, 2003). To generate FISH probes, cosmids, plasmid, or PCR-derived DNA fragments were labeled by incorporating Cy3-dCTP or Cy5-dCTP (GE Healthcare, Waukesha, WI) using a random primer DNA labeling kit (Takara, Tokyo, Japan). The cosmid clones and the plasmid pRS140 were used for preparing FISH probes specific to the respective genomic loci and centromeres, respectively. To generate FISH probes specific to the *tRNA* and *5S rRNA* genes, the DNA fragments were first amplified by PCR using genomic DNA as template. A single primer pair that amplifies most of *5S rRNA* genes (31 of 33 members in the genome) was used for the PCR (Wood *et al.*, 2002). DNA fragments corresponding to an individual *tRNA* gene family were prepared using two or three primer pairs that amplify almost all *tRNA* genes of the *tRNA* gene family, *tRNA^{ala}* (11 of 12 members in the genome), *tRNA^{gly}* (12 members), or *tRNA^{pro}* genes (8 of 9 members). Although the DNA fragments amplified by using the primer pairs for *tRNA^{ala}*, *tRNA^{pro}* and *5S rRNA* genes lack a few members, every member of those genes should be detected by FISH due to high sequence homology among the family members. After electrophoresis, the DNA fragments corresponding to *tRNA* and *5S rRNA* genes were cut from agarose gel according to their sizes and were subjected to DNA labeling. IF experiments were followed by the FISH procedure as described (Sadaie *et al.*, 2003). More than 100 cells were analyzed for each experiment if not otherwise specified.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) was carried out as described previously (Noma *et al.*, 2001) with slight modifications. Chromatin was fixed with 3% paraformaldehyde followed by further cross-linking with 10 mM dimethyl adipimidate (DMA). Proteins tagged with Myc and TAP were immunoprecipitated using anti-Myc polyclonal antibody (Novus Biologicals) and IgG Sepharose (Amersham, Piscataway, NJ), respectively. ChIP samples were subjected to PCR analysis, and PCR products were separated on a 4% polyacrylamide gel. The gel was stained by SYBR Green I (Invitrogen, Carlsbad, CA) and scanned by Typhoon 9410 Variable Mode Imager (GE Healthcare).

RNA Analysis

Total RNA containing genomic DNA was extracted from cells as described previously (Volpe *et al.*, 2002). Total RNA sample (~5 μg) was treated with 10 U of DNase I (Promega, Madison, WI) at 37°C for 40 min and then purified by phenol/chloroform extraction. The resultant RNA sample was subjected to RT-PCR (Onestep RT-PCR kit, Qiagen, Chatsworth, CA). RT-PCR samples were separated on a 4% polyacrylamide gel. The gel was stained by SYBR Green I and scanned by Typhoon 9410 Variable Mode Imager.

Immunoprecipitation

Exponentially growing cells in YEA medium were harvested from 300 ml culture (OD 0.5). Cell lysate was prepared by grinding frozen cell pellet in 500 μl IP buffer (50 mM HEPES, pH 7.6, 75 mM KCl, 0.1% NP-40, 20% Glycerol, 1 mM EDTA, 1 mM PMSF), supplemented with protease inhibitor cocktail (Roche, Indianapolis, IN). Flag-tagged proteins were recovered from soluble fractions of cell extracts by incubation with anti-Flag M2 agarose beads (Sigma). The beads were washed with 500 μl IP buffer five times and subsequently boiled with 60 μl 1× Laemmli sample buffer before SDS-PAGE analysis.

RESULTS

Localization of Pol III Transcription Machinery in the Nucleus

Genomic binding sites for the Pol III transcription machinery, which consists of TFIIIC, TFIIIB, Pol III, and TFIIIA, are dispersed throughout the fission yeast genome (Supplemental Figure S1). We hereon refer to any component derived

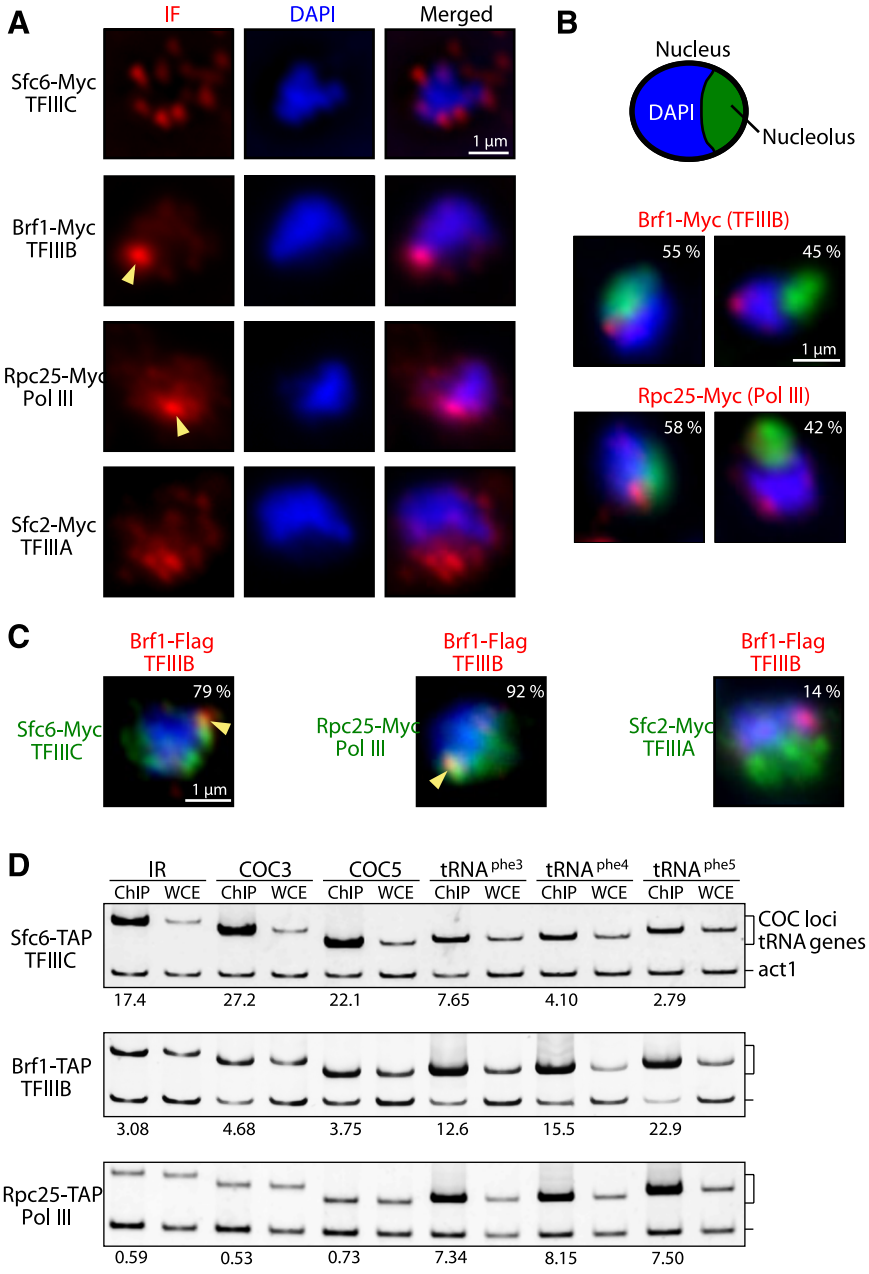

Figure 1. Localization of Pol III factors within the nucleus. (A) Deconvolved immunofluorescent images of cells stained for Sfc6-Myc (TFIIIC component), Brf1-Myc (TFIIIB), Rpc25-Myc (Pol III), and Sfc2-Myc (TFIIIA) proteins (red) were merged with DAPI signals (blue). Arrowheads indicate focal localization of Brf1 and Rpc25 proteins. (B) Localization of Brf1 and Rpc25 near the nucleolus. Schematic representation of fission yeast nucleus is shown on top. Localizations of Brf1-Myc and Rpc25-Myc (red) are shown in the middle and bottom panels, respectively. Localization of Nop1, a fibrillarin protein, is shown as nucleolar marker (green). Nucleoplasm was visualized by DAPI staining (blue). The percentage with which Brf1 or Rpc25 foci associate (left) and do not associate (right) with the nucleolus is noted in each image. (C) Colocalization of Pol III factors. Pairs of Pol III factors, selected from Sfc6, Brf1, Rpc25, and Sfc2 carrying either Myc (green) or Flag (red) epitope, were immunostained and merged with DAPI signals (blue). Arrowheads indicate the positions at which foci derived from two different Pol III factors overlap in the percentage of cells noted in each image. (D) Binding of Pol III factors to distant genomic loci. ChIP was performed using strains expressing Sfc6-TAP, Brf1-TAP, and Rpc25-TAP. DNA isolated from ChIP or whole-cell extract (WCE) fractions was subjected to multiplex PCR to amplify DNA fragments from *COC* loci (including the *IR* boundary at the mating-type region) and *tRNA*^{phe} genes (top bands), as well as an *act1* fragment (bottom bands) as an internal amplification control. The ratios of top and bottom bands present in WCE were used to calculate the relative enrichment of precipitated samples. ChIP experiments were repeated at least twice for each factor, and representative results are shown.

from the Pol III transcription machinery as a Pol III factor. Although we have recently shown that TFIIIC plays important roles in higher-order genome organization (Noma *et al.*, 2006), it was unclear whether other components of the Pol III machinery might also be involved in the organization of higher-order genome structure. To determine whether this is the case, we first performed immunofluorescence (IF) analysis, to visualize the locations of the various Pol III factors within the nucleus. We found that different Pol III factors have distinct localization patterns (Figure 1A). Consistent with our previous finding (Noma *et al.*, 2006), Sfc6 protein, a subunit of TFIIIC, preferentially localized to 5–10 discrete spots associated with the nuclear periphery, with a few Sfc6 spots always present near the nucleolus (Figure 1A and Supplemental Figure S2A). Staining of the Sfc2 protein, a subunit of TFIIIA, showed a diffuse localization pattern with distinct signals present in the nucleolus (Figure 1A and Supplemental Figure S2A). Remarkably, we also observed the focal localizations of Brf1 (TFIIIB) and Rpc25 (Pol III) proteins at the nuclear periphery (Figure 1A). Brf1 primarily displayed dot-like nucleoplasmic signals, whereas diffuse signals of Rpc25 were detected in the nucleoplasm. We further investigated the intranuclear positioning of Brf1 and Rpc25 foci. Interestingly, Brf1 and Rpc25 foci were located at the surface boundary between the nucleoplasm and the nucleolus in more than 50% of the cells (Figure 1B). To determine whether the Pol III factors colocalize in the nucleus, we performed coIF experiments with selected pairs of Pol III factors. We found that Brf1 and Rpc25 colocalized at the nuclear periphery (Figure 1C). Similarly, Brf1 foci overlapped with one of the Sfc6 spots present at the nuclear periphery. One of the Sfc6 spots also overlapped with the Sfc2 signal (Supplemental Figure S2B). We did not, however, observe significant colocalization between Brf1 and Sfc2,

although faint signals for Sfc2 were present at Brf1 foci in most cases.

To examine associations of Pol III factors with genomic regions, we carried out ChIP analysis and investigated binding of the Pol III factors at the *COC* loci (including the *IR* boundary at the mating-type region), *tRNA,* and *5S rRNA* genes. Sfc6 localized at the *tRNA* genes and the *COC* loci (Figure 1D). Enrichment of Sfc6 at the *COC* loci was significantly higher than at the *tRNA* genes (Figure 1D). Because TFIIIC dissociates from the *tRNA* genes during transcription and the *COC* loci are not transcribed by Pol III, this result suggests that TFIIIC binds more stably to the *COC* loci than to actively transcribed *tRNA* genes. We could not detect Sfc6 localization at the *5S rRNA* genes (Supplemental Figure S2C), suggesting that the association of TFIIIC with the *5S rRNA* genes is more transient than TFIIIC binding to the *tRNA* genes. Alternatively, our ChIP analysis is not sufficiently sensitive to detect Sfc6 localization at the *5S rRNA* genes. Furthermore, we found that Brf1 localized at all tested loci including the *COC* loci, *tRNA,* and *5S rRNA* genes, but the levels of Brf1 binding were consistently higher at the *tRNA* and *5S rRNA* genes than at the *COC* loci (Figure 1D and Supplemental Figure S2C). These results suggest that Brf1 associates more stably with actively transcribed Pol III genes than with the *COC* loci, probably because TFIIIB remains associated with Pol III genes, directing multiple rounds of Pol III transcription (Kassavetis *et al.,* 1990). In support of this hypothesis, we found that Rpc25 (Pol III) localized at the *tRNA* and *5S rRNA* genes, but not at the *COC* loci (Figure 1D and Supplemental Figure S2C).

Focal Localization of TFIIIC, TFIIIB, and Pol III at Centromeres

It has been shown that heterochromatic loci including centromeres and telomeres are bound by HP1/Swi6 heterochromatin proteins and are located at the nuclear periphery in fission yeast (Funabiki *et al.,* 1993; Hall *et al.,* 2003). To investigate whether TFIIIC, TFIIIB, and Pol III foci, which we have shown to be present at the nuclear periphery, colocalize with heterochromatic loci, we covisualized the Pol III factors and Swi6 proteins by IF. One of the Swi6 spots clearly colocalized with Sfc6, Brf1, and Rpc25 foci (Figure 2A). In fission yeast, IF staining for Swi6 proteins consistently shows Swi6 foci associating with centromeres and telomeres (Hall *et al.,* 2003). To determine whether TFIIIC, TFIIIB, and Pol III foci also associate with either centromeres or telomeres, we performed fluorescent in situ hybridization (FISH) analysis combined with IF in order to visualize both centromeres and Pol III factors (Figure 2B). The FISH result showed that centromeres of all three chromosomes clustered at the nuclear periphery, as indicated by the presence of a single centromeric spot. Our results in Figure 2B showed that Sfc6, Brf1, and Rpc25 foci also colocalized with centromeres. These results indicate that Pol III factors are significantly enriched near centromeres.

Centromeric Localization of Dispersed Pol III Genes

In fission yeast, 52 of 174 *tRNA* genes are distributed at the centromeric regions, whereas the remaining *tRNA* genes and all (33 members) of the *5S rRNA* genes are dispersed throughout the three chromosomes (Takahashi *et al.,* 1991; Wood *et al.,* 2002). To examine whether the noncentromeric Pol III genes can relocate and cluster at centromeres, we performed FISH analyses using probes specific to three individual *tRNA* gene families ($tRNA^{ala}$, $tRNA^{gly}$, and $tRNA^{pro}$) as well as to the *5S rRNA* genes. We found that a prominent FISH signal corresponding to multiple *tRNA* genes colocalized with centromeres (Figure 2C). Because of the utilization of short specific probes, only clusters of *tRNA* and *5S rRNA* genes can be visualized as a focal spot, because the combined signal from multiple probes is required for visualization. The $tRNA^{ala}$ gene signal was most strongly colocalized with centromeres among the three *tRNA* gene families, probably because 7 members of 12 $tRNA^{ala}$ genes are encoded at centromeres. However, we also found that the $tRNA^{gly}$ and $tRNA^{pro}$ genes colocalized with centromeres in more than 50% of the cells, even though only 2 of 12 $tRNA^{gly}$ genes and 0 of 9 $tRNA^{pro}$ genes are encoded at centromeres, supporting the colocalization of these genes to centromeres from their remote chromosomal locations. We also found that foci corresponding to *5S rRNA* genes were present within the nucleus, and that one of the *5S rRNA* gene foci was associated with centromeres in ~50% of the cells (Figure 2C). These FISH results strongly suggest that Pol III genes at dispersed genomic locations can cluster at or in close proximity to centromeres.

Frequent Localization of the Pol III Gene Locus near Centromeres

It remains unclear how frequently Pol III genes localize at centromeres. It is possible that only a subset of Pol III genes localized at centromeres could be visualized by the above FISH experiments, because only the signals from clustered *tRNA* and *5S rRNA* genes provide a sufficient signal to form prominent FISH foci. To further explore the centromeric localization of individual Pol III gene loci, we also performed FISH analysis using cosmid probes, c417 and c162, which correspond to Pol III and non-Pol III gene loci, respectively. The Pol III gene locus (c417) contains three *tRNA* and two *5S rRNA* genes, whereas the non-Pol III gene locus (c162) located only 120 kb away from the Pol III gene locus (c417) does not contain any Pol III genes. These two loci could be detected as distinct spots in the nucleus by FISH analysis, because 120-kb DNA occupies ~0.5 μm of the interphase chromatin fiber (Bystricky *et al.,* 2004). The Pol III gene locus was more frequently located in the vicinity of centromeres than the non-Pol III gene locus (Figure 2D). The difference in the two localization patterns of the genomic loci is highly significant ($p < 0.001$), as evaluated by Mann-Whitney U test. This statistical test does not involve any cutoff parameters, but compares the entire distribution pattern. We found that the Pol III gene locus and centromeres were positioned within <0.3 μm in ~35% of the cells, whereas the non-Pol III gene locus and centromeres were positioned within <0.3 μm in only ~5% of cells.

To further support these observations, we investigated whether several other genomic loci consisting of Pol III genes also localize near centromeres. We observed in Supplemental Figure S3A that the Pol III gene loci (c27D7, c354, and c10H11) frequently localized near centromeres, whereas the non-Pol III gene loci (c110 and c887) rarely localized near centromeres. One Pol III gene locus (c343) did not exhibit the centromeric localization. The reason for this is not clear, but we speculate that the centromeric localization of the c343 locus might be inhibited by another genome-organizing mechanism that influences the genome structure around the c343 locus and overrides the Pol III gene-dependent mechanism. The observation that four of five dispersed Pol III gene loci frequently localize near centromeres, and that all three non-Pol III gene loci investigated rarely localize near centromeres strongly suggests that Pol III genes contribute to the centromeric localization of distant genomic loci. We also note that not all the *tRNA* gene loci frequently localize near centromeres and that additional genome organizing mechanisms must be considered. We also asked how frequently the Pol III gene

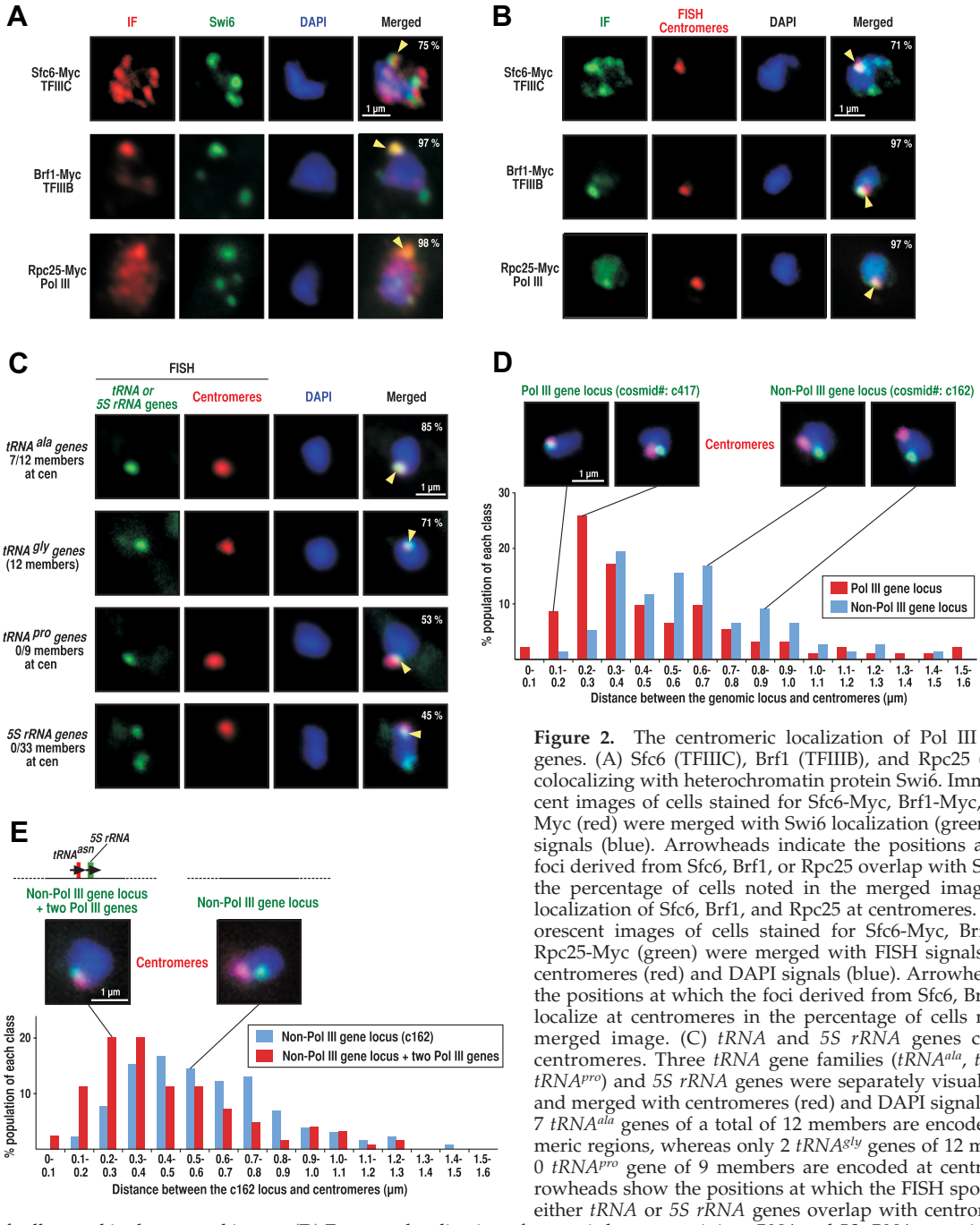

Figure 2. The centromeric localization of Pol III factors and genes. (A) Sfc6 (TFIIIC), Brf1 (TFIIIB), and Rpc25 (Pol III) foci colocalizing with heterochromatin protein Swi6. Immunofluorescent images of cells stained for Sfc6-Myc, Brf1-Myc, and Rpc25-Myc (red) were merged with Swi6 localization (green) and DAPI signals (blue). Arrowheads indicate the positions at which the foci derived from Sfc6, Brf1, or Rpc25 overlap with Swi6 spots in the percentage of cells noted in the merged image. (B) Focal localization of Sfc6, Brf1, and Rpc25 at centromeres. Immunofluorescent images of cells stained for Sfc6-Myc, Brf1-Myc, and Rpc25-Myc (green) were merged with FISH signals visualizing centromeres (red) and DAPI signals (blue). Arrowheads indicate the positions at which the foci derived from Sfc6, Brf1, or Rpc25 localize at centromeres in the percentage of cells noted in the merged image. (C) *tRNA* and *5S rRNA* genes clustering at centromeres. Three *tRNA* gene families ($tRNA^{ala}$, $tRNA^{gly}$, and $tRNA^{pro}$) and *5S rRNA* genes were separately visualized (green) and merged with centromeres (red) and DAPI signals (blue). The 7 $tRNA^{ala}$ genes of a total of 12 members are encoded at centromeric regions, whereas only 2 $tRNA^{gly}$ genes of 12 members and 0 $tRNA^{pro}$ gene of 9 members are encoded at centromeres. Arrowheads show the positions at which the FISH spots indicating either *tRNA* or *5S rRNA* genes overlap with centromeres in the percentage of cells noted in the merged image. (D) Frequent localization of genomic locus containing *tRNA* and *5S rRNA* genes in the vicinity of centromeres. The two genomic loci (green) were visualized by FISH using the cosmid clones (c417 and c162), and merged with centromeres (red) and DAPI signals (blue). The chromosomal positions of the cosmids are shown in Supplemental Figure S1. Several typical images are shown on top. In each image, measurement of the distance between the genomic locus and centromeres is indicated by linking the image with the histogram. The distance was measured between two focal centers. A histogram of distributions of observed distances between the genomic loci and centromeres is shown at the bottom. The percent populations of the observed distances between the genomic loci and centromeres were binned into 0.1 μm. (E) The insertion of Pol III genes into the non-Pol III gene locus results in frequent localization of the locus near centromeres. The non-Pol III gene locus (green) was visualized by FISH using the cosmid clone (c162) and merged with centromeres (red) and DAPI signals (blue). Schematic representations of the c162 locus with (left) and without (right) the insertion of the Pol III genes are shown on top. The Pol III genes ($tRNA^{asn}$ and *5S rRNA*) are derived from the c417 locus (Supplemental Figure S4A). The microscopic images typified intranuclear positioning of the c162 locus and centromeres. The plotting in the histogram was carried out as described in D.

locus, c417, localized near a second Pol III gene locus, c354. We found that the colocalization between the c417 locus and the c354 locus was highly infrequent, similar to the colocalization pattern between the non-Pol III gene locus (c162) and the c354 locus (Supplemental Figure S3B). This result suggests that Pol III gene loci primarily

localize near centromeres rather than to other Pol III gene loci present along the chromosome arms.

We further investigated whether Pol III genes can impart the capacity for the centromeric localization to a genomic locus. If Pol III genes are a critical driver for the centromeric localization of genomic locus, then a non-Pol III gene locus, which normally does not associate with centromeres, should localize near centromeres more frequently if Pol III genes are inserted into the non-Pol III gene locus. Indeed, we found that when two Pol III genes are inserted into the non-Pol III gene locus (c162), the c162 locus localizes near centromeres more frequently than when the Pol III genes are not present (Figure 2E). The two localization patterns, plus and minus the Pol III genes, are statistically significantly different ($p < 0.001$, Mann-Whitney U test). Taken together, we conclude that Pol III genes can drive the centromeric localizations of genomic loci.

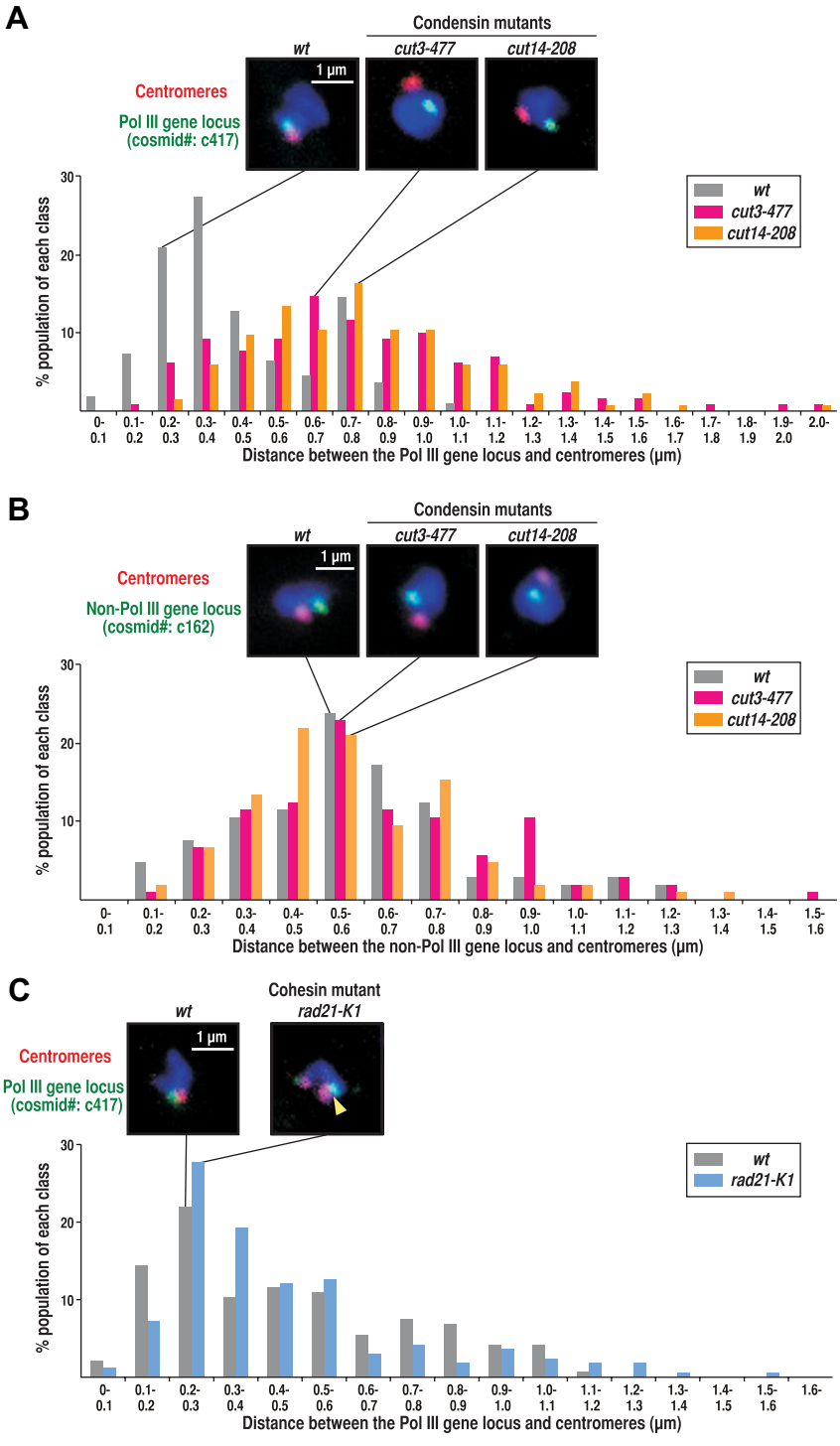

Figure 3. Condensin, but not cohesin, mutations disrupt the centromeric localization of Pol III genes. (A) The centromeric localization of Pol III genes was defective in the condensin mutants. The Pol III gene locus (green) was visualized using a FISH probe specific to the cosmid clones (c417) and merged with centromeres (red) and DAPI signals (blue). The *cut3-477*, *cut14-208*, and wild-type (wt) cells grown at 26°C were subsequently cultured at the restricted temperature (36°C) for 2 h for *cut3-477* and wt cells and 1 h for *cut14-208* cells and then subjected to FISH analysis. Most cells used for FISH analysis were in interphase. The microscopic images on top typified intranuclear positioning of the c417 locus and centromeres in the respective strains. The quantitative measurements of distances between the c417 locus and centromeres, and their plotting in the histogram, were carried out as described in Figure 2D. (B) Positioning of the non-Pol III gene locus relative to centromeres was not affected by the condensin mutations. The non-Pol III gene locus (green) was visualized using a FISH probe specific to the cosmid clones (c162) and merged with centromeres (red) and DAPI signals (blue). The experiment was performed as described in A. (C) The centromeric localization of the Pol III gene locus was not visibly impaired in the cohesin mutant. The wt and cohesin mutant (*rad21-K1*) cells grown at 26°C were subsequently cultured at the restricted temperature (36°C) for 4 h and subjected to FISH analysis. The cohesin mutant typically contains two foci for the c417 locus (green) and centromeres (red), respectively (top).

Centromeric Localization of Pol III Genes Is Diminished in Condensin Mutants

We next explored the molecular mechanism by which dispersed Pol III genes localize near centromeres. It has been shown that two protein complexes, condensin and cohesin, which localize at different portions of centromeres in fission yeast, are involved in various chromosome behaviors including sister chromatid cohesion and mitotic chromosome condensation (Tomonaga *et al.*, 2000; Nakazawa *et al.*, 2008). Furthermore, the cohesin and condensin complexes are believed to mediate interchromosomal (sister chromatids) and intrachromosomal interactions, respectively (Losada and Hirano, 2001). In budding yeast, condensin mutations disrupt the clustering of *tRNA* genes in the nucleolus (Haeusler *et al.*, 2008), and cohesin loading onto *tRNA* genes is involved in sister chromatid cohesion (Dubey and Gartenberg, 2007), suggesting that condensin and cohesin complexes might mediate higher-order genome organization through Pol III genes (Gartenberg and Merkenschlager, 2008). Therefore, we asked whether condensin and cohesin might be involved in the centromeric localization of Pol III genes. To test this possibility, we used coFISH analysis to determine whether the centromeric localization of the Pol III gene locus (c417) is affected by temperature-sensitive (ts) mutations in the condensin and cohesin genes. In *cut3-477* and *cut14-208* condensin mutants, the centromeric localization of the c417 locus was significantly compromised at the restrictive temperature ($p < 0.001$ in both mutants, Mann-Whitney U test; Figure 3A), exhibiting centromeric localization patterns resembling that of the non-Pol III gene locus c162 (Figure 2D). By contrast, the condensin mutations did not affect the intranuclear positioning of the non-Pol III gene locus (c162) relative to centromeres (Figure 3B), suggesting that the condensin mutations specifically affect the centromeric localization of the Pol III genes. We also asked whether a cohesin ts mutation affects the colocalization between the Pol III gene locus (c417) and centromeres. We observed that the cohesin mutation, *rad21-K1*, did not affect the colocalization of the Pol III gene locus and centromeres (Figure 3C). We thus concluded that condensin, but not cohesin, is necessary for the centromeric localization of Pol III genes.

Condensin Associates with Pol III Genes during Both Interphase and Mitosis

The condensin-mediated localization of Pol III genes near centromeres might involve interactions between the condensin complex and Pol III genes. We further examined this possibility. Indeed, we found that the condensin subunit, Cut3, was enriched at Pol III genes at the c417 locus, where both Pol III factors Brf1 (TFIIIB) and Rpc25 (Pol III) were also enriched (Supplemental Figure S4, A and B). This observation is consistent with similar findings of condensin enrichment at *tRNA* genes in budding and fission yeasts (D'Ambrosio *et al.*, 2008; Haeusler *et al.*, 2008).

In mitosis, condensin is known to associate with the kinetochore portion of the centromere, including the *cnt1* region (Nakazawa *et al.*, 2008). Our results indicated that, in mitosis and interphase, Cut3 was significantly enriched both at the *cnt1* region and at the Pol III genes (Supplemental Figure S4, A and C). We next investigated whether condensin binding to a genomic locus is dependent on the presence of Pol III genes. We found that when two Pol III genes are inserted into a non-Pol III gene locus (c162), Cut3 was more enriched at the c162 locus than when the Pol III genes are not present (Supplemental Figure S4D). We also performed CoIP analysis and asked whether condensin physically interacts with Pol III factors. We detected an association between the condensin subunit, Cut3, and the Pol III component, Rpc25. The association between condensin and Pol III was less stable compared with the association between Rpc25 and Brf1 (TFIIIB; Figure 4), although the significant association between Cut3 and Rpc25 remained after DNaseI treatment (data not shown). The Cut3 association with Rpc25 might indicate either a direct interaction between condensin and the Pol III subunit or an interaction between the condensin subunit and other Pol III factors derived from TFIIIB, TFIIIC, and TFIIIA. Together, our results support a role for condensin in the centromeric localization of dispersed Pol III genes during interphase and mitosis.

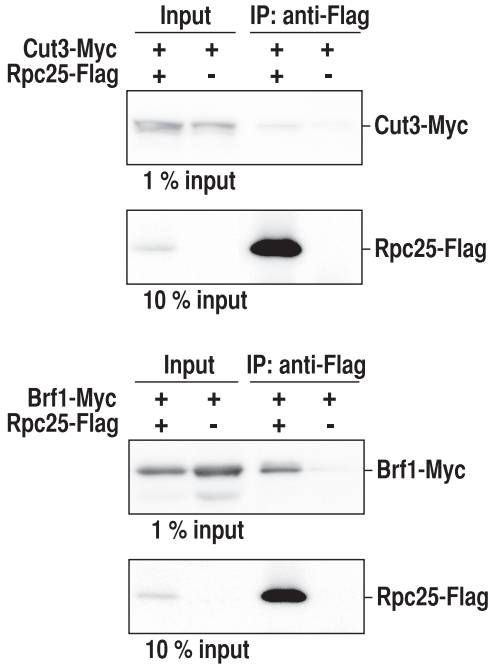

Figure 4. Association of Cut3-Myc with Rpc25-Flag. Top, extracts prepared from strains expressing tagged proteins were incubated with anti-Flag antibody, and immunoprecipitated (IP) fractions were analyzed by Western blotting using anti-Myc and anti-Flag antibodies. Bottom, the control experiment showing the interaction between Brf1-Myc (TFIIIB) and Rpc25-Flag (Pol III). IP experiments were repeated at least twice, and representative results are shown.

Functional Relationship between the Centromeric Localization of Pol III Genes and the Assembly of Condensed Mitotic Chromosomes

We examined whether Pol III factor(s) participates in the centromeric localization of Pol III genes. To this end, we first constructed a strain carrying a mutation in the *sfc3* gene encoding the TFIIIC subunit. It has been shown that a mutation of the *sfc3* orthologous gene in budding yeast (*tfc3*) causes thermosensitivity and reduces Pol III transcription (Lefebvre *et al.*, 1994). The same mutation substituting an amino acid (G361E) in the *sfc3* gene of fission yeast, *sfc3-1*, resulted in ts growth (Figure 5A). Surprisingly, we found that the *sfc3-1* mutation suppressed the ts phenotype of the *cut3-477* mutant, as indicated by growth of the *cut3-477 sfc3-1* double mutant at the elevated temperature (Figure 5A). Considering that the *cut3-477* mutation resulted in the disruption of the centromeric localization of Pol III genes (Figures 3A and 5B), it is possible that the *sfc3-1* mutation might alleviate the disruption observed in the condensin

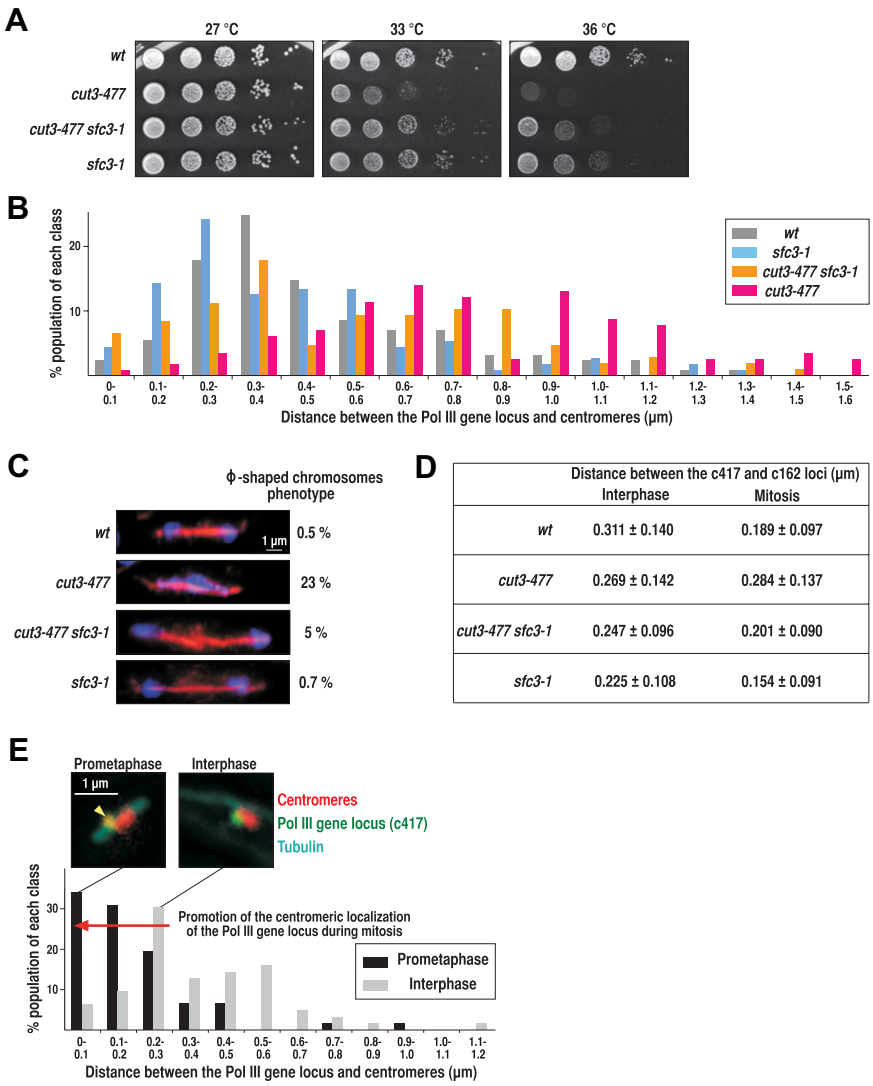

Figure 5. The centromeric localization of Pol III genes is linked to mitotic chromosome condensation. (A) The temperature sensitivity phenotype of the condensin mutant, *cut3-477*, is suppressed by the *sfc3-1* mutation. Logarithmically growing cells (OD ~ 0.5) in YEA liquid medium at 27°C were serially diluted by 10-fold and spotted onto nonselective YEA plates that were incubated at indicated temperatures for 2–3 d. (B) The *sfc3-1* mutation promotes the centromeric localization of Pol III genes. Wild-type (wt), *cut3-477*, *cut3-477 sfc3-1*, and *sfc3-1* cells grown at 26°C were subsequently cultured at the restricted temperature (36°C) for 2 h and then subjected to FISH analysis. Most cells used for FISH analysis were in interphase. The Pol III gene locus was visualized using a FISH probe specific to the cosmid clone (c417), whose signal was merged with that of centromeres. The quantitative measurements of distances between the c417 locus and centromeres, and their plotting in the histogram, were carried out as described in Figure 2D. (C) The *ϕ*-shaped chromosomes phenotype of the *cut3-477* mutant is suppressed by the *sfc3-1* mutation. The indicated strains were cultured at 36°C for 2 h. Immunofluorescent images of cells stained for tubulin (red) were merged with DAPI signals (blue). The percentage of the *ϕ*-shaped chromosomes phenotype in each strain is shown on the right. More than 300 cells were counted for each strain. (D) The *sfc3-1* mutation facilitates mitotic chromosome condensation. The indicated strains were cultured at 36°C for 2 h. The Pol III genes locus (c417) and non-Pol III gene locus (c162) visualized by FISH were merged with IF images of cells stained for tubulin, and distances between the two loci were measured in interphase cells ($n > 100$) and in mitotic cells with short spindles ($n > 20$). (E) The centromeric localization of the Pol III gene locus becomes prominent during prometaphase compared with interphase. For cell-cycle synchronization, exponentially growing cells were arrested in S phase by culturing in YEA medium containing 11 mM hydroxyurea (HU) at 30°C for 4 h, released by further culturing without HU for 1.5 h, and then subjected to IF-FISH experiments. Immunofluorescent images of cells stained for tubulin (cyan) were merged with FISH signals visualizing the c417 locus (green) and centromeres (red). During prometaphase, a few centromeric signals attached with short spindles were observed. Arrowheads indicate the positions at which foci derived from centromeres completely overlap with the c417 locus. Measurements of the distance between the c417 locus and centromeres in the respective images were indicated by linking the image with the histogram.

mutant. We observed that the c417 locus localized more frequently near centromeres in the *sfc3-1* mutant compared with wild-type ($p < 0.05$, Mann-Whitney U test; Figure 5B). The FISH results further indicated that the centromeric localization of the Pol III gene locus (c417) was relatively restored in the *cut3-477 sfc3-1* double mutant compared with the *cut3-477* single mutant (Figure 5B), with a highly significant p-value ($p < 0.001$, Mann-Whitney U test). These results revealed that the *sfc3-1* mutation results in more frequent localization of Pol III genes near centromeres.

It has been shown that the *cut3-477* condensin mutation results in the *ϕ*-shaped chromosomes phenotype during anaphase because of the compromised chromosome condensation (Saka *et al.*, 1994). If the centromeric localization of Pol III genes mediated by condensin is involved in the assembly of condensed mitotic chromosomes, the *sfc3-1* mutation promoting the centromeric localization of Pol III genes might suppress the *ϕ*-shaped chromosomes phenotype of the condensin mutant. Indeed, the mutant phenotype of the *cut3-477* was suppressed by the *sfc3-1* mutation (Figure 5C). To directly investigate mitotic chromosome condensation, we measured the physical distance between the Pol III gene locus (c417) and the non-Pol III gene locus (c162) in interphase and mitotic cells. In wild-type cells, the distance between the two loci was shorter in mitosis than interphase ($p < 0.001$, Mann-Whitney U test; Figure 5D), indicating that mitotic chromosome condensation can be quantified by this assay. In the *cut3-477* mutant, the mitotic chromosome condensation was compromised ($p < 0.05$, Mann-Whitney U test). The condensation level of mitotic chromosome was significantly improved in the *cut3-477 sfc3-1* double mutant compared with the *cut3-477* mutant ($p < 0.05$, Mann-Whitney U test; Figure 5D). Moreover, the *sfc3-1* single mutation significantly increased chromosome condensation in interphase compared with wild-type ($p < 0.001$, Mann-Whitney

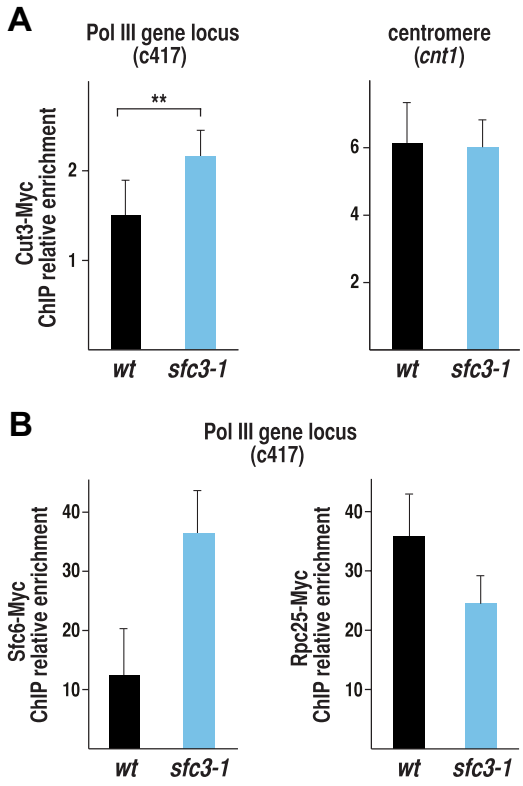

Figure 6. Binding of condensin and Pol III factors to the Pol III gene region in the *sfc3-1* mutant. (A) The *sfc3-1* mutation results in the increased level of Cut3 (condensin) binding to the Pol III gene region. The *wt* and *sfc3-1* cells were cultured at 36°C for 2 h and subjected to ChIP analysis. Cut3-Myc levels at the Pol III gene region (c417) and the *cnt1* region of centromere 1 were determined by ChIP. Quantitative measurements of ChIP results were carried out as described in Figure 1D. ChIP analyses were repeated three times. The difference in the ChIP relative enrichment levels between the *wt* and *sfc3-1* mutant is significant; $^{**}p < 0.05$, two-tailed *t* test. (B) Binding of Sfc6 (TFIIIC) and Rpc25 (Pol III) to the Pol III gene region (c417) in the *sfc3-1* mutant. ChIP was performed using strains expressing Sfc6-Myc and Rpc25-Myc in the wt and *sfc3-1* mutant.

U test; Figure 5D). We also observed that the centromeric localization of Pol III genes becomes prominent during prometaphase, when chromosomes are condensed (Figure 5E). These results suggest that the centromeric localization of Pol III genes mediated by condensin might participate in the assembly of the condensed mitotic chromosomes.

Promotion of Condensin Binding to the Pol III Gene Region in the sfc3-1 Mutant

The centromeric localization of Pol III genes is promoted in the *sfc3-1* mutant. This might involve increased binding of condensin to Pol III genes. Indeed, binding of the condensin subunit, Cut3, to the Pol III gene region (c417) was increased in the *sfc3-1* mutant, whereas condensin binding to the centromeric region (*cnt1*) was not affected (Figure 6A). We next analyzed binding of Pol III factors to the Pol III gene region in the *sfc3-1* mutant. Interestingly, binding of Sfc6 (TFIIIC) to the Pol III gene region was increased in the *sfc3-1* mutant (Figure 6B). We speculate that the *sfc3-1* mutation reduces the level of Pol III transcription and stabilizes the binding of TFIIIC complex to Pol III genes. In support of this hypothesis, the binding level of Rpc25 (Pol III) was decreased in the *sfc3-1* mutant compared with the wild-type, although we still detected the high enrichment of Pol III binding at the Pol III gene region in the *sfc3-1* mutant (Figure 6B). These results suggest that condensin loading onto Pol III genes might contribute to the centromeric localization of Pol III genes.

Pol III Transcription Affects the Centromeric Localization of Pol III Genes

To examine whether Pol III transcription influences the centromeric localization of Pol III genes, we quantified Pol III transcription by the phenotypic assay. Briefly, in this assay, if the *ade6-704* mutation is suppressed by expression of the suppressor *tRNA* gene (*tRNAmSer7T*), then yeast colonies growing on an adenine-limiting plate have a white appearance. If expression of the suppressor *tRNA* gene is reduced, the colonies appear red in color (Huang *et al.*, 2005). The phenotypic assay indicated that the *sfc3-1* mutation reduced Pol III transcription (Figure 7A). The *cut3-477* mutation resulted in slightly elevated Pol III transcription, as reflected by the whiter colony compared with wild type (Figure 7A). Pol III transcription that was reduced in the *sfc3-1* mutant was restored to wild-type levels when combined with the *cut3-477* mutation, indicating that condensin negatively affects Pol III transcription (Figure 7A). We next analyzed the transcript levels of 6 *tRNA* gene families by RT-PCR. The RT-PCR results also revealed that the *sfc3-1* and *cut3-477* mutations, respectively, reduced and enhanced *tRNA* gene expression (Figure 7B). Moreover, the *cut3-477 sfc3-1* double mutant showed the increased levels of tRNA transcripts compared with the *sfc3-1* single mutant. As described above, the centromeric localization of Pol III genes was promoted and diminished by the *sfc3-1* and *cut3-477* mutations, respectively (Figure 5B). Thus, our data indicate that Pol III transcription is negatively correlated with the centromeric localization of Pol III genes. To further examine whether Pol III transcription could affect the centromeric localization of Pol III genes, we next investigated the centromeric localization of Pol III genes in the cells treated with a Pol III transcription inhibitor ML-60218 (Wu *et al.*, 2003). We found that inhibitor treatment with ML-60218 (50 and 200 μM) reduced Pol III transcription and significantly promoted the centromeric localization of Pol III genes ($p < 0.05$ for 50 μM and $p < 0.001$ for 200 μM, Mann-Whitney U test; Figure 7, C and D). Together, these results suggest that transcription of the Pol III genes might interfere with their centromeric localization.

DISCUSSION

The molecular process by which the Pol III machinery transcribes Pol III genes such as *tRNA* and *5S rRNA* genes has been intensely studied. Our study reveals a novel role for the Pol III transcription machinery in genome organization. Results described here indicate that Pol III factors from different protein complexes (TFIIIA, TFIIIB, TFIIIC, and Pol III) exhibit distinct localization patterns. The TFIIIB and Pol III foci localize at the nuclear periphery at the surface boundary between the nucleoplasm and the nucleolus in more than 50% of the cells. Approximately 97% of these foci associate with centromeres that are frequently positioned at the nuclear periphery adjacent to the nucleolus. In fission yeast, the interphase centromeres localize adjacent to the spindle pole body (SPB), which is attached by microtubules (Funabiki *et al.*, 1993; Ding *et al.*, 1997). It is likely that microtubule-mediated positioning of SPBs as well as the centromeres directs the intranuclear location of Pol III factors and transcription. We show a global genome organization, by which

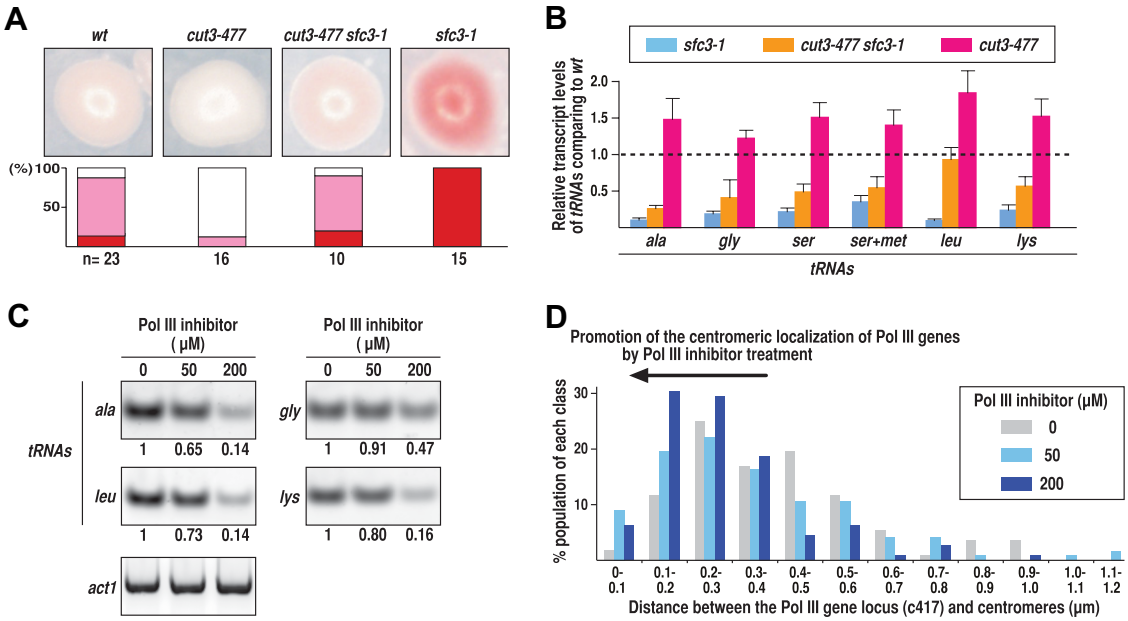

Figure 7. Transcription levels of Pol III genes are negatively correlated with the centromeric localization of Pol III genes. (A) Pol III transcription is affected by the *cut3-477* and *sfc3-1* mutations. Transcription of *tRNA* gene is monitored by the assay as described (Huang *et al.*, 2005). The typical colony phenotypes (top) and percent frequencies of white, pink, and red colonies derived from the same genotypes after genetic cross (bottom) are shown. (B) Pol III transcription is decreased and increased in the *sfc3-1* and *cut3-477* mutants, respectively. The *sfc3-1*, *cut3-477 sfc3-1* mutants and their control wt cells were cultured at 36°C for 10 h. The *cut3-477* mutant and its control wt cells were cultured at 36°C for 2 h. Harvested cells were lysed to extract nucleic acids containing total RNA and genomic DNA. These mutations might affect the total RNA amount in a cell. Thus, RNA samples were first normalized based on the genomic DNA concentration in nucleic acid fractions before being subjected to RT-PCR analysis. Genomic DNA concentration in the nucleic acid fraction was quantified by PCR and normalized against *act1* gene. Normalized nucleic acid fractions were treated with RNase-free DNaseI, and subjected to RT-PCR. The transcript levels from six distinct *tRNA* genes in the mutants and wt cells were measured, and the relative transcript levels (mutants vs. wt) are shown. The RNA preparation and RT-PCR were repeated three times, and average transcript levels are shown. The *tRNA* gene for *ser+met* indicates that these two *tRNA* genes are known to be transcribed as a dimeric transcript (Johnson *et al.*, 1989). (C) Treatment with a Pol III inhibitor reduces transcript levels from *tRNA* genes in fission yeast. The Pol III inhibitor treatment was performed as described previously (Wu *et al.*, 2003). The cells were cultured in YEA liquid medium containing 0, 50, and 200 μM of the Pol III inhibitor (ML-60218) for 4 h. RNA samples were prepared and subjected to RT-PCR analysis. The transcript levels from four *tRNA* genes and *act1* gene were measured. The tRNA levels were normalized against the expression levels from *act1* gene. The relative transcript levels of tRNAs are shown below each lane. (D) Treatment with the Pol III inhibitor promotes the centromeric localization of Pol III genes. Cells were cultured in YEA liquid medium containing 0, 50, and 200 μM of the Pol III inhibitor (ML-60218) for 4 h and then subjected to FISH analysis. The Pol III gene locus was visualized by FISH using the cosmid clone (c417) and merged with the centromeric signal. The quantitative measurements of distances between the c417 locus and centromeres, and their plotting in the histogram, were carried out as described in Figure 2D.

Pol III genes dispersed throughout the fission yeast chromosomes localize near centromeres with a statistically significant frequency. The centromeric localization of dispersed Pol III genes likely impacts the global three-dimensional genome structure in which linear DNA fibers are arranged into chromatin loops emanating from centromeres.

How is this centromere-centered genome structure organized? We observed that condensin binds to Pol III genes and Pol III transcription machinery and that the centromeric localization of Pol III genes is compromised in condensin mutants. We suggest that condensin is an important mediator for the centromeric localization of dispersed Pol III genes. In fission yeast, ~50 *tRNA* genes are encoded at centromeres. Condensin likely binds to both the centromeric *tRNA* genes and the noncentromeric Pol III genes. It has been shown by Nakazawa *et al.* (2008) that condensin also localizes at the kinetochore portions of the centromeres. It is possible that Pol III genes present in the chromosomal arms associate with either the centromeric *tRNA* genes or the kinetochore portions of centromeres through interaction between condensin complexes, as it has been suggested that condensin mediates interactions between two DNA duplexes through interactions with other condensin complexes (Hirano, 2006). Thus, condensin may mediate tethering of Pol III genes to centromeres. It has been shown that some *tRNA* genes serve as heterochromatin boundaries, which prevent heterochromatin from spreading into neighboring euchromatic regions (Noma *et al.*, 2006; Scott *et al.*, 2006, 2007). The centromeric localization of Pol III genes might have a role in boundary formation. In budding yeast, *tRNA* genes bound by condensin are clustered in the nucleolus, and the nucleolar clustering is compromised by the condensin mutations (Thompson *et al.*, 2003; D'Ambrosio *et al.*, 2008; Haeusler *et al.*, 2008), further supporting a role for condensin in intranuclear localization of *tRNA* genes (Gartenberg and Merkenschlager, 2008). The centromeric localization of Pol III genes in fission yeast and the nucleolar clustering of *tRNA* genes in budding yeast might be governed by similar mechanisms. It has been known that *5S rRNA* genes often localize at the nucleolar periphery in several higher eukaryotes, including mammals, suggesting that the global genome organization by Pol III genes might be conserved in some form among eukaryotes (Haeusler and Engelke, 2006).

We have recently shown that more than 60 *COC* loci, dispersed across the fission yeast genome, containing bound

TFIIIC without Pol III association, participate in organizing a higher-order genome structure in fission yeast (Noma *et al.,* 2006). TFIIIC binding to specific DNA sequences is critical for boundary function demarcating chromosomal domains. These *COC* sites are occupied by high concentrations of TFIIIC that localizes to ~5–10 bodies at the nuclear periphery. By contrast, the Pol III genes preferentially localize near centromeres. These two genome-organizing mechanisms, dependent on Pol III genes and the *COC* loci, might share some common mechanisms, as Pol III genes are also bound by TFIIIC. Other Pol III machinery such as TFIIIB and Pol III might participate in the primary localization of Pol III genes to centromeres.

Chromosome condensation is essential for proper chromosome segregation during mitosis and meiosis. This compaction of the chromosomes is mediated by a condensin complex consisting of five subunits, structural maintenance of chromosomes (SMC), and non-SMC proteins (Losada and Hirano, 2005). In fission yeast, the SMC proteins are Cut3 and Cut14, and the non-SMC proteins are Cnd1, Cnd2, and Cnd3 (Sutani *et al.,* 1999). Studies from different systems have led to models that explain how chromosomes are condensed in mitosis (Laemmli *et al.,* 1992; Koshland and Strunnikov, 1996; Yanagida, 1998; Hirano, 2000; Hagstrom and Meyer, 2003; Nasmyth and Haering, 2005; Hirano, 2006). It has recently been reported that condensin may only be partially responsible for the mitotic chromosome condensation process (Gassmann *et al.,* 2004; Belmont, 2006). It is thought that condensin mediates numerous interactions between DNA duplexes residing within a chromosome and functions in some of the steps of chromosome compaction. However, the molecular mechanism governing the higher-order structure of the condensed chromosome and the molecular processes underlying chromosome compaction, remain elusive. In fission yeast, condensin mutants show a severe chromosome segregation defect, called ϕ-shaped chromosomes (Saka *et al.,* 1994). We show that the *sfc3-1* mutation promotes the centromeric localization of Pol III genes and chromosome condensation, whereas the *cut3-477* condensin mutation results in the opposite effects. We find that the centromeric localization of Pol III genes becomes prominent during prometaphase when chromosomes are condensed. We also find that the ϕ-shaped chromosomes phenotype, due to the defective chromosome condensation in the *cut3-477* cells, is suppressed by the *sfc3-1* mutation that promotes the centromeric localization of Pol III genes. Taken together, these results suggest a functional link between the centromeric localization of Pol III genes and chromosome condensation. However, we cannot eliminate the possibility that the centromeric localization of Pol III genes might function in other biological processes, which facilitate chromosome condensation during mitosis. Our analyses also suggest that Pol III transcription might interfere with the centromeric localization of Pol III genes. It has been shown that TFIIIC dissociates from *tRNA* genes during Pol III transcription. In a similar manner, condensin might be released from Pol III genes during their transcription, leading to the dissociation of the Pol III genes from centromeres. Thus, Pol III transcription can inhibit the centromeric localization of Pol III genes. On the basis of the sum of these observations, we suggest that the centromeric localization of Pol III genes, interfered with by Pol III transcription, might be a part of the assembly processes for the condensed mitotic chromosomes. It has been shown that Pol III transcription is repressed during mitosis in human (Fairley *et al.,* 2003), implicating that the biological significance of Pol III transcription in chromosome condensation might be generally conserved among eukaryotes. The centromere is the chromosomal domain where kinetochore microtubules attach and where the pulling force is generated. Therefore, tethering chromosomal arm regions to the centromere might facilitate chromosome movement along the spindle microtubules during anaphase. Our study illuminates the roles of Pol III genes and their transcription machinery in interphase genome organization that might be functionally connected to mitotic chromosome condensation essential for faithful chromosome segregation.

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