Centromeric motion facilitates the mobility of interphase genomic regions in fission yeast

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Summary
Dispersed genetic elements such as retrotransposons and Pol III-transcribed genes, including tRNA and 5S rRNA, cluster and associate with centromeres in fission yeast through the condensin function. However, the dynamics of these condensin-mediated genomic associations remains unknown. We have examined the 3D motions of genomic loci including the centromere, telomere, ribosomal DNA (rDNA) repeat locus, and the loci carrying Pol III-transcribed genes or LTR retrotransposons in live cells at as short as 1.5-sec intervals. Treatment with carbendazim (CBZ), a microtubule-destabilizing agent, not only prevents centromeric motion, but also reduces the mobility of the other genomic loci during interphase. Further analyses demonstrate that condensin-mediated associations between centromeres and the genomic loci are clonal, infrequent, and transient. However, when associated, centromeres and the genomic loci migrate together in a coordinated fashion. In addition, a condensin mutation that disrupts associations between centromeres and the genomic loci results in a concomitant decrease in the mobility of the loci. Our study suggests that highly mobile centromeres pulled by microtubules in cytoplasm serve as “genome mobility elements” by facilitating physical relocations of associating genomic regions.
**Introduction**

Genome organizations have been characterized in terms of several aspects, including chromosomal territories, nuclear bodies, and genomic associations involving transcriptional regulatory elements and their target genes (Cremer and Cremer, 2010; Sexton et al., 2009; Tanizawa and Noma, 2012; Williams et al., 2010; Zhao et al., 2009). It is becoming clear that three-dimensional genome organization is connected to various nuclear processes such as transcription, DNA replication and repair, and chromatin domain formation (Cook, 1999; Labrador and Corces, 2002; Misteli, 2007). For example, enhancer-promoter association has been demonstrated to function in gene activation (Deng et al., 2012; Mahmoudi et al., 2002; Mueller-Storm et al., 1989). Using the latest genomics method, referred to as Hi-C, which combines chromosome conformation capture (3C) and next-generation sequencing, genome-wide associations have been comprehensively captured in several organisms (Duan et al., 2010; Hou et al., 2012a; Lieberman-Aiden et al., 2009; Sexton et al., 2012; Tanizawa et al., 2010). These studies demonstrate that eukaryotic genomes are highly organized into functional structures. Although genomic associations have been comprehensively mapped for several genomes, these genomic data cannot directly represent the stability of genomic associations. For example, stable genomic associations in a few cells cannot be distinguished from transient association in many cells. Therefore, an important problem in the current chromatin/genome biology fields is to determine the dynamics of genomic associations using cell biological approaches (Baker, 2011).

We have recently developed the ELP (Enrichment of Ligation Products) genomic approach, similar to Hi-C, and identified genome-wide associations in fission yeast (Tanizawa et al., 2010; Tanizawa and Noma, 2012). This genomic analysis revealed the existence of chromosomal territories and significant associations among highly transcribed genes, coregulated genes, and functionally related genes, respectively. Moreover, our microscopic studies, employing a fluorescent *in situ* hybridization (FISH) assay, showed that dispersed genetic elements such as retrotransposons and RNA polymerase III-transcribed genes, including tRNA and 5S rRNA, referred to as Pol III genes in this article, cluster and associate with centromeres (Iwasaki et al., 2010; Tanaka et al., 2012). We also showed that condensin functions as a molecular connector among chromatin fibers and is responsible for centromeric clustering of those dispersed genetic elements. Together, these studies demonstrated that the fission yeast
genome is organized into a highly elaborate functional structure through genome-wide associations. Concurrent with the rising problem in the chromatingenome biology fields, the dynamics of genomic associations in fission yeast remains unexplored.

In this study, we have investigated the spatiotemporal associations of genomic loci in live fission yeast cells. We begin by elucidating the 3D motion of the centromeres, the telomere, the ribosomal DNA (rDNA) repeat locus, several genomic loci carrying Pol III genes or retrotransposons, and a control locus. Our analysis reveals that treatment with CBZ, a microtubule-destabilizing agent, not only strongly affects centromeric motion but also impedes movement of the other genomic regions. Our studies also demonstrate that condensin-mediated associations between centromeres and genomic loci carrying Pol III genes and retrotransposons are transient and infrequent. However, once they associate, highly mobile centromeres and the genomic regions migrate together. In addition, we show that the condensin mutation that disrupts those associations reduces the mobility of the genomic regions. Since Pol III genes and retrotransposons are dispersed across the genome, centromeric motion coupled to microtubule polymerization can impact the mobility of non-centromeric genomic regions through condensin-mediated associations. Our studies may have identified a new role of centromeres and cytoplasmic microtubules in genome-wide mobility.
Results

Non-random positioning of fission yeast genomic loci

For the initial step into the examination of the dynamics of genome organization, we made several fission yeast strains carrying lacO repeats (Fig. S1A, B). We employed strains carrying lacO or tetO repeats at the centromeres (cen1, cen2, and cen3), the telomere (tel2R), the rDNA locus, the genomic loci consisting of Pol III genes (c417 and c10H11) and retrotransposons (c947), and a control locus (c887). The c887 control locus does not contain retrotransposons nor Pol III-transcribed genes within 80 kb from the locus, and also does not have condensin-binding sites, as determined by the ChIP-seq analysis (Tanaka et al., 2012). The FISH analysis indicated that the insertions of lacO repeats into the c417 Pol III gene locus and the c887 control locus did not affect the positioning of those genomic loci in relation to the nuclear periphery, suggesting that a lacO insertion is unlikely to drastically affect endogenous positioning of genomic regions (Fig. S1C). We also observed that the centromeric insertions of lacO/tetO repeats did not cause a chromosome segregation defect, suggesting that the lacO/tetO insertions probably do not affect centromeric function (Fig. S1D).

It has previously been shown that several genomic loci in budding yeast tend to localize within respective nuclear domains rather than randomly distribute throughout the nucleus (Berger et al., 2008). This study elegantly examined the frequent positioning of genomic loci in relation to the nuclear membrane and the nucleolus used as nuclear landmarks. The Nucloc software used in that study processed hundreds of 3D images and calculated the tendency of intra-nuclear positioning of a genomic locus in cell populations as a probabilistic density. We have employed the same approach to study spatial localization of genomic regions in fission yeast. We visualized genomic loci carrying lacO repeats in addition to nuclear landmarks such as the nuclear membrane and the nucleolus. Probability density was calculated for the centromeres (cen1 and cen2), the telomere (tel2R), the rDNA locus, the Pol III gene locus (c417), the LTR retrotransposons locus (c947), and the control locus (c887) (Fig. S2). The probability density maps revealed that the genomic regions we examined tend to localize within limited subnuclear compartments. For example, the centromeres (cen1 and cen2) were frequently positioned in proximity to the nuclear membrane but were distant from the nucleolus. The telomere (tel2R) was present near the nuclear periphery but often located away from the centromere-occupied domain. These observations suggest that the fission yeast genome might be organized into a
Rabl-like chromosome conformation, which was predicted for budding yeast (Berger et al., 2008; Bystricky et al., 2005; Spector, 2003). The locus adjoining the rDNA tandem array tends to localize near the nucleolus. Consistently, rDNA repeats themselves were shown to be present in the fission yeast nucleolus (Uzawa and Yanagida, 1992). Moreover, the c417 Pol III gene locus, as well as the c947 LTR retrotransposon locus and the c887 control locus, localized within the interior domain of the nucleus, typically between the subnuclear domains occupied by the rDNA-flanking locus and the peripheral loci (cen1, cen2, and tel2R). The c887 control locus was distributed more evenly across the nucleus compared to the retrotransposon locus. We observed that the Pol III gene locus and the retrotransposon locus were present near the centromere-occupied nuclear domain in the limited cell population, while this positioning was not detected for the control locus, suggesting that the Pol III gene locus and the retrotransposon locus associate with centromeres in some populations (Iwasaki et al., 2010; Tanaka et al., 2012) (Fig. S2B). These data demonstrate that the respective genomic loci tend to localize within specific subnuclear domains in fission yeast.

**Movement of genomic loci in live fission yeast cells**

We visualized several genomic loci and the nuclear landmarks, such as the nuclear membrane and nucleolus, in live fission yeast cells at as short as 1.5-sec intervals for 5 min. At each time point, the position of the genomic loci was plotted in the 3D nuclear space, determined by using the nuclear landmarks. It is important to note that effects from cell migration and movement of the nucleus within the cytoplasm were filtered out using the nuclear landmarks, allowing us to examine only intra-nuclear migration of genomic loci. We observed that the centromeres (cen1 and cen2) and the telomere (tel2R) were typically located between 1.0 and 1.2 μm from the nuclear center (Fig. S3A). The Nup61-mCherry NPC signals were also located at the same distance from the nuclear center, suggesting that centromeres and telomeres were both located at the nuclear periphery. In clear contrast, the rDNA-flanking locus resided between 0.2 and 0.6 μm from the nuclear center and just outside of the nucleolus. These data are consistent with the probability density maps (Fig. S2B). Moreover, the Pol III gene loci (c417 and c10H11) and the LTR retrotransposons locus (c947) typically migrated between 0.8 and 1.0 μm from the nuclear center (Fig. S3A). This positioning, which is relatively close to the nuclear membrane, might reflect potential associations between these genomic loci and centromeres present at the nuclear center.
periphery. Consistently, we observed that the c887 control locus, which does not associate with centromeres, was typically positioned between 0.6 and 0.8 μm from the nuclear center and a little farther away from the nuclear periphery.

Interestingly, we observed that the cen1 was sometimes positioned more than 1.2 μm away from the nuclear center (Fig. S3A). To further examine centromeric behavior, we next visualized the centromere, cen1, with microtubules in live cells. Three-dimensional imaging in live cells demonstrated that the centromere dynamically moves along microtubules (Fig. 1A). The centromere was typically located near the tip of the microtubule fiber and shifted position in the direction of microtubule polymerization. Live-cell imaging also showed that the centromere moves along the nuclear periphery and that the nuclear morphology was sometimes transformed from the normal round to the “teardrop” shape in association with this movement (Fig. 1B).

Treatment with CBZ interrupted the dynamic motion of the centromere (Fig. 1C). Together, these results suggest a model that cytoplasmic microtubules pull/move centromeres along the nuclear membrane, and that nuclear morphology can be deformed to accommodate the physical force when microtubules attempt to pull centromeres beyond the nuclear space.

We also investigated the movement velocities of the respective genomic loci based on 3D time-lapse imaging. We did not find a clear difference among velocities estimated from the 3D movement of the centromere (cen1), the telomere (tel2R), the Pol III gene locus, the LTR retrotransposon locus, and the control locus when we estimated migration velocities at 1.5-sec intervals (Fig. S3B). Velocities of those loci were distributed around 0.1 μm/sec. This is probably because these velocities mainly reflect random motion of the genomic loci, which has been referred to as Brownian-like or diffusional motion detected from budding yeast to man (Chubb et al., 2002; Heun et al., 2001; Marshall et al., 1997). Therefore, we predict that fission yeast genomic loci are also subjected to Brownian-like motion. We next investigated velocities of the respective loci at prolonged intervals ranging from 3 to 36 sec. Average velocities were reduced from 0.06 to 0.01 μm/sec with the longer intervals, suggesting that the genomic loci typically migrate without directional movement. Interestingly, we observed that the velocity of the cen1 was enhanced to 0.02-0.03 μm/sec at 24- and 36-sec long intervals in some populations, while typical velocities for other genomic loci were around 0.01 μm/sec (Fig. S3B). Considering that centromeres are pulled by microtubules and move along the nuclear periphery (Fig. 1), this
directional motion of centromeres is mediated by the microtubules at an approximate velocity of 0.02-0.03 μm/sec.

CBZ treatment reduces 3D motion of centromeres and other genomic loci
Our analyses demonstrated that the fission yeast genomic loci were mobile, yet typically restricted within distinct subnuclear domains. We next examined tracking of the respective genomic loci in terms of 3D nuclear volume using a new approach with our custom algorithm (Fig. 2A; see Materials and Methods). This approach allowed us to estimate 3D nuclear domains, referred to here as moving volumes, in which different genomic loci migrate for 5 min. We observed that the moving volumes of the centromeres (cen1 and cen2) were around 0.3 μm³ (Fig. 2B, C). Since the nuclear volume excluding the nucleolus is approximately 6.8 μm³, the centromere movement is confined to 4.4% of the nuclear volume. The average nuclear volume was estimated from 3D images visualizing the nuclear landmarks in 800 cells (Fig. S2). The other genomic loci also migrated within relatively similar nuclear volumes (~0.3 μm³), while the telomere (tel2R) was seemingly restricted to a slightly smaller nuclear volume (0.2 μm³), which is estimated as 2.9% of the entire nuclear volume (Fig. 2C).

It has been shown that inhibition of metabolic processes by ATP depletion affects rapid movement of budding yeast genomic loci (Heun et al., 2001). We analyzed whether ATP depletion, by treatment with CCCP (Carbonyl cyanide m-chlorophenyl hydrazone) and NaN₃ (sodium azide), has an effect on the moving volumes of the fission yeast genomic loci. We observed that ATP depletion severely reduced the moving volume of every genomic locus we examined. For example, the CCCP treatment reduced the moving volumes of the respective genomic loci to 0.05 μm³, which is estimated as only 0.74% of the nuclear volume and 16.7% of the original moving volumes. These data suggest that energy-dependent cellular activities promote motion of the interphase genomic loci (Fig. 2). To relate this phenomenon to a specific cellular process, we next focused on microtubule polymerization, an important cellular activity utilizing the energy of GTP hydrolysis (Desai and Mitchison, 1997). Remarkably, the CBZ treatment affected the moving volumes of not only the centromere, but also all other genomic loci we investigated (Fig. 2). The centromeric moving volume was reduced from 0.3 to 0.15 μm³, and the moving volumes of the other genomic loci were also decreased to 0.2 μm³, which is estimated as around 66% of the original moving volumes. Since our analyses only reflect intra-
nuclear migration of the genomic loci, the data suggest that microtubule polymerization in the cytoplasm promotes the mobility of centromeres and the other genomic loci.

To compare these results with previous studies in the other organisms, we also analyzed mean squared change in distance ($<\Delta d^2>$) based on the 3D time-lapse data (Fig. S4) (Chubb et al., 2002; Heun et al., 2001; Marshall et al., 1997). A $<\Delta d^2>$ value serves as an index for diffusional motion of a genomic locus. The $<\Delta d^2>$ plots for the respective genomic loci showed that the $<\Delta d^2>$ values reached a range of 0.08 and 0.15 μm$^2$ after 50-75 sec, and the slopes of the $<\Delta d^2>$ plots typically decayed for the longer time points. These results suggest that genomic loci are mobile but tend to position within restricted subnuclear volumes, which agrees with the probability density maps (Fig. S2B). This constrained motion of genomic loci has also been observed in other eukaryotes (Chubb et al., 2002; Heun et al., 2001; Marshall et al., 1997), suggesting that similar characteristics of the dynamic motion of genomic regions are probably conserved among eukaryotes. Moreover, we observed that ATP depletion severely decreased the $<\Delta d^2>$ values, and the CBZ treatment also affected the $<\Delta d^2>$ values to a lesser extent.

Coordinated motion of centromeres and their associated loci

To begin to address the dynamics of condensin-mediated genomic associations, we decided to examine associations between centromeres and the three representative genomic loci carrying Pol III genes and LTR retrotransposons in live cells. We performed a FISH analysis and confirmed that the lacO insertion into the c417 Pol III gene locus did not affect association frequency between centromeres and the loci ($p > 0.05$, Mann-Whitney U test; Fig. S5A). We next investigated the cen2-cen3 association as a positive control for genomic associations, as fission yeast centromeres are known to stably interact with one another during interphase (Funabiki et al., 1993). The live-cell imaging data showed that the 3D distances between cen2 and cen3 foci were almost always between 0.2 and 0.6 μm during interphase (Fig. S5B, C). Moreover, the relative positioning of cen1 and Mis12 (kinetochore) foci was similar to that of cen2-cen3 (Fig. S5D). Therefore, we accepted the potential technical limitation of this imaging approach by which genomic associations cannot be detected as perfect co-localization of two foci. From this point, we decided to follow the criterion that two foci positioned within 0.6 μm reflect potential associations.
We also visualized centromeres and the c417 Pol III gene locus in live cells and monitored the 3D distances between two foci continuously for 5 min. We observed that two foci were temporarily positioned in proximity (Fig. 3A, B). Based on the above criterion, centromeres potentially associate with the Pol III gene locus for approximately 20 sec around the 156-sec time point. In clear contrast, the c887 control locus was constantly separated from the centromeric foci. We observed that the control locus was rarely positioned within 0.9 μm from centromeres, while the c417 Pol III gene locus was frequently detected within 0.9 μm from centromeres (Fig. 3A-C). Moreover, another Pol III gene locus (c10H11) and the retrotransposon locus (c947) showed essentially the same results as the c417 Pol III gene locus (Fig. 3C). Therefore, we speculated that the genomic loci positioned within 0.9 μm from centromeres might also reflect association with centromeres. In this case, centromeres and their associating genomic loci should migrate in a coordinated fashion. Indeed, we observed that the c417 Pol III gene locus positioned between 0.6 and 0.9 μm from centromeres, with which we believe the locus does not directly associate according to the above criterion, was found to still shift along the x and y axes as centromeres moved (Fig. S5E). The coordinated motion was also observed in cells where the c417 locus migrated within 0.6 μm from centromeres (Fig. 3D). However, the coordinated motion was not observed in cells with centromeres and the c417 locus continuously separated by more than 0.9 μm (Fig. S5F). To evaluate the coordinated movement between centromeres and the c417 locus, we calculated the mean correlation coefficient for each cell. This value represents similarity between the movements of centromeres and the locus. The same approach was previously employed for telomere associations in budding yeast (Bystricky et al., 2005). We found that the mean correlation coefficient for cells with centromeres and the locus always separated by more than 0.9 μm was near 0 (Fig. 3E). However, the mean correlation coefficient was increased in the cases where the c417 Pol III gene locus migrated within 0.6-0.9 μm from centromeres at least once during the 5-min observation (Fig. 3E). These results suggest that the locus positioned between 0.6 and 0.9 μm from centromeres moves in synchrony with centromeres to some extent. Our current hypothesis is that the flanking region of the c417 Pol III gene locus likely associates with centromeres and, as a result, the locus and centromeres move in a coordinated fashion. We suggest that the coordinated movement of two foci with an intervening distance of 0.6-0.9 μm reflects an indirect association. The coordinated motion reflecting direct associations was further promoted in cells where the c417 locus migrated within
0.6 μm from centromeres (Fig. 3D, E). Moreover, the mean correlation coefficient for cen2-cen3 was around 0.61, probably reflecting continuous association between the centromeres.

**Genomic associations in live fission yeast cells**

We analyzed 3D live-cell movies (~ 5 min) co-visualizing centromeres and various genomic loci, such as the Pol III gene loci (c417 and c10H11), the LTR retrotransposon locus (c947), and the control locus. We examined more than 35 independent live cells for each locus and calculated the mean distance between centromeres and the genomic locus in each cell. Distributions of mean distances between centromeres and the respective genomic loci showed that the Pol III gene loci and the retrotransposon locus were positioned in proximity to centromeres in some cell populations, which was significantly different from the distribution with the c887 control locus (p < 0.05, Mann-Whitney U test; Fig. 4A). It is noteworthy that a significant difference in distributions of mean distances was not observed when distances between centromeres and the respective genomic loci were measured in 2D images (p > 0.05, Mann-Whitney U test; Fig. S6A), suggesting that genomic associations are preferably examined in 3D.

We noticed that mean distances between centromeres and the control locus were always more than 1.0 μm, implying that in cells with mean distances below 1.0 μm, the genomic loci might associate with centromeres. In about 20-30% of cells, mean distances between centromeres and the loci were below 1.0 μm (Fig. 4B). The coordinated motion between centromeres and the genomic loci was promoted in these cells comparing to the remaining cell population with mean distances above 1.0 μm (Fig. 4C). Moreover, we observed that the Pol III gene loci and retrotransposon locus migrated within 0.6 μm from centromeres in the similar percentages of cells (Fig. 4B). Therefore, these results suggest that genomic associations occur in approximately 20-30% of cells.

Next, we estimated association frequency and duration between centromeres and the genomic loci (Fig. 4D, E). The Pol III gene loci and LTR retrotransposon locus potentially associated with centromeres at frequencies of 1-2 times for every 10 min when the entire cell population was considered (Fig. 4D). Since associations only occurs in 20-30% of cells (Fig. 4B), associations in those cells were re-estimated to occur at frequencies of 4-6 times per 10 min. Association duration was typically less than 30 sec, although the genomic associations were sustained for 30-80 sec in the limited cell population (Fig. 4E). These results collectively suggest
that associations between centromeres and the genomic loci are clonal, infrequent, and transient, but sufficient for their coordinated migration once they associate.

**Role of condensin in tethering the genomic loci to centromeres**

We also investigated the effects of the condensin mutations on the association between centromeres and the c417 Pol III gene locus in live cells. We observed that the mean distances between centromeres and the Pol III gene locus were significantly affected by the *cut3-477* and *cut14-208* condensin mutations (p < 0.05, Mann-Whitney U test), while the c887 control locus was not affected (p > 0.05, Mann-Whitney U test; Fig. 5A). Interestingly, positioning of the Pol III gene locus and retrotransposon locus in relation to the nuclear periphery was affected by the *cut3-477* condensin mutation, while cen1 remained at the periphery (Fig. 5B). Moreover, the probability density maps suggested that the positioning of cen1 was not affected by the condensin mutation (Fig. S2B). Therefore, these data suggest that condensin mediates associations between centromeres and the genomic loci and, once associations are disrupted, the loci shift toward the interior domain of the nucleus.

Based on the moving volume data (Fig. 2), we hypothesized that centromeric motion influences genome-wide mobility through associations among centromeres and other genomic regions. To test this hypothesis, we investigated moving volumes of the genomic loci in the *cut3-477* condensin mutant and observed that the Pol III gene loci (c417 and c10H11) and the retrotransposon locus (c947) became more static compared to the wild-type cells, while centromeres (cen1 and Mis12) were not affected (Fig. 5C). The moving volumes of the genomic loci, such as the Pol III gene loci (c417 and c10H11) and the retrotransposon locus (c947), were decreased from 0.4-0.5 μm³ to 0.25-0.35 μm³ in condensin mutant cells, which is estimated as 60-70% of the original moving volumes. Therefore, these results suggest that condensin tethers the genomic loci to centromeres, thereby allowing centromeric motion to impact the mobility of the associated loci.

**Centromeric motion and the mobility of other genomic loci**

If centromeric motion influences the mobility of other genomic loci, we should observe that genomic loci associating with centromeres migrate with centromeres, while genomic loci might become more static and independent of centromeric movement when they do not associate with
centromeres. We analyzed the live-cell data (Fig. 4A) and observed a clear correlation in the moving volumes of centromeres and the genomic loci in cells with mean distances below 1.0 μm (Fig. 6A). In contrast, the moving volumes of centromeres and the genomic loci were not well correlated in cells with mean distances above 1.0 μm. Interestingly, 30 of 46 cells (~ 65%) with mean distances above 1.0 μm showed a moving volume of less than 0.4 μm³ for the genomic loci, while centromeres moved independently (Fig. 6A). Moreover, we observed that, in cells with mean distances below 1.0 μm, the c417 Pol III gene locus was positioned in proximity to centromeres and they migrated in a coordinated fashion (Fig. 6B). In cells with mean distances above 1.0 μm, the Pol III gene locus became relatively more static and did not move with centromeres. All together, these studies suggest that highly mobile centromeres, pulled by microtubules, contribute to the mobility of other genomic loci through condensin-mediated associations (Fig. 6C).

**Intra-nuclear positioning of Pol III genes and their transcription**

It has previously been shown that Pol III genes, dispersed across the fission yeast genome, cluster and associate with centromeres, and that this genome organization is coupled to the transcriptional repression of Pol III genes (Iwasaki et al., 2010). We examined whether the mobility of the Pol III gene locus affects Pol III transcription. Fission yeast cells were subjected to the CBZ treatment, and the mean distances between centromeres and the genomic loci, such as the c417 Pol III gene locus and the c887 control locus, were calculated in more than 20 cells. Distributions of the mean distances demonstrated that the CBZ treatment did not affect the positioning of the Pol III gene locus nor the control locus in relation to centromeres (p > 0.05, Mann-Whitney U test), although it reduced the mobility of centromeres and the genomic loci (Fig. 2, S6B). We also observed that tRNA expression was not affected by the CBZ treatment (Fig. S6C). Therefore, these results suggest that the mobility of the Pol III gene locus is unlikely to be involved in the transcriptional regulation of Pol III genes.

The ChIP results indicated that the Cut14 condensin subunit was still localized at the Pol III gene locus (c417) and the centromere (cnt1) in cut3-477 mutant cells, suggesting that the condensin complex or a subset of the complex can bind to the Pol III gene locus and centromeres in cut3-477 condensin mutant cells (Fig. S6D). However, associations between centromeres and the Pol III gene locus were significantly compromised by the cut3-477 mutation, and Pol III
transcription is enhanced (Fig. 5A; Iwasaki et al., 2010). Therefore, our current hypothesis is that condensin binding might not be sufficient to regulate Pol III genes, but their transcriptional regulation probably entails condensin-mediated positioning of the genes. Together, these results suggest that centromeric localization of Pol III genes, but not their mobility, contributes to their transcriptional regulation. It is possible that a subnuclear environment around centromeres, densely occupied by heterochromatin components, is unfavorable for optimal Pol III transcription (Kniola et al., 2001).
Discussion

Dynamics of condensin-mediated genomic associations in fission yeast

We have previously shown that centromeres associate with Pol III genes and LTR retrotransposons through condensin function (Iwasaki et al., 2010; Tanaka et al., 2012). We decided to elucidate the dynamics of the condensin-mediated associations between centromeres and the genomic loci carrying Pol III genes and retrotransposons using live-cell imaging tools developed in our lab. For this purpose, we first needed to define genomic associations in live cells and developed the criteria that positioning of two foci within 0.6 μm, and in a range between 0.6 and 0.9 μm, reflects direct and indirect genomic associations, respectively. These initial observations in fission yeast offer the standards to examine the dynamics of genomic associations in other organisms.

Based on these criteria, we estimated association frequency and duration between centromeres and the genomic loci carrying Pol III genes and retrotransposons. Our data suggest that associations between centromeres and the genomic loci investigated, potentially occur at frequencies of 1-2 times per 10 min when the entire cell population is considered. About 20-30% of cells show that mean distances between centromeres and the genomic loci are below 1.0 μm. In these cells, association frequencies are enhanced to 4-6 times per 10 min, indicating that there is a clonal variation for genomic associations. These estimations are likely reliable, because the control locus hardly associates with centromeres according to the same criteria. Moreover, our data also suggest that association duration between centromeres and the genomic loci is typically less than 30 sec, suggesting that condensin-mediated genomic associations are dynamic. However, prolonged associations were also observed in the limited cell population. Our previous study demonstrated that the Pol III gene locus tightly associates with centromeres during mitosis, thereby resulting in the proper assembly of mitotic chromosomes (Iwasaki et al., 2010). Therefore, the dynamics of condensin-mediated genomic associations are most likely regulated during the cell cycle. Namely, condensin-mediated associations are typically unstable during interphase as observed in this study, but probably become more static during mitosis. It is intriguing to understand the mechanism that regulates the association stability.

Protein complexes consisting of structural maintenance of chromosomes (SMC) proteins are essential for the faithful segregation of chromosomes. Two of the best-studied SMC complexes are condensin and cohesin, which are required for mitotic chromosome assembly and
for holding sister chromatids together, respectively (Hagstrom and Meyer, 2003; Hirano, 2000; Koshland and Strunnikov, 1996; Laemmli et al., 1992; Nasmyth and Haering, 2005; Yanagida, 1998). These SMC complexes function as connectors between two chromatin fibers (Hirano, 2006). Recent studies have revealed that cohesin mediates functionally distinct genomic associations, including interactions between enhancers and promoters (Hadjur et al., 2009; Hou et al., 2010; Kagey et al., 2010; Mishiro et al., 2009; Nativio et al., 2009). Despite their functional importance, the dynamic nature of SMC-mediated genomic associations had remained uncharacterized. This study has examined SMC-mediated genomic associations in live cells for the first time and demonstrated that those associations are transient in the fission yeast model organism. It is possible that SMC-mediated associations in higher eukaryotes might be dynamic, as observed in fission yeast.

Role of centromeres and cytoplasmic microtubules in genome-wide mobility
Among the genomic loci we examined, centromeres most widely migrate along the nuclear membrane. In fission yeast, the interphase centromeres are known to localize adjacent to the spindle pole body (SPB), which is attached by microtubules (Ding et al., 1997; Funabiki et al., 1993). Microtubules-mediated movement of the SPB was previously observed in fission yeast (King et al., 2008). The intricate interactions among the SPB present at the cytoplasmic side of the nuclear surface, the SUN-KASH complex integrated in the nuclear membrane, and the Csi1 protein, connect cytoplasmic microtubules to centromeres (Chikashige et al., 2010; Hou et al., 2012b). We find that centromeric foci present near the tip of the microtubule fiber migrate along the nuclear membrane with microtubule polymerization (Fig. 1A). CBZ treatment severely inhibits centromeric motion. The migration velocity of centromeres is around 0.02-0.03 μm/sec. This coincides with the rate of microtubule polymerization in fission yeast (0.024 μm/sec) (Tran et al., 2001). Therefore, it is likely that microtubule polymerization is a major contributor to centromeric motion. We also demonstrate that centromeric movement sometimes alters nuclear morphology into the “teardrop”-shape. This suggests that cytoplasmic microtubules pull centromeres along the nuclear membrane, and this pulling force is sufficient to deform the nuclear shape when centromeres are pulled beyond the nuclear volume. It has also been shown that an overproduction of the separase C-terminal fragment causes a nuclear morphological defect (Nakamura et al., 2002). These studies support the idea that the nuclear membrane is
deformable. On the other hand, interphase microtubules also function in the proper positioning of the nucleus in cytoplasm, revealing some stiffness of the nuclear membrane (Sawin and Tran, 2006; Tran et al., 2001). Therefore, rigidity/flexibility of the nuclear membrane is tuned to accommodate centromeric motion, nuclear deformation, and central positioning of the nucleus in cytoplasm.

Hundreds of Pol III genes and retrotransposons, which are distributed across the fission yeast genome, mediate global genome organization by associating with centromeres (Bowen et al., 2003; Iwasaki et al., 2010; Tanaka et al., 2012). Our results indicate that centromeres and their associating genomic loci move together. Therefore, centromeric motion can facilitate the mobility of other genomic regions. In other words, once centromeric motion is inhibited, it should reduce the mobility of many other genomic loci. Indeed, we find that CBZ treatment prevents centromeric motion and impedes movement of the other genomic loci (Fig. 2). We also find that disruption of the genomic associations between centromeres and the genomic loci by the condensin mutation reduces the moving volumes of the loci. Therefore, we propose that centromeres serve as “genome mobility elements” by connecting highly mobile centromeres to dispersed genomic regions. Our current model is that centromeric motion, driven by microtubule polymerization, can promote the mobility of other genomic regions through condensin-mediated associations (Fig. 6C). Since microtubule polymerization is a coordinated cellular process with cell growth, cell cycle, and stress response (Chang and Martin, 2009; Robertson and Hagan, 2008), this study may have identified a new mechanism by which cytoplasmic signals reflecting important cellular activities are transmitted to the nucleus and moderate the mobility of interphase genomic regions through centromeric motion coupled to microtubule polymerization. For instance, genetic materials maintain mobility while cells grow and microtubules are actively polymerized. The high mobility of genetic materials potentially facilitates associations between the transcriptional regulatory elements and their target genes, as well as DNA interactions related to recombination. Therefore, our studies shed light on condensin-mediated coordination between cellular/cytoplasmic activities and genome-wide mobility that can potentially contribute to various nuclear processes.
Materials and Methods

Strain construction and culture conditions

Strains carrying the insertions of lacO repeats were generated using a cloning-free, PCR-based method (Rohner et al., 2008). The lacO repeats were inserted into the two Pol III gene loci (c417 and c10H11), the LTR retrotransposons locus (c947), the rDNA locus, the tel2R telomere locus, and the control locus (c887). Strains carrying lacO repeats at the different genomic loci were subjected to genetic crosses to introduce LacI-GFP expressed from the his7 locus under control of the disl1 promoter. Nup61 and Rpa49 proteins were tagged with mCherry at the C termini of their proteins using a PCR-based module method (Bahler et al., 1998). Other strain constructions were performed using conventional genetic crosses. Yeast cells were cultured in Edinburgh Minimal Medium (EMM) at 26°C, unless otherwise noted.

Live-cell imaging

The genomic loci were visualized by the insertions of lacO and tetO repeats bound by LacI-GFP and TetR-tdTomato. The nuclear membrane, nucleolus, microtubules, and centromeres were visualized by mCherry fused to Nup61 (NPC), Rpa49 (RNA polymerase III), Atb2 (α-tubulin), and Mis12 (kinetochore protein), respectively. Exponentially growing cells in liquid EMM were mounted on an agar pad that contained EMM plus 2% electrophoresis-grade agarose (Tran et al., 2004). Cells were maintained at 26°C in an environmental chamber during image acquisition.

For the CBZ treatment, cells were cultured for 15 min in liquid EMM containing 50 μg/ml CBZ and applied to the agar pad with the same concentration of CBZ. Images were captured using a Leica TCS SP5 II AOBS spectral laser scanning confocal microscope (Leica Microsystems, Inc.) mounted on a DMI6000 inverted microscope. The system includes a 405 nm diode, multi-line Argon, and 561 nm DPSS lasers with three standard PMTs and two Leica HyD detectors. Images were captured at 1400 Hz with a 63X HCX PL APO CS oil immersion objective (NA = 1.4). Multi-channel image stacks were acquired at each time point. Time-lapse images were typically taken continuously, with a stack interval of 1.5 or 3.0 sec., for a total of 5 min. Construction of 3D images and analyses on 3D data were carried out using Volocity 6.1.1 software (PerkinElmer).
Estimation of the moving volume of the genomic locus

The genomic locus (lacO, green) was co-visualized with the nuclear landmarks such as NPC (Nup61-mCherry) and the nucleolus (Rpa49-mCherry). Time-lapse images were captured at 3.0-sec intervals for 5 min. Using the NPC foci, the center of the nucleus was estimated and the position of the genomic locus was normalized according to the nuclear center. The tracking line of the genomic locus was built by spline interpolation, resulting in a 3D tracking line at 0.3-sec intervals. The nucleus, excluding the nucleolus, was divided into ~850 cubes (0.2 μm of each side, 0.008 μm³ in volume for each cube) with a lattice arrangement. The tracking line of the genomic locus was used to assign the time-lapse occupancy of the genomic locus to the cubes. The moving volume (MV) was defined as the accumulated number of cubes occupied by the genomic locus.

\[ MV = \text{Number of cubes occupied by the genomic locus} \times \text{Volume of a cube (0.008 μm}^3) \]

\[ \% \text{ of MV in the nucleus} = \frac{MV}{\text{Volume of the nucleus (6.8 μm}^3) \times 100} \]
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Figure legends

**Fig. 1. Centromeric motion coupled to microtubule polymerization in cytoplasm.** (A) Selected frames from a time-lapse series of the live fission yeast cell showing microtubules (red) and the centromere (cen1-lacO, green). Microtubules were visualized by mCherry fused to Atb2 (α-tubulin). Tracking of the cen1 locus is shown at the end of the time-lapse sequence. (B) Nuclear morphology and centromeric position. The centromere (cen1-lacO, green), the NPC (Nup61-mCherry, red), and the nucleolus (Rpa49-mCherry, red) were visualized in live cells. Selected frames from a time-lapse series and 3D distances between the nuclear centers and centromeric foci are shown (left). Schematic nuclear morphology was depicted next to the microscopic images. (C) CBZ treatment affects centromeric mobility. Cells were treated with 50 μg/ml CBZ in EMM liquid medium for 15 min and applied to microscopic slides with a mounting medium containing CBZ. Images were captured in 3D at 3.0-sec intervals for 5 min. Scale bars indicate 1 μm.

**Fig. 2. Moving volume analysis for the genomic loci in several culturing conditions.** (A) Estimation of the moving volume of the genomic locus. This schematic illustrates how occupancy of the genomic locus was estimated based on 3D time-lapse images. The moving volume of the genomic locus was defined as an accumulated number of cubes. Also see the Materials and methods for details. (B) Moving volume analysis for the centromere (cen1). Cells were treated with 50 μg/ml CBZ, 40 mM CCCP, and 15 mM NaN3 in liquid EMM for 15 min and applied to microscopic slides. The centromere (cen1-lacO, green), the NPC (Nup61-mCherry, red), and the nucleolus (Rpa49-mCherry, red) were co-visualized in live cells, and tracking of the centromere was shown (left). Images were captured in 3D at 3.0-sec intervals for 5 min. Position of the centromere was normalized by the center of the nucleus. The moving volume of the centromere was estimated by counting the cubes (middle). The moving volume of the centromere was analyzed in five cells and data are represented as mean ± s.d. (right). (C) Moving volume analyses for the centromere (cen2), the Pol III gene loci (c417 and c10H11), the LTR retrotransposon locus (c947), the tel2R telomere locus, and the control locus (c887). Typical tracking images are shown as insets. Scale bars indicate 1 μm.
**Fig. 3. Coordinated migration between centromeres and their associating genomic loci.** (A) The genomic locus (green), either the Pol III gene locus (c417, top) or the control locus (c887, bottom), was co-visualized with centromeres (Mis12-mCherry, red). Images were captured in 3D at 3.0-sec intervals for 5 min and selected frames are shown. (B) Distances between centromeres and the genomic loci (c417 and c887) were measured in 3D time-lapse images used in panel (A) and plotted against time. Distances below 0.6 μm and between 0.6 and 0.9 μm were highlighted with different colors. (C) Distances between centromeres and the genomic loci such as the Pol III gene locus (c417 and c10H11), the LTR retrotransposon locus (c947), and the control locus (c887) were measured in 3D time-lapse images. Images were captured at 3.0-sec intervals for 5 min in more than 35 cells, and 10 examples for each locus are shown in a graph. (D) The c417 Pol III gene locus and centromeres show the coordinated movement in the case where two foci transiently migrate within 0.6 μm during the 5-min investigation. Distances between the Pol III gene locus and centromeres were plotted against time (left). Tracking of the Pol III gene locus (green) and centromeres (red) along the x and y axes is shown (right). Coordinates of the c417 Pol III gene locus and centromeres in the x and y axes were used to calculate Δx and Δy at 3.0-sec intervals. The Δx and Δy values were plotted against time and used to calculate Pearson’s correlations (bottom). (E) The coordinated motion between the c417 Pol III gene locus and centromeres. Distances between centromeres and the c417 Pol III gene locus were measured in 3D time-lapse images (n=42). Based on the following criteria, cells were classified into three groups. In 7 and 13 cells, the Pol III gene locus migrated within 0.6 μm and between 0.6-0.9 μm from centromeres, respectively, at least once during the 5-min investigation. In 22 cells, two foci were always separated more than 0.9 μm. The mean correlation coefficient is calculated for each cell as the mean of Pearson’s correlations in x and y directions. The averages of the mean correlation coefficients in the three cell populations are shown. Scale bars indicate 1 μm.

**Fig. 4. Estimation of the association dynamics between centromeres and the genomic loci.** (A) Distributions of mean distances between centromeres and the indicated genomic loci in the cell population. Distances between centromeres and the genomic loci (c417, c10H11, c947, and c887) were measured at 3.0-sec intervals for 5 min (n=42, 39, 36, and 35, respectively). Mean distance was calculated for each cell. Distributions of mean distances were plotted in the histogram. (B) Estimation of association frequencies in the cell population. Data in panel (A)
were used to calculate the percent populations of cells, in which mean distances between the centromeres and the indicated genomic loci were below 1.0 μm (left) and the genomic loci migrated within 0.6 μm from centromeres at least once during the 5-min investigation (right). (C) The coordinated motion between centromeres and the genomic loci is promoted when mean distances between two foci are below 1.0 μm. Based on mean distances shown in panel (A), cells were classified into two groups, in which mean distances between centromeres and the genomic loci were below or above 1.0 μm. The mean correlation coefficient is calculated for every cell as described in Fig. 3E, and the averages of the mean correlation coefficients in the respective groups are shown. (D) Association frequencies between centromeres and the genomic loci. The number of times that 3D distances between centromeres and the genomic loci fell below 0.6 μm was counted to estimate their potential associations in the entire cell population and in cells showing that mean distances between centromeres and the genomic loci were below 1.0 μm. (E) Associating duration between centromeres and the genomic loci. Associating durations were estimated for potential associations predicted in panel (D), and distributions of associating durations were plotted.

**Fig. 5. Condensin is involved in the mobility of the genomic loci.** (A) Distances between centromeres and the genomic loci, such as the c417 Pol III gene locus and the c887 control locus, were measured in cut3-477 and cut14-208 condensin mutant cells (n > 35). Wild-type and cut3-477 cells were cultured at the restrictive (36°C) temperature for 2 hr and subjected to live-cell imaging analysis within a temperature-controlled chamber. The cut14-208 cells were cultured at 36°C for 1 hr. Most cells used for the analyses were in interphase. Images were captured in 3D at 3.0-sec intervals for 3 min. Distributions of mean distances are summarized in a graph. (B) The c417 Pol III gene locus and c947 retrotransposon locus tend to localize in the interior domain of the nucleus in the cut3-477 condensin mutant. The genomic loci (lacO, green), the NPC (Nup61-mCherry, red), and the nucleolus (Rpa49-mCherry, red) were co-visualized in wild-type and cut3-477 cells. Images were captured in 3D at 6.0-sec intervals for 5 min. Distance from the nuclear center was divided into three zones based on the criteria depicted on the left. Mean distance between the nuclear center and the indicated genomic loci was binned into one of the assigned zones. (C) The moving volumes of the indicated genomic loci were estimated for the wild type and cut3-477 condensin mutant as described in Fig. 2A. The moving volumes of the
respective loci were analyzed in more than 10 cells, and data are represented as mean ± s.d. (right).

**Fig. 6. The potential role of centromeres in genome-wide mobility.** (A) Comparison between the moving volumes of centromeres and the genomic loci. Based on mean distances shown in Fig. 4A, cells were classified into two groups, in which mean distances between centromeres and the genomic loci were below or above 1.0 μm. In individual cells, the moving volumes of centromeres and the genomic loci were estimated as described in Fig. 2A. The moving volumes of centromeres and the genomic loci at the last time-point (5 min) were plotted. The dotted circle represents cells with a moving volume of less than 0.4 μm³ for the genomic loci. (B) Representative data showing 5-min tracking of centromeres (Mis12-mCherry, red) and the c417 Pol III gene locus (green) in cells with mean distances below (left) and above 1.0 μm (right). The scale bar indicates 1 μm. (C) A model for the role of centromeric motion in the mobility of other genomic regions. Microtubule polymerization in cytoplasm actively pushes centromeres in nucleoplasm. Centromeres transiently associate with genomic loci carrying Pol III genes and retrotransposons. These associations are mediated by condensin. Centromeres and their associating genomic loci migrate in a coordinated fashion. Since Pol III genes and retrotransposons are dispersed across the genome, centromeric motion regulated by cytoplasmic microtubules can potentially impact genome-wide mobility.
Figure 1

A

\( \text{cen}1\text{-lacO} \) -tubulin (Atb2-mCherry)

B

\( \text{cen}1\text{-lacO} \) NPC (Nup61-mCherry) Nucleolus (Rpa49-mCherry)

C

\( \text{cen}1\text{-lacO} \) NPC (Nup61-mCherry) Nucleolus (Rpa49-mCherry)
Figure 2

A

Figure 2A shows the nuclear pore complex (NPC) labeled with Nup61-mCherry (cen1-lacO) and the nucleolus labeled with Rpa49-mCherry. The volume of the nucleus is 6.8 µm³, and the number of cubes is ~850.

B

Table 2B summarizes the movement of different loci under various conditions, including control, CBZ, CCCP, and NaN3. The figures depict the moving volume of each locus over time.

- cen1-lacO NPC (Nup61-mCherry) with nuclei and nucleoli labeled.
- Control locus (c887) with moving volume graphs showing no significant changes.
- Pol III gene locus (c417) with changes in moving volume under different treatments.
- LTR locus (c947) with minimal movement.
- tel2R with consistent movement.

Graphs show the moving volume in µm³ over time (sec) for each condition.
Figure 3

(A) Pol III gene locus (c417)-lacO Centromeres (Mis12-mCherry) and Control locus (c887)-lacO Centromeres (Mis12-mCherry).

Distance between the genomic locus and centromeres (µm):

- Pol III gene locus (c417) Centromeres
- Control locus (c887) Centromeres

Time (sec): 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216, 228, 240, 252, 264, 276

Bivariate linear regression analysis reveals:

- The closest distance (<0.6 µm)
- 0.6~0.9 µm
- >0.9 µm

(E) Mean correlation coefficient:

- <0.6 µm
- 0.6~0.9 µm
- >0.9 µm

Time (sec): 0, 150, 300

Y axis:

- X axis:

Correlation coefficient r:

- 0.341
- 0.365
Figure 5

A

![Histograms showing mean distance between loci and centromeres](image)

- Mean distance between the c417 locus and centromeres (µm)
- Mean distance between the c887 locus and centromeres (µm)

B

- Pol III gene locus (c417)
- LTR locus (c947)
- Centromeres (Mis12)
- Control locus (c887)

C

- Graphs showing moving volume over time for different conditions (wt, cut3-477, cut14-208)
Figure 6

A

Below 1.0 µm

Over 1.0 µm

Moving volume of the genomic locus (µm³)

Moving volume of centromeres (µm³)

Correlation = 0.47

R² = 0.23

B

Pol III gene locus (c417)-lacO Centromeres (Mis12-mCherry)

Below 1.0 µm

Over 1.0 µm

C

Microtubule

Spg

Nuclear membrane

Condensin

Pol III-transcribed genes

or Retrotransposons

SUN/KASH

Association

Indirect Association