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Global genome organization mediated by RNA polymerase III-transcribed genes in fission yeast

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Abstract

Eukaryotic genomes exist as an elaborate three-dimensional structure in the nucleus. Recent studies have shown that this higher-order organization of the chromatin fiber is coupled to various nuclear processes including transcription. In fission yeast, we demonstrated that RNA polymerase III (Pol III)-transcribed genes such as *tRNA* and *5S rRNA* genes, dispersed throughout chromosomal arm regions, localize to centromeres in interphase. This centromeric association of Pol III genes, mediated by the condensin complex, becomes prominent during mitosis. Here, we discuss potential roles of the Pol III gene-mediated genome organization during interphase and mitosis, and hypothesize that the interphase genome structure serves as a scaffold for the efficient assembly of condensed mitotic chromosomes and that tethering of chromosomal arm regions to centromeres allows chromosomes to properly segregate along the spindle microtubules during anaphase.

Keywords

Genome organization; RNA polymerase III; chromosome condensation

1. Introduction

Large-scale sequencing for various eukaryotes has provided complete genomic sequences accompanied by lists of genes and their regulatory elements. Chromatin fibers that carry those genomic sequences are in fact present in the nucleus as a complex three-dimensional (3D) structure. At the chromosomal level, it has been shown that DNA fibers are non-randomly arranged into chromosomal territories (Lancot et al., 2007; Fraser and Bickmore, 2007). It is becoming clear that the 3D structure of the genome is linked to numerous nuclear processes such as transcription (Cook, 1999; Misteli, 2007). For instance, it is well known that the tandemly repeated ribosomal RNA genes are transcribed by RNA polymerase I (Pol I) in a subnuclear compartment, the nucleolus, where Pol I and *rRNA* genes are concentrated (Long and Dawid, 1980; Shaw and Jordan, 1995). Classes of Pol II-transcribed genes are enriched at transcriptional factories or nuclear speckles, likely contributing to efficient transcription and processing of the transcripts (Cook, 1999; Lamond and Spector, 2003; Chakalova et al., 2005; Sutherland and Bickmore, 2009). This is also true for Pol III-transcribed genes such as *tRNA* and *5S rRNA* genes. *5S rRNA* genes arranged as contiguous repeats in many eukaryotes often localize to the nucleolar periphery (Matera et

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al., 1995; Haeusler and Engelke, 2006). While 5S *rRNA* genes are dispersed in fission yeast and a few other organisms, they still co-localize at the nucleolar periphery (Iwasaki et al., 2010). Moreover, *tRNA* genes cluster in the nucleolus of budding yeast (Thompson et al., 2003). DNA replication and repair also involve higher-order genome organization through the establishment of replication factories and repair centers, respectively (Cook, 1999; Lisby et al., 2003; Kitamura et al., 2006; Misteli, 2007). Although the observed higher-order genome structures in the nucleus have been shown to be linked to various nuclear processes, the mechanisms that organize the functional genome structures have remained unknown.

We have used fission yeast as a model system to investigate the relationships between the global genome organization and genomic functions, and have modeled the 3D structure of the fission yeast genome using the latest genomics approach that combines the molecular biology procedure called chromosome conformation capture (3C) and massively parallel DNA sequencing (Tanizawa et al., 2010). This study suggests the existence of chromosomal territories and transcription factories in this model organism. Moreover, we have shown that Pol III-transcribed genes can localize to centromeres, thereby contributing to the global genome organization (Iwasaki et al., 2010). In this article, we focus on the molecular mechanism that drives the Pol III gene-mediated genome organization in fission yeast, and the roles of this organization during interphase and during mitosis.

2. Overview of the Pol III gene-mediated global genome organization

The molecular process by which the Pol III machineries transcribe Pol III genes such as *tRNA* and 5S *rRNA* genes has been intensely studied (Willis, 1993; Roeder, 1996; Paule and White, 2000; Huang and Maraia, 2001). The Pol III transcription involves 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). The TFIIB and TFIIC transcription factor complexes are sufficient to recruit Pol III to *tRNA* genes, although an additional transcription factor, TFIIA, is required for loading Pol III onto 5S *rRNA* genes. These Pol III transcription machineries are conserved from yeast to human (Huang and Maraia, 2001; Geiduschek and Kassavetis, 2001).

It has recently been shown that Pol III transcription machineries are not evenly distributed throughout the nucleus of fission yeast (Iwasaki et al., 2010). While TFIIA is enriched in the nucleolus, TFIIC is preferentially localized to 5-10 discrete spots associated with the nuclear periphery. Moreover, TFIIB and Pol III are concentrated at centromeres. Besides the localization of these Pol III transcription machineries, Pol III genes themselves, in dispersed locations throughout the fission yeast genome, frequently localize to centromeres. This centromeric association of Pol III genes is mediated by the condensin complex (Figure 1).

Condensin has been shown to function in chromosome condensation during mitosis (Laemmli et al., 1992; Koshland and Strunnikov, 1996; Yanagida, 1998; Hirano, 2000; Hagstrom and Meyer, 2003; Nasmyth and Haering, 2005; Hirano, 2006). The condensin complex consists of five subunits, two structural maintenance of chromosomes (SMC) proteins and three non-SMC proteins, which are conserved from yeast to human (Koshland and Strunnikov, 1996; Hagstrom and Meyer, 2003; Losada and Hirano, 2005). In addition to its known role in chromosome compaction, there is accumulating evidence that the condensin complex participates in other nuclear processes including transcriptional regulation (Hagstrom and Meyer, 2003; Wood et al., 2010). Recent observations in fission yeast have demonstrated an additional role for condensin in the interphase global genome organization that associates Pol III genes with centromeres. Moreover, the nucleolar clustering of *tRNA* genes in budding yeast is disrupted in the condensin mutants, indicating

that this role of condensin in the Pol III gene-mediated genome organization is not unique to fission yeast (Haeusler et al., 2008). Considering that Pol III gene-mediated genome organization in yeast involves evolutionarily conserved protein complexes such as condensin and Pol III transcription machineries, a similar genome organization might be conserved in higher eukaryotes.

3. Mechanism of the Pol III gene-mediated global genome organization

A series of results argues that condensin is recruited to Pol III genes through its binding to a component of the Pol III transcription machineries, likely TFIIC (D'Ambrosio et al., 2008; Haeusler et al., 2008; Iwasaki et al., 2010). Condensin can also be targeted to the kinetochore portions of the centromeres through a mechanism involving Mis6 kinetochore protein (Nakazawa et al., 2008). In fission yeast, while 50 *tRNA* genes are encoded at centromeres, more than 100 non-centromeric *tRNA* genes are dispersed along the chromosomal arms and approximately 30 *5S rRNA* genes are also scattered across the genome. Condensin likely binds to both the centromeric *tRNA* genes as well as the non-centromeric Pol III genes. Condensin mediates associations between two DNA duplexes through interactions with other condensin complexes (Hirano, 2006). Therefore, it is likely that the centromeric association of Pol III genes is mediated by interaction between the condensin complexes present at centromeres and those distributed through the chromosomal arms, although it is not yet clear whether the non-centromeric Pol III genes are just targeted to the kinetochore portions of centromeres, or just the centromeric *tRNA* genes, or both. In any case, relatively more condensin molecules localize at centromeres than individual non-centromeric Pol III gene loci, which might explain why Pol III genes are frequently tethered to centromeres rather than other Pol III gene regions. It is still possible that association among non-centromeric Pol III genes might occur infrequently and also be mediated by the same mechanism.

Unexpectedly, Pol III transcription was shown to have an inhibitory effect on the centromeric localization of Pol III genes. For instance, inhibition of Pol III transcription promotes the centromeric association of Pol III genes. This was further confirmed by the observation that the *sfc3-1* TFIIC mutation which reduces Pol III transcription enhances the centromeric association of Pol III genes. Binding of TFIIC and condensin to Pol III genes is also increased in the *sfc3-1* mutant. It has been shown that TFIIC dissociates from *tRNA* genes during Pol III transcription (Kassavetis et al., 1990; Roberts et al., 2003). We speculate that a reduction in the transcription frequency of Pol III genes might stabilize the association of the TFIIC complex carrying the mutant Sfc3 protein with the Pol III genes, and that this in turn might result in the recruitment of condensin molecules and enhance the centromeric association of Pol III genes. In other words, TFIIC together with the condensin complex might be released from Pol III genes during their transcription, leading to the dissociation of the Pol III genes from centromeres. Thus, increased Pol III transcription can inhibit the centromeric localization of Pol III genes.

4. Roles of the Pol III gene-mediated global genome organization during interphase

Although Pol III genes are dispersed throughout the fission yeast genome, many of them dynamically associate with centromeres, which are often positioned at the nuclear periphery near the surface boundary between the nucleoplasm and the nucleolus (Iwasaki et al., 2010). Therefore, the linear DNA fiber is folded into various chromatin loops emanating from centromeres in the fission yeast nucleus (Figure 2). The biological functions of this global genome organization involving Pol III genes and condensin complexes during interphase are not well understood. There are several possibilities that we can propose based on recent

observations from our lab and others. The first possibility is that the interphase genome structure might be used as a scaffold for the efficient assembly of condensed mitotic chromosomes. This possibility is supported by observations that the centromeric association of Pol III genes becomes prominent during mitosis, indicating that global genome organizations during interphase and mitosis are at least not completely independent.

Tethering of Pol III genes to centromeres might be required for boundary activity which prevents heterochromatin spreading into neighboring euchromatin domains. *tRNA* gene situated between heterochromatin and euchromatin domains functions as a chromatin boundary in budding yeast and fission yeast (Donze and Kamakaka, 2001; Oki and Kamakaka, 2005; Scott et al., 2006; Noma et al., 2006). *Alu* and another SINE element, *B2*, which are transcribed by Pol III, are also involved in forming chromatin boundaries in mammals (Willoughby et al., 2000; Lunyak et al., 2007). It is possible that tethering of Pol III genes to centromeres might create a physical barrier for heterochromatin proteins to encroach beyond the boundaries, resulting in separation of heterochromatin and euchromatin domains present in distinct chromatin loops.

The Pol III gene-mediated genome organization might be linked to a global transcriptional regulation of Pol III genes. This hypothesis is supported by the observation that the decrease and increase in Pol III transcription are connected to the more and less frequent associations of Pol III genes with centromeres, respectively. Concentration of Pol III genes and their transcription machineries at a specific subnuclear domain, such as centromeres in fission yeast, may facilitate Pol III transcription (Iwasaki et al., 2010). This is also observed in human cells where Pol III transcription occurs in about 2000 discrete sites accumulating numerous Pol III molecules (Pombo et al., 1999). Enrichment of ongoing Pol III transcription at a subnuclear domain, often near or in the nucleolus, might also be beneficial for efficient processing of the Pol III transcripts. It has been shown that several components of RNaseP, the splicing complex for tRNA molecules, are nucleolar in yeast and in mammals (Bertrand et al., 1998; Jarrous et al., 1999).

In fission yeast, centromeres are often positioned at the nucleolar periphery where Pol III genes are frequently located. Enrichment of Pol III genes near the nucleolus may facilitate the coordination of Pol III transcription with cellular processes in the nucleolus, especially Pol I transcription which synthesizes rRNA molecules. It has been shown that Pol I and Pol III transcription processes are co-regulated by the extracellular signal-regulated kinase (ERK) and the retinoblastoma protein (RB) (White, 2005). Considering that 5S rRNA, synthesized by Pol III, as well as other rRNA molecules synthesized by Pol I, are finally assembled into the ribosome within the nucleolus, it is probably important for efficient ribosome assembly that these two types of transcription are located close to each other. In human cells, the 5S rRNA gene array is also localized to the nucleolar periphery (Matera et al., 1995).

In budding yeast, the nucleolar clustering of active *tRNA* genes represses nearby Pol II genes, a process referred to as *tRNA* gene-mediated (tgm) silencing (Hull et al., 1994; Wang et al., 2005a). Similarly, in fission yeast, Pol II genes present near Pol III genes are often down-regulated in the *cut3-477* condensin mutant where Pol III transcription is enhanced (Figure 3). We speculate that Pol III transcription or its favored chromatin environment might inhibit efficient Pol II transcription occurring in the vicinity of the Pol III gene.

Another interesting aspect of the Pol III gene-mediated global genome organization is that centromeres associating with Pol III genes are connected to the spindle pole body (SPB) which in turn is attached to cytoplasmic microtubules (Funabiki et al., 1993; Ding et al., 1997; King et al., 2008). Therefore, cytoplasmic contexts can influence the global genome

organization in the nucleus through the microtubule-dependent positioning of SPB and centromeres.

5. Role of the centromeric association of Pol III genes in the assembly of condensed mitotic chromosomes

Recent findings in fission yeast demonstrate that the centromeric association of Pol III genes becomes prominent during mitosis (Figure 2; Iwasaki et al., 2010). This Pol III gene-mediated genome organization is mediated by the interaction between Pol III transcription machinery and the condensin complex, which is essential for chromosome condensation during mitosis and meiosis. 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 (Hirano, 2006). However, the exact molecular processes underlying chromosome compaction remain elusive (Gassmann et al., 2004; Belmont, 2006). We propose that the condensin-mediated global genome organization through Pol III genes accounts for a part of the assembly process for the condensed mitotic chromosomes.

In anaphase cells of fission yeast, Pol III genes distributed through the chromosomal arms are tethered to centromeres, and chromosomes are segregated along the spindle microtubules (Figure 4). 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 can facilitate chromosome movement along spindle microtubules during anaphase. In condensin mutant cells, centromeres are pulled by spindle microtubules, but chromosomal arms are left behind with the Pol III genes, called ϕ -shaped chromosomes, further suggesting that condensin-mediated tethering of chromosomal arms to centromeres is an architectural requirement for mitotic chromosome segregation (Figure 4; Saka et al., 1994). If an association occurs between Pol III genes and centromeres present on different chromosomes or sister chromatids, mitotic chromosomes may not segregate properly, as mitotic chromosome fibers may become entangled during anaphase. Therefore, a molecular mechanism, which has remained elusive, likely directs Pol III genes present at chromosomal arms to interact with a centromere on the same chromosome and the same sister chromatid.

Accumulation of the condensin complex at the centromere was shown in many organisms including budding yeast, fission yeast, fly, nematode, and human (Steffensen et al., 2001; Hagstrom et al., 2002; Ono et al., 2004; Wang et al., 2005b; Nakazawa et al., 2008). In addition, loss of condensin function results in similar chromosome segregation defects such as ϕ -shaped chromosomes and lagging chromosomes in various organisms (Saka et al., 1994; Freeman et al., 2000; Lavoie et al., 2000; Steffensen et al., 2001; Hagstrom et al., 2002; Hudson et al., 2003; Ono et al., 2004). It has recently been reported in budding yeast that condensin associates with *tRNA* genes and that condensin mutations affect clustering of *tRNA* genes in the nucleolus (D'Ambrosio et al., 2008; Haeusler et al., 2008). Furthermore, molecular mechanisms of chromosome segregation are generally well-conserved from fission yeast to human (Yanagida, 2005). Therefore, it is conceivable that chromosome compaction mediated through the associations among Pol III genes and centromeres are likely to be conserved among eukaryotes.

6. Conclusion

Pol III genes associate with centromeres during interphase and those associations become prominent during mitosis. The Pol III gene-dependent genome organization mediated by condensin is likely to be a part of the assembly process for the condensed chromosomes during mitosis. The exact mechanism of this global genome organization remains unclear, but it certainly appears to involve interactions between condensin and the Pol III

transcription machinery, likely TFIIC. We previously showed that TFIIC participates in organizing higher-order genome structure in fission yeast (Noma et al., 2006). More than 60 loci dispersed across the fission yeast genome contain bound TFIIC without Pol III association. These loci are referred to as *COC* (Chromosome-Organizing Clamps), based on the observation that these sites are occupied by high concentrations of TFIIC that are tethered to the nuclear periphery. In budding yeast, there are 8 loci called extra TFIIC (ETC) that are bound by TFIIC without Pol III binding (Moqtaderi and Struhl, 2004). TFIIC binding to specific DNA sequences is critical for boundary function demarcating chromosomal domains in both fission yeast and budding yeast (Noma et al., 2006; Simms et al., 2008; Valenzuela et al., 2009). Pol III transcription machineries have been mapped throughout the human genome and *COC*/*ETC* loci are identified in human cells (Raha et al., 2010; Barski et al., 2010; Moqtaderi et al., 2010; Oler et al., 2010; Canella et al., 2010). Human *ETC* sites are often positioned near CTCF binding sites (Moqtaderi et al., 2010). CTCF is known to function in insulator activity and in global genome organization in higher eukaryotes (Wallace and Felsenfeld, 2007; Phillips and Corces, 2009). Thus, TFIIC that can bind to both Pol III genes as well as *COC*/*ETC* loci might have a general role in chromatin domain formation through higher-order genome organization in eukaryotes.

Genomes of single cell eukaryotes such as fission yeast display a specific functional architecture. For instance, active genes, functionally-related genes, LTR retrotransposons, telomeres, and centromeres tend to associate (Funabiki et al., 1993; Cam et al., 2008; Tanizawa et al., 2010). As described above, Pol III genes and *COC*/*ETC* loci also participate in organizing the higher-order genome structure (Noma et al., 2006; Iwasaki et al., 2010), suggesting that the 3D genome structure in the nucleus is generated by the coexistence of various genome-organizing mechanisms. Recent observations in fission yeast have shed light on an additional complexity in the global genome-organizing mechanism, linking the interphase genome structure to the mitotic chromosome architecture. To understand the fundamental importance of global genome organizations in various nuclear processes, further elucidation of the molecular mechanisms that direct the relevant genome organizations is essential. The existence of genetic, genomic, cell biology, and biochemical tools in the model organism fission yeast, the simplicity of its genome, and similarities of global genome organizations with mammals, make this system ideal for exploring the genome-organizing mechanisms conserved among eukaryotes.

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Abbreviations

Pol III	RNA polymerase III
TFIIIA	Transcription factor IIIA
TFIIIB	Transcription factor IIIB
TFIIIC	Transcription factor IIIC

Figure 1. The centromeric association of Pol III genes

A number of Pol III genes such as *tRNA* and *5S rRNA* genes dispersed throughout the fission yeast genome associate with centromeres. Red and blue vertical bars indicate *tRNA* and *5S rRNA* genes, respectively. The thick arrow indicates more frequent localization of Pol III genes at centromeres. The current hypothesis is that Pol III transcription machinery binding to Pol III genes interacts with condensin, which in turn mediates tethering of Pol III genes to centromeres through interaction between condensin complexes.

Figure 2. A schematic model for global chromosome organization by the centromeric association of Pol III genes

In interphase, the centromeric association of Pol III genes at the nuclear periphery near the surface boundary between the nucleoplasm and the nucleolus is mediated by condensin interacting with Pol III transcription machinery (top panel). The centromeric association of Pol III genes likely influences global higher-order chromosome structure, possibly helping mediate formation of numerous chromatin loops derived from centromeres. Pol III transcription is negatively correlated with the centromeric association of Pol III genes. In mitosis, the centromeric association of Pol III genes contributes to chromosome condensation essential for faithful chromosome segregation (bottom panel).

Figure 3. Reduction of transcript levels of Pol II genes located near *tRNA* genes in the condensin mutant (*cut3-477*)

The graph indicates proportions of significantly down-regulated Pol II genes comparing the *cut3-477* condensin mutant to wild-type (wt) in two groups, those positioned less than or more than 4 kb away from a *tRNA* gene. Significance of the difference is $p < 0.01$.

Figure 4. Role of the centromeric association of Pol III genes in the assembly of condensed mitotic chromosomes

Immunofluorescent images of cells stained for tubulin (red) were merged with DAPI signals (blue; top panels). The *c417* Pol III gene locus (green) and centromeres (magenta) visualized by FISH were merged with DAPI signals (blue; middle panels). Anaphase cells were subjected to the microscopic analysis. Intra-nuclear positioning of centromeres and the Pol III gene locus is depicted in the schematic diagrams (bottom panels). In wild-type cells (wt), centromeres associate with Pol III genes dispersed through the chromosomal arm regions and are pulled by spindle microtubules, and as a result chromosomes are properly segregated along the spindle microtubules during anaphase (left). In the *cut3-477* condensin mutant, the centromeric association of Pol III genes is diminished and centromeres are segregated without holding the chromosomal arms (right). We hypothesize that tethering of Pol III genes to centromeres is a part of the proper assembly process for the condensed mitotic chromosomes, which is essential for faithful chromosome segregation.