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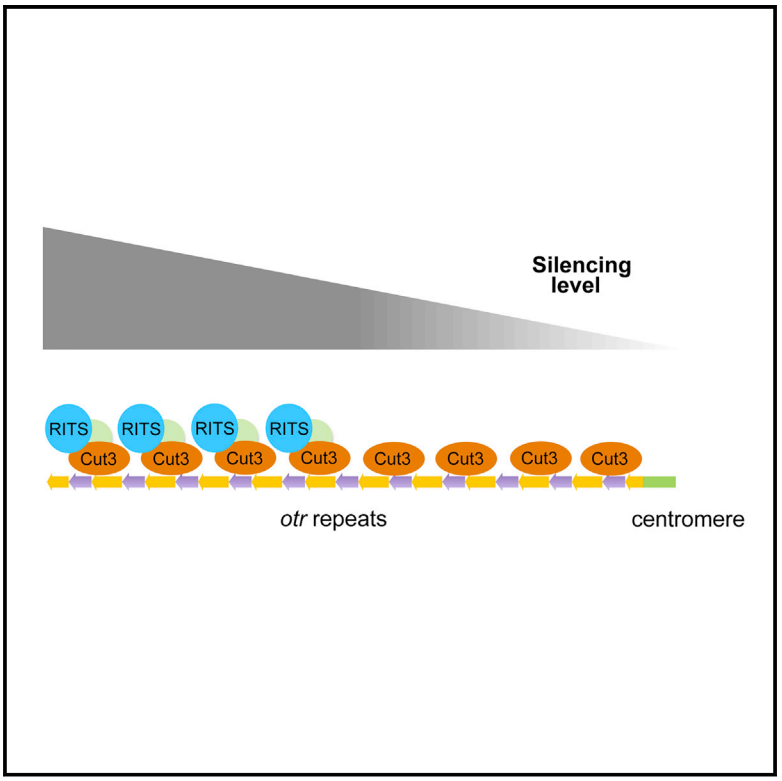
Report

Condensin Promotes Position Effects within Tandem DNA Repeats via the RITS Complex

Graphical Abstract



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In Brief

How the individual sequences within tandem repeats behave remains poorly understood. He et al. show that the heterochromatin silencing between individual units within a peri-centromeric tandem array can vary significantly. They further show that condensin and RNAi components are important for the distinct silencing states in individual repeats.

Highlights

- Silencing between individual units in a tandem repeat array can vary significantly
- ClrC complex and RNAi are essential for the position effect within the *otr* repeats
- Cut3 regulates the position effect in *otr* repeats and CENP-A localization
- Replication timing for individual *otr* repeats also differs significantly

He et al., 2016, Cell Reports *14*, 1018–1024
February 9, 2016
http://dx.doi.org/10.1016/j.celrep.2016.01.006

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Condensin Promotes Position Effects within Tandem DNA Repeats via the RITS Complex

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http://dx.doi.org/10.1016/j.celrep.2016.01.006

SUMMARY

Tandem repetitive DNA is highly abundant in eukaryotic genomes and contributes to transcription control and genome stability. However, how the individual sequences within tandem repeats behave remains largely unknown. Here we develop a collection of fission yeast strains with a reporter gene inserted at different units in a tandem repeat array. We show that, contrary to what is usually assumed, transcriptional silencing and replication timing among the individual repeats differ significantly. RNAi-mediated H3K9 methylation is essential for the silencing position effect. A short hairpin RNA of *ura4*$^{+}$ induces silencing in *trans* within the tandem array in a position-dependent manner. Importantly, the position effect depends on the condensin subunit, *cut3*$^{+}$. Cut3 promotes the position effect via interaction with the RNA-induced transcriptional silencing (RITS) complex. This study reveals variations in silencing within tandem DNA repeats and provides mechanistic insights into how DNA repeats at the individual level are regulated.

INTRODUCTION

In eukaryotic cells, tandem repetitive DNA sequences occupy a substantial fraction of the genome. For example, tandem repetitive DNA arrays make up about 10% of mammalian genomes (Richard et al., 2008; Warburton et al., 2008). Initially considered "junk" DNA, repetitive DNA sequences now have been implicated in transcription control, genome stability, and cancer development (Martienssen et al., 2004; Richard et al., 2008; Shapiro and von Sternberg, 2005). However, due to their repetitive nature, it is difficult to study tandem DNA sequences. As a result, tandem repeat arrays remain among the most poorly understood structures in the genome.

DNA repeats often are organized into heterochromatin, the highly condensed and transcriptionally silenced chromatin domain (Martienssen et al., 2004). Heterochromatin structure and function have been extensively studied. Yet, very little is known about how heterochromatin at the individual repeat level is regulated. It has been assumed that the silenced heterochromatin state is uniformly distributed within a tandem repeat array.

In many eukaryotes, including humans, peri-centromeres are heterochromatic and consist of long arrays of tandem repetitive DNA (Allshire and Karpen, 2008; Martienssen et al., 2004). The peri-centromeric heterochromatin has been linked to centromere function and chromosome segregation (Boyarchuk et al., 2014; Folco et al., 2008; Gonzalez et al., 2014; Smith et al., 2011). Like many other eukaryotes, peri-centromeres in fission yeast (*Schizosaccharomyces pombe*) comprise arrays of large heterochromatic DNA repeats that have been used widely to study heterochromatin. Fission yeast has three chromosomes, each containing a single centromere, ranging from 35 kb to 110 kb (Wood et al., 2002). The core region of the centromeres (*cnt*, centromere core domain) is enriched with the CENP-A homolog Cnp1 (CENP-A^{cnp1}), a centromeric-specific histone H3 variant that defines centromere identity (Allshire and Karpen, 2008; Takahashi et al., 2000). Immediately flanking *cnt* are the innermost repeat regions (*imr*), which include imperfect inverted repeat elements. Outside of the *imr* region are the outermost repeat regions (*otr*). The *otr* regions contain large repeats, each of which consists of *dg* and *dh* elements and spans approximately 6.7 kb (Wood et al., 2002). Both *imr* and *otr* regions are heterochromatic (Allshire et al., 1994), and they are enriched for methylation at histone H3 lysine 9 (H3K9me), the conserved epigenetic mark of heterochromatin. H3K9me is regulated by the ClrC complex, which contains Clr4 (H3K9 methyltransferase), Rik1, Cul4, Dos1, and Dos2 (Hong et al., 2005; Horn et al., 2005; Jia et al., 2005; Li et al., 2005; Thon et al., 2005). RNA interference (RNAi) is also required for H3K9me and heterochromatin silencing. Fission yeast contains a single copy of Argonaute (Ago1), Dicer (Dcr1), and the RNA-dependent RNA polymerase (Rdp1). Ago1, together with the chromo-domain protein Chp1 and Tas3, is assembled into the RITS (RNA-induced transcriptional silencing) complex (Motamedi et al., 2004; Volpe et al., 2002). During the S phase of the cell cycle, the DNA Pol epsilon subunit Cdc20 promotes the transcription of peri-centromeric heterochromatin. RNAi subsequently processes the peri-centromeric transcripts into siRNAs, which in turn facilitate recruitment of the ClrC complex to the heterochromatin region (Chen et al., 2008; Kloc et al., 2008; Li et al., 2011a; Zaratiegui et al., 2011).

To directly probe the behavior of individual repeats in tandem repeat arrays, we developed a collection of strains carrying a reporter gene in different *otr* repeats. Our results demonstrate that heterochromatin silencing and replication timing between different *otr* repeats can vary significantly. The position effect is dependent on RNAi and Cut3, a subunit of the condensin complex. Cut3 mutation also results in mislocalization of CENP-A^{cnp1}. This study

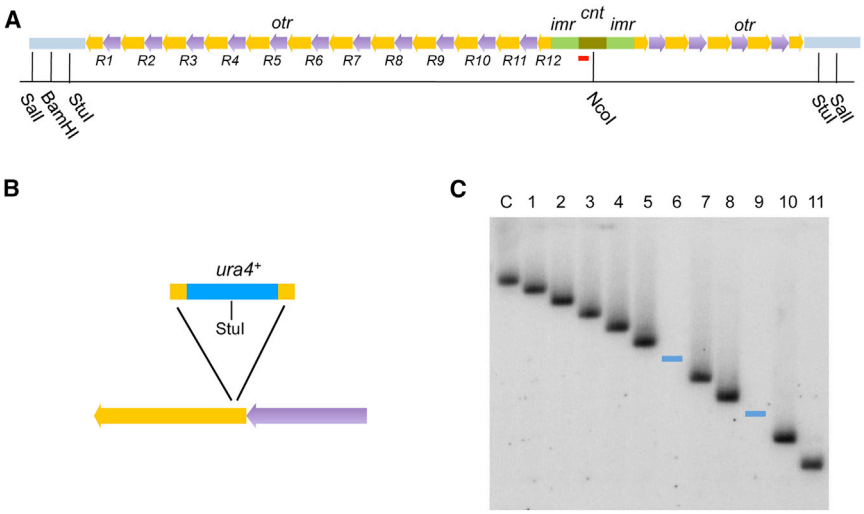

Figure 1. Construction of Strains Carrying a *ura4*$^+$ Reporter Inserted at Different Repeat Units within the *otr* Tandem Array

(A) Schematic structure of centromeric core (*cnt*) and peri-centromeric DNA repeats in fission yeast Chr3. Each outer repeat (*otr*) contains a *dg* (yellow) and a *dh* (purple) element. The red lines indicate the position of probes used for the Southern blot analysis.

(B) Schematic diagram of insertion of a *ura4*$^+$ gene into a single *otr* repeat.

(C) Southern blot analysis of transformants carrying *ura4*$^+$ at individual *otr3L* repeats. DNA of the transformants was digested with NcoI and StuI and probed for the core centromeric region. *ura4*$^+$ was successfully inserted at each repeat in *otr3L* region except repeats 6, 9, and 12. The blue lines mark the position of the predicted bands for digestion of DNA from cells carrying *ura4*$^+$ in repeat 6 or 9 of the *otr3L* region. C, control strain without *ura4*$^+$ at the *otr* region.

reveals previously unknown position effects within tandem DNA repeats, and it suggests a mechanism for how DNA repeats at the individual level are regulated. Our study also implicates the position effects at peri-centromeric regions as a possible contributor in the CENP-A positioning at the centromere.

RESULTS

Construction of Strains for Analyzing Silencing in Individual *otr* Repeats

To examine the level of silencing in each individual DNA repeat within the *otr* tandem array of chromosome 3 (Chr3), we created a collection of strains, in which the *ura4*$^+$ reporter was inserted in a single *otr* repeat in each strain (Figure 1A). The *otr* region in Chr3 has the largest number of repeats among the three centromeres. The *otr* region at the right side of the Chr3 centromere contains four full-length 6.7-kb repeats. The number of *otr* repeats at the left side (*otr3L*) is not well defined, with estimated numbers ranging from 7 to 11 (Ellermeier et al., 2010; Wood et al., 2002). However, it is known that the first and last of the *otr3L* repeats contain only a partial *otr* sequence (Wood et al., 2002).

Using a recently described two-step process (Vader et al., 2011), we successfully obtained strains carrying *ura4*$^+$ reporter inserted at each repeat in the *otr3L* region except repeats 6, 9, and 12 (Figures 1B and 1C). Based on this analysis, we concluded that the left arm of Chr3 contains a total of 12 *otr* repeats, including the first and last incomplete repeats (Figure 1C). Further Southern blotting analysis indicated that, in a portion of the transformants, the total length of the *otr3L* array was different than the size of the *otr* region in wild type (Figure S1), likely resulting from aberrant, non-allelic recombination. In this study, we only focused on the transformants carrying the wild-type length of the *otr* region.

otr Tandem Repeats Exhibit Position-Dependent Silencing

To evaluate the level of silencing at each individual *otr* repeat, strains from our collection were analyzed by growth assays on medium lacking uracil (−ura). The growth rate of these strains differs on −ura medium (Figure 2A), indicating that different *otr* repeats exhibit variation in heterochromatin silencing. We found that cells carrying the *ura4*$^+$ reporter inserted in the repeats distal to the centromere core, including repeats 2–8, have the slowest growth on −ura medium, indicating that these repeats are strongly silenced. Interestingly, repeat 1, which contains a partial *otr* repeat, also displays strong silencing. On the other hand, silencing of repeats close to the centromere is substantially reduced. Repeat 11, the rightmost repeat carrying the reporter, appears to exhibit the weakest silencing (Figure 2A). The position-dependent silencing within the *otr* region was further confirmed by northern blotting (Figures 2B and S2A). Our results thus demonstrate that silencing in the centromeric *otr* repeats is profoundly position-dependent.

To assess whether the silencing state of individual repeats can be inherited through generations, we backcrossed the strains carrying *ura4*$^+$ in *otr* repeat 2, 8, or 11 multiple times to a parental strain lacking the reporter. Our growth assays indicate that the specific silencing state in different *otr* repeats is stably inherited (Figures S2B and S2C).

H3K9 Methylation and RNAi Are Essential for the Position Effect in *otr* Repeats

H3K9me plays an important role in heterochromatin silencing (Nakayama et al., 2001). Our chromatin immunoprecipitation (ChIP) assays showed that H3K9me was detected in all repeats examined; however, repeats 2 and 8 contained relatively higher level of H3K9me than repeats 10 and 11 (Figure 3A). These results indicate that the H3K9me and chromatin silencing level in the repeats are highly correlated. Furthermore, deletion of Dos1, a key component of the ClrC complex (Li et al., 2005), abolishes the position effect in the *otr* repeats (Figure 3B), indicating that the position-dependent regulation of heterochromatic silencing at the *otr* repeats acts upstream of H3K9me.

Assembly of peri-centromeric heterochromatin requires the RNAi machinery (Volpe et al., 2002). Our growth assays showed

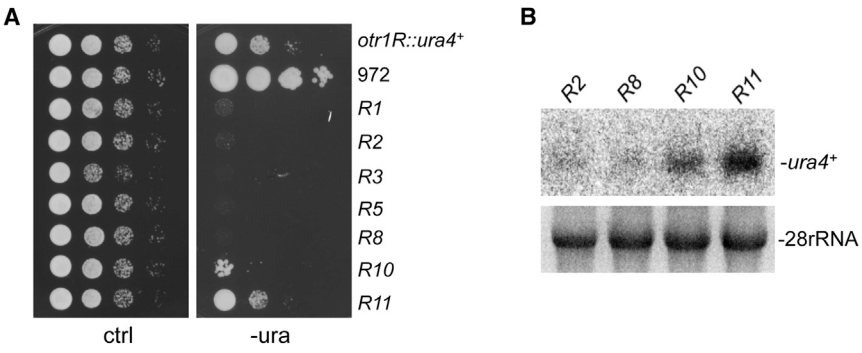

Figure 2. Silencing Level in *otr* Tandem Repeats Is Position Dependent

(A) Serial dilutions of cells harboring *ura4*$^+$ inserted in the indicated *otr3L* repeats were plated on minimal medium without uracil (−ura). Strain 972, wild-type strain harboring *ura4*$^+$ at the endogenous locus. *otr1R::ura4*$^+$, wild-type control strain carrying a *ura4*$^+$ at the *otr* region in Chr1 (Allshire et al., 1995).

(B) Total RNA was prepared from cells carrying *ura4*$^+$ inserted in the indicated *otr3L* repeats and was analyzed by northern blot assay with a probe specific for *ura4*$^+$. 28S rRNA was used as a loading control.

that, similar to the *dos1Δ* background, position-dependent silencing in *dcr1Δ* strains with *ura4*$^+$ in different repeats is eliminated (Figure S3A). We also observed a similar loss of position effect in the deletion mutant of Chp1, a component of the RITS complex (Figure 3C). Furthermore, our ChIP analysis demonstrated that Chp1 is highly enriched in repeats 2 and 8, but decreased in repeats 10 and 11 (Figure 3D), a pattern consistent with the silencing state in these individual repeats.

A *ura4*$^+$ Hairpin Induces Silencing within *otr* Repeats In *trans* in a Position-Dependent Manner

A hairpin structure of *ura4*$^+$ in fission yeast can induce heterochromatin silencing in *trans* at a target locus near heterochromatin, but this has only minor effects on silencing within the single *otr* repeat in Chr1 (Iida et al., 2008; Simmer et al., 2010). To determine whether heterochromatin silencing can be induced by the *ura4*$^+$ hairpin in *trans* in the *otr* tandem repeats, we used a hairpin that contains a sequence complementary to 200bp of *ura4*$^+$ under the *nmt1* promoter (U-HP) and was integrated on chromosome 1 (Simmer et al., 2010). We found that expression of U-HP can induce strong silencing in *trans* in repeat 11, but it has little effect on silencing in *otr* repeat 2 or 8 (Figure 3E). We also found that expression of U-HP in the *dcr1Δ* mutant cells did not result in silencing in the *otr* repeats, indicating that the hairpin-mediated *trans*-silencing requires RNAi (Figure S3B). Together, our result supports the idea that siRNAs generated by the *ura4*$^+$ hairpin induce silencing in *otr* repeats in *trans*, and that this construct can overcome the position-dependent regulation of heterochromatic silencing in the *otr* repeats. The weak effect of *ura4*$^+$ hairpin expression on silencing in repeats 2 and 8 may be due to the fact that these regions already form a highly condensed heterochromatin structure.

Replication Timing Varies between Different *otr* Repeats

DNA replication contributes to peri-centromeric heterochromatin formation in fission yeast (Li et al., 2011a). Our collection of repeat-specific reporters provides us the opportunity to analyze how replication timing differs between individual repeats. Using BrdU-IP, we observed that the incorporation of BrdU into repeats 10 and 11 is severely delayed compared to its incorporation into repeat 2 using BrdU-ChIP (Figures 3F and S3C). These results indicate that the strongly silenced repeats in *otr* region (i.e., repeat 2) replicate earlier than the weakly silenced repeats 10 and 11.

Cut3, a Condensin Subunit, Promotes the Position Effect in *otr* Repeats via the RITS Complex

Condensin, a protein complex that is essential for chromosome condensation during mitosis (Hudson et al., 2009), has been implicated in heterochromatin function (Chen et al., 2008; Oliveira et al., 2005). To determine whether condensin is involved in the position effect on silencing in the *otr* repeats, we analyzed the silencing states in *otr* repeats in a condensin mutant, *cut3-477*. *cut3-477* is a temperature-sensitive mutant that is unable to grow at 37°C (Saka et al., 1994). We found that the position-dependent silencing pattern across the *otr* region is severely disrupted in *cut3-477* mutant even at 32°C. However, unlike *dos1Δ* and RNAi mutants, which exhibit a total loss of silencing in all repeats tested, the effect of the *cut3-477* mutation on silencing varied between repeats. Although silencing is only mildly reduced in the mutant in repeat 2, silencing in repeat 8 is significantly lost. In contrast, the mutation has little effect on the silencing in repeat 11 (Figure 4A). Consistent with this, H3K9me is drastically reduced in repeat 8 in the *cut3-477* mutant (Figure 4B). We also observed that heterochromatin silencing is substantially decreased in repeats 1 and 5 (Figure S4A). Furthermore, we found that overexpression of Cut3 can enhance silencing in the repeat 11, but it has no obvious effect on silencing in repeats 2 and 8 (Figure 4C). These results indicate that Cut3 is a key regulator of the position effect in *otr* repeats. However, we found that the association of Cut3 with repeats 2, 8, and 11 is not significantly different (Figure S4B). In addition, disruption of centromere structure using a mutant of Mis6, an essential centromere protein, results in no obvious effect on Cut3 distribution between different repeats (Figure S4C). These data suggest that additional factors may be required for the position effect.

To determine how Cut3 contributes to the position-dependent silencing in the *otr* repeats, we investigated how the recruitment of the RITS complex to different *otr* repeats is affected in *cut3-477* mutants by ChIP. We found that the level of Chp1 in repeat 8 is greatly reduced in the mutant, whereas its level in repeats 2 and 11 is only mildly affected (Figure 4D), indicating that Cut3 contributes to the position-dependent recruitment of RITS. Furthermore, our co-IP assays showed that Cut3-myc is

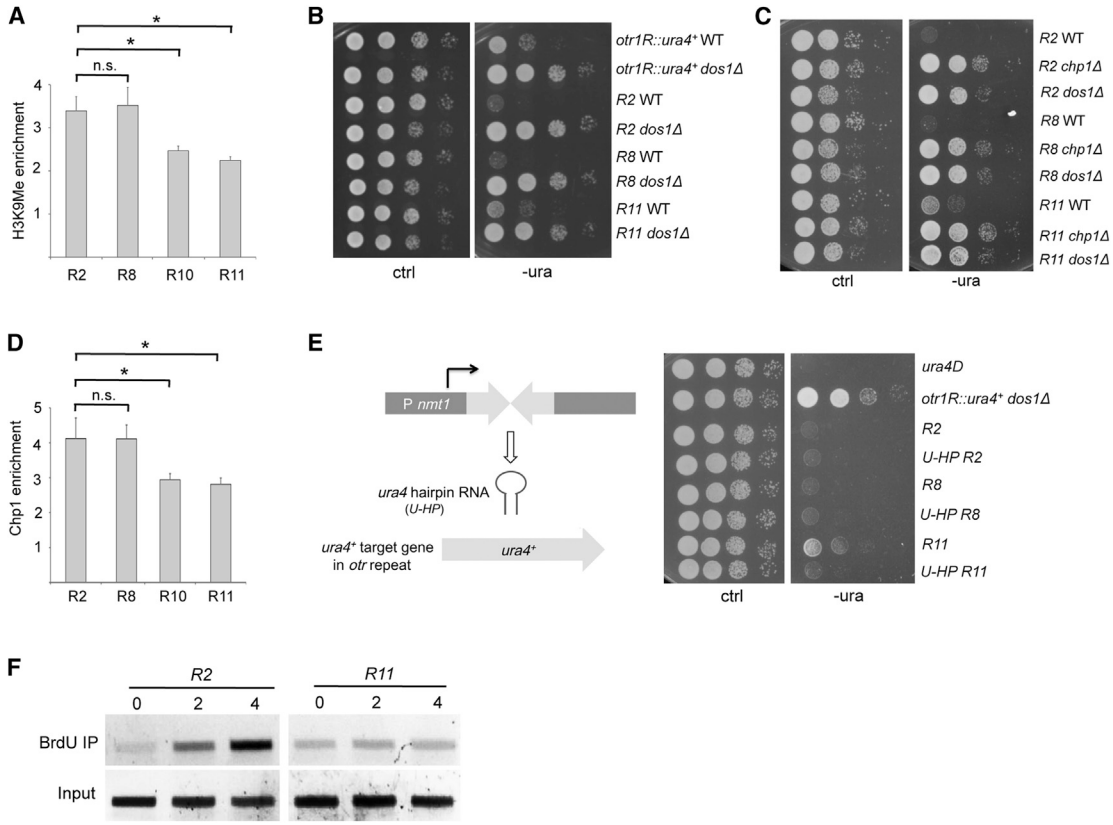

Figure 3. Position Effect in *otr* Repeats Depends on H3K9 Methylation and RNAi

(A) Analysis of H3K9 methylation in repeat 2, 8, 10, or 11 in the *otr* region by ChIP. Wild-type cells carrying *ura4*$^+$ in the indicated repeats were used. ChIP assays were performed using an antibody against H3K9me and primers specific for *ura4*$^+$. *act1*$^+$ was used as a control. Three independent experiments were performed. Error bars indicate SD. (*) $p < 0.05$. n.s., not significant.

(B) Serial dilutions of *dos1Δ* mutant cells with *ura4*$^+$ in *otr3L* repeat 2, 8, or 11 were spotted on −ura medium, and they were incubated at 25°C.

(C) Growth assay for *dcr1Δ* mutant carrying *ura4*$^+$ in *otr3L* repeats 2, 8 or 11. Serial dilution of *dcr1Δ* strains was plated on −ura medium.

(D) ChIP analysis of Chp1-GFP in *otr* repeat 2, 8, 10, or 11. Wild-type cells carrying Chp1-GFP and the *ura4*$^+$ in indicated repeats were used. Error bars indicate SD.

(E) A hairpin of *ura4*$^+$ (U-HP) (Simmer et al., 2010) induces silencing in *otr* repeats in *trans* in a position-dependent manner. Cells carrying the *ura4*$^+$ hairpin and the *ura4*$^+$ reporter in the indicated repeats were analyzed by growth assays in −ura medium.

(F) Cells carrying *ura4*$^+$ in *otr3L* repeat 2 or 11 were analyzed by BrdU-IP. Cells were collected at the indicated time points (hr).

co-immunoprecipitated with Chp1-GFP (Figure 4E), demonstrating that Cut3 interacts with the RITS complex.

CENP-A^{cnp1} Is Delocalized in *cut3-477*

The core centromere is enriched for CENP-A^{cnp1} (Allshire and Karpen, 2008; Takahashi et al., 2000). To investigate whether the position effect at the peri-centromeric *otr* repeats may play a role in the positioning of CENP-A^{cnp1}, we examined CENP-A^{cnp1}-GFP in *cut3-477* cells. In wild-type *S. pombe* cells, centromeres are clustered together at the nuclear envelope. Thus, a single fluorescent focus is observed in most cells expressing CENP-A^{cnp1}-GFP at the endogenous level (Gonzalez et al., 2014). However, we found that 36% of *cut3-477* cells incubated at 34°C for 5 hr exhibit multiple foci or a diffused CENP-A^{cnp1}-GFP pattern (Figure 4F), indicating that association of CENP-A with centromeres is severely perturbed in the mutant. Western blotting demonstrated that the level of CENP-A^{cnp1}-GFP is not affected in *cut3-477* cells (Figure S4D).

DISCUSSION

Despite the importance of tandem DNA repeats (Martienssen et al., 2004; Shapiro and von Sternberg, 2005), how the individual sequences within the tandem repeats behave and how their function is regulated remain poorly understood. Using a collection of repeat-specific reporters, Vader et al. (2011) have shown that the edges of a repetitive ribosomal DNA (rDNA) array in budding yeast are more susceptible to homologous recombination during meiosis. Here, using a similar approach, we demonstrated that transcriptional silencing in the different repeat units within the peri-centromeric tandem *otr* repeats can be strikingly different. An accompanying study in this issue of *Cell Reports* by Wang et al. (2016) also revealed a transcriptional position effect in the repetitive rDNA regions in budding yeast, suggesting that position effects in tandem repeat arrays are conserved.

We show that the *otr* tandem repeats in fission yeast Chr3 exhibit striking position-dependent silencing. These findings

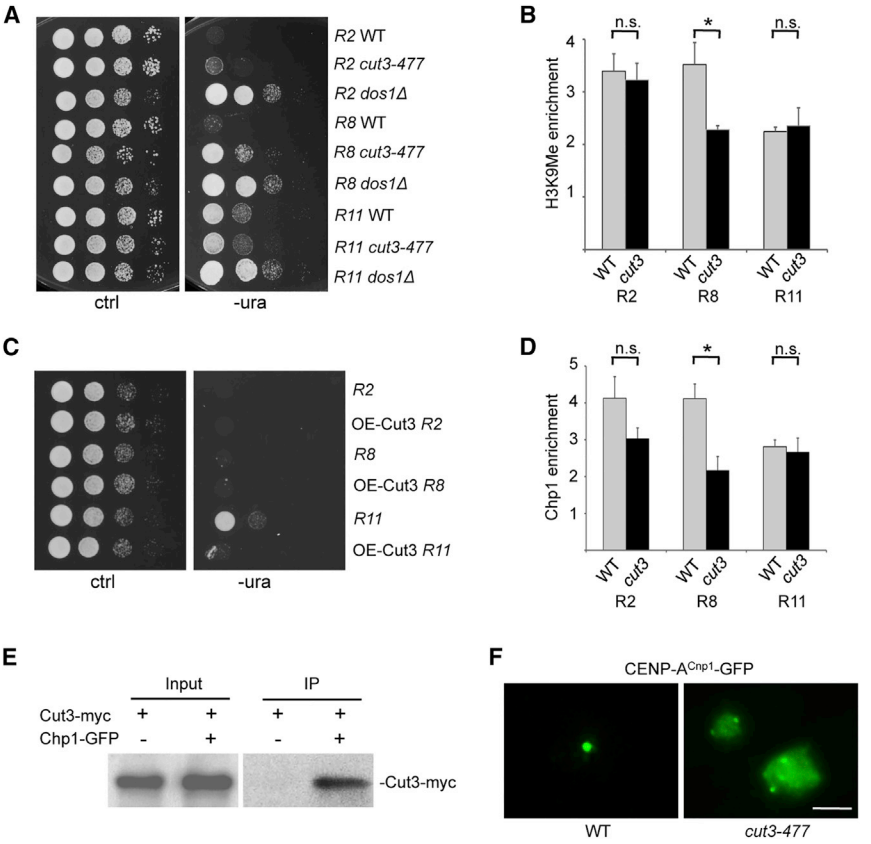

Figure 4. Condensin Regulates the Position Effect within *otr* Repeats via RITS and Is Essential for CENP-A Centromeric Localization

(A) *cut3-477* mutant cells with *ura4*$^+$ in *otr* repeat 2, 8, or 11 were serially diluted and spotted on the −ura medium, and they were incubated at 32°C.

(B) ChIP analysis of H3K9 methylation in *otr* repeat 2, 8, and 11 in *cut3-477*. Error bars indicate SD. (*) $p < 0.05$. n.s., not significant.

(C) Overexpression of Cut3 perturbs the position-dependent silencing in *otr* repeats. Serial dilution of wild-type cells carrying *ura4*$^+$ in the indicated *otr* repeat and *nmt1-cut3*$^+$ were incubated in −ura medium lacking thiamine. OE, overexpression.

(D) ChIP analysis of Chp1-GFP in *otr3L* repeat 2, 8, or 11 in *cut3-477*. Error bars indicate SD. (*) $p < 0.05$. n.s., not significant.

(E) Cut3-myc is co-immunoprecipitated with Chp1-GFP. Extracts from cells carrying Cut3-myc and Chp1-GFP were immunoprecipitated with a GFP antibody. The input and immunoprecipitate were analyzed by western blotting using the anti-body against the myc tag.

(F) CENP-A^{cnp1} is mislocalized in the *cut3-477* mutant. *cut3-477* cells expressing CENP-A^{cnp1}-GFP at an endogenous level were incubated at 34°C for 5 hr. Scale bar, 2 μm.

suggest that, although different repeats in the tandem array share the same sequence, each one is organized into a specific higher order structure. We demonstrated that the H3K9me and RNAi components are key effectors of the observed positional differences. Moreover, we found that condensin is essential for the formation of the position-dependent epigenetic states. In particular, the specific pattern of the RITS complex associated with different individual *otr* repeats is perturbed in the *cut3* mutant. These results suggest that condensin acts as the upstream instructor to recruit the proper level of silencing effectors, including the RITS complex, to individual repeats, and to establish the unique heterochromatin state in the particular position. Nevertheless, we did not observe any significant difference in the level of Cut3 associated with different peri-centromeric repeats, suggesting that additional factor(s) may be required to function together with Cut3 to promote the position effect.

What is the biological relevance of the position effect at the peri-centromeric repeats? Centromeres are responsible for kinetochore assembly, and they play a key role in chromosome segregation (Allshire and Karpen, 2008). In most eukaryotes, centromere identity is believed to be predominantly epigenetically specified (Fukagawa and Earnshaw, 2014). CENP-A is the most likely candidate for the epigenetic mark used to define centromeres. How CENP-A is incorporated to centromeric regions remains poorly understood, but mis-regulation of CENP-A adversely affects chromosome segregation, resulting in aneuploidy and cancer (Allshire and Karpen, 2008). Our studies suggest that the distinct three-dimensional architecture created by the tandem arrays at peri-centromeres may contribute to proper positioning of CENP-A. Indeed, RNAi components and Clr4 have been shown to be required for establishment of CENP-A^{cnp1} chromatin at centromere in fission yeast (Folco et al., 2008; Gonzalez et al., 2014). Previous studies also demonstrate that peri-centromeric heterochromatin is important for centromeric localization of CENP-A in *Neurospora crassa* and mouse cell lines (Boyarchuk et al., 2014; Smith et al., 2011). Here, we show that the key regulator of the position effect in peri-centromeric repeats, Cut3, is also required for the centromeric localization of CENP-A^{cnp1} (Figure 4F). Condensin has been previously implicated in the assembly of CENP-A chromatin in *Xenopus* and human cells (Bernad et al., 2011; Samoshkin et al., 2009), but its precise role in the process is still unclear. We propose that condensin mediates the organization of peri-centromeric repeats into a specific higher-order structure, which in turn helps restrict CENP-A to centromeres.

We demonstrated that replication timing among the individual units in a tandem array can vary significantly. Replication of an *otr* repeat near the centromere is severely delayed compared to more strongly silenced repeats close to the chromosome arm. Although heterochromatin in general is associated with late replication, centromeric heterochromatin in fission yeast and a few of other organisms replicates early in the S phase (Kim et al., 2003). The reason for the early replication of these heterochromatic regions remains unclear. During DNA replication, CENP-A chromatin is disassembled and CENP-A must be faithfully reincorporated into centromeres after replication (Allshire

and Karpen, 2008; Gonzalez et al., 2013). It has been shown that DNA replication is important for heterochromatin assembly at peri-centromeric regions in fission yeast (Chen et al., 2008; Kloc et al., 2008; Li et al., 2011a; Zaratiegui et al., 2011). We propose that early replication of strongly silenced peri-centromeric repeats allows the establishment of the position effect at an early stage so as to create a chromatin environment that can ensure the proper positioning of CENP-A. Consistent with this idea, the core region of centromeres, *cnt*, and the flanking *imr* regions, both of which are weakly silenced, replicate later than the highly heterochromatic *otr* regions (Li et al., 2011b).

Our findings reveal previously unrecognized position effects within tandem repeat arrays, and they support the concept that the position effects at peri-centromeric repeats promote the epigenetic specification of centromeres. In addition, our studies uncover condensin and RNAi components as key factors involved in position-dependent epigenetic regulation of tandem DNA repeats, and they provide insight into how DNA repeats at an individual level are controlled. It will be important in future studies to identify other key regulators of the position effects. It will also be interesting to explore how tandem repeats are spatially organized. These studies will shed light on regulation of this important but poorly studied part of the genome.

EXPERIMENTAL PROCEDURES

Strains, Media, and Genetic Analysis

The fission yeast strains used in this study are listed in Table S1. Standard media and genetic analysis for fission yeast were used (Moreno et al., 1991).

Strain Construction

The repeat-specific reporters were created using a recently described process with minor modifications (Vader et al., 2011), and further details are available in the Supplemental Information.

CHEF Gel Electrophoresis and Southern Blot

CHEF gel electrophoresis and Southern blotting were performed as described (Vader et al., 2011) with minor modifications, and further details are available in the Supplemental Information.

Co-immunoprecipitation and Western Blot Analysis

Immunoprecipitation was performed using an anti-GFP antibody (Abcam, ab290). Eluates were analyzed by standard western blotting protocols using an anti-myc antibody (Sigma, C3956). For western blot analysis of the *cut3* mutant, blots were probed with anti-GFP (Roche, 11 814 460 001) or α-tubulin (Abcam, ab6160) antibodies.

ChIP

ChIP was performed as described (Li et al., 2008). The primers used are listed in Table S2. Quantifications were performed using ImageJ 1.46r software. All experiments were independently repeated three times. A two-tailed Student's t test was used to determine the statistical significance between different experimental groups.

BrdU IP

BrdU IP was essentially performed as described previously (Li et al., 2011b). The primers used in this study are listed in Table S2.

Northern Blot

Northern blot was performed according to the standard protocol. Briefly, RNA samples were separated and transferred to an Amersham Hybond-N+ membrane in 2X SSC buffer. After UV cross-linking, the membranes were hybridized by ^{32}P-labeled probe recognizing *ura4*$^{+}$ or the 28S rRNA as a control and exposed to film for autoradiography.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.01.006.

AUTHOR CONTRIBUTIONS

H.H. performed the experiments with assistance from S.Z. and D.W.; F.L. designed the study and wrote the manuscript with input from A.H.

ACKNOWLEDGMENTS

We are grateful to Robin Allshire, Mitsuhiro Yanagida, and The Japan Yeast Genetic Resource Center for kindly providing strains. We thank members of the F.L. laboratory, particularly Marlyn Gonzalez, for critical reading of the manuscript. F. L. is a Pew Scholar in the Biomedical Sciences, supported by The Pew Charitable Trusts. This project was supported by NIH grant 1R01 GM106037 (to F.L.) and NSF grant MCB-1330557 (to F.L.).

Received: May 20, 2015
Revised: October 30, 2015
Accepted: December 29, 2015
Published: January 28, 2016

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