Monopolin recruits condensin to organize centromere DNA and repetitive DNA sequences

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ABSTRACT The establishment and maintenance of higher-order structure at centromeres is essential for accurate chromosome segregation. The monopolin complex is thought to cross-link multiple kinetochore complexes to prevent merotelic attachments that result in chromosome missegregation. This model is based on structural analysis and the requirement that monopolin execute mitotic and meiotic chromosome segregation in Schizosaccharomyces pombe, which has more than one kinetochore–microtubule attachment/centromere, and co-orient sister chromatids in meiosis I in Saccharomyces cerevisiae. Recent data from S. pombe suggest an alternative possibility: that the recruitment of condensin is the primary function of monopolin. Here we test these models using the yeast Candida albicans. C. albicans cells lacking monopolin exhibit defects in chromosome segregation, increased distance between centromeres, and decreased stability of several types of repeat DNA. Of note, changing kinetochore–microtubule copy number from one to more than one kinetochore–microtubule/centromere does not alter the requirement for monopolin. Furthermore, monopolin recruits condensin to C. albicans centromeres, and overexpression of condensin suppresses chromosome segregation defects in strains lacking monopolin. We propose that the key function of monopolin is to recruit condensin in order to promote the assembly of higher-order structure at centromere and repetitive DNA.

INTRODUCTION
Centromere DNA is organized into higher-order chromatin, and this organization is critical for proper chromosome segregation. Numerous protein complexes, including cohesin, condensin, and monopolin, contribute to centromere organization (reviewed in Poon and Mekhail, 2011). Cohesin binds to regions flanking centromere DNA and promotes chromatin condensation and accurate chromosome segregation (Eckert et al., 2007). Condensin binding is enriched near centromeres in both Saccharomyces cerevisiae and Schizosaccharomyces pombe (D’Ambrosio et al., 2008; Tada et al., 2011). The monopolin complex is important for meiotic and mitotic chromosome segregation in multiple fungal species. It was originally identified in S. cerevisiae and found to be essential for co-orienting kinetochores on sister chromatids in meiosis I (Toth et al., 2000). The S. cerevisiae mitotic monopolin complex includes the monopolin core complex (Csm1/Lrs4), Hrr25, and Mam1 (Rabitsch et al., 2003; Petronczki et al., 2006). Monopolin and condensin cooperate to establish centromere structure in meiosis I (Brito et al., 2010b). The monopolin core complex also localizes to kinetochores and enhances plasmid segregation accuracy during mitosis (Brito et al., 2010a). S. cerevisiae CSM1 and LRS4 exhibit synthetic genetic interactions with genes critical for mitotic chromosome segregation, further supporting a mitotic, as well as a meiotic, role for the monopolin core complex (Pan et al., 2006). In S. pombe, homologues of the monopolin core complex (Pcs1/Mde4) function during both...
mitosis and meiosis II to prevent merotelic attachments (Gregan et al., 2007). Cells lacking either the monopolin core complex or proteins required to establish centromeric heterochromatin have high frequencies of merotelic attachments (Rumpf et al., 2010). The functions of monopolin and condensin are closely linked in S. pombe. Both complexes promote kinetochore orientation, faithful sister chromosome segregation, and ribosomal DNA (rDNA) separation during mitosis (Nakazawa et al., 2008; Tada et al., 2011). Furthermore, monopolin recruits condensin to the centromere, and tethering of condensin to the centromere rescues many phenotypes of a monopolin-deletion mutant (Tada et al., 2011). Together these results highlight the importance of the monopolin core complex, and its recruitment of condensin, in accurate chromosome segregation.

The chromosome segregation phenotype of monopolin mutants is related to centromere structure. S. cerevisiae has small, point centromeres with a single kinetochore microtubule attachment per centromere (Winey et al., 1995; Joglekar et al., 2008). S. pombe has larger, regional centromeres, each with more than one kinetochore-microtubule attachment (Ding et al., 1993; Joglekar et al., 2008). S. cerevisiae monopolin mutants have stronger phenotypes in meiosis than in mitosis, whereas the monopolin complex in S. pombe is required for both mitotic and meiotic chromosome segregation. These observations led to the hypothesis that monopolin is primarily required to coordinate the segregation of centromeres with multiple kinetochore-microtubule attachments (Rabitsch et al., 2003).

Monopolin also functions at repetitive DNA other than centromeres. Repetitive DNA regions are packaged into higher-order chromatin structures that repress sister chromatin exchange and other mechanisms of recombination. In S. cerevisiae vegetative cells, the monopolin core complex, also known as cohbin, is important for rDNA stability and telomere maintenance (Huang et al., 2006; Mekhail et al., 2008; Chan et al., 2011). Tof2, a nucleolar component important for rDNA silencing, associates with Csm1 and Lrs4 to enhance condensin binding to rDNA repeats. In addition, LRS4 deletion results in subtelomeric DNA instability (Chan et al., 2011), and the CSM1 deletion strain exhibits increased telomere length (Askree et al., 2004). The role of the monopolin core complex at repetitive DNA, both rDNA and telomeres, has only been tested in S. cerevisiae and has not been explored in other organisms.

Several models have been proposed for the mechanisms of monopolin function. One model, based on structural data, is that it functions as a cross-linker. The S. cerevisiae Csm1/Lrs4 core complex has a V-shaped structure, with the globular domain of Csm1 containing a conserved patch that interacts with kinetochore proteins and the rDNA-associated protein Tof2 in vitro (Corbett et al., 2010). This V-shaped structure suggests that the monopolin core complex may function as a molecular clamp to cross-link kinetochores and proteins bound to rDNA repeats. However, the proposal that monopolin is required for the alignment of multiple kinetochore-microtubule attachments has not been directly tested. An alternative model, that the primary function of monopolin is to recruit condensin, which in turn provides higher-order structure to DNA within centromere regions, was proposed recently based on experiments in S. pombe demonstrating that tethering of condensin bypasses the requirement for monopolin in chromosome segregation (Dudas et al., 2011; Tada et al., 2011).

We exploited the flexibility of kinetochore size in the yeast Candida albicans to test the requirement for the monopolin complex as a function of one or more than one kinetochore-microtubule/centromere. C. albicans has small, regional centromeres that span ~4 kb and are inherited epigenetically (Baum et al., 2006). Normally, C. albicans centromeres associate with one kinetochore-microtubule attachment (Joglekar et al., 2008). On overexpression of CSE4, the C. albicans gene encoding the homologue of the centromere-specific histone H3 CENP-A, the majority of centromeres bind more than one kinetochore-microtubule complex (Burrack et al., 2011). The C. albicans genome also has a highly repetitive region found in one or two copies on all but one chromosome. Each of these major repeat sequences (MRSs) is composed of tandem repeats that span 10–50 kb and undergo shifts in repeat length, presumably due to recombination between tandem repeats and/or unequal sister chromatid recombination (Chibana and Magee, 2009).

Here we find that deletion or repression of monopolin in C. albicans causes defects in chromosome segregation, an increase in metaphase sister centromere separation, and increased recombination at rDNA, telomeres, and the MRSs. If monopolin was required specifically to cross-link chromosomes with multiple microtubules, then we would expect an increased requirement for monopolin in C. albicans cells having more than one kinetochore-microtubule/centromere. This was not the case: increasing the kinetochore-microtubule copy number did not affect the requirement for monopolin. Instead, the results support a model in which the primary role of the monopolin complex is to recruit condensin and organize DNA at regional centromeres. We also demonstrate that the monopolin core complex member Csm1 has critical roles in maintaining the integrity of a range of repetitive DNA tracts, including rDNA, telomeres, and MRSs, indicating that the role of monopolin in the maintenance of repetitive DNA tracts, which was previously observed in S. cerevisiae, is more widely conserved in fungi.

**RESULTS**

*C. albicans* Csm1, a monopolin core complex homologue, localizes to centromeres and kinetochores in actively dividing *C. albicans* cells

Csm1 (ORF19.7663) is the *C. albicans* gene most similar to the *S. cerevisiae* CSM1 monopolin complex subunit gene. We constructed a Csm1–green fluorescent protein (GFP) fusion protein to track the localization pattern of Csm1 in cells also stained with 4′,6-diamidino-2-phenylindole (DAPI) to demarcate the nucleus. Csm1-GFP localized both as a diffuse pattern coincident with the nucleus (Figure 1A, top) and a localized focus within the nucleus (Figure 1A, bottom). The localization was cell cycle dependent. The diffuse localization was seen in 84% of unbudded (G1) cells and 68% of cells with segregated nuclei (late anaphase/telophase); the focal localization was more prevalent in budded cells, with 56% of small-budded cells (S) and 58% of medium-budded cells (G2/M) having Csm1-GFP foci (Figure 1A). Thus the Csm1 localization pattern is consistent with monopolin binding throughout the nuclear DNA in unbudded cells and with more specific monopolin binding to kinetochores in actively dividing cells.

We used colocalization of Csm1-GFP with other proteins tagged with mCherry to more carefully map the intracellular localization of Csm1. Because Csm1 colocalizes with the nucleolus in *S. cerevisiae* and *S. pombe* (Rabitsch et al., 2003), we examined the localization of Csm1 relative to the nucleolar marker Nop1-mCherry (Lavoie et al., 2008). Of interest, Csm1-GFP colocalized with Nop1-mCherry in 91% of cells with diffuse Csm1-GFP localization (Figure 1B). The diffuse Csm1-GFP signal usually extended beyond the Nop1-mCherry signal (Figure 1B, top right) and was similar to the DAPI localization patterns (Figure 1A). Therefore Csm1-GFP appears to be distributed throughout the nucleus, including the nucleolus in *C. albicans*.
During mitosis, monopolin binds to kinetochores in both *S. cerevisiae* (Brito et al., 2010a) and *S. pombe* (Rabitsch et al., 2011). We next asked whether Csm1 associated with centromere DNA during mitosis using chromatin immunoprecipitation (ChiP). In G1 phase (15 min after release from stationary phase, >90% unbudded cells), only weak binding of Csm1-GFP to centromere DNA was detected by ChiP (Figure 1D, light green). In contrast, in S and G2/M phases (105 min after release from stationary phase, >80% budded cells) we detected strong binding of Csm1-GFP to centromere DNA (Figure 1D, dark green). Csm1-GFP binding was restricted to the centromere core region, which is bound by inner and outer kinetochore proteins (Burrack et al., 2011). Therefore Csm1 associates with the core region of centromere DNA in *C. albicans*, and this is especially evident in actively dividing S- and G2/M-phase cells.

**Csm1 is required for accurate chromosome segregation and cell cycle progression**

Having established that *C. albicans* Csm1 is present at kinetochores and centromere DNA in actively dividing cells, we asked whether the monopolin core complex is required for chromosome segregation or for cell cycle progression in wild-type *C. albicans*, which have a single kinetochore–microtubule. We followed marker loss using two counterselectable markers: loss of GAL1 was detected by resistance to 2-deoxygalactose (2-DOG), and loss of URA3 was detected by resistance to 5-fluoroorotic acid (5-FOA). Deletion of both copies of *CNS1* resulted in increased loss of the heterozygous GAL1 marker on Chr1 (Figure 2A), suggesting an increased rate of chromosome loss. Similar results were obtained with conditional repression of *CSM1*, using the MET3 promoter to drive *CSM1* expression in a strain with the other copy deleted. Growth of this strain under inducing conditions (media lacking methionine and cysteine) permitted expression of Csm1, whereas expression was dramatically reduced under repressing conditions (media with high levels of methionine and cysteine; Supplemental Figure S1A). Conditional depletion of *CSM1* resulted in increased marker loss, detected with GAL1 on Chr1 and with the URA3 marker gene inserted on Chr3 (Supplemental Figure S1B). In addition, we measured chromosome segregation defects by observing segregation patterns of TetR-GFP bound to TetO sequences inserted immediately adjacent to *CEN7*. Abnormal segregation patterns, such as no TetR-GFP signal or >2 TetR-GFP foci/cell, were increased 11-fold in *csm1Δ/Δ* cells compared with control cells (Supplemental Figure S2).

**FIGURE 1:** Csm1 colocalizes with kinetochores in actively dividing cells. (A) Csm1-GFP (green) exhibited both diffuse (top) and focal (bottom) localization patterns within DAPI-stained nuclei (blue). Cells were imaged by fluorescence microscopy at 1000× total magnification. The percentage of cells in each cell cycle stage with each localization pattern was quantified. Data shown are mean ± SEM of three biological replicates. Scale bar, 5 μm. (B) Csm1-GFP (green) and Mtw1-mCherry (red) were coexpressed and imaged by fluorescence microscopy at 1000× total magnification. The percentage of cells in each cell cycle stage exhibiting colocalization of the Csm1-GFP diffuse region with Mtw1-mCherry was quantified. Data shown are mean ± SEM of three biological replicates. Scale bar, 5 μm. (C) Csm1-GFP (green) and Mtw1-mCherry (red) were coexpressed and imaged by fluorescence microscopy at 1000× total magnification. The percentage of cells in each cell cycle stage exhibiting colocalization of the Csm1-GFP focus with Mtw1-mCherry was quantified. Data shown are mean ± SEM of three biological replicates. Scale bar, 5 μm. (D) Anti-GFP ChIP analyzed with primers amplifying CEN5 for a Csm1-GFP strain released from stationary phase into YPA-glucose for 15 min (G1, light green) and 105 min (M, dark green). Data shown are mean ± SEM of three biological replicates.
Marker loss was due to increase in whole-chromosome loss rather than increase in recombination events, as determined by single nucleotide polymorphism–restriction fragment length polymorphism (SNP-RFLP) analysis of a subset of strains. Whole-chromosome loss of heterozygosity occurred in 40 of 55 (73%) of csm1Δ/Δ 2-D0G⁵ isolates compared with 5–10% whole-chromosome loss of heterozygosity previously observed in wild-type strains (Forche et al., 2011). Similarly, 17 of 24 (71%) of 2-D0G⁵ isolates (chr1) and 12 of 24 (50%) of 5-FOA⁶ isolates (chr3) underwent whole-chromosome loss of heterozygosity when CSM1 was repressed, which is significantly more than in cells in which CSM1 was expressed, in which 0 of 24 (0%) of 2-D0G⁵ (chr1) isolates and 2 of 23 (9%) of 5-FOA⁶ isolates (chr3) had whole-chromosome loss. Thus strains lacking monopolin, as well as strains that expressed very low amounts of monopolin, underwent whole-chromosome loss of similar in csm1Δ/Δ strains overexpressing CSE4 and csm1Δ/Δ deletion strains with normal levels of Cse4. Thus increased kinetochore–microtubule copy number did not affect the chromosome loss defect in cells lacking the monopolin complex in C. albicans (Figure 2A). This result is consistent with the idea that coordination of multiple kinetochore–microtubules per centromere is not a critical function of the monopolin core complex.

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The number of kinetochore–microtubule attachments does not alter the requirement for monopolin C. albicans chromosomes normally have 1 kinetochore–microtubule attachment/centromere. In C. albicans, Csm1 was required for accurate mitotic chromosome segregation; therefore this suggests that the monopolin complex function facilitates mitotic chromosome segregation even when there is only 1 kinetochore–microtubule/centromere. We tested the hypothesis by manipulating the number of assembled kinetochore complexes and kinetochore–microtubules using a conditional promoter system previously developed to increase C. albicans kinetochore copy number (Burrack et al., 2011). If Csm1 contributes primarily to the cross-linking of kinetochore–microtubules, we would expect to see a more severe chromosome segregation defect in strains over-expressing CSE4, which results in increased number of kinetochore–microtubules per centromere (Figure 2B). Growth of the cells in succinate activated the PCK1 promoter driving CSE4, resulting in high levels of CSE4 expression (Supplemental Figure S3A), increased binding of Cse4 (CaCENP-A) protein to centromere DNA (Supplemental Figure S3B), increased recruitment of the kinetochore protein Mtw1 to centromeres, and increased number of spindle microtubules per centromere (Supplemental Figure S3C). Approximately 60% of centromeres have two kinetochore–microtubule attachments when CSE4 is overexpressed (Supplemental Figure 3C; Burrack et al., 2011). As a control, growth in glucose (repressing the PCK1 promoter upstream of one copy of CSE4) yielded levels of CSE4 similar to unmodified controls and did not change the association of other kinetochore proteins with centromere DNA (Burrack et al., 2011).

Of importance, marker loss rates were similar in csm1Δ/Δ strains overexpressing CSE4 and csm1Δ/Δ deletion strains with normal levels of Cse4. Thus increased kinetochore–microtubule copy number did not affect the chromosome loss defect in cells lacking the monopolin complex in C. albicans (Figure 2A). This result is consistent with the idea that coordination of multiple kinetochore–microtubules per centromere is not a critical function of the monopolin core complex.

In addition to marker loss, we tracked nuclear segregation and cell cycle progression in DAPI-stained csm1Δ/Δ strains. As expected of cells with defects in chromosome segregation, deletion of the monopolin complex was associated with a significant increase in the fraction of large-budded cells with elongated nuclei stretched along the mother–bud axis, indicating that monopolin mutants are delayed in anaphase (Figure 2C). In addition, cells with abnormal morphologies, including cells with missegregated nuclei (Figure 2D,
The metaphase separation distance between sister centromeres has been characterized as an indicator of chromosome stiffness, with increased separation distance suggestive of decreased chromosome stiffness (Ribeiro et al., 2009). To assess whether monopolin could affect chromosome stiffness during mitosis, we measured the separation distance between sister centromeres in wild-type and csm1Δ/Δ strains. We did this by inserting TetO sequences immediately adjacent to CEN7 in wild-type and csm1Δ/Δ strains and then expressing a TetR-GFP fusion protein from an intergenic region. We imaged metaphase cells (Figure 3A) and measured the distance between TetR-GFP spots on sister chromosomes, which approximates the distance between sister centromeres. The sister centromere separation distance was ~30% longer in csm1Δ/Δ than in wild-type cells (587 ± 13 nm vs. 448 ± 12 nm, mean ± SEM, p << 0.001; Figure 3B). This suggests that deletion of CSM1 caused a decrease in chromosome stiffness during mitosis.

Loss of monopolin leads to increased sister centromere separation

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Monopolin recruits condensin to centromeres and kinetochores

Condensin is believed to be an important contributor to chromosome stiffness (Ribeiro et al., 2009; Stephens et al., 2011). One explanation for decreased chromosome stiffness in the csm1Δ/Δ mutant is that condensin recruitment to the chromosomes might be impaired. Thus we asked whether monopolin recruits condensin in C. albicans by tracking condensin using ChIP of a hemagglutinin (HA) tag on Smc4, an ATPase subunit of the condensin complex. The DNA binding pattern and timing of Smc4-HA binding to centromere sequences mirrored the binding of Csm1-GFP (Figures 4A and 1D). Smc4-HA bound to the core centromere region, and the extent of Smc4-HA binding was higher in actively dividing S-phase and G2/M cells (105 min after release from stationary phase) than in G1 cells (15 min after release from stationary phase; Figure 4A). Of importance, Smc4-HA binding to centromeres in actively...
Significance between inducing and noninducing conditions was determined by two-tailed paired *t* test. *p* < 0.05. (B) Strain: CSM1/CSM1 MET3-SMC4/SMC4

Figure 5: Overexpression of SMC4 suppresses the monopolin-deletion phenotype. (A) Fluctuation analysis of loss of GAL1 in CSM1/CSM1 and csm1Δ/Δ MET3p-SMC4/SMC4 strains during growth in repressing conditions (SDC+Met+Cys, normal expression) and activating conditions (SDC+Met+Cys, overexpression). Loss of GAL1 was quantified by plating cells on nonselective media and media containing 2-DOG to select for loss of GAL1. Colony counts were used to calculate the rate of loss per cell division. Results are mean ± SEM of rates calculated from at least three experiments, each with eight cultures per condition. Significance between inducing and noninducing conditions was determined by two-tailed paired *t* test. *p* < 0.05. (B) Strain: CSM1/CSM1 MET3-SMC4/SMC4

Eight cultures per condition. Significance between inducing and noninducing conditions was determined by two-tailed paired *t* test. *p* < 0.05. (C) Strain: CSM1/CSM1 MET3-SMC4/SMC4

Deletion of monopolin had no detectable effect on Cse4 incorporation at C. albicans centromeres (Supplemental Figure S4), consistent with observations in S. pombe.

In S. pombe, targeting of condensin to centromeres via a fusion protein rescued monopolin-deletion phenotypes including growth defects and high rates of lagging chromosomes (Tada et al., 2011). Therefore we hypothesized that if a primary function of monopolin is to recruit condensin to centromeres, then overexpression of condensin may rescue the observed chromosome segregation defects. We constructed conditional CSM1/CSM1 and csm1Δ/Δ strains in which one copy of SMC4 was placed under control of the MET3 promoter. Growth of these strains under inducing conditions (media lacking methionine and cysteine) resulted in overexpression of SMC4 relative to noninducing conditions (media with high levels of methionine and cysteine) or a control strain (Supplemental Figure S5). Remarkably, overexpression of SMC4 significantly suppressed chromosome segregation defects in the monopolin-deletion strain: excess Smc4 reduced the rate of GAL1 marker loss relative to a strain with lower Smc4 levels (Figure 5A). When CSM1 was present, chromosome loss rates were not affected by changes in SMC4 expression. In addition, SMC4 overexpression reduced the proportion of csm1Δ/Δ cells that exhibited nuclear segregation defects and abnormal cell morphologies, measured by microscopy of DAPI-stained cells (Figure 5B). Furthermore, growth of csm1Δ/Δ cells, as measured by colony size, was increased in cells overexpressing SMC4 (Figure 5C). Overexpression of a single subunit of condensin was sufficient to counteract many of the defects in chromosome segregation and cell cycle progression characteristic of monopolin mutants, whereas cells with an intact monopolin complex did not exhibit sensitivity to changes in SMC4 expression. Thus an important function of monopolin is to promote assembly of higher-order chromosome structure in cooperation with, or via recruitment of, condensin.

Expression) and activating conditions (SDC-Met-Cys, overexpression) for 6 h. Cells were stained with DAPI and imaged at 1000× total magnification with a DAPI filter set. The percentage of cells with abnormal nuclear segregation and/or morphology was determined as in Figure 2. Results are mean ± SEM of three biological replicates. Significance between inducing and noninducing conditions was determined by two-tailed paired *t* test. *p* < 0.05. (C) Colony size was measured after 48 h incubation. Data shown are mean ± SEM of three experiments. Significance between inducing and noninducing conditions was determined by two-tailed paired *t* test. *p* < 0.05.
Monopolin is essential for maintenance of repetitive DNA tracts in the genome

Monopolin is required for the maintenance of rDNA and telomere repeats in *S. cerevisiae* (Johzuka and Horiuchi, 2009), where it binds to the NTS1 region of rDNA (Huang et al., 2006). The function of monopolin at repetitive DNA in other organisms, however, has not been studied. To ask whether monopolin is also required to maintain repeats in *C. albicans*, we compared rDNA repeat tract length in *csm1Δ/Δ* monopolin-deletion strains relative to control *CSM1* strains. Fragments containing the entire rDNA tract were digested with *Kpn*I, separated by clamped homogeneous electric field (CHEF) gel electrophoresis, and detected by Southern blot. Two independently constructed *csm1Δ/Δ* strains both had shorter rDNA tracts relative to control strains (Figure 6A). Of importance, the rDNA was shorter and more variable within a population of *csm1Δ/Δ* cells than rDNA observed in wild-type cells, regardless of whether the culture was initiated from a mixed population or a single colony. This suggests that rDNA length changes more frequently in monopolin mutants.

Because Csm1-GFP colocalizes with the nucleolus, especially in unbudded G1 cells (Figure 1B), we next asked whether monopolin or condensin interacts with rDNA sequences. Both Csm1-GFP and Smc4-HA bound to the NTS1 region of rDNA as detected by ChIP of chromatin prepared from unbudded G1 cells (Supplemental Figure S6). This indicates that, at least during the early part of the cell cycle, monopolin and condensin associate with the rDNA repeats. These results are consistent with the idea that monopolin is nucleolar and its role in maintaining rDNA length is conserved from *S. cerevisiae* to *C. albicans*.

We next measured telomere stability by Southern blot with a probe specific to telomere repeat sequences in *C. albicans*. Genomic DNA from two independently constructed *csm1Δ/Δ* strains and a control *CSM1* strain was digested, separated by gel electrophoresis, and analyzed by Southern blot with probes to the *C. albicans* telomere repeat (McEachern and Blackburn, 1994). Telomere length in *C. albicans* differs between chromosomes, such that telomeres from control cells appear as several discrete bands. In *csm1Δ/Δ* deletion strains, telomere length was increased and was also more variable, appearing more heterogeneous both visually (Figure 6B, left) and on densitometry scans of the gel (Figure 6B, right), suggesting altered regulation of recombination at telomeres (Basenko et al., 2011).

In addition to rDNA and telomeres, *C. albicans* has another class of nested repeats, which presents a unique opportunity to examine the role of the monopolin complex at different types of repetitive DNA sequences. One or two MRS tracts of 20–150 kb are present on seven of the eight *C. albicans* chromosomes in derivatives of SC5314, the progenitor of all *C. albicans* strains used in this study (Lephart et al., 2005). This variability in MRS length is derived from changes in

**FIGURE 6**: Monopolin is required for repeat tract length stability. (A) CHEF Southern blot of *Kpn*I-digested genomic DNA plugs prepared from control and *csm1Δ/Δ* deletion strains was probed with a DIG probe targeting *RDN18*. (B) Southern blot of *Nla*III- and *Alu*I-digested genomic DNA prepared from control and *csm1Δ/Δ* deletion strains was probed with a DIG probe targeting telomeric repeat sequences. The intensity pattern of each lane from top to bottom was quantified (right). (C) CHEF Southern blot of *Xho*I-digested genomic DNA plugs prepared from control and *csm1Δ/Δ* deletion strains was probed with a DIG probe targeting chromosome-specific regions flanking the MRS on ChrR, Chr4, and Chr5.
the copy number of nested repeats within the MRS. Changes in MRS tract length, including the appearance of heterogeneity in MRS length between homologous chromosomes, as well as homoygosis of the two alleles, have been detected occasionally in wild-type strains (Chibana and Magee, 2009). We analyzed MRS stability by following the ChrR, Chr4, and Chr5 MRS tracts using CHEF Southern blots and probes specific to the unique sequences that flank each MRS tract over ~100 generations of successive culturing. Of interest, csm1Δ/Δ monopolin-deletion strains had an increased frequency of repeat tract length changes on at least one of the six alleles (22 of 48 samples) compared with control cells (4 of 42 samples; Figure 6C). Thus monopolin affects the stability of repetitive tract lengths at the rDNA repeats, at the telomeres, and also at the internal, long tandem MRS repeats. Taken together, these results imply that monopolin is important for the maintenance of all types of repetitive DNA and is not specific to only rDNA or telomere repeats.

**Monopolin has a more dramatic effect on the segregation of chromosomes carrying rDNA repeats**

We considered the possibility that excess repeat tract recombination caused lagging chromosomes due to failure to resolve the recombination junctions. To ask whether, in monopolin mutants, the mechanisms that cause increased repeat tract recombination also impair chromosome segregation, we measured marker loss rates using URA3 inserted on ChrR, which contains all of the rDNA repeats in the *C. albicans* genome. Of note, the csm1Δ/Δ strain exhibited a 27-fold increase in the loss rate (5-FOA<sup>+</sup>) relative to control strains when URA3 had been inserted on ChrR (Figure 7A). This is in contrast to the fourfold increase in the loss of GAL1 on Chr1 (non-rDNA-containing chromosome) in a csm1Δ/Δ strain relative to control CSM1 strains (Figure 7A). The increase in marker loss on ChrR was primarily due to recombination rather than whole-chromosome loss, as measured by SNP-RFLP analysis: only 1 of 55 (2%) of 5-FOA<sup>+</sup> isolates had undergone whole-chromosome loss of heterozygosity.

Furthermore, the increased loss of heterozygosity of ChrR (containing the rDNA repeat tracts) in a csm1Δ/Δ strain was suppressed approximately sixfold in a strain overexpressing condensin (Figure 7B). Therefore it appears that the role of monopolin in suppressing excessive recombination involves cooperation with, or recruitment of, condensin. Of interest, we also observed defects in nucleolar segregation in csm1Δ/Δ strains (Supplemental Figure S7), suggesting that the cell cycle defects observed in monopolin-deletion strains may be due to difficulty in resolving rDNA recombination.

To ask whether marker loss rates were increased for the chromosome carrying the rDNA in other yeasts, we inserted URA3 into intergenic regions on *S. cerevisiae* ChrXII (containing the rDNA repeat tract) and ChrX (non-rDNA-containing) and compared the loss rates in the two strains. The ChrXII URA3 loss rates were 10-fold higher in csm1Δ/Δ strains than in control strains, whereas the ChrX URA3 loss rates were similar in csm1Δ/Δ and control CSM1 strains (Supplemental Figure S8). Consistent with these observations, a large-scale screen for loss of heterozygosity in diploid *S. cerevisiae* also detected increased rates of marker loss on ChrXII (containing the rDNA repeat tract) in monopolin-deletion strains, whereas marker loss rates on other chromosomes were very similar to those in controls. Approximately 50% of the increase in marker loss on ChrXII was due to reciprocal recombination (Andersen et al., 2008). Thus, as in *C. albicans*, monopolin reduces the frequency of recombination specifically on the rDNA-containing chromosome in *S. cerevisiae*.

**DISCUSSION**

The monopolin complex structure suggests that it may function as a molecular clamp to cross-link kinetochore components and rDNA repeats (Corbett et al., 2010). An alternative model however, posits that the primary function of monopolin is to recruit condensin (Tada et al., 2011). Here we tested the effect of increasing the number of kinetochore–microtubule attachments on the requirement for the monopolin core complex in mitosis. We found that monopolin mutants have a strong phenotype even during mitosis with a single kinetochore–microtubule attachment and that increasing kinetochore...
Monopolin promotes DNA organization

Figure 8: Monopolin organizes DNA at centromeres and repetitive sequences. (A) At centromeres, monopolin (dark green) functions through the recruitment of condensin (purple) to organize centromere DNA. (B) Monopolin functions at repetitive DNA sites throughout the C. albicans genome, including rDNA repeats, telomere repeats, and the major repeat sequence, to maintain repeat stability.

Copy number does not alter the requirement for the monopolin complex (Figure 2). Deletion of monopolin decreased apparent chromosome stiffness during mitosis (Figure 3) and reduced the recruitment of condensin to centromere regions (Figure 4). These results support a model in which monopolin recruits condensin to organize centromere DNA into the appropriate higher-order structure, irrespective of the number of kinetochore–microtubule attachments (Figure 8A). In addition, monopolin is required for the stability of different types of repetitive DNA sequences by preventing high levels of recombination (Figure 8B).

In other yeasts, monopolin complex members interact physically with condensin in two-hybrid assays (Wysocka et al., 2004; Johzuka and Horiuchi, 2009; Tada et al., 2011). Furthermore, overexpression of a single condensin subunit bypassed chromosome segregation defects associated with knockdown of the telomere-associated Ccq1 protein, a protein that cooperates with condensin at S. cerevisiae telomeres (Motwani et al., 2010). On the basis of the reduced amount of condensin at centromere DNA in csm1ΔΔ mutants (Figure 4), we infer that monopolin is required to recruit condensin in C. albicans. Of importance, overexpression of C. albicans condensin also suppressed the chromosome segregation and cell cycle progression defects associated with loss of monopolin (Figure 5). Taken together, the genetic and phenotypic results here are consistent with the idea that monopolin recruits condensin to the centromere to maintain proper chromosome segregation dynamics.

Condensin proteins are components of the chromatin spring necessary for chromosome segregation (Stephens et al., 2011) and are hypothesized to contribute to the stiffness of the chromatin spring (Haase et al., 2012; Stephens et al., 2013). Increased distance between centromeres during metaphase is believed to reflect reduced stiffness of centromeric chromatin or loss of sister centromere cohesion (Goshima et al., 1999). This occurs in csm1ΔΔ strains (Figure 3) and is consistent with the elongation of S. pombe spindles in monopolin mutants at the metaphase-to-anaphase transition (Choi et al., 2009). Reduced stiffness correlates with decreased chromosome segregation accuracy. This is believed to be due to reduced structural integrity at the centromere, which in turn can arise from multiple types of defects. For example, reduced stiffness could cause defects in the positioning of multiple centromeric nucleosomes, kinetochore assembly, or the efficiency of checkpoint or error correction proteins, such as Aurora B.

The increased mitotic requirement for the monopolin complex in S. pombe compared with S. cerevisiae was proposed to be due to the presence of more than one kinetochore–microtubule attachment/centromere (Gregan et al., 2007; Brito et al., 2010a). This model, however, is inconsistent with the data we obtained in C. albicans mitosis. Although we cannot exclude the possibility that monopolin functions as a clamp in some situations, such as meiosis (which has not been observed to occur in C. albicans; Butler et al., 2009), our data are more consistent with a model in which monopolin organizes DNA at centromeres and repetitive DNA by recruiting condensin.

We posit that the increased requirement for monopolin during mitosis in C. albicans and S. pombe relative to S. cerevisiae is due to the need for correct positioning of multiple centromeric nucleosomes within the centromeric core and/or organization of longer centromere DNA sequences, perhaps including their flanking repeats. Consistent with this, the severity of the mitotic phenotype in monopolin mutants increases with increased centromere DNA length: S. cerevisiae has short (~<200 base pair) point centromeres, C. albicans has regional centromeres of intermediate length (~3- to 5-kb core), and S. pombe has longer regional centromeres (~10-kb core). Although the exact number of Cse4/CENP-A nucleosomes per centromere remains controversial (Collman et al., 2011; Lawrimore et al., 2011; Henikoff and Henikoff, 2012; Aravamudhan et al., 2013), the idea that there are more CENP-A nucleosomes per centromere in C. albicans and S. pombe than in S. cerevisiae is quite clear (Joglekar et al., 2008). Another difference between centromeres in these three organisms is the degree of repetitive DNA. S. cerevisiae centromeres are located within nonrepetitive DNA, whereas S. pombe centromeres are flanked by long tracts of tandem repeats (up to ~45 kb/arm; Allshire and Karpen, 2008). C. albicans centromeres again have an intermediate structure: they are flanked by short (120 base pairs to 2.2 kb) direct and/or inverted repeats (Supplemental Figure S7). Given the role of monopolin at other repeat sequences (Figure 6; Huang et al., 2006; Johzuka and Horiuchi, 2009), its role at centromeres could be to organize repeats into a higher-order chromatin structure necessary for centromere function during mitosis.

Monopolin restrains from a diffuse distribution in G1 cells to a sharp focus that colocalizes with Mtw1 specifically in actively dividing cells suggests that monopolin is actively recruited to kinetochores. This localization pattern is consistent with work in other yeasts. In S. cerevisiae, monopolin localizes to the nucleolus throughout the cell cycle and to kinetochores beginning at S phase and continuing through anaphase (Bachellier-Bassi et al., 2008). In S. pombe, condensin localizes to the nucleus throughout the cell cycle and is enriched in binding to centromeric chromatin during mitosis when Aurora B is active (Tada et al., 2011). We assume that the diffuse nuclear and nucleolar monopolin should be available to bind rDNA, telomeres, and MRS repeats; indeed, monopolin and condensin bind to rDNA repeats by ChIP, although binding levels were lower than at centromeres. Differences in the affinity of monopolin for centromeres (where it forms a bright, focused spot) and rDNA (where binding appears more diffuse) could account for the reduced ChIP signal. Monopolin may bind to only a subset of the repeat sequences, and the ChIP signal may be diluted by unbound
rDNA repeat copies. Consistent with this, in S. pombe monopolin (Pcs1/Mde4) bound more tightly to centromere DNA than to nucleolar chromatin (Gregan et al., 2007). Thus, the localization of monopolin and condensin is cell cycle regulated to promote accurate chromosome segregation and maintenance of repetitive sequences.

The monopolin core complex is critical for maintenance of the length of rDNA repeats, telomeres, and the MRS. In S. cerevisiae, the monopolin core complex is important for rDNA stability and telomere maintenance (Huang et al., 2006; Mekhail et al., 2008; Chan et al., 2011). Our observation that deletion of the monopolin complex also resulted in repetitive DNA instability in C. albicans implies that the function of monopolin at repetitive DNA is evolutionarily conserved beyond the Saccharomyces clade. Furthermore, the role of monopolin in maintenance of the MRS, a repetitive sequence not found in C. albicans, supports the idea that monopolin has a general role at repetitive DNA sequences and not just at rDNA and telomeres.

We found that the monopolin organization of repetitive DNA is important to prevent recombination. Marker loss rates were significantly higher for markers on the rDNA-containing chromosome in C. albicans (Figure 7) and also in S. cerevisiae (Supplemental Figure S8; Andersen et al., 2008) than for markers on other chromosomes, and in both organisms, the marker loss rate was due to increased recombination rather than chromosome loss. Nonetheless, defects in nucleolar segregation were also evident in the csm1Δ/Δ mutant, suggesting that segregation of rDNA and the rDNA-containing chromosome was also impaired (Supplemental Figure S7). This differs from what was seen in S. pombe, in which all three chromosomes are approximately equally likely to lag when monopolin is disrupted (Gregan et al., 2007). Condensin mutants also do not show rDNA-specific chromosome segregation defects in S. pombe (Tada et al., 2011) or vertebrate cells (Vagnarelli et al., 2006). The much longer repeat regions flanking each S. pombe centromere may have a more dramatic effect on segregation of each chromosome, thus reducing the relative effect of rDNA seen in S. cerevisiae and C. albicans. Furthermore, we were unable to detect significant changes in rDNA length in S. pombe Δpcs1 and Δmde4 strains. One possibility is that differences in chromatin structure, such as the presence of canonical heterochromatin, reduce the requirement for the monopolin complex in the maintenance of rDNA repeats in S. pombe.

In C. albicans, recombination at the very long (~2 Mb) rDNA repeats impaired chromosome segregation, whereas recombination at the shorter (~50–100 kb) MRS sequences did not have a detectable chromosome segregation effect. To ask whether the MRS affected chromosome loss in a monopolin-dependent manner, we compared loss rates for Chr1 (a chromosome that contains an MRS) and Chr3 (a chromosome that does not contain an MRS) in conditional CSM1 depletion strains and controls. Monopolin depletion resulted in segregation defects for both chromosomes, and the severity of the defect was similar for the MRS-containing and the non-MRS-containing chromosome (Supplemental Figure S1). This result supports the idea that monopolin is important for kinetochore function and that the role of monopolin in maintenance of shorter repeat sequences, such as the MRS and telomeres, does not make a major contribution to chromosome stability.

Taken together, our results support a model in which monopolin organizes centromeric DNA to promote accurate chromosome segregation and proper centromere stiffness via the recruitment of condensin to regional centromeres regardless of the number of kinetochore–microtubule attachments (Figure 8A). In addition, monopolin is required for the maintenance of repetitive DNA sequences in C. albicans (Figure 8B) by preventing excess recombination, and this has implications for genome stability as well.

MATERIALS AND METHODS

Strain construction

Strains are listed in Supplemental Table S1. Lithium acetate transformation of linearized plasmids or PCR products with at least 70 base pairs of homology to the targeted gene was used for strain construction. Briefly, strains to be transformed were inoculated in YPA (yeast extract–peptone–adenine)–glucose and grown at 30°C for 16–18 h. Cultures were then diluted 1:166 in YPA-glucose and grown at 30°C for 3–4 h. Cells were washed with water, and then TELiAc (10 mM Tris, pH 7.5, 1 mM EDTA, 100 mM lithium acetate [LiAc]) and incubated in TELiAc with transformation DNA and 50 μg of sheared salmon sperm DNA (Ambion, Austin, TX) for 30 min. Four volumes of PLATE mix (40% polyethylene glycol, 10 mM Tris, pH 7.5, 1 mM EDTA, 100 mM LiAc) were then added, and the transformation mix was incubated for 16–18 h at 20–24°C. Transformations were heat shocked at 42°C for 1 h and then plated on selective media, with the exception of NAT1 marker transformations, which were recovered on nonselective media for 6 h before replica plating to selective media containing 400 μg/ml nourseothricin (Werner BioAgents, Jena, Germany). Strains were checked by PCR of genomic DNA.

For the construction of the UAU knockouts of CSM1 the URA3- ARG4-URA3 (UAU) construct was amplified via PCR from plasmid pBME101 (Enloe et al., 2000) using primers with flanking 5′ and 3′ ends containing 70–base pair homology to the targeted genes of interest. Insertion of the UAU cassette at the correct location was confirmed by PCR. Recombination events resulting in Arg+Ura+ cells were selected for by streaking colonies of confirmed transformants onto SDC (synthetic dextrose complete)-Arg-Ura. Recombination and absence of native CSM1 gene were verified with PCR and by Southern blot. To construct the PCK1p-CSE4 strains, either the URA3-PCK1p-CSE4 plasmid (Sanyak and Carbon, 2002) was linearized with EcoRV or the HIS1-PCK1p-CSE4 plasmid was linearized with HindIII. Integration into the CSE4 upstream region was confirmed by PCR. Nop1-GFP, Csm1-GFP, Mtw1-GFP, and Tub1-GFP strains were tagged at the C-terminus with PCR-mediated transformation. Primers containing at least 70–base pair homology to the targeted gene were used to amplify the GFP and marker from the C-terminal GFP-tagging plasmids with PCR from plasmid pBME101 (Enloe et al., 2000) using primers with flanking 5′ and 3′ ends containing 70–base pair homology to the targeted genes of interest. Insertion of the UAU cassette at the correct location was confirmed by PCR. Recombination and absence of native CSM1 gene were verified with PCR and by Southern blot. To construct the PCK1p-CSE4 strains, either the URA3-PCK1p-CSE4 plasmid (Sanyak and Carbon, 2002) was linearized with EcoRV or the HIS1-PCK1p-CSE4 plasmid was linearized with HindIII. Integration into the CSE4 upstream region was confirmed by PCR. Nop1-GFP, Csm1-GFP, Mtw1-GFP, and Tub1-GFP strains were tagged at the C-terminus with PCR-mediated transformation. Primers containing at least 70–base pair homology to the targeted gene were used to amplify the mCherry and marker from the C-terminal mCherry-tagging plasmids (Gerami-Nejad et al., 2009). For MET3p conditional CSM1 strains, one copy of CSM1 was deleted by amplifying HIS1 with primers containing homology to the regions immediately 5′ and 3′ to the gene of interest. The other copy of CSM1 was placed under control of the MET3p by amplifying the NAT1 marker and MET3p region only from a NAT1-MET3p-GFP plasmid (similar to the URA3-MET3p-GFP plasmid described in Gerami-Nejad et al., 2004). For the MET3p conditional SMC4 overexpression strains, one copy of SMC4 was left intact and the other copy was placed under control of the MET3p by amplifying the NAT1 marker and MET3p region only from a NAT1-MET3p-GFP plasmid.
To construct TetO-CEN7/TetR-GFP strains, TetR under the control of the SNU114 promoter was fused with GFP into the plasmid pD153 (Gerami-Nejad et al., 2013) by in vivo recombination in S. cerevisiae. The plasmid was recovered, digested with SfiI to isolate the targeting sequences and insert, and transformed into strain SN76. Insertion of the TetR-GFP-NAT1 cassette into the intergenic region between ORF19.1963 and ORF19.1961 in the C. albicans genome was confirmed by PCR. URA3 and surrounding plasmid sequences were inserted adjacent to CEN7. pSR8 (Rohner et al., 2008) was modified for use in C. albicans by swapping out the S. cerevisiae HIS3 gene and replacing it with C. albicans HIS1. This plasmid was linearized with Ascl and transformed into the URA3-CEN7/TetR-GFP strain. Transformants were selected on media lacking histidine and then replica plated to media lacking uridine to confirm loss of the URA3 marker flankered by the targeting sequences. Insertions were also confirmed by PCR. Approximately 3 kb of TetO sequences were inserted adjacent to CEN7. CSM1 deletion by UAU was performed in this strain as described.

S. cerevisiae strains BY4743 and csm1Δ/Δ were obtained from the S. cerevisiae diploid deletion collection (www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html). URA3 was targeted to intergenic regions on ChrX and ChrXII by PCR-mediated transformation. Primers containing at least 40–base pair homology to the targeted region were used to amplify the URA3 marker from pRS316 (Sikorski and Hieter, 1989).

Microscopy

For localization studies, strains were inoculated into SDC-glucose and grown at 30°C for 16–18 h. Cultures were then washed and diluted 1:100 into SDC-glucose. For strains with PCK1 conditional promoters, cultures were washed and diluted into repressing conditions (SDC-glucose) and activating conditions (SDC-succinate) and grown at 30°C for 6 h. For conditional MET3 strains, cultures were washed and diluted 1:50 into SDC-glucose without methionine and cysteine (-Met-Cys) or SDC-glucose with added methionine and cysteine (+Met+Cys) and grown at 30°C for 6 h. DNA was stained with 15 μg/ml DAPI in phosphate-buffered saline (PBS) for 10 min before imaging. Cells were imaged with a Nikon Plan Apo 100×/1.4 numerical aperture (NA) objective on a Nikon E600 microscope (Nikon, Melville, NY) with differential interference contrast, DAPI, GFP, and Texas red filter sets as appropriate, using constant exposure times and scaling for each image. Images were acquired using MetaMorph software (Molecular Devices, Sunnyvale, CA). Quantification of Mtw1-GFP and Tub1-GFP in csm1Δ/Δ strains was performed as previously described (Burrack et al., 2011).

To compare centromere separation distances during metaphase between wild-type and csm1Δ/Δ cells, we imaged TetO-CEN7/TetR-GFP cells using a Nikon Eclipse Ti microscope equipped with a Nikon CFI Apochromat 100×/1.49 NA oil objective and an iXon3 EMCCD camera (Andor, Belfast, United Kingdom). Log-phase cells were adhered to concanavalin-coated glass coverslips and imaged in SDC media at 30°C. We chose metaphase cells by including only the distance between centromeres, using a custom Matlab script, with nanometer precision by taking advantage of the fact that the location of a fluorescent particle can be precisely determined by fitting a Gaussian distribution to the captured point-spread function of the particle (Jaqaman et al., 2008). To conduct this fitting in each image, we rotated the image so that the centromeres were aligned on the horizontal axis. Next fluorescence was integrated in a 15-pixel column for each location along the horizontal vector on which the centromeres were aligned. The resulting one-dimensional vector of fluorescence intensities were fitted with a two-mixture Gaussian mixture model using the Matlab function gmdistribution.fit(), which returned the locations of each centromere. We compared the distances between centromeres in wild-type and csm1Δ/Δ metaphase cells using a Welch’s two-sample, two-tailed t-test conducted in R.

ChIP of Cse4, Csm1-GFP, and Smc4-HA

ChIP was performed essentially as described in Ketel et al. (2009), with the exception that an additional fixation step using 10 mM dimethyl adipimidate (Pierce Biotechnology, Rockford, IL) in PBS for 45 min was added before fixation in 1% formaldehyde. ChIP was performed using rabbit anti-Cse4 (CaCENP-A) antibodies (Ketel et al., 2009), mouse anti-GFP antibodies (Roche Applied Science, Indianapolis, IN), and mouse anti-HA antibodies. DNA pull-down efficiency was measured by quantitative PCR using the Universal Probe Library (Roche Applied Science) with a LightCycler 480 PCR machine (Roche Applied Science) according to the manufacturer’s instructions. Enrichment was calculated as relative quantification of (+Ab/Input) – (−Ab/Input) using the second-derivative maximum to determine C values and corrections for primer efficiency values with the LightCycler 480 software (Roche Applied Science).

Southern blot

To confirm the loss of CSM1 from csm1Δ/Δ UAU deletion strains, genomic DNA was digested with SpeI for 18–20 h at 37°C. The digested genomic DNA was run on a 0.8% agarose gel, transferred to a nylon membrane (GE Osmonics, Minnetonka, MN), and probed with digoxigenin (DIG) probes binding the wild-type copy of CSM1 as described in Ketel et al. (2009).

We measured rDNA length, MRS length, and telomere length by inco culturing in 2 ml of YPA-glucose with strains directly cultured from storage at −80°C and with strains passaged on YPA-glucose every day for 10 d. The cultures were grown for 16–18 h at 30°C.

To measure rDNA length and MRS length, we prepared plugs for CHEF gel electrophoresis. A total of 0.5 ml of a stationary-phase culture in YPA-glucose was harvested and washed once with 1 ml of 50 mM EDTA, pH 8.0, and resuspended in 20 μl of EDTA containing 1% low-melting-point agarose every day for 10 d. The cultures were grown for 16–18 h at 30°C.

To confirm Cse4, Csm1-GFP, and Smc4-HA ChIP homogeneity, the conditions for separation were as follows: 1% Megabase agarose gel, 3–8 s, 6 V/cm, 120° included angle for 14 h, and then 8–30 s, 5 V/cm, 120° included angle for 6 h. DNA was transferred to a nylon membrane (GE Osmonics) and probed with DIG probes binding RDN18 (rDNA) and chromosome-specific regions flanking MRS DNA (MRS), and hybridized as described in Ketel et al. (2009).

To measure telomere length, we prepared genomic DNA and digested the DNA with Nial1 and Alu1 for 2 h at 37°C (Hsu et al.,
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REFERENCES


