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The Monopolin Complex Crosslinks Kinetochore Components to Regulate Chromosome-Microtubule Attachments

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SUMMARY

The monopolin complex regulates different types of kinetochore-microtubule attachments in fungi, ensuring sister chromatid co-orientation in *Saccharomyces cerevisiae* meiosis I and inhibiting merotelic attachment in *Schizosaccharomyces pombe* mitosis. In addition, the monopolin complex maintains the integrity and silencing of ribosomal DNA (rDNA) repeats in the nucleolus. We show here that the *S. cerevisiae* Csm1/Lrs4 monopolin subcomplex has a distinctive V-shaped structure, with two pairs of protein-protein interaction domains positioned ~10 nm apart. Csm1 presents a conserved hydrophobic surface patch that binds two kinetochore proteins: Dsn1, a subunit of the outer-kinetochore MIND/Mis12 complex, and Mif2/CENP-C. Csm1 point-mutations that disrupt kinetochore-subunit binding also disrupt sister chromatid co-orientation in *S. cerevisiae* meiosis I. We further show that the same Csm1 point-mutations affect rDNA silencing, probably by disrupting binding to the rDNA-associated protein Tof2. We propose that Csm1/Lrs4 functions as a molecular clamp, crosslinking kinetochore components to enforce sister chromatid co-orientation in *S. cerevisiae* meiosis I and to suppress merotelic attachment in *S. pombe* mitosis, and crosslinking rDNA repeats to aid rDNA silencing.

INTRODUCTION

Mitosis and meiosis are related processes in which duplicated eukaryotic chromosomes segregate to daughter cells or gametes (Lee and Amon, 2001; Marston and Amon, 2004; Nasmyth, 2001). In mitosis, chromosomes replicate and the resulting sister-chromatid pairs are held together along their length by cohesin complexes. Associated with the centromere of each chromatid is a kinetochore, a specialized protein assembly that captures microtubules (MTs) of the mitotic spindle. In early mitosis, each sister chromatid pair becomes "bi-oriented" when its kinetochores capture MTs extending from opposite spindle poles. Once all chromatid pairs are properly attached, cleavage of the cohesin links between sisters allows chromosome segregation and subsequent cell division.

DNA replication and cell division strictly alternate in mitosis, but in meiosis, DNA replication is followed by two successive divisions to yield four haploid gametes. During meiotic prophase, homologous chromosomes align and form crossovers that hold them together. This organization allows for bi-orientation and segregation of homologs in meiosis I, followed by segregation of sister chromatids in meiosis II (Lee and Amon, 2001; Marston and Amon, 2004; Nasmyth, 2001). Thus, although sister chromatids bi-orient and segregate from each other in mitosis and meiosis II, they instead co-orient and segregate together in meiosis I.

In the budding yeast *Saccharomyces cerevisiae*, sister chromatid co-orientation in meiosis I depends on the four-protein monopolin complex (Mam1, Csm1, Lrs4, and Hrr25/casein kinase 1), which localizes to centromeres from meiotic prophase through metaphase I (Monje-Casas et al., 2007; Petronczki et al., 2006; Rabitsch et al., 2003; Toth et al., 2000). Mam1 is expressed specifically in meiosis (Toth et al., 2000) and associates at centromeres with the ubiquitous kinase Hrr25 (Petronczki et al., 2006). The remaining subunits, Csm1 and Lrs4, form a complex that resides in the nucleolus during interphase and relocalizes to centromeres during meiotic prophase, accompanied by phosphorylation of Lrs4 (Huang et al., 2006; Katis et al., 2004; Lo et al., 2008; Matos et al., 2008; Rabitsch et al., 2003). Robust centromeric localization of Csm1/Lrs4 requires Mam1 (Rabitsch et al., 2003). It has been proposed that the monopolin complex crosslinks and/or co-orients sister kinetochores in meiosis I, so that they attach to MTs extending from the same spindle pole (Monje-Casas et al., 2007). Although monopolin complex subunits have not been identified outside of fungi, the concept of sister kinetochore "fusion" in meiosis I may have parallels in higher eukaryotes: in maize meiosis I, for example, inner kinetochores of sister chromatids can be

Table 1. Molecular Mass Determinations from Sedimentation Equilibrium Centrifugation

Protein/Complex	Observed MW (kDa)	Calculated MW (kDa)[a]	Oligomeric State
Csm1 full-length	43.2 ± 0.9	43.5	2 Csm1
Csm1 1–181	43.4 ± 1.2	41.2	2 Csm1
Csm1 69–190	29.1 ± 2.3	28.2	2 Csm1
Pcs1 full-length	53.4 ± 1.7	51.8	2 Pcs1
Pcs1 85–222	30.1 ± 2.3	32.2	2 Pcs1
Csm1/Lrs4 full-length	172.3 ± 4.7	165.6	4 Csm1 + 2 Lrs4
Csm1/Lrs4 1–130	120.4 ± 3.1	117.6	4 Csm1 + 2 Lrs4
Csm1/Lrs4 1–102	108.5 ± 3.0	111.0	4 Csm1 + 2 Lrs4
Csm1 1-181/Lrs4 2–30	91.3 ± 3.1	89.7	4 Csm1 + 2 Lrs4
Pcs1/Mde4 full-length	164.2 ± 7.8	198.9	4 Pcs1 + 2 Mde4
Pcs1/Mde4 1–231	160.2 ± 3.6	156.9	4 Pcs1 + 2 Mde4
Pcs1/Mde4 1–125	127.0 ± 2.7	132.5	4 Pcs1 + 2 Mde4
Pcs1/Mde4 1–77	108.2 ± 4.1	121.0	4 Pcs1 + 2 Mde4

[a] Calculated MW is the expected molecular mass of a complex with the stoichiometry listed in "Oligomeric State."

resolved by fluorescence microscopy, whereas their outer kinetochores appear fused (Li and Dawe, 2009).

Orthologs of the monopolin subunits Csm1 and Lrs4 are present in the fission yeast *Schizosaccharomyces pombe* (Pcs1 and Mde4, respectively) and also cycle between the nucleolus and kinetochores (Gregan et al., 2007; Rabitsch et al., 2003). These proteins inhibit merotelic attachment (capture of a single kinetochore by MTs from opposite spindle poles) during mitosis, but they do not contribute to sister chromatid co-orientation in meiosis I. (An unrelated protein, Moa1, is important for ensuring meiosis I sister co-orientation in *S. pombe*, probably by modifying cohesin-complex function near centromeres [Yokobayashi and Watanabe, 2005]). Although *S. cerevisiae* kinetochores capture a single MT, *S. pombe* and higher eukaryotes assemble larger kinetochores that capture multiple MTs (2–4 in *S. pombe* [Ding et al., 1993] and 15–30 in metazoans [McEwen et al., 1997]). In this context, Pcs1 and Mde4 have been proposed to organize *S. pombe* kinetochores by clamping together adjacent MT-binding sites (Gregan et al., 2007). In addition, Pcs1 and Mde4 have recently been shown to localize to the mitotic spindle in anaphase, revealing another potential function for monopolin in anaphase spindle elongation and stability (Choi et al., 2009).

During interphase, *S. cerevisiae* Csm1/Lrs4 and *S. pombe* Pcs1/Mde4 are both in the nucleolus, where they have been shown in *S. cerevisiae* to be important for maintaining the ribosomal DNA (rDNA) (Gregan et al., 2007; Huang et al., 2006; Mekhail et al., 2008). The repetitive rDNA array (100–200 copies of a 9.1-kb repeat in *S. cerevisiae*) is normally kept in a silenced, heterochromatin-like state by a network of rDNA-associated proteins, including Fob1, Tof2, Csm1/Lrs4, and the RENT complex (Regulator of nucleolar silencing and telophase exit), which contains Net1/Cfi1, Cdc14, and the Sir2 histone deacetylase (Huang et al., 2006; Huang and Moazed, 2003). The rDNA is also protected from unequal sister chromatid exchange (USCE), which can lead to addition or deletion of repeats within the rDNA (Sinclair and Guarente, 1997). USCE is suppressed by Csm1/Lrs4 (Huang et al., 2006), the inner-nuclear membrane proteins Heh1 and Nur1 (Mekhail et al., 2008), and the condensin complex (Johzuka and Horiuchi, 2009), in addition to Sir2 (Huang et al., 2006; Smith and Boeke, 1997; Smith et al., 1999). With the exception of Sir2, which independently contributes to USCE suppression, these proteins appear to tether rDNA repeats to the nuclear periphery, sequestering them from recombination factors (Mekhail et al., 2008), and they may also clamp sister chromatids together in register (Brito et al., 2010; Johzuka and Horiuchi, 2009). Thus, although Csm1/Lrs4 contributes to both rDNA silencing and USCE suppression, it acts with distinct sets of proteins in these different processes, raising the question of whether a common mechanism underlies these activities.

The regulatory functions of monopolin described above suggest that Csm1/Lrs4 and the orthologous Pcs1/Mde4 are molecular crosslinkers, joining MT-binding elements at kinetochores and rDNA repeats in the nucleolus. We report here that *S. cerevisiae* Csm1 and Lrs4 form a complex with a distinctive "V" shape, which positions two pairs of protein-protein interaction domains ~10 nm apart. We find that a conserved surface patch on these domains binds two kinetochore subunits: Dsn1, a subunit of the outer-kinetochore MIND/Mis12 complex, and Mif2/CENP-C. Point-mutations in this conserved surface disrupt both Dsn1 and Mif2 binding in vitro and cause bi-orientation of sister chromatids in meiosis I. These data are consistent with a model of monopolin as a crosslinker that clamps kinetochores together to enforce co-orientation in *S. cerevisiae* meiosis I and inhibit merotelic attachment in *S. pombe* mitosis. We also find that Csm1 interacts with the nucleolar protein Tof2 through the same conserved surface that interacts with Dsn1 and Mif2, and that mutating the Csm1 surface patch also disrupts rDNA silencing. These mutations do not, however, affect the rate of unequal sister chromatid exchange, demonstrating that Csm1/Lrs4 has two biochemically separate roles in the maintenance of rDNA. Overall, our data show that Csm1/Lrs4 is a molecular crosslinker that regulates kinetochore-microtubule attachment and helps preserve rDNA integrity.

RESULTS

Structure of Csm1

We purified full-length *S. cerevisiae* Csm1 and *S. pombe* Pcs1 proteins, as well as truncations lacking the bulk of their N-terminal regions, which are predicted to form coiled-coils (Gregan et al., 2007; Rabitsch et al., 2003). By sedimentation equilibrium analytical ultracentrifugation, we found that both constructs of Csm1 and Pcs1 are homodimers in solution (Table 1). We obtained crystals of both full-length *S. cerevisiae* Csm1 and its isolated C-terminal domain (residues 69–181 of 190). We determined the structure of the C-terminal domain to 2.35 Å resolution using anomalous diffraction methods with selenomethionine-derivatized protein (see Table S1, available online, for crystallographic statistics), and we then determined the structure of the full-length protein to 3.4 Å resolution by molecular replacement. The structures show that Csm1 has a 12-nm long, N-terminal coiled-coil (residues 3–82), and a