Molecular Biology of the Cell
Vol. 20, 3818–3827, September 1, 2009

Pericentromeric Sister Chromatid Cohesion Promotes Kinetochore Biorientation

Tessie M. Ng,*† William G. Waples,‡ Brigitte D. Lavoie,‡ and Sue Biggins*

*Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; †Molecular and Cellular Biology Program, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA 98195; and ‡Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada M5S 1A8

Submitted April 27, 2009; Revised June 25, 2009; Accepted July 2, 2009
Monitoring Editor: Kerry S. Bloom

Accurate chromosome segregation depends on sister kinetochores making bioriented attachments to microtubules from opposite poles. An essential regulator of biorientation is the Ipl1/Aurora B protein kinase that destabilizes improper microtubule–kinetochore attachments. To identify additional biorientation pathways, we performed a systematic genetic analysis between the *ipl1-321* allele and all nonessential budding yeast genes. One of the mutants, *mcm21*Δ, precociously separates pericentromeres and this is associated with a defect in the binding of the Scc2 cohesin-loading factor at the centromere. Strikingly, Mcm21 becomes essential for biorientation when Ipl1 function is reduced, and this appears to be related to its role in pericentromeric cohesion. When pericentromeres are artificially tethered, Mcm21 is no longer needed for biorientation despite decreased Ipl1 activity. Taken together, these data reveal a specific role for pericentromeric linkage in ensuring kinetochore biorientation.

INTRODUCTION

The precise regulation of chromosome segregation ensures that each daughter cell receives an entire complement of the genome. During replication, cohesion is established between sister chromatids. Microtubules (MTs) bind to these sister chromatids through kinetochores, the specialized protein complexes that assemble on centromeric DNA. To ensure that sister chromatids segregate away from each other, kinetochores must biorient and attach to MTs originating from opposite poles. If sister kinetochores make syntelic attachments to MTs from the same pole, the spindle checkpoint halts the cell cycle, allowing time for the defect to be corrected.

Although the precise mechanism by which sister kinetochores biorient is not known, tension generated by MT-pulling forces on linked sister chromatids appears to be required (for review, see Pinsky and Biggins, 2005). Micromanipulation experiments demonstrated that artificially applying tension on sister kinetochores both stabilizes and increases the number of MT–kinetochore attachments (Nicklas and Ward, 1994; King and Nicklas, 2000). These observations suggest that syntelic attachments are unstable due to a lack of tension. Consistent with this, a key regulator of biorientation and the spindle checkpoint is the conserved protein kinase Ipl1/Aurora B, which destabilizes improper MT–kinetochore attachments (for review, see Ruchaud *et al.*, 2007).

This article was published online ahead of print in *MBC in Press* (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E09–04–0330) on July 15, 2009.

Address correspondence to: Sue Biggins (sbiggins@fhcrc.org).

Abbreviations used: MT, microtubule; NC-Mcd1, noncleavable Mcd1; SGA, systematic genetic analysis.

Sister chromatid cohesion is also essential for biorientation because it keeps sisters physically associated and allows tension to be generated by MTs that pull on the linked sister kinetochores (Tanaka *et al.*, 2000). When two kinetochores were physically connected on a single DNA molecule, they were able to biorient in an Ipl1-dependent manner (Dewar *et al.*, 2004). These studies led to the conclusion that the critical role of cohesion was to physically link sister chromatids instead of orienting sister kinetochores toward opposite poles as previously suggested. However, these experiments did not eliminate the possibility that cohesion has an additional role in biorientation or that other biorientation pathways exist (for review, see Hauf and Watanabe, 2004).

The bulk of sister chromatid cohesion is mediated by the cohesin complex that consists of four subunits: Smc1, Smc3, Mcd1/Scc1, and Scc3 (for review, see Peters *et al.*, 2008). Cohesin appears to form a ring-like structure that encircles and traps sister chromatids together. Although it is still not mechanistically understood how cohesion is established between sister chromatids, this multistep process is normally coupled to replication and requires cohesin-loading by the Scc2/Scc4 complex (Toth *et al.*, 1999; Ciosk *et al.*, 2000; Lengronne *et al.*, 2006) and acetylation of Smc3 by the Eco1/Ctf7 acetyltransferase during S phase (Michaelis *et al.*, 1997; Skibbens *et al.*, 1999; Toth *et al.*, 1999; Ben-Shahar *et al.*, 2008; Unal *et al.*, 2008; Zhang *et al.*, 2008). Cohesion is then maintained until metaphase when all kinetochores come under tension and biorient. At this time, the anaphase-promoting complex degrades the anaphase inhibitor Pds1/securin, liberating the Separase protease to cleave Mcd1 and release cohesin from DNA.

Cohesin is not randomly distributed throughout the genome. In budding yeast, cohesin complexes are enriched at ~10–15-kb intervals called cohesin-associated regions (CARs) that tend to be A+T rich or occur at sites of convergent transcription (Blat and Kleckner, 1999; Megee *et al.*, 1999; Tanaka *et al.*, 1999; Laloraya *et al.*, 2000; Lengronne

et al., 2004). A conserved feature of cohesin localization is a strong enrichment at pericentromeres that coincides with the large heterochromatic regions that flank the regional centromeres in most organisms (for review, see Grewal and Jia, 2007). Although budding yeast lack pericentromeric heterochromatin, cohesin is enriched in a domain that extends ~50 kb around the 125-base pair point centromere in a kinetochore-dependent manner (Megee *et al.*, 1999; Tanaka *et al.*, 1999; Weber *et al.*, 2004). The enrichment of cohesin around centromeres appears paradoxical because biorientation causes centromeres to split before anaphase (Goshima and Yanagida, 2000; He *et al.*, 2000; Pearson *et al.*, 2001), although cohesin may mediate intramolecular rather than intermolecular interactions in this region (Yeh *et al.*, 2008). Regardless, the enrichment of pericentromeric cohesin appears to ensure the fidelity of chromosome segregation in most organisms. In fission yeast, the major function of pericentromeric heterochromatin in mitotic chromosome segregation is to recruit cohesin (Bernard *et al.*, 2001; Yamagishi *et al.*, 2008). A budding yeast chromosome that lacks pericentromeric cohesion exhibits chromosome segregation defects despite retaining cohesin along the arm (Eckert *et al.*, 2007). Furthermore, budding yeast respond to decreased tension between sister chromatids by recruiting cohesin specifically around pericentromeres (Eckert *et al.*, 2007; Ocampo-Hafalla *et al.*, 2007). Taken together, these data suggest that pericentromeric cohesin may have a specific function in chromosome segregation beyond simply holding sisters together.

We reasoned that there might be pathways that facilitate biorientation whose roles have been masked by the strength of the Ipl1 error correction mechanism. We therefore isolated mutants that are required for viability when the function of the budding yeast Ipl1/Aurora B kinase is reduced. Three of the mutants identified (Mcm16, Mcm21, and Mcm22) encode components of the 12-member CTF19 kinetochore complex that was originally identified in budding yeast (Ortiz *et al.*, 1999; Poddar *et al.*, 1999; Cheeseman *et al.*, 2002). COMA, a four-subunit subcomplex of the larger CTF19 complex that consists of Ctf19, Mcm21, Okp1, and Ame1, was later isolated (De Wulf *et al.*, 2003). Here, we further analyze the function of the conserved Mcm21 protein that is a member of both complexes and find that it is required to enrich cohesin factors at pericentromeres. Strikingly, Mcm21 becomes essential for biorientation when Ipl1 function is reduced, and this appears to be related to its role in pericentromeric cohesion. When pericentromeres are artificially tethered, Mcm21 is no longer needed for biorientation despite decreased Ipl1. Taken together, these data are consistent with a specific requirement for cohesin enrichment at pericentromeres to facilitate kinetochore biorientation.

MATERIALS AND METHODS

Systematic Genetic Analysis Screen

Systematic genetic analysis (SGA) was performed at 23°C, using an array of viable yeast deletion strains in the S288c strain background (*MATa xxxΔ::KAN*) as previously described (Tong *et al.*, 2001; Waples *et al.*, 2008). The query strain YBL165b (*MATα ipl1-321::NAT can1Δ*) was generated by backcrossing the temperature-sensitive (ts) *ipl1-321* allele nine times into the Y2454 parent strain (Tong *et al.*, 2001), followed by integration of a NatR-MX4 cassette immediately downstream of the Ipl1 stop codon. The correct integration was verified by colony PCR and linkage analysis between the ts and NatR resistance genes before performing screens. *Ipl1-321::NatR xxxΔ::KAN* double mutants were systematically generated in quadruplicate, by mating the query strain in parallel to duplicate arrays containing each viable yeast gene deletion. After sporulation on plates, haploid progeny (wild type [WT], single, and double mutant) were selected through two sequential rounds of growth on SD-HIS+CAN plates based on the presence of the *MFA1pr-HIS3* marker. Double mutant (*ipl1-321::NatR xxxΔ::KAN*) progeny were selected under the same media conditions plus antibiotics. Synthetic growth defects were identified by comparing colony sizes between haploid versus double mutant selection plates as well as versus an *YCG1::NatR ΔxxxΔ::KAN* control (in the presence of antibiotics). Under stringent conditions (23°C), ~200 double mutant combinations exhibited slow or no growth phenotypes at least two to four times on the double mutant plates. Of these, 23 interactions were confirmed by standard tetrad dissection and linkage analysis to have a synthetic lethal or sick growth phenotype, and these interactions are reported in this article. Double mutant combinations with poor sporulation efficiencies or low spore viability were not considered further. Genetic interactions between *ipl1-321* and *mcm21*, *ctf8*, *dcc1*, and *bim1* were also confirmed in the w303 strain background but the others have not been analyzed in w303.

Microbial Techniques and Plasmids

Media and microbial techniques were as described (Sherman *et al.*, 1974; Rose *et al.*, 1990). In all synchronous cell cycle experiments reported, α-factor was used to arrest cells in G1 and cell cycle progression was monitored by scoring the budding index. For the relevant experiments, doxycycline (25 μg/ml) and nocodazole (10–15 μg/ml, 2.5–3 h) were added upon G1 release. Checkpoint arrest was confirmed by the presence of a single DNA mass. For the noncleavable-Mcd1 experiment, cells were released into media containing galactose to induce expression. Cells were shifted to 37°C after bud emergence. Yeast strains are listed in Supplemental Table S1.

The *deg-ipl1 plasmid* (pSB244) was constructed by PCR amplification of the *IPL1* ORF using primers SB89 and SB90 with PstI and NotI sites engineered and ligated into pSB230 digested with the same enzymes. The plasmid is integrated at the *ADE2* locus after digestion with AflII. The tetramerizing LacI (pSB1591) was constructed by ligating the EagI/MluI fragment from pAFS55 into pSB116. Primer sequences are available upon request.

Microscopy

Analysis of GFP-LacI was performed as described (Biggins *et al.*, 1999). Indirect immunofluorescence was performed as described (Rose *et al.*, 1990) with antibodies that recognize the myc tag (9E10, Covance, (Princeton, NJ) and Alexa Fluor 488–conjugated anti-green fluorescent protein (GFP; Molecular Probes, Eugene, OR) at a 1:500 dilution. Chromosome spreads were performed as described (Michaelis *et al.*, 1997; Loidl *et al.*, 1998) using the Alexa Fluor GFP antibody (1:1000) and anti-Cse4 (1:250; Pinsky *et al.*, 2003). For all microscopy experiments, more than 200 cells were scored unless otherwise noted. The scale bar in all images equals 5 μm. The Bernoulli distribution was used to assess statistical significance at 95% confidence.

Protein and Immunological Techniques

Protein extracts were made and immunoblotted as described (Minshull *et al.*, 1996). Antibodies that recognize the myc tag (9E10) and the hemagglutinin (HA) tag (12CA5) were obtained from Covance and used at a 1:10000 dilution. Anti-tubulin immunoblotting (Accurate Chemical and Scientific, Westbury, NY) was used at 1:1000 dilution as a loading control.

Chromatin Immunoprecipitation and Kinase Assays

Immunoprecipitations were performed using 3F10 anti-HA antibodies (Roche, Indianapolis, IN) or M2 anti-Flag antibodies (Sigma, St. Louis, MO). Chromatin immunoprecipitation (ChIP) was performed, and samples were quantified as described previously (Collins *et al.*, 2005). Sequences of PCR primers (Eckert *et al.*, 2007) are available upon request. Quantification of bound DNA was calculated as the percentage of total chromatin isolated before immunoprecipitation. ChIP experiments were performed at least three times. The Ipl1 kinase assay was performed as described (Buvelot *et al.*, 2003; Kotwaliwale *et al.*, 2007).

RESULTS

Ipl1-321 SGA Screen Identifies Novel Genetic Interactions

To identify additional biorientation pathways, we analyzed mutants in nonessential genes for interactions with *ipl1-321*, an allele that accumulates syntelic attachments due to decreased kinase activity at the nonpermissive temperature (37°C; Biggins *et al.*, 1999). Ipl1-321 activity is also substantially reduced at lower temperatures, but these cells remain viable due to redundant pathways (Kotwaliwale *et al.*, 2007). We therefore performed an SGA at 23°C by crossing the *ipl1-321* allele to a genome-wide deletion set of all nonessential yeast genes (Tong *et al.*, 2001; Waples *et al.*, 2008). We confirmed 23 genetic interactions with mutants in genes broadly involved in the spindle checkpoint, chromatin structure/genome stability, and the cytoskeleton (Figure 1). Be-

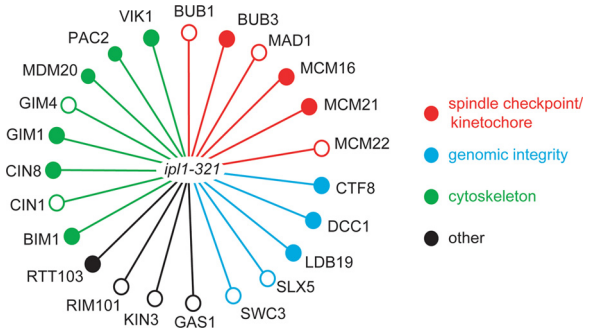

Figure 1. *IPL1* genetic interaction profiles. Synthetic genetic interactions between the hypomorphic allele *ipl1-321*::NAT (YBL165b) and indicated yeast deletion strains. Filled and open circles denote synthetic lethal and sick interactions, respectively.

cause Ipl1 has a number of functions, the genetic interactions could represent a variety of cellular defects. It will therefore be critical to directly study each interaction to understand its requirement for viability when Ipl1 function is reduced.

Mcm21 Has a Role in Pericentromeric Sister Chromatid Cohesion

Because we were interested in pathways that regulate biorientation, we focused on the three kinetochore mutants identified in the screen that all encode components of the CTF19 kinetochore complex (*mcm21*Δ, *mcm16*Δ, and *mcm22*Δ). We began by monitoring the segregation of a pair of sister chromatids in an *mcm21*Δ strain. ChrIV was marked by the binding of a GFP-LacI fusion to Lac operator sequences inserted 12 kb from the centromere (pericentromeric), a locus that remains linked when sisters biorient (Straight *et al.*, 1996). WT and *mcm21*Δ cells were released from G1 and analyzed for sister separation at 20-min intervals after bud emergence (Figure 2A). In both strains, sister separation was first detected 60 min after G1 release. Surprisingly, despite similar kinetics of bud emergence, 23% of *mcm21* cells had separated sisters compared with only 10% of WT cells at this time point. The phenotype was not due to aneuploidy because two GFP foci were only observed in 2% of G1-arrested cells. We performed a similar analysis on *mcm22*Δ cells but found that the sister separation in *mcm22*Δ cells was only slightly increased over WT cells (15 vs. 10%, Figure 2B). Because *mcm21*Δ cells had a larger sister separation defect and a stronger genetic interaction with *ipl1-321*, we decided to further pursue characterization of the *mcm21* mutant strain.

We noticed that the separated sister foci in *mcm21* mutant cells were always closely spaced within the mother cell at the early time points (Supplemental Figure S1), a phenotype that is rarely seen in WT cells. Because these data suggested that the cohesion defect may be specific to pericentromeres, we monitored sister separation at various loci on ChrIV and ChrV. To eliminate potential differences in MT-pulling forces and cell cycle progression, cells were released from G1 into nocodazole to depolymerize the MTs and arrest cells in metaphase. Although there was a significant increase in separated GFP foci in *mcm21* cells at the ChrIV pericentromeric locus, there was no detectable defect at the telomere (Figure 2C, left). The lower percentage of separation in the *mcm21* cells observed in this experiment was presumably due to the lack of MT-pulling forces that enhance the ability to resolve separated pericentromeres. The sister separation defect was also observed with a GFP mark 13 kb from the centromere on ChrV that decreased as it was moved further away to 18 and 35 kb (Figure 2C, right). In all of these experiments, the percentage of cells with separated foci during the G1 arrest never exceeded 3%. Taken together, these data strongly suggest that there is a cohesion defect specific to all pericentromeres in *mcm21*Δ cells.

To test whether the pericentromeric sister separation in *mcm21* cells was due to the premature initiation of anaphase, we performed immunofluorescence microscopy to localize GFP-LacI and the Pds1 protein that is degraded at anaphase onset. WT and *mcm21*Δ cells were quantified 100 min after release from G1 for Pds1 staining and sister separation (Fig-

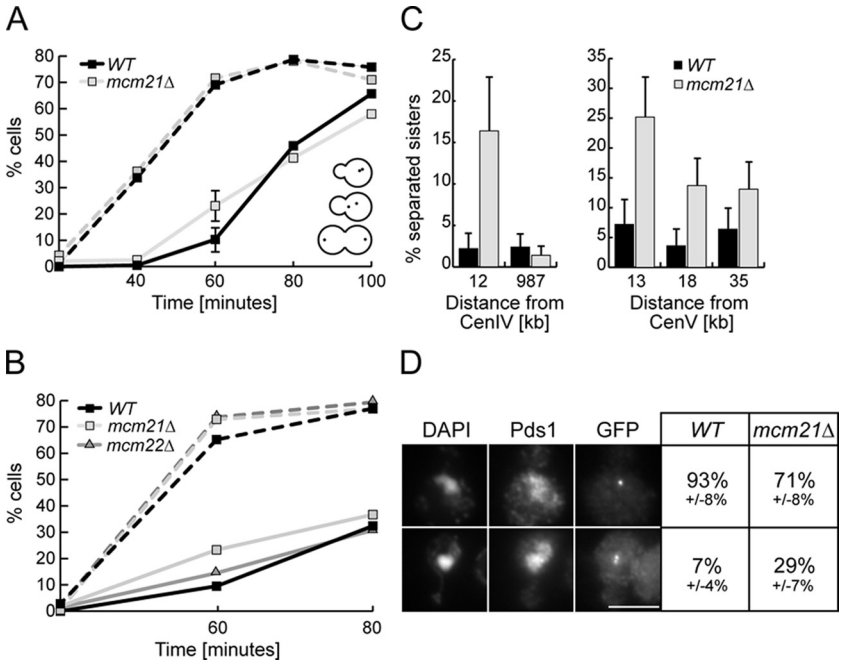

Figure 2. Mcm21 is required for pericentromeric cohesion. (A) Total sister chromatid separation (includes all categories shown in representative depictions of cells) was monitored during a synchronous cell cycle after release from G1 in WT and *mcm21*Δ cells (SBY818, SBY1897) at a ChrIV pericentromeric locus. Dotted lines indicate percent of budded cells. (B) Total separation of the ChrIV pericentromere was monitored in WT, *mcm21*Δ, and *mcm22*Δ cells (SBY818, SBY1897, SBY1983) after release from G1. Budding index shown as dotted lines. (C) Sister separation was monitored in nocodazole-arrested cells at various loci on ChrIV and ChrV (SBY818, SBY1897, SBY6133, SBY6134, SBY7876, SBY7877, SBY7878, SBY7879, SBY7880, and SBY7881). (D) Immunofluorescence microscopy was performed on strains in A. Cells shown are 100 min after G1 release ($n > 100$).

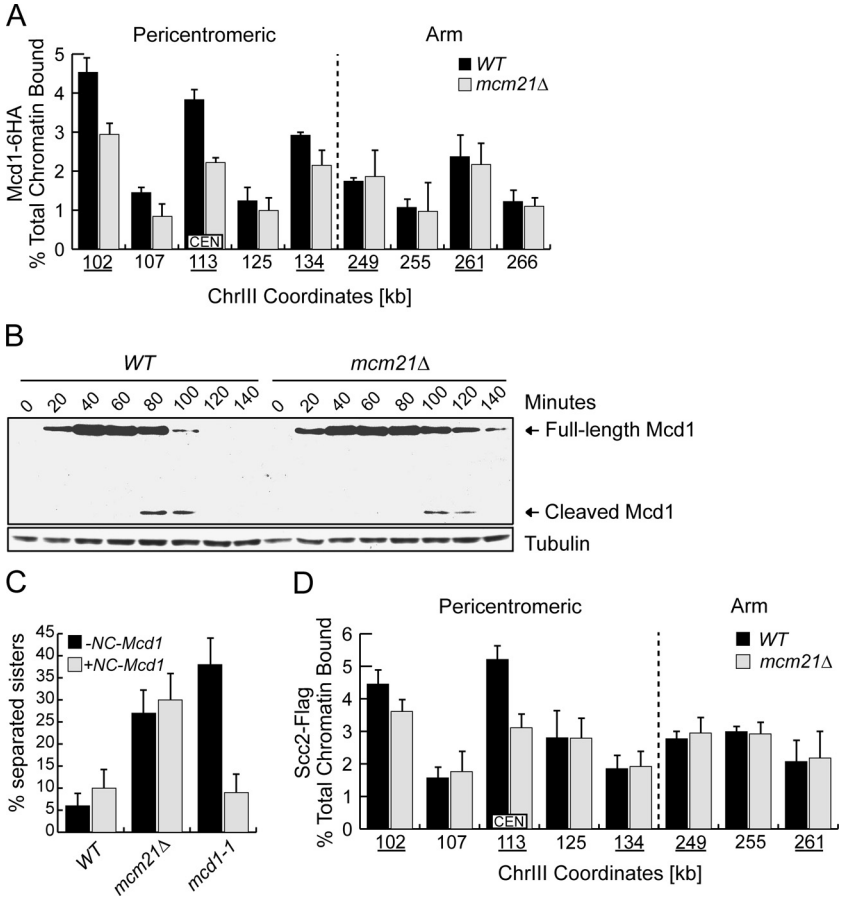

Figure 3. Mcm21 is required for cohesin loading at pericentromeres. (A) ChIP was carried out on nocodazole-arrested WT and *mcm21*Δ cells containing Mcd1-6HA (SBY7002 and SBY7007). CAR (underlined) and non-CAR sites were assessed for Mcd1-6HA binding at pericentromeric and arm loci on ChrIII as shown. (B) Lysates of strains in A were immunoblotted with anti-HA antibody during a synchronous cell cycle. Tubulin is shown as a loading control. (C) Sister separation was monitored at the pericentromeric locus in WT, *mcm21*Δ, and *mcd1-1* cells that contained (SBY6998, SBY6999, and SBY7847) or did not contain (SBY818, SBY1897, and SBY7846) NC-Mcd1 during a nocodazole arrest. (D) ChIP analysis of nocodazole-arrested WT and *mcm21*Δ cells containing Scc2-Flag (SBY7651 and SBY7652) was performed as in A.

ure 2D). As expected, a single GFP-LacI focus was observed in the majority of WT cells that also had high levels of Pds1 (93%). In contrast, 29% of *mcm21*Δ cells with strong Pds1 staining had two GFP foci in close proximity, indicating that the pericentromeric locus prematurely separated in metaphase. These data are consistent with our ability to detect separated sisters in *mcm21* cells arrested in nocodazole. In addition, more than 22% of *mcm21* cells arrested in metaphase by the overexpression of a nondegradable version of Pds1 or repression of the Cdc20 activator of anaphase also exhibited separated pericentromeres (data not shown).

Cohesin Loading at Pericentromeres Is Perturbed in mcm21Δ Cells

Because the Ctf19 protein is required for pericentromeric cohesin recruitment in response to nocodazole treatment that decreases kinetochore tension (Eckert *et al.*, 2007), we examined the localization of the Mcd1 cohesin protein in *mcm21* cells by ChIP. We arrested *WT* and *mcm21*Δ cells containing Mcd1-6HA in nocodazole to reduce tension and also to eliminate any cell cycle variation between the strains. The chromatin samples were analyzed by PCR at known CAR and non-CAR sites both proximal and distal to the centromere on ChrIII. Consistent with our observation that a locus near *CEN3* prematurely separates (data not shown), Mcd1 binding decreased at the three CAR sites tested in the pericentromeric region of *mcm21*Δ cells (Figure 3A). However, there were no differences between WT and *mcm21*Δ cells at CAR or non-CAR sites on the chromosomal arm.

To determine whether the decrease in Mcd1 at pericentromeres could be due to premature cleavage in the *mcm21* mutant cells, we monitored the appearance of the Mcd1 C-terminal cleavage fragment that results from Separase cleavage as cells were released from G1 (Uhlmann *et al.*, 1999). Although Mcd1 cleavage was first apparent in WT cells at 80 min, it was not detected until 100 min in *mcm21*Δ cells (Figure 3B). Consistent with this, the full-length Mcd1 protein was retained longer in *mcm21*Δ cells than in WT cells. The delay in cohesin cleavage and degradation is most likely due to the transient activation of the spindle checkpoint in *mcm21*Δ cells (see below, Figure 5E).

Because a population of cleaved cohesin might have escaped detection, we also tested whether the expression of an ectopic copy of noncleavable Mcd1 (NC-Mcd1) could suppress the cohesion defect (Uhlmann *et al.*, 1999). WT and *mcm21*Δ cells with or without NC-Mcd1 were arrested in G1 and released into nocodazole under conditions that induce NC-Mcd1. Sister separation was quantified at the pericentromeric locus on ChrIV (Figure 3C). Although NC-Mcd1 was sufficient to link sisters in *mcd1-1* cells, it did not suppress the separation defect in *mcm21*Δ cells, consistent with the data indicating that cohesin is not prematurely cleaved (Figure 3B).

We therefore asked whether cohesion establishment was defective in *mcm21* cells by analyzing the binding of the Scc2 cohesin-loading factor by ChIP. Cells containing Scc2-Flag were arrested in nocodazole and the chromatin bound to Scc2 was analyzed by PCR at the same CAR and non-CAR loci analyzed for Mcd1 binding. Similar to our findings for Mcd1-HA binding, Scc2 enrichment at the centromeric CAR site decreased in the absence of Mcm21 but was unperturbed at arm sites (Figure 3D). However, in contrast to Mcd1, we

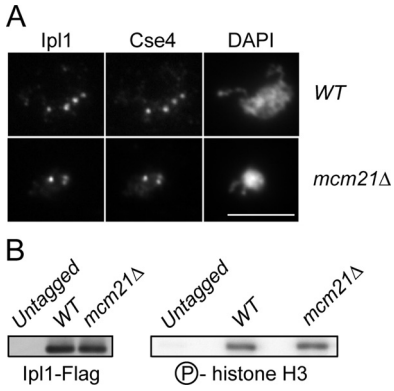

Figure 4. Ipl1 localization and function are not altered in the absence of Mcm21. (A) Chromosome spreads were performed on nocodazole-arrested WT and *mcm21*Δ cells (SBY8352, SBY8353) to localize Cse4 and Ipl1-GFP. (B) Ipl1-FLAG was immunoprecipitated from untagged, WT, and *mcm21*Δ cultures (SBY3, SBY7018, and SBY7019). The immunoprecipitates were immunoblotted with anti-Flag (left) or subjected to in vitro kinase assays (right).

did not observe changes in Scc2 binding at the pericentromeric CAR sites we assayed. We also analyzed the Eco1 cohesion establishment factor by ChIP but did not detect any differences in enrichment around centromeres in *mcm21* cells under similar growth conditions (data not shown). Therefore, the absence of Mcm21 may prevent proper pericentromeric cohesion due to the inability to fully recruit the Scc2 cohesin-loading factor to the centromere.

Mcm21 Has a Function in Kinetochore Biorientation

We next addressed the nature of the defect that leads to the synthetic lethality between *mcm21* and *ipl1-321*. We considered the possibility that the loss of Mcm21 alters Ipl1 localization, resulting in synthetic lethality between *mcm21* and *ipl1* mutants. To test this, we carried out chromosome spreads to visualize kinetochores on nocodazole-arrested WT and *mcm21*Δ cells containing Ipl1-GFP. Ipl1 colocalized with the Cse4 kinetochore protein in both WT and *mcm21*Δ cells (Figure 4A). In addition, we did not detect any obvious changes in GFP intensity in the absence of Mcm21, suggesting that Ipl1 localizes to kinetochores normally. Similar results were observed for chromosome spreads performed on cells from asynchronous cultures (data not shown), as well as when we visualized Ipl1-GFP by live microscopy (Supplemental Figure S2). To further analyze Ipl1 localization to centromeres, we attempted ChIP. However, Ipl1-Flag was not enriched at the centromere in WT cells compared with an untagged control strain (data not shown), consistent with previously reported ChIP data (He *et al.,* 2001). Taken together, our data suggest that Ipl1 localizes to kinetochores normally in the absence of Mcm21.

We next tested whether Ipl1 kinase activity was altered in the absence of Mcm21. Ipl1-Flag was immunoprecipitated from asynchronously growing cultures of WT and *mcm21*Δ cells and incubated with histone H3 and ^{32}P-ATP in a kinase assay in vitro. We did not detect any change in histone H3 phosphorylation in *mcm21*Δ cells compared with WT cells (Figure 4B), indicating that overall Ipl1 kinase activity is not altered in *mcm21*Δ cells.

To understand why *mcm21 ipl1* mutants are inviable, we constructed a conditional Ipl1 allele by fusing a degron tag to its N-terminus (Deg-Ipl1) to destabilize the protein and target it for degradation via the proteasome (Cormack and Struhl, 1992). The expression of the allele is also repressed by doxycycline, so we analyzed the growth of WT, *mcm21*Δ, *deg-ipl1*, and *mcm21*Δ *deg-ipl1* cells in the presence and absence of doxycycline. All of the strains grew similarly on plates without doxycycline, indicating that the cells retain enough Ipl1 function for viability (Figure 5A). As expected, WT and *mcm21*Δ cells also grew normally in the presence of doxycycline. However, doxycycline did not inhibit the growth of *deg-ipl1* cells, so these cells retain enough Ipl1 function to support viability even when its expression is inhibited. In contrast, the *mcm21*Δ *deg-ipl1* strain was inviable on doxycycline. These data indicate that *deg-ipl1* depends on *MCM21* function for viability, fortuitously creating a conditional strain. We confirmed that the *mcm21*Δ *deg-ipl1* cells lose viability upon release from G1 (Supplemental Figure S3).

Although the precise reason why the *deg-ipl1* allele is hypomorphic is not known, it gave us a way to analyze the requirement for Mcm21 in these cells. First, we assayed cohesion at the pericentromeric locus 12 kb from *CEN4* in *mcm21*Δ *deg-ipl1* cells. We performed these experiments in WT, *mcm21*Δ, *deg-ipl1*, and *mcm21*Δ *deg-ipl1* cells treated with nocodazole to eliminate potential differences in MT–kinetochore interactions and cell cycle progression (Figure 5B). Consistent with previous data suggesting that Ipl1 is not required for cohesion in budding yeast (Biggins *et al.,* 1999), we did not detect any exacerbation of the *mcm21*Δ cohesion defect in the double mutant.

We then assessed whether *mcm21* mutant cells activate the spindle checkpoint in an Ipl1-dependent manner. We analyzed Pds1 levels in WT, *mcm21*Δ, *deg-ipl1,* and *mcm21*Δ *deg-ipl1* cells that were released from G1 into doxycycline (Figure 5C). There was a transient delay in Pds1 destruction in *mcm21* cells that was not abolished when *deg-ipl1* was repressed. These data suggest that the inviability of these cells is not due to a defect in spindle checkpoint activation, so we directly examined the requirement of the checkpoint for the viability of *mcm21* mutant cells by constructing an *mcm21 mad3* double mutant. This strain grew normally (Figure 5D), but completely abolished the delay in Pds1 destruction in *mcm21* cells (Figure 5E). Therefore, *mcm21* cells transiently activate the spindle checkpoint but do not require checkpoint activity for viability. These data are consistent with the observation that cohesin mutants also transiently activate the spindle checkpoint (Skibbens *et al.,* 1999; Biggins *et al.,* 2001). Although it was previously concluded that the *mcm21 mad3* cells are viable because Mad3 is not required to detect defects in tension at kinetochores, the status of the spindle checkpoint was never analyzed in the double mutant cells (Lee and Spencer, 2004). Instead, our data show that Mad3 is required for the spindle checkpoint delay induced by a lack of the Mcm21 protein.

Because the essential function of Ipl1 is to biorient kinetochores, we tested whether there is an increased dependency on Mcm21 for biorientation when Ipl1 function is reduced. We were unable to monitor biorientation by directly analyzing sister centromere splitting at metaphase because we could not distinguish it from the pericentromeric cohesion defect in *mcm21*Δ cells. As a result, we analyzed sister chromatid segregation at anaphase by releasing WT, *mcm21*Δ, *deg-ipl1*, and *mcm21*Δ *deg-ipl1* cells from G1 into media containing doxycycline. Cells were analyzed for ChrIV segregation when >50% of the cells had proceeded into anaphase and segregated DNA masses to opposite poles (Figure 5F). Consistent with the viability data, sister chromatids segregated to opposite poles in all of the WT, *mcm21*Δ, and *deg-ipl1* cells. However, both copies of ChrIV

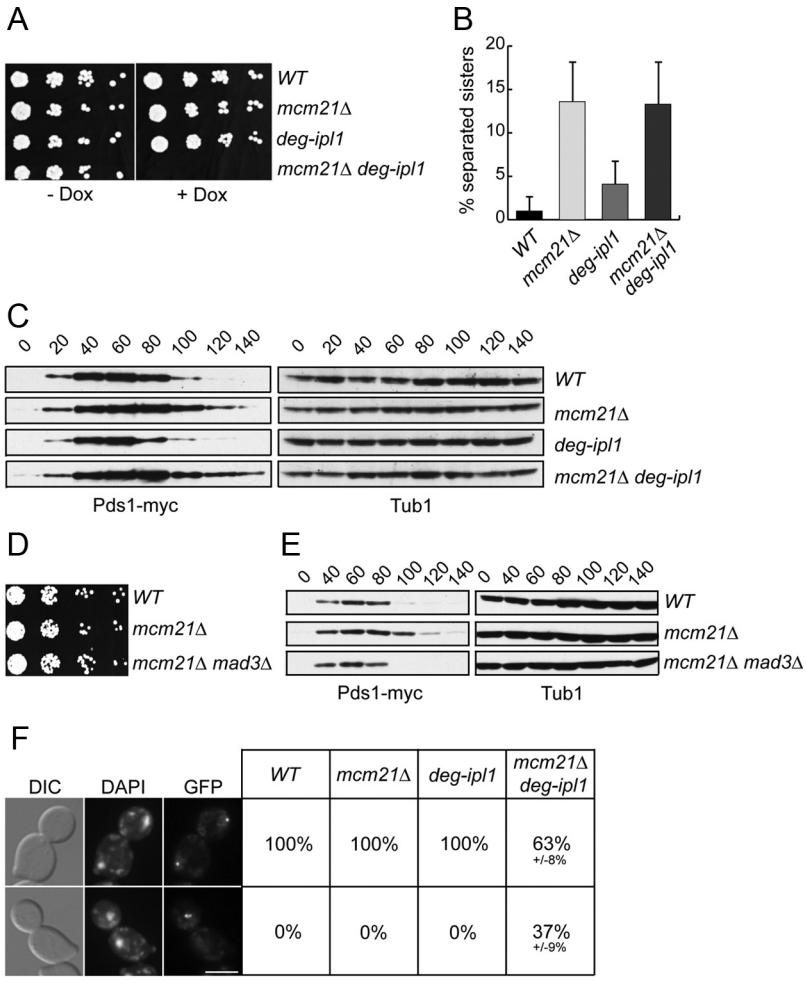

Figure 5. Mcm21 is required for kinetochore biorientation when Ipl1 function is impaired. (A) Serial dilutions (fivefold) of WT, *mcm21*Δ, *deg-ipl1*, and *mcm21*Δ *deg-ipl1* (SBY818, SBY1897, SBY6940, and SBY5551) cells were plated in the presence or absence of doxycycline. (B) Sister separation of the ChrIV pericentromeric locus was monitored in strains in A released from G1 into a nocodazole arrest in the presence of doxycycline. (C) Lysates from strains in A were immunoblotted against Pds1-myc and tubulin during a synchronous cell cycle. (D) Fivefold dilutions of WT, *mcm21*Δ, and *mcm21*Δ *mad3*Δ cells (SBY818, SBY1897, and SBY7656) were plated for viability. (E) Lysates of cells in D were immunoblotted for Pds1-myc and tubulin during a synchronous cell cycle. (F) ChrIV segregation was assessed in strains in A that reached anaphase during a synchronous cell cycle in the presence of doxycycline ($n > 100$).

segregated to the same pole in ~37% of the *mcm21*Δ *deg-ipl1* cells, suggesting that the kinetochores were mono-oriented. In support of this, the sisters were always in close proximity to the spindle poles, and we sometimes observed cells where both sisters were pulled into the bud (see Figure 5F). Assuming that all 16 chromosomes in budding yeast have an equal probability of mono-orienting in *mcm21*Δ *deg-ipl1* double mutant cells, cells would rarely segregate all of their chromosomes properly. Therefore, although we were not able to directly visualize the biorientation process, these data are most consistent with the possibility that Mcm21 is required for proper biorientation when Ipl1 function is downregulated.

Pericentromeric Sister Chromatid Cohesion Facilitates Biorientation

Although a variety of data suggest that pericentromeric cohesin is important for chromosome segregation, its precise role has not yet been elucidated (Allshire *et al.*, 1995; Kellum and Alberts, 1995; Peters *et al.*, 2001; Eckert *et al.*, 2007). While our work was in preparation, data were published indicating that it facilitates biorientation in fission yeast (Sakuno *et al.*, 2009). We had also been working on the possibility that pericentromeric cohesin aids biorientation during mitosis. In this case, a decrease in pericentromeric cohesin should reduce the efficiency of biorientation and therefore increase the dependency on Ipl1 to correct mal-oriented attachments. To test this, we utilized the *I-CEN-I* strain that prevents cohesin recruitment around *CEN3* (Eckert *et al.*, 2007). WT, *I-CEN-I*, *deg-ipl1*, and *I-CEN-I deg-ipl1* cells were plated for viability in the presence and absence of doxycycline (Figure 6A). Although all of the strains grew similarly in the absence of doxycycline (Eckert *et al.*, 2007), *I-CEN-I deg-ipl1* cells grew more slowly when Ipl1 function was reduced by addition of doxycycline. Therefore, the disruption of pericentromeric cohesin on a single chromosome sensitizes cells to a slight reduction in Ipl1 activity, supporting the idea that pericentromeric cohesion aids biorientation.

As an alternative test of the relationship between pericentromeric cohesion and biorientation, we asked whether the biorientation defect in *mcm21*Δ *deg-ipl1* cells could be suppressed when pericentromeric linkage is restored. To this end, we utilized a tetramerizing version of LacI ($LacI_4$) that is sufficient to hold sister chromatids together in the absence of MT-pulling forces and was used to argue that kinetochore geometry has an important role during meiosis I (Straight *et al.*, 1996; Lacefield and Murray, 2007). Nontetramerizing GFP-LacI used in the previous experiments ($LacI_2$) or tetramerizing GFP-LacI ($LacI_4$) were expressed in WT, *mcm21*Δ, *deg-ipl1*, and *mcm21*Δ *deg-ipl1* cells that contained LacO sequences 12 kb from *CEN4*. Sister separation was quantified 80 min after release from G1, a time when *mcm21* cells exhibit premature separation at the pericentromere (Figure 6B). Strikingly, the cohesion defect decreased in both

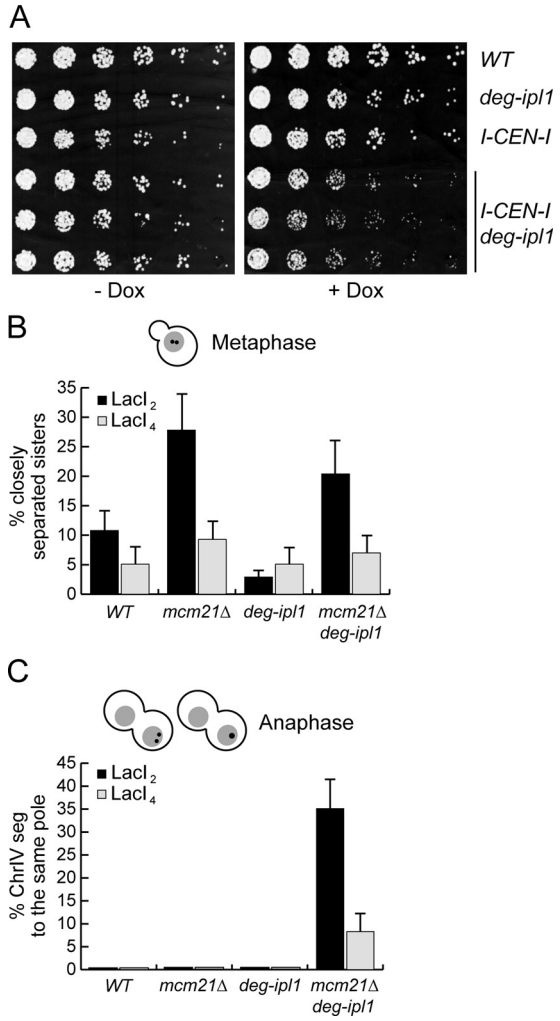

Figure 6. Pericentromeric linkage promotes biorientation. (A) Serial dilutions (threefold) of WT, *deg-ipl1*, *I-CEN-I* and three independent spores of *I-CEN-I deg-ipl1* cells (SBY3, SBY6993, SBY8102, SBY8103, SBY8104, and SBY8105) were plated in the presence and absence of doxycycline. (B) WT, *mcm21*Δ, *deg-ipl1*, and *mcm21*Δ *deg-ipl1* cells containing $LacI_2$ (SBY818, SBY1897, SBY6940, and SBY5551) or $LacI_4$ (SBY7871, SBY7872, SBY7873, and SBY7874) were monitored for closely separated sisters 80 min after G1 release in cells containing a single nucleus. (C) ChrIV segregation was monitored in the same experiment when the majority of cells had entered anaphase and segregated DNA masses to opposite poles ($n > 100$). The corresponding budding index is reported in Supplemental Figure S7.

*mcm21*Δ and *mcm21*Δ *deg-ipl1* cells, indicating that the tetramerizing LacI fusion can restore pericentromeric linkage.

We next analyzed biorientation in the same experiment by monitoring sister chromatid segregation at anaphase. We quantified GFP foci at the time point in each strain where the majority of cells had pulled their DNA to opposite poles (Figure 6C). As expected, WT, *mcm21*Δ, and *deg-ipl1* cells did not exhibit a biorientation defect and always segregated ChrIV to opposite poles. Remarkably, although ~35% of the *mcm21*Δ *deg-ipl1* cells segregated sisters to a single pole in the presence of $LacI_2$, only ~8% of the cells exhibited this phenotype when $LacI_4$ was expressed. Therefore, restoring pericentromeric linkage in *mcm21*Δ *deg-ipl1* cells was sufficient to partially suppress the biorientation defect. As a control, we analyzed biorientation in an *mcm21*Δ *deg-ipl1* mutant where the LacO sequences were moved to the telomere and compared that with the pericentromeric strain used in the previous experiment (Supplemental Figure S4). Although there was slight variation in the biorientation defects within the pericentromeric-marked strain between experiments, the differences are not significant. More importantly, artificially linking the telomeric locus did not suppress the biorientation defect, strongly suggesting that tethering the pericentromeres, specifically, restores proper biorientation in the absence of normal levels of pericentromeric cohesion.

DISCUSSION

We performed an SGA analysis with the *ipl1-321* allele and found that at least one component of the CTF19 kinetochore subcomplex becomes important for biorientation when Ipl1 function is impaired. Our characterization of the *mcm21* mutant revealed that it is involved in establishing pericentromeric cohesion. The role of Mcm21 in kinetochore biorientation can be partially bypassed if linkage is restored to pericentromeres, strongly suggesting that the enrichment of sister chromatid cohesin at pericentromeres serves to physically link them together to facilitate kinetochore biorientation.

The CTF19 Complex Ensures Proper Pericentromeric Cohesion

It was previously shown that the Ctf19 protein contributes to cohesin enrichment around centromeres when MTs are depolymerized (Eckert *et al.*, 2007). Our work and others (A. Marston, personal communication) extend these observations by showing that components of the CTF19 complex (Mcm21, Iml3, and Chl4) are not only required for this enrichment, but also contribute to physically linking pericentromeres together, consistent with the strong genetic interactions between mutants in CTF19 components and an allele of the *MCD1* gene (Supplemental Figure S5). Furthermore, Smc3-GFP fluorescence at pericentromeres was diminished in the absence of Mcm21 during a normal cell cycle (K. Bloom, personal communication), indicating that the loss of pericentromeric cohesin enrichment we observed by ChIP is not an artifact of the nocodazole arrest.

We analyzed the possible causes of the cohesion defect and found that *mcm21* cells do not prematurely cleave cohesin, but that there is a decrease in the Scc2 loading factor at the centromere, suggesting that cohesion establishment may be defective. To date, we have not detected a physical interaction between Mcm21 and Scc2 (unpublished data), so it is not clear how Mcm21 contributes to cohesin establishment. The CTF19 complex may specify a chromatin modification around centromeres that enriches cohesin, similar to the γ-H2AX phosphorylation that surrounds a DNA double-strand break and serves to signal the de novo loading of cohesin there (Strom *et al.*, 2004; Unal *et al.*, 2004). Another possibility is that Mcm21 ensures a higher order pericentromeric chromosome structure that involves cohesin, such as the proposed cruciform structure (Yeh *et al.*, 2008). The localization of Mcm21 is restricted to within 2 kb of the centromere (Supplemental Figure S6), so it is unlikely to directly mediate pericentromeric cohesion. Because budding yeast lack pericentromeric heterochromatin, the CTF19 kinetochore complex may fulfill this role in establishing pericentromeric cohesion. Mcm21 is conserved (McAinsh *et al.*, 2006; McClelland *et al.*, 2007), so it will be interesting to determine if it and other conserved CTF19 components are involved in the establishment of cohesin domains around

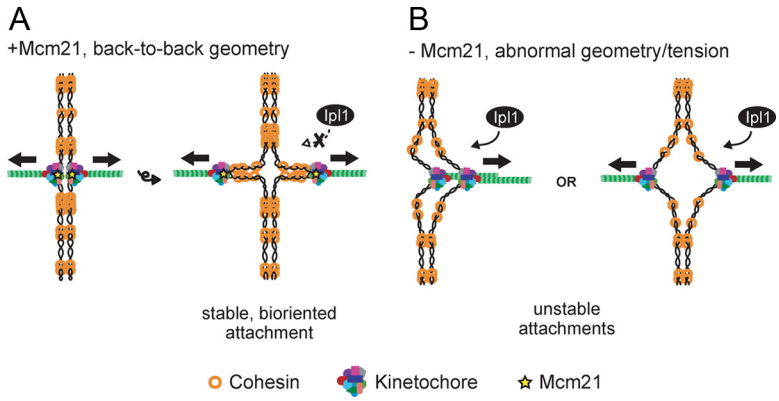

Figure 7. Model for the role of pericentromeric cohesion in biorientation. (A) Cohesion establishment at pericentromeres mediated in part by Mcm21 helps to impose steric constraints on kinetochore orientation to ensure biorientation. Ipl1 senses tension and the bioriented attachments are stabilized. The pericentromeric region is depicted as the looped out regions of the chromosome. (B) In the absence of Mcm21, the decrease in pericentromeric cohesion may alter kinetochore geometry, making it more difficult for kinetochores to biorient and increasing the requirement for Ipl1 (left). The defect in pericentromeric cohesion may also reduce the cell's ability to sense tension properly and affect the regulation of Ipl1 and/or the stability of the MT–kinetochore attachments (left and right).

neocentromeres that lack heterochromatin in multicellular eukaryotes (for review, see Cheeseman and Desai, 2008).

Pericentromeric Cohesion Is Important for Biorientation

We analyzed the requirement for Mcm21 function when Ipl1 function was decreased and found a defect in kinetochore biorientation. The missegregation in the *mcm21*Δ *deg-ipl1* strain allowed us to specifically assess the role of pericentromeric sister chromatid cohesion in biorientation. Remarkably, artificially linking the pericentromeres was sufficient to suppress both the cohesion and the biorientation defects in *mcm21*Δ *deg-ipl1* cells. Consistent with this, we found a genetic interaction between a small reduction in Ipl1 levels and a defect in pericentromeric cohesion on just a single chromosome. Although we cannot eliminate the possibility that Mcm21 directly regulates Ipl1 function in some manner, the simplest interpretation of this data is that pericentromeric cohesion directly aids biorientation, consistent with recent work in fission yeast (Sakuno *et al.*, 2009). Although we did not detect a biorientation defect in *mcm21*Δ cells, the mutant was originally identified due to an increase in the nondisjunction of a nonessential minichromosome (Poddar *et al.*, 1999). We assume that the strength of the Ipl1 correction system ensures that chromosomes biorient in *mcm21* mutant cells. Furthermore, a number of the genes we identified in the SGA screen have been previously implicated in sister chromatid cohesion (Mayer *et al.*, 2001; Kenna and Skibbens, 2003; Mayer *et al.*, 2004), underscoring the critical connection between Ipl1 and cohesion.

We were unable to test whether suppression of the cohesion defect was sufficient to restore viability to *mcm21 deg-ipl1* cells because we could not establish a way to restore linkage to all pericentromeres. Therefore, additional defects in *mcm21* mutant cells may contribute to creating lethality with *ipl1-321*. However, many other kinetochore mutants do not exhibit synthetic interactions with *ipl1-321* (Pinsky *et al.*, 2006), suggesting specific connections to the CTF19 complex. In conclusion, our data strongly suggest that the biorientation defect in the double mutant cells is due to the lack of proper pericentromeric cohesion.

Models for the Role of Pericentromeric Cohesion in Biorientation

Although any linkage between sister chromatids may be sufficient to generate the tension required for biorientation (Dewar *et al.*, 2004), our data reveal that pericentromeric cohesion significantly enhances the fidelity of biorientation. We propose two possible models that are not mutually exclusive (Figure 7). First, pericentromeric cohesion might impose a kinetochore orientation that facilitates proper chromosome segregation, consistent with recent evidence suggesting a special kinetochore geometry (Indjeian and Murray, 2007; Loncarek *et al.*, 2007; Yeh *et al.*, 2008; Sakuno *et al.*, 2009). This geometry would aid in biorientation and result in stable, bioriented attachments that are not substrates for Ipl1 (Figure 7A). In the absence of this geometry, there might be an enhanced requirement for Ipl1 function to achieve biorientation, or Ipl1 could have an unidentified role in kinetochore geometry (Figure 7B). Second, a certain level of pericentromeric cohesion may be required to satisfy the tension-sensing mechanism (Figure 7B). This could be through a variety of mechanisms, such as attaining the proper spatial regulation of Ipl1 from its substrates (Tanaka *et al.*, 2002; Liu *et al.*, 2009; Shimogawa *et al.*, 2009) or achieving the appropriate interkinetochore or intrakinetochore stretch (Maresca and Salmon, 2009; Uchida *et al.*, 2009). Because defects in pericentromeric cohesion could alter these properties, MT attachments may not be properly stabilized in *mcm21* mutant cells. In this case, MTs may detach from kinetochores even when they biorient, consistent with our observation that *mcm21* mutant cells transiently activate the spindle checkpoint and other reports that the CTF19 complex contributes to MT–kinetochore interactions (Hyland *et al.*, 1999; De Wulf *et al.*, 2003; Pot *et al.*, 2005; Tanaka *et al.*, 2005; McAinsh *et al.*, 2006). Therefore, *mcm21* mutant cells may become very sensitive to slight decreases in Ipl1 function due to difficulty in detecting appropriate levels of tension.

In sum, although tension may be sufficient for biorientation, pericentromeric cohesion facilitates biorientation to ensure proper chromosome segregation, and thereby reduces the need for Ipl1 function. Future studies of the other genes that are required for viability when Ipl1 function is decreased may lead to the identification of other biorientation pathways or functions for the Ipl1/Aurora kinase. In the long-term, the requirement for these genes to maintain viability when Ipl1/Aurora function is reduced could be exploited to make cancer cells more sensitive to Aurora kinase inhibitors.

ACKNOWLEDGMENTS

We are indebted to Brenda Andrews and Charlie Boone (University of Toronto) for use of their pinning robots and analysis software during SGA. We thank Igor Kogut and Paul Megee for information about Scc2-Flag ChIP and helpful discussions. We thank Andrew Murray (Harvard University), Peter Sorger (Harvard Medical School), and Frank Uhlmann (Cancer Research UK) for strains. We thank Adele Marston, Josefin Fernius, Kerry Bloom, and Yoshi Watanabe for communicating results and helpful comments, and the

Biggins lab, Chitra Kotwaliwale, and Sue Jaspersen for critical reading of the manuscript and discussions. T.M.N. was supported in part by Public Health Service, National Research Service Award, T32 GM07270, from the National Institute of General Medical Sciences. This work was supported by National Institutes of Health grant GM064386 to S.B. and funded by Canadian Institutes of Health Research operating (FRN 57913) and salary (MSH 63646) grants to B.D.L. S.B. is a Scholar of the Leukemia and Lymphoma Society.

REFERENCES

Allshire, R. C., Nimmo, E. R., Ekwall, K., Javerzat, J. P., and Cranston, G. (1995). Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. Genes Dev. *9*, 218–233.

Ben-Shahar, T. R., Heeger, S., Lehane, C., East, P., Flynn, H., Skehel, M., and Uhlmann, F. (2008). Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. Science *321*, 563–566.

Bernard, P., Maure, J. F., Partridge, J. F., Genier, S., Javerzat, J. P., and Allshire, R. C. (2001). Requirement of heterochromatin for cohesion at centromeres. Science *294*, 2539–2542.

Biggins, S., Bhalla, N., Chang, A., Smith, D. L., and Murray, A. W. (2001). Genes involved in sister chromatid separation and segregation in the budding yeast *Saccharomyces cerevisiae*. Genetics *159*, 453–470.

Biggins, S., Severin, F. F., Bhalla, N., Sassoon, I., Hyman, A. A., and Murray, A. W. (1999). The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. Genes Dev. *13*, 532–544.

Blat, Y., and Kleckner, N. (1999). Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. Cell *98*, 249–259.

Buvelot, S., Tatsutani, S. Y., Vermaak, D., and Biggins, S. (2003). The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. J. Cell Biol. *160*, 329–339.

Cheeseman, I. M., Anderson, S., Jwa, M., Green, E. M., Kang, J., Yates, J. R., Chan, C. S., Drubin, D. G., and Barnes, G. (2002). Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. Cell *111*, 163–172.

Cheeseman, I. M., and Desai, A. (2008). Molecular architecture of the kinetochore-microtubule interface. Nat. Rev. Mol. Cell Biol. *9*, 33–46.

Ciosk, R., Shirayama, M., Shevchenko, A., Tanaka, T., Toth, A., and Nasmyth, K. (2000). Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. Mol. Cell *5*, 243–254.

Collins, K. A., Castillo, A. R., Tatsutani, S. Y., and Biggins, S. (2005). De novo kinetochore assembly requires the centromeric histone H3 variant. Mol. Biol. Cell *16*, 5649–5660.

Cormack, B. P., and Struhl, K. (1992). The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell *69*, 685–696.

De Wulf, P., McAinsh, A. D., and Sorger, P. K. (2003). Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. Genes Dev. *17*, 2902–2921.

Dewar, H., Tanaka, K., Nasmyth, K., and Tanaka, T. U. (2004). Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. Nature *428*, 93–97.

Eckert, C. A., Gravdahl, D. J., and Megee, P. C. (2007). The enhancement of pericentromeric cohesin association by conserved kinetochore components promotes high-fidelity chromosome segregation and is sensitive to microtubule-based tension. Genes Dev. *21*, 278–291.

Goshima, G., and Yanagida, M. (2000). Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. Cell *100*, 619–633.

Grewal, S. I., and Jia, S. (2007). Heterochromatin revisited. Nat. Rev. Genet *8*, 35–46.

Hauf, S., and Watanabe, Y. (2004). Kinetochore orientation in mitosis and meiosis. Cell *119*, 317–327.

He, X., Asthana, S., and Sorger, P. K. (2000). Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. Cell *101*, 763–775.

He, X., Rines, D. R., Espelin, C. W., and Sorger, P. K. (2001). Molecular analysis of kinetochore-microtubule attachment in budding yeast. Cell *106*, 195–206.

Hyland, K. M., Kingsbury, J., Koshland, D., and Hieter, P. (1999). Ctf19p: a novel kinetochore protein in *Saccharomyces cerevisiae* and a potential link between the kinetochore and mitotic spindle. J. Cell Biol. *145*, 15–28.

Indjeian, V. B., and Murray, A. W. (2007). Budding yeast mitotic chromosomes have an intrinsic bias to biorient on the spindle. Curr. Biol. *17*, 1837–1846.

Kellum, R., and Alberts, B. M. (1995). Heterochromatin protein 1 is required for correct chromosome segregation in *Drosophila* embryos. J. Cell Sci. *108*(Pt 4), 1419–1431.

Kenna, M. A., and Skibbens, R. V. (2003). Mechanical link between cohesion establishment and DNA replication: Ctf7p/Eco1p, a cohesion establishment factor, associates with three different replication factor C complexes. Mol. Cell. Biol. *23*, 2999–3007.

King, J. M., and Nicklas, R. B. (2000). Tension on chromosomes increases the number of kinetochore microtubules but only within limits. J. Cell Sci. *113*(Pt 21), 3815–3823.

Kotwaliwale, C. V., Frei, S. B., Stern, B. M., and Biggins, S. (2007). A pathway containing the Ipl1/aurora protein kinase and the spindle midzone protein Ase1 regulates yeast spindle assembly. Dev. Cell *13*, 433–445.

Lacefield, S., and Murray, A. W. (2007). The spindle checkpoint rescues the meiotic segregation of chromosomes whose crossovers are far from the centromere. Nat. Genet. *39*, 1273–1277.

Laloraya, S., Guacci, V., and Koshland, D. (2000). Chromosomal addresses of the cohesin component Mcd1p. J. Cell Biol. *151*, 1047–1056.

Lee, M. S., and Spencer, F. A. (2004). Bipolar orientation of chromosomes in *Saccharomyces cerevisiae* is monitored by Mad1 and Mad2, but not by Mad3. Proc. Natl. Acad. Sci. USA *101*, 10655–10660.

Lengronne, A., Katou, Y., Mori, S., Yokobayashi, S., Kelly, G. P., Itoh, T., Watanabe, Y., Shirahige, K., and Uhlmann, F. (2004). Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature *430*, 573–578.

Lengronne, A., McIntyre, J., Katou, Y., Kanoh, Y., Hopfner, K. P., Shirahige, K., and Uhlmann, F. (2006). Establishment of sister chromatid cohesion at the *S. cerevisiae* replication fork. Mol. Cell *23*, 787–799.

Liu, D., Vader, G., Vromans, M. J., Lampson, M. A., and Lens, S. M. (2009). Sensing chromosome bi-orientation by spatial separation of Aurora B kinase from kinetochore substrates. Science *323*, 1350–1353.

Loidl, J., Klein, F., and Engebrecht, J. (1998). Genetic and morphological approaches for the analysis of meiotic chromosomes in yeast. Methods Cell Biol. *53*, 257–285.

Loncarek, J., Kisurina-Evgenieva, O., Vinogradova, T., Hergert, P., La Terra, S., Kapoor, T. M., and Khodjakov, A. (2007). The centromere geometry essential for keeping mitosis error free is controlled by spindle forces. Nature *450*, 745–749.

Maresca, T. J., and Salmon, E. D. (2009). Intrakinetochore stretch is associated with changes in kinetochore phosphorylation and spindle assembly checkpoint activity. J. Cell Biol. *184*, 373–381.

Mayer, M. L., Gygi, S. P., Aebersold, R., and Hieter, P. (2001). Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in *S. cerevisiae*. Mol. Cell *7*, 959–970.

Mayer, M. L., *et al.* (2004). Identification of protein complexes required for efficient sister chromatid cohesion. Mol. Biol. Cell *15*, 1736–1745.

McAinsh, A. D., Meraldi, P., Draviam, V. M., Toso, A., and Sorger, P. K. (2006). The human kinetochore proteins Nnf1R and Mcm21R are required for accurate chromosome segregation. EMBO J. *25*, 4033–4049.

McClelland, S. E., Borusu, S., Amaro, A. C., Winter, J. R., Belwal, M., McAinsh, A. D., and Meraldi, P. (2007). The CENP-A NAC/CAD kinetochore complex controls chromosome congression and spindle bipolarity. EMBO J. *26*, 5033–5047.

Megee, P. C., Mistrot, C., Guacci, V., and Koshland, D. (1999). The centromeric sister chromatid cohesion site directs Mcd1p binding to adjacent sequences. Mol. Cell *4*, 445–450.

Michaelis, C., Ciosk, R., and Nasmyth, K. (1997). Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell *91*, 35–45.

Minshull, J., Straight, A., Rudner, A., Dernburg, A., Belmont, A., and Murray, A. W. (1996). Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast. Curr. Biol. *6*, 1609–1620.

Nicklas, R. B., and Ward, S. C. (1994). Elements of error correction in mitosis: microtubule capture, release, and tension. J. Cell Biol. *126*, 1241–1253.

Ocampo-Hafalla, M. T., Katou, Y., Shirahige, K., and Uhlmann, F. (2007). Displacement and re-accumulation of centromeric cohesin during transient pre-anaphase centromere splitting. Chromosoma *116*, 531–544.

Ortiz, J., Stemmann, O., Rank, S., and Lechner, J. (1999). A putative protein complex consisting of Ctf19, Mcm21, and Okp1 represents a missing link in the budding yeast kinetochore. Genes Dev. *13*, 1140–1155.

Pearson, C. G., Maddox, P. S., Salmon, E. D., and Bloom, K. (2001). Budding yeast chromosome structure and dynamics during mitosis. J. Cell Biol. *152*, 1255–1266.

Peters, A. H., *et al.* (2001). Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell *107*, 323–337.

Peters, J. M., Tedeschi, A., and Schmitz, J. (2008). The cohesin complex and its roles in chromosome biology. Genes Dev. *22*, 3089–3114.

Pinsky, B. A., and Biggins, S. (2005). The spindle checkpoint: tension vs. attachment. Trends Cell Biol. *15*(9), 486–493.

Pinsky, B. A., Kung, C., Shokat, K. M., and Biggins, S. (2006). The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. Nat. Cell Biol. *8*, 78–83.

Pinsky, B. A., Tatsutani, S. Y., Collins, K. A., and Biggins, S. (2003). An Mtw1 complex promotes kinetochore biorientation that is monitored by the Ipl1/Aurora protein kinase. Dev. Cell *5*, 735–745.

Poddar, A., Roy, N., and Sinha, P. (1999). *MCM21* and *MCM22*, two novel genes of the yeast *Saccharomyces cerevisiae* are required for chromosome transmission. Mol. Microbiol. *31*, 349–360.

Pot, I., Knockleby, J., Aneliunas, V., Nguyen, T., Ah-Kye, S., Liszt, G., Snyder, M., Hieter, P., and Vogel, J. (2005). Spindle checkpoint maintenance requires Ame1 and Okp1. Cell Cycle *4*, 1448–1456.

Rose, M. D., Winston, F., and Heiter, P. (1990). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Ruchaud, S., Carmena, M., and Earnshaw, W. C. (2007). Chromosomal passengers: conducting cell division. Nat. Rev. Mol. Cell Biol. *8*, 798–812.

Sakuno, T., Tada, K., and Watanabe, Y. (2009). Kinetochore geometry defined by cohesion within the centromere. Nature *458*, 852–858.

Sherman, F., Fink, G., and Lawrence, C. (1974). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Shimogawa, M. M., Widlund, P. O., Riffle, M., Ess, M., and Davis, T. N. (2009). Bir1 is required for the tension checkpoint. Mol. Biol. Cell *20*, 915–923.

Skibbens, R. V., Corson, L. B., Koshland, D., and Hieter, P. (1999). Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev. *13*, 307–319.

Straight, A. F., Belmont, A. S., Robinett, C. C., and Murray, A. W. (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Curr. Biol. *6*, 1599–1608.

Strom, L., Lindroos, H. B., Shirahige, K., and Sjogren, C. (2004). Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol. Cell *16*, 1003–1015.

Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E. K., Prescott, A. R., Antony, C., and Tanaka, T. U. (2005). Molecular mechanisms of kinetochore capture by spindle microtubules. Nature *434*, 987–994.

Tanaka, T., Cosma, M. P., Wirth, K., and Nasmyth, K. (1999). Identification of cohesin association sites at centromeres and along chromosome arms. Cell *98*, 847–858.

Tanaka, T., Fuchs, J., Loidl, J., and Nasmyth, K. (2000). Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. Nat. Cell Biol *2*, 492–499.

Tanaka, T. U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M. J., and Nasmyth, K. (2002). Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell *108*, 317–329.

Tong, A. H., *et al.* (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science *294*, 2364–2368.

Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., and Nasmyth, K. (1999). Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev. *13*, 320–333.

Uchida, K. S., Takagaki, K., Kumada, K., Hirayama, Y., Noda, T., and Hirota, T. (2009). Kinetochore stretching inactivates the spindle assembly checkpoint. J. Cell Biol. *184*, 383–390.

Uhlmann, F., Lottspeich, F., and Nasmyth, K. (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature *400*, 37–42.

Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J. E., and Koshland, D. (2004). DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol. Cell *16*, 991–1002.

Unal, E., Heidinger-Pauli, J. M., Kim, W., Guacci, V., Onn, I., Gygi, S. P., and Koshland, D. E. (2008). A molecular determinant for the establishment of sister chromatid cohesion. Science *321*, 566–569.

Waples, W. G., Chahwan, C., Ciechonska, M., and Lavoie, B. D. (2008). Putting the brake on FEAR: Tof2 promotes the biphasic release of Cdc14 phosphatase during mitotic exit. Mol. Biol. Cell *20*, 245–255.

Weber, S. A., Gerton, J. L., Polancic, J. E., DeRisi, J. L., Koshland, D., and Megee, P. C. (2004). The kinetochore is an enhancer of pericentric cohesin binding. PLoS Biol. *2*, E260.

Yamagishi, Y., Sakuno, T., Shimura, M., and Watanabe, Y. (2008). Heterochromatin links to centromeric protection by recruiting shugoshin. Nature *455*, 251–255.

Yeh, E., Haase, J., Paliulis, L. V., Joglekar, A., Bond, L., Bouck, D., Salmon, E. D., and Bloom, K. S. (2008). Pericentric chromatin is organized into an intramolecular loop in mitosis. Curr. Biol. *18*, 81–90.

Zhang, J., *et al.* (2008). Acetylation of Smc3 by Eco1 is required for S phase sister chromatid cohesion in both human and yeast. Mol. Cell *31*, 143–151.