Current Biology *16*, 902–906, May 9, 2006 DOI 10.1016/j.cub.2006.03.060

Report

Sister-Chromatid Telomere Cohesion Is Nonredundant and Resists Both Spindle Forces and Telomere Motility

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Summary

It is well documented that inactivation of essential cohesion proteins results in precocious sister-chromatid separation. On average, however, only ~55% of cohesin-deficient budding yeast cells arrested prior to anaphase contain separated sister chromatids [1–4], suggesting that cohesin-independent factors also contribute to sister-chromatid pairing. Recently, redundant pairing mechanisms were found to occur at both rDNA and centromeres [5, 6]. Here, we tested whether redundant mechanisms also function to pair telomeres or whether cohesins provide sole pairing activity. Results from both *mcd1* and *ctf7* mutant cells show that nearly 100% of telomeres separate prior to anaphase, twice the cohesion defect reported for centromeres. Such complete loci separation reveals that cohesins are singularly responsible for maintaining telomere cohesion, in contrast to other loci. We also found that sister telomeres moved 141% farther apart than centromeres. Telomere separation occurred in the absence of spindle microtubules and an actin cytoskeleton and persisted in cells abrogated for Mps3p function—an integral nuclear envelope protein previously shown to function in cohesion [7–9]. These findings are consistent with numerous studies that telomeres translocate along the nuclear periphery [10–14] and provide new evidence that telomere dynamics can contribute to sister-chromatid separation, independent of centromere motility.

Results and Discussion

To ascertain the nature of telomere pairing and whether chromosome-end dynamics can contribute to sister-chromatid separation, we generated a telomere-proximal GFP assay strain in which Lac operator repeats are integrated only 9.7 Kb from the end of chromosome IV. Telomere detection is provided by expression of GFP-tagged Lac repressor and identification of preanaphase cells provided by epitope-tagged Pds1p. We found that telomeres remained tightly paired in preanaphase cells (Figure 1), consistent with a previous study on telomere-proximal dynamics in wild-type cells [15].

The finding that telomeres remain tightly paired until anaphase allowed us to address two critical issues: (1) are telomeres paired by cohesin complexes alone or by redundant pairing mechanisms and (2) in the absence of cohesins, will sister telomeres separate independent of centromeres? To assess the effect of abrogating cohesin function on telomere pairing, we crossed our telomere GFP strain into *ctf7-203* (cohesion establishment) and *mcd1-1* (structural cohesin) mutant strains [1–4]. Log phase wild-type, *ctf7-203,* and *mcd1-1* mutant strains containing either centromere or telomere-GFP cassettes were synchronized in G_1 at 23°C with α-factor, released into 37°C rich medium supplemented with nocodazole, and arrested prior to anaphase onset. For each cell culture, parallel samples were harvested and assessed for DNA content, cell morphology, Pds1p content (an inhibitor of anaphase onset [16]), and disposition of sister-chromatid loci.

Wild-type cells contained tightly paired telomeres such that very few (2.5%) sister chromatids were dissociated. In fact, this minimal background level of separated sisters is significantly less than reported for centromere-proximal loci in wild-type cells (frequencies of centromere separation range from 7% to 22% with an average separation of 17%) [1–4]. As expected, strains harboring *ctf7-203* or *mcd1-1* alleles exhibited cohesion defects at both centromere-proximal and telomere-proximal loci. Surprisingly, however, we found a tremendous increase in the frequency of separated telomeres in preanaphase cells. Results from two independent studies revealed that telomeres were separated 95% of the time in *ctf7-203* preanaphase cells and 86% of the time in *mcd1-1* preanaphase cells—nearly double the frequency of separated centromeres previously reported (45% for *ctf7* cells and 55% for *mcd1* cells; averages of values reported in [1–4]) (Figure 1). To confirm this loci-specific effect, we repeated our analyses so that both telomere- and centromere-separation frequencies could be directly compared in *mcd1-1* cells. Results from two independent experiments confirm that 85% of telomeres were separated while only 46% of centromeres were separated in cells abrogated for Mcd1p function. This is the first report of a near 100% cohesion defect associated with loss of essential cohesion factors, revealing that the combined effects of establishment and structural cohesin pathways promote the only structure required to resist sister-telomere separation. Moreover, that telomeres separate at much higher frequencies than centromeres suggests that a centromere-independent mechanism promotes telomere separation.

Formally, it is possible that telomeres contain reduced cohesin levels and thus are only weakly paired in wild-type cells, relative to other cohesion-association regions, and that this decreased cohesin level exacerbates the cohesion defects found at telomeres in *ctf7* and *mcd1* cells. To test whether our telomere-cohesion assay strain in general produces elevated cohesion defects, we repeated our analyses with *mps3* mutant cells previously shown to produce intermediate levels of cohesion defects, relative to *ctf7* and *mcd1* [9]. Importantly, *mps3* cells exhibit telomere-cohesion defects nearly identical to those previously quantified for

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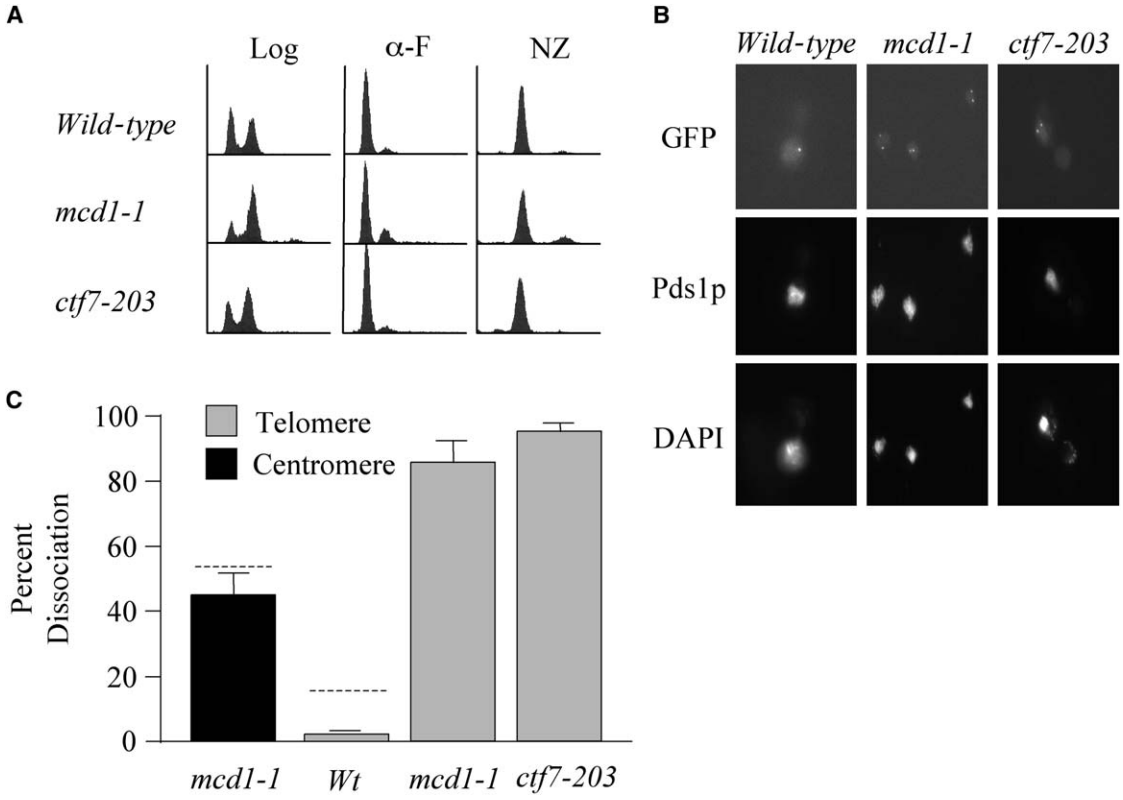

Figure 1. Essential Cohesion Factor Mutant Strains Exhibit Defects in Telomere Cohesion

(A) DNA profiles of asynchronous (Log), G_1 synchronized at 23ºC (α-F) and released into 37ºC fresh medium containing nocodazole (*NZ*) to arrest cells prior to anaphase of wild-type, *mcd1-1*, and *ctf7-203* mutant strains.

(B) Micrographs of wild-type, *mcd1-1*, and *ctf7-203* mutant strains in which sister-chromatid loci (GFP) and Pds1p (Pds1p) are visualized within the DNA mass (DAPI).

(C) Quantification of telomere-cohesion defects of *mcd1-1* and *ctf7-203* averaged (mean ± SD) from two independent studies (100 cells for each strain were counted per study). Dashed line represents percentage of separated sisters in *mcd1-1* or *ctf7-203* averaged from multiple studies [1–4].

centromere-proximal loci (14% for *msp3-5* and 17% for *mps3-3* at telomeres compared to 14% at centromeres [9]) (Figure 2). Thus, our telomere-cohesion assay strain does not generally exacerbate cohesion defects. Moreover, a simple reduced cohesin decoration model predicts that sister telomeres would exhibit greater separation frequencies not only in *mps3* cells, but also in wild-type cells. This is clearly not the case: telomeres exhibit lower levels of separation than centromeres in preanaphase wild-type cells (Figures 1 and 2).

We also noted that telomeres in preanaphase *ctf7* and *mcd1* mutant cells were overtly separated—often to opposite sides of the nucleus. This spatial separation contrasts that of centromeres, which are typically closely apposed ([1–4], unpublished data). We decided to quantify this loci-specific effect. *mcd1-1* mutant cells harboring centromere-proximal or telomere-proximal GFP tags were synchronized in G_1 prior to release into fresh 37ºC media containing nocodazole as described above. Cells that contained separated sister chromatids were photographed, and intercentromere and intertelomere distances were measured according to the following criteria. First, we included in our analyses only cells containing GFP-marked loci that occurred in roughly the same focal plane (~0.4 μm Z-axial resolution). Second, we excluded any cells in which GFP sister loci were positioned to opposite sides of the mother-bud neck (observed in telomere-marked *mcd1-1* cells but less often in centromere-marked *mcd1-1* cells). Thus, our analyses most likely underrepresent spatial separation effects that occur between sister telomeres, relative to sister centromeres. Following these parameters, combined results from two independent analyses show that centromere-proximal loci were separated by 9.7 ± 5.8 units (112 *mcd1-1* cells) while telomere-proximal loci were separated by 13.7 ± 7.3 units (111 *mcd1-1* cells). These results reveal that sister telomeres are separated 141% more than the distance measured between sister centromeres. This distance between sister telomeres is not unique to *mcd1* cells: *ctf7-203* alleles placed into the telomere cohesion strain contained telomeres separated by 13.3 ± 6.4 units (54 cells measured). Thus, in the absence of cohesin function, sister telomeres exhibit a significant increase in both the frequency of cohesion defects and in their spatial separation, relative to sister centromeres.

Telomere separation in the absence of microtubules suggests that telomeres can directly contribute to sister-chromatid separation. In fact, numerous reports documented that telomeres can cluster near spindle bodies and also move along the nuclear periphery—possibly by hopping along nuclear envelope anchor

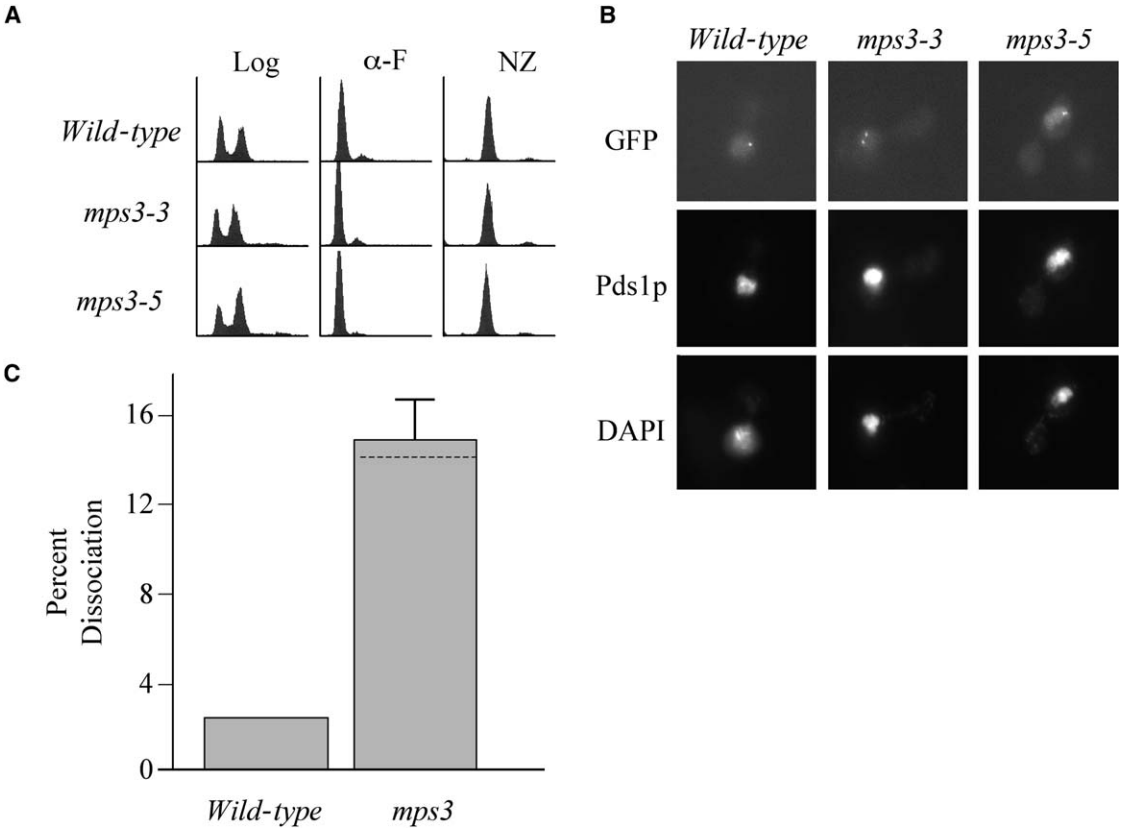

Figure 2. Nonessential Cohesion Factor Mutant Strains Exhibit Defects in Telomere Sister-Chromatid Cohesion

(A) DNA profiles of cell-cycle arrests. Asynchronous log phase cultures (*Log*) of wild-type, *mps3-3,* and *mps3-5* mutant strains were synchronized in G_1 (α-factor) at 23ºC and released into 37ºC fresh medium containing nocodazole (*NZ*) to arrest cells prior to anaphase.

(B) Micrographs of wild-type, *mps3-3*, and *mps3-5* mutant strains in which sister-chromatid loci (GFP) and Pds1p (Pds1p) are visualized within the DNA mass (DAPI).

(C) Quantification (mean ± SD) of cohesion defects exhibited by wild-type and *mps3* mutant strains (14% for *msp3-5* and 17% for *mps3-3*). Dashed line represents 14% of centromere-proximal cohesion defects previously described in preanaphase *mps3-3* cells [9].

sites [10–14]. Among other possibilities, such anchor sites could involve either an actin meshwork proximal to the nuclear inner envelope or Mps3p—an integral nuclear envelope transmembrane protein recently shown to function in sister-chromatid cohesion [9, 17]. We first tested whether telomere separation involved an association with spindle pole bodies. Wild-type, *ctf7-203,* and *mcd1-1* cells were arrested in mitosis as described above and processed to visualize both sister telomeres (GFP) and spindle pole bodies (Tub4p). The results show that telomeres did not colocalize with spindle pole bodies but instead became positioned away from each other and from the spindle pole body (Figure 3). We tested the efficacy of our microtubule-depolymerization regime and found that almost 100% of the spindle poles had collapsed back together into a single foci (Figure 3). Thus, sister-chromatid separation in cohesion mutants occurs independent of both spindle microtubules and spindle pole body attachments.

We next tested whether telomere separation required an actin cytoskeleton. Wild-type and *mcd1-1* cells synchronized in early S-phase (hydroxyurea) were released into fresh 37ºC medium containing nocodazole and 100 μM Latrunculin B, the latter to poison actin-assembly reactions. Rhodamine phalloidin staining confirmed the loss of the actin cytoskeleton. As expected, telomeres in wild-type cells remained tightly paired, such that all separated sisters (5%) remained closely apposed in the absence of both microtubules and actin. Telomeres in *mcd1-1* cells exhibited high separation frequencies both in the absence of either microtubules (93% of cells contained separated telomeres, 41% of these dramatically separated) or microtubules and actin (92% of cells contained separated telomeres, 52% of these dramatically separated). The sister-telomere separations observed here are consistent with prior studies revealing that interphase telomere dynamics are both microtubule and actin independent but diminished upon ATP depletion [12]. Finally, we tested whether Mps3p provided telomere anchor sites that promote sister-chromatid separation. Cohesion-defective *mps3-3* and *mps3-5* alleles were crossed into the telomere-cohesion assay strain harboring *ctf7-203* as the sole source of Ctf7p function. Cells harboring either *ctf7* or *ctf7 mps3* alleles were synchronized in G_1 and released into 37ºC fresh media containing nocodazole for 3 hr prior to quantifying distances between sister-telomere loci according to the criteria described above. Results from independent analyses show that sister telomeres separated to nearly identical distances in *ctf7* mutant cells regardless of Mps3p function (13.3 ± 6.4 for *ctf7* mutant cells versus 13.6 ± 8.3 for *ctf7 mps3* double mutant cells).

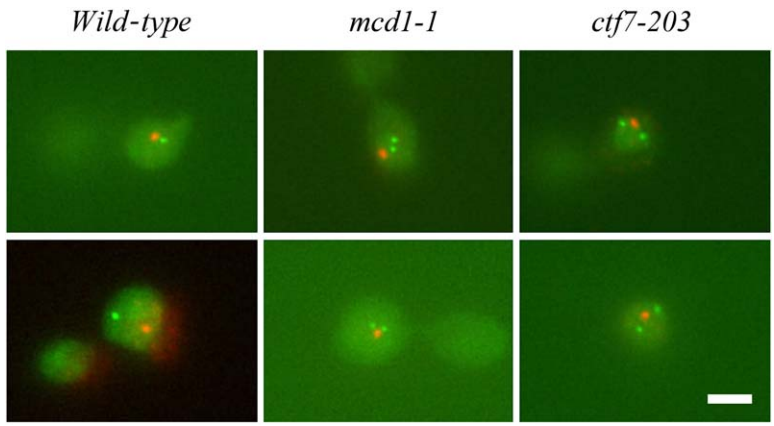

Figure 3. Spindle Pole Body Localization Compared to Telomere GFP Dots

Nocodazole-arrested mitotic wild-type, *mcd1*, and *ctf7* cells contain closely apposed spindle pole bodies (single red dots) that do not colocalize with separated sister telomeres (green). Scale bar equals 2 μm.

Here, we provide new evidence that cohesins are solely responsible for resisting sister-chromatid separation forces at telomeres. Our findings contrast those of other loci in which only moderate cohesion defects were obtained in cohesin-deficent cells [1–4]. In fact, cohesin-independent factors now appear to participate in sister pairing at those loci [5, 6]. The majority of those studies relied on either rDNA loci or centromere-proximal loci positioned no more than ~40 kb from centromeres. Given that telomeres can be 1100 kb away from the centromere, the frequency and spatial separation of telomeres observed here highlights the importance of assessing dynamics of individual loci along the entire chromosome length.

We further provide novel evidence that sister-chromatid telomeres, possibly via interactions with the nuclear envelope, experience a separation force that is resisted by cohesins until anaphase onset. Here, telomere-dependent separation was observable in budding yeast only in the absence of cohesins and spindle microtubules. Whether sister-telomere motility in yeast is directional and, as such, can contribute to bona fide chromosome segregation remains untested. However, our findings are consistent with previous reports that find telomeres are dynamic and motile organelles that reproducibly cluster into 4–6 discrete foci in association with the nuclear envelope in a cell-cycle-dependent manner and redistribute along the nuclear periphery during meiosis [10–14]. Images of vertebrate cell kinetochores that lead chromosome movement to spindle poles during anaphase have dominated the field of chromosome segregation for more than 100 years. In response, a wealth of information now exists regarding kinetochore components, assembly, and contributions of both kinetochore and spindle microtubules to poleward-directed chromosome movement [18–20]. Much less is known regarding telomere-envelope interactions and their functions. It is tempting to speculate that, if correct, the telomere-nuclear envelope-based separation mechanism proposed here might be a remnant of some ancient prokaryotic membrane-linked chromosome-segregation system. It is also interesting to note that telomeres and nonkinetochore regions can lead poleward chromosome motion in some plant cells, suggesting that telomere motility may not be limited to the closed mitosis of yeast [21, 22] (see also http://www.bio.unc.edu/faculty/salmon/lab/mitosis/Bloodlily.mov, a time-lapse motion picture by A.S. Bajer and J. Mole-Jaber filmed between 1959 and 1962).

Supplemental Data

Supplemental Data include one table and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/9/902/DC1/.

Acknowledgments

For their generous gifts, the authors thank Drs. V. Guacci for *mcd1-1* mutant cells, D. Sherratt and I. Lau (provided by D. Koshland) for LacO arrays, and M. Winey and S. Jaspersen for the anti-Tub4p antibodies. The authors also thank Drs. Lynne Cassimeris, Alex Brands, Frank Luca, Linda Lowe-Krentz, and Meg Kenna and lab members Laura Loder, Marie Maradeo, and Jill McCane for stimulating conversation and comments on this manuscript. This research was supported by an award to R.V.S. from the National Science Foundation (MCB-0212323). Any opinions, conclusions, or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

Received: September 26, 2005
Revised: February 6, 2006
Accepted: March 8, 2006
Published: May 8, 2006

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