Eukaryotic chromosome segregation is an essential component of the cell division process, ensuring equal partitionings of duplicated genetic material to daughter cells. Canonical, conserved, and well-studied molecular mechanisms ensure accurate segregation of chromosomes for most genomic regions. Briefly, following duplication of chromosomes, replicated sister chromatids are held together by a ring-shaped complex of cohesin proteins (20). Duplicated sisters aligned along their lengths are axially compacted by the condensin complex into discrete, rod-shaped chromosomes. This process of chromosome assembly, a cytologically striking transformation of chromatin structure, converts an amorphous mass of chromatin into condensed units that can be faithfully segregated during mitosis (22). At anaphase onset, the protease separase promotes removal of cohesin proteins tethering bioriented sisters (6), thus allowing the spindle apparatus to pull apart replicated chromatids (48).

While the above-described mechanisms segregate most genomic regions, recent work with several systems, especially *Saccharomyces cerevisiae*, implicates specialized mechanisms for the segregation of repeat-rich regions, such as nucleolar ribosomal DNA (rDNA) and telomeric DNA at linear chromosome ends (36). These regions remain connected until mid-anaphase, at which point specialized mechanisms drive their segregation. Distinct segregation mechanisms are warranted for rDNA and telomere regions, given that both are comprised of highly repetitive sequences with increased potential for recombination (4, 37). In *S. cerevisiae*, such a mechanism is facilitated by the FEAR (Cde fourteen early anaphase release) pathway (43, 44). Pathway components, including the phosphatase Cdc14p and the enzyme separase, recruit condensins to rDNA regions of nucleoli (9, 50). This sets the stage for a unique, condensation-based mechanism, acting independently of the mitotic spindle, which drives rDNA segregation by "unzipping" the rDNA locus from its centromere-proximal region to its centromere-distal region (26). This function of condensins is separate from their more traditional function in chromosome compaction in preparation for mitosis. Although much less work has focused on telomere segregation, conditional *cdc14* mutants fail to segregate both rDNA and telomeres, indicating that specialized segregation mechanisms target both regions (9). Specialized condensation may promote aspects of telomere dynamics, including their segregation, given reports of condensin binding at telomeres of budding yeast (49). Although *Schizosaccharomyces pombe* does not contain a FEAR pathway resembling that of budding yeast (5), specialized mechanisms may also promote segregation of repeat-rich regions in this divergent yeast. Intriguingly, certain mutations of the fission yeast *cut1*+ gene, which encodes *S. pombe* separase, produce a phenotype in which most DNA regions, but not telomeres, segregate during anaphase (16). This phenotype is very similar to that of conditional *cdc14* mutants in budding yeast as described above, perhaps indicating distantly related pathways for telomere segregation in these two divergent yeasts.

Data from more complex eukaryotic systems also point to a specialized telomere segregation system. In *Drosophila melanogaster*, mutations in the *SMC4/gluon* locus, which encodes the conserved condensin subunit SMC4p, cause telomeric chromatin bridges (40). In human cells expressing mutant forms of

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**Ccq1p and the Condensin Proteins Cut3p and Cut14p Prevent Telomere Entanglements in the Fission Yeast**

Schizosaccharomyces pombe†‡

Tina Motwani, Rosemarie Doris, Scott G. Holmes,* and Mark R. Flory#

Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06459

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The Schizosaccharomyces pombe telomere-associated protein Ccq1p has previously been shown to participate in telomerase recruitment, heterochromatin formation, and suppression of checkpoint activation. Here we characterize a critical role for Ccq1p in mitotic transit. We show that mitotic cells lacking Ccq1p lose minichromosomes at high frequencies but that conditional knockdown of Ccq1p expression results in telomere bridging within one cell cycle. Elevating Ccq1p expression resolves the telomere entanglements caused by decreased Taz1p activity. Ccq1p affects telomere resolution in the absence of changes in telomere size, indicating a role for Ccq1p that is independent of telomere length regulation. Using affinity purification, we identify the condensin proteins Cut3p and Cut14p as candidate Ccq1p interactors in this activity. Condensin loss-of-function disrupts Ccq1p telomeric localization and normal intertelomere clustering, while condensin overexpression relieves the chromosome segregation defects associated with conditional Ccq1p knockdown. These data suggest that Ccq1p and condensins collaborate to mediate resolution of telomeres in mitosis and regulate intertelomere clustering during interphase.

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*Corresponding author. Mailing address: Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT 06459. Phone: (860) 685-3557. Fax: (860) 685-2141. E-mail: sholmes@wesleyan.edu.
† Present address: Mendel Biotechnology, Inc., Hayward, CA 94545-3720.
‡ Supplemental material for this article may be found at http://ec.asm.org/.
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the ADP-ribosylase tankyrase, the majority of genomic material separates during mitosis, but telomeres remain joined by apparent proteinaceous bridges (11). Notably, telomere-mediated anaphase bridges in colorectal cancers appear to serve as a primary facilitator of the physical and numerical changes in chromosomes associated with genomic instability (41). Thus, in addition to revealing a fundamental aspect of chromosome segregation, molecular dissection of telomere segregation mechanisms has the potential to shed light on pathways of tumorigenesis.

In this report, we provide evidence that a telomeric protein in fission yeast, Ccq1p, plays a critical and unique role in chromosome segregation. Ccq1p was initially identified through quantitative proteomic analysis of affinity-enriched tumorigenesis. We also show that these proteins are critical for normal intertelomeric clustering interactions during mitotic interphase.

MATERIALS AND METHODS

Media and strains. Schizosaccharomyces pombe was grown with supplements, as appropriate, in rich yeast extract medium with supplements (YES) or in synthetic Edinburgh minimal medium (EMM), as described previously (14). For routine plasmid shuttling and molecular cloning methods, Escherichia coli cells of strain DH5α (Invitrogen, Carlsbad, CA) were grown and transformed according to the manufacturer's recommendations.

Fission yeast strains used in this study are listed in Table 1. Fission yeast chromosome manipulations were done using PCR-mediated gene-tagging methods and reagents (1, 39). Creation of a cut3Δ strain 99 and digested with restriction enzymes BamHI and NotI (all restriction enzymes were from New England Biolabs, Beverly, MA). The resulting fragment was inserted into the BamHI and NotI sites of the S. pombe expression plasmid pREP-NTAP (19), creating pREP-NTAP-ccq1+. The cut3Δ and the cut14Δ open reading frames were amplified, and similar strategies were used to introduce digested PCR products into the unique AscI and SacII sites of pREP-NTAP, resulting in plasmids pREP-NTAP-cut3Δ and pREP-NTAP-cut14Δ, respectively.

Microscopy. For analysis of cell length and DNA morphology, cells were collected at mid-logarithmic growth phase and fixed with formaldehyde (3.7%, cold-sensitive strain MFP131 (permissive, 30°C; restrictive temperature, 37°C) and the temperature-sensitive strains FY8026 and FY8033 (38) (permissive temperature, 25°C; restrictive temperature, 37°C) and the cut14Δ cold-sensitive strain MFP131 (permissive, 30°C; restrictive, 20°C).

Plasmids. A genomic fragment containing the ccq1+ open reading frame was amplified by PCR from wild-type S. pombe strain 99 and digested with restriction enzymes BamHI and NotI (all restriction enzymes were from New England Biolabs, Beverly, MA). The resulting fragment was inserted into the BamHI and NotI sites of the S. pombe expression plasmid pREP-NTAP (19), creating pREP-NTAP-ccq1+. The cut3Δ and the cut14Δ open reading frames were amplified, and similar strategies were used to introduce digested PCR products into the unique AscI and SacII sites of pREP-NTAP, resulting in plasmids pREP-NTAP-cut3Δ and pREP-NTAP-cut14Δ, respectively.

Microscopy. For analysis of cell length and DNA morphology, cells were collected at mid-logarithmic growth phase and fixed with formaldehyde (3.7%, wt/vol) and their DNA was stained with 1 μg/ml 4,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma Chemical Co.) in phosphate-buffered saline (PBS). Cells were washed with PBS and mounted on polylysine-coated coverslips in Citifluor glycerol (Ted Pella Inc., Redding, CA) for analysis by fluorescence microscopy. The frequency of chromosomal bridge events was assessed independently by two individuals, yielding essentially identical results. For analysis of green fluorescent protein (GFP) and monomeric red fluorescent protein (mRFP) localization patterns in live cells, cells from an overnight streak or from a culture at mid-logarithmic phase were suspended in 2 μl sterile water on a
microscope slide, onto which a coverslip was placed. Light and fluorescence microscopy was done using a Zeiss Axioplan epifluorescence microscope (Zeiss Plan Apochromat with a 100× oil immersion objective) and a monochrome AxiosCam HRm digital camera (Carl Zeiss MicroImaging Inc., Thornwood, NY). Cell length measurements of fixed cells and quantitative measurements of GFP and mRFP signal intensity in live cells were done using Axiovision digital imaging software (Carl Zeiss MicroImaging Inc., Thornwood, NY). Cell length was defined as the tip-to-tip distance along the long axis of cells. For fluorescence intensity measurements, pixel intensities in matching regions of interest (ROI) for the GFP and mRFP channels were averaged for each cell. A background correction corresponding to the average pixel intensity in a similarly sized ROI from a peripheral cellular region was applied to each measurement. At least 100 cells for cell length measurements, or 100 foci, were assayed at each condition or time point. Images were processed in Adobe Photoshop (Adobe, San Jose, CA) and cell length measurements analyzed in Microsoft Excel (Microsoft, Redmond, WA).

Chromosome loss and telomere length assays. Linear minichromosome loss analysis was performed as previously described (28). A minimum of 100 well-separated colonies were examined for the presence of pink or red sectors for each strain. Southern blotting of telomere length with digoxigenin-labeled PTELPROBE oligonucleotide probe has been described previously (14).

Immunoaffinity purification and mass spectrometry. Four- to eight-liter samples of appropriate strains were cultured to late logarithmic growth phase in YES liquid medium. Cells were collected by centrifugation, and pellets were either processed immediately or stored at −80°C. Cell pellets, resuspended in NP-40 buffer containing protease inhibitors as described above, were subjected to glass bead lysis in a bead beater instrument (19). Protein complexes were isolated on IgG-Sepharose 6 fast-flow resin (GE Healthcare, Piscataway, NJ) for Ccq1p-tandem-affinity protein (TAP) or anti-hemagglutinin (HA) Affinity Matrix (clone 3F10; Roche Diagnostics Corporation, Indianapolis, IN) for 3X/HA-tagged Ccq1p for 1 h at 4°C with rocking. Complexes were washed three times with lysis buffer, eluted in 1.2% SDS, 50 mM Tris (pH 8.3), and precipitated with trichloroacetic acid (TCA; Sigma Chemical Co.). TCA pellets were washed in acetone, lyophilized completely, and resuspended in 10 μl 8 M urea. Following a 10-fold addition of 20 μM Tris-HCl, pH 8.3, 5 mM EDTA, complexes were digested with 20 ng/μl sequencing-grade trypsin (V5113; Promega Corporation, Madison, WI).

Resulting peptides were then analyzed by reversed-phase electrospray ionization tandem mass spectrometry (RP-ESI-MS/MS) essentially as previously described (23) on an LCO Deca XP ion trap instrument fitted with a packed-tip column source for nanospraying (Thermo Fisher Scientific, Waltham, MA) and coupled to an HP 1100 binary pump system and degasser (Agilent Technologies, Santa Clara, CA). Three collision-induced dissociation (CID) scans followed each survey scan, and the S. pombe proteome was searched using SEQUEST software (12) embedded in the Bioworks (Thermo Fisher Scientific, Waltham, MA) package. Analysis was done in duplicate for each strain, including strains 99 (wild type) and MFP 102 (ccq1Δ), which served as controls for nonspecific protein binding.

RESULTS

Cells lacking Ccq1p exhibit dramatic loss of marked minichromosomes. We previously observed a cell cycle delay in cells lacking the Ccq1 protein. To determine if this delay was caused by defects in chromosome transmission, we analyzed linear minichromosome loss frequencies using strains containing ade6-marked linear minichromosome derivatives (28). We found an extraordinarily high frequency of loss; approximately 86% of ccq1Δ colonies (strain MFP115) were pink or red, indicative of loss of the ade6-marked minichromosome (Fig. 1), while only ~1% colonies were red/pink in the wild-type background (strain HM248). This loss rate was much higher than that associated with the deletion of genes coding for the telomere-associated Taz1 (strain MFP116) (Fig. 1) or Pku70 (strain MFP117) (data not shown) protein. We considered the hypothesis that the striking chromosome loss rate specifically associated with ccq1Δ results from deleterious intertelomeric recombination-dependent repair (RDR) initiated to restore telomere length (13). However, we noted that red sectoring colonies were immediately apparent, even after the first replica platings for integrant selection (data not shown), when the ccq1Δ deletion was introduced in the linear minichromosome loss strain background to create strain MFP115. This is inconsistent with the phenotypic lag normally required before telomere length defects become apparent (25), suggesting that another mechanism causes the primary ccq1Δ loss-of-function (LOF) phenotype.

Rapid, primary ccq1Δ mitotic defects occur independently of telomere shortening. To more specifically pinpoint the primary defects associated with ccq1Δ loss of function, we created and analyzed a ccq1Δ promoter repression strain. We used PCR-mediated gene tagging (1) to introduce the attenuated nmt1(81°) (no message in thiamine) thiamine-repressible promoter and GFP (green fluorescent protein) immediately upstream of the ccq1Δ ORF at the endogenous ccq1Δ chromosomal locus. The GFP moiety, while not affecting Ccq1p function (14), allowed us to assess Ccq1p levels via fluorescence microscopy and anti-GFP Western blotting. When cells of the resulting strain, MFP122, were grown in medium lacking thiamine, the level of GFP-Ccq1p driven by nmt1(81°) was sufficient to allow cells to divide at normal lengths and with efficient chromosome segregation indistinguishable from that of strain 99 wild-type cells. Upon addition of thiamine to the medium, Western blotting revealed that Ccq1 protein levels were reduced approximately 5-fold at 9 h after thiamine-induced promoter repression (Fig. 2A). This decrease in Ccq1 protein was accompanied by a striking increase in cell length observable as early as the 6-hour time point (T6) following thiamine addition (Fig. 2C). An increase in linear cell length, easily discernible given the rod-like shape of S. pombe, is a classic indicator of cell cycle delay in this yeast (35). The effect becomes more prominent at 9 h (Fig. 2C). Decreasing Ccq1p levels were also associated with a significant increase in the fraction of cells undergoing cell division, suggesting that cells may have experienced delayed transit through mitosis (Fig. 2C).

In many of the cells expressing low levels of Ccq1p, we also observed cells with continuous DNA staining between segre-
gating nuclei (Fig. 2B and E). The presence of one or more clearly intact DAPI-stained chromatin strands connecting separating mitotic nuclei has been defined as an anaphase bridge (2, 27, 30). Anaphase bridges are associated with dicentric chromosomes (2), which can result from abnormal telomere-telomere interactions (see, e.g., reference 30). Applying this definition, we determined the frequency of cells exhibiting anaphase bridges under conditions of limiting Ccq1p. By the criteria that we applied, the T6 and T9 cells shown in Fig. 2B and the T9 cells in the left and middle panels of Fig. 2E exhibit anaphase bridges, while the other cells in these figures do not. Examining populations of cells at each time point, we found a significant increase in the frequency of anaphase bridges as a consequence of decreased Ccq1p levels (Fig. 2B). Analysis of telomere length via Southern blotting at T0 versus T9 using methods previously described (14) revealed telomere lengths indistinguishable from those of wild-type cells (Fig. 2D). These data indicated that a rapid, primary defect in chromosome segregation is associated with the loss of Ccq1p and that the phenotypes that we observed were independent of telomere shortening.

Ccq1p loss of function causes telomeric anaphase bridging as a primary defect. To analyze telomere position following ccq1 promoter repression, we engineered an nmt1(81)-HA-ccq1 allele in strain FY15617 (10), containing LacI-GFP bound to chromosome I telomere-proximal lac operator sites. Anal-

FIG. 2. Loss-of-function ccq1 characterization via promoter repression analyses. (A) Anti-GFP Western blotting of GFP-Ccq1p from normalized protein extracts of strain MFP122 collected at time points 0, 3, 6, and 9 h following promoter repression, indicating a gradual, ~5-fold decrease in protein concentration. (B) Representative cells from 0-, 6- and 9-hour time points following nmt1(81)-GFP-ccq1+ promoter repression in strain MFP122; DNA stained with DAPI and white cell outlines based on corresponding phase images were added for reference. Bar, 5 μm (applies to all images of panel B). (C) Quantification of cell length, cell division, and chromosome bridging events following ccq1 promoter repression. The graph indicates the percentages of nmt1(81)-GFP-ccq1+ MFP122 cells at time points 0, 3, 6, and 9 h following promoter repression with long-axis lengths greater than the median length (10.36 μm) (white bars), the percentage of cells undergoing cell division at each time point (grey bars), and the percentages of cells containing bridging chromosomal DNA (black bars); at least 100 cells were assessed for each time point. Differences in cell length and frequency of bridged-chromosome figures at T3, T6, and T9 versus those at T0 were found to be highly significant (P < 0.001, pairwise chi-square analysis), except with the T3 bridged-chromosome count, which difference was moderately significant (P < 0.02). Differences between T0 cell lengths and those of a ccq1+/GFP control strain, MFP104 (in which expression is controlled by an endogenous ccq1+ promoter [14]), were not significantly different (0.1 < P < 0.5), as expected. (D) Analysis of telomere length by Southern blotting in haploid strains containing chromosomal ccq1+ (WT, strain 99), ccq1Δ (MFP102), and nmt1(81)-GFP-ccq1+ (MFP122) prior to repression (T0) and 9 h postrepression (T9). Telomere lengths are as indicated. (E) Representative cells from the 0 h (all T0) and 9 h (all T9) time points following nmt1(81)-HA-ccq1+ promoter repression in strain MFP126 are shown. (Top row) DAPI-stained images of cells exhibiting segregating DNA masses and/or anaphase bridges; (bottom row) position of the chromosome I telomere (visualized using LacI-GFP and LacO-sod2) in cells exhibiting segregating DNA masses and/or in anaphase bridged cells. Bar, 2.5 μm (applies to all images of panel E).
ysis of cells in the resulting strain, MFP126, cultured in the presence of thiamine for 9 h revealed one or two telomere foci at the center of bridged chromosomes in all cases (28 bridges) (Fig. 2E, T9, left and center panels). In rare cells exhibiting chromosome separation, telomere spots were found at the trailing edges of segregating masses, as expected for late-segregating regions (Fig. 2E, T9). When telomere location was examined in T0 cells undergoing mitosis, we observed no bias in position (Fig. 2E, left panels). These data suggested that abnormally low levels of Ccq1p impair the effective resolution of telomeric end regions. The rapid kinetics of telomere bridge formation suggested a role for Ccq1p in actively segregating telomeres and/or prevention of deleterious telomeric entanglements prior to anaphase transit.

Ccq1p overexpression promotes resolution of abnormally elongated telomeres. To further examine whether Ccq1p specifically affected telomere resolution, we tested whether increased expression of ccq1+ is sufficient to resolve hyper-elongated telomeres prone to entanglement. Cells lacking Taz1p, a highly conserved telomere-binding protein, exhibit massively elongated telomeres; when grown at 20°C, these cells exhibit cell lengthening and telomeric entanglements (31). We hypothesized that increased levels of Ccq1p might compensate for these abnormally elongated telomeres by alleviating their entanglements, thus allowing mitotic chromosome segregation. To test this, we used the pREP-NTAP expression vector (45) to express a functional N-terminal fusion of the tandem-affinity protein tag (N-TAP) to Ccq1p under the control of the full-strength (nonattenuated) nmt1 promoter in taz1Δ MFP131 cells. We found that overexpression of N-TAP–Ccq1p in taz1Δ cells at 20°C diminishes the number of elongated cells stalled in mitosis and reduces the frequency of telomere entanglements (Fig. 3A). This effect was seen with both moderate and high N-TAP–ccq1+ overexpression, as controlled by adjusting the thiamine level in the medium (15) but not in control taz1Δ MFP131 cells treated identically but that express N-TAP alone (Fig. 3A). In Ccq1p-rescued taz1Δ cells, cells transiting anaphase frequently exhibited tail-like extensions at the trailing end of segregating chromosomal masses. DNA “tails” (asterisks) possibly indicate elongated telomeres trailing main chromosomal masses. White cell outlines based on corresponding phase images are added for reference. Bar, 5 μm (applies to both images of panel B).

Analysis of telomere length by Southern blotting in haploid strains containing the chromosomal taz1+ (control), NTAP, and N-TAP-ccq1+ expression plasmid in the wild-type (strain 99) and taz1Δ (MFP 131) backgrounds.

**FIG. 3.** Overexpression of Ccq1p prevents telomeric entanglements associated with taz1Δ at 20°C. (A) Percentages of cells with long-axis lengths greater than the median taz1Δ strain cell length (14.11 μm) (white bars) and percentages of cells containing bridged chromosomal DNA (black bars). OL, Ccq1p overexpressed at relatively low levels in defined medium containing thiamine; OH, Ccq1p overexpressed at relatively high levels in medium lacking thiamine (15). At least 100 cells were assessed for each strain. Differences in cell length and frequency of bridged-chromosome figures in taz1Δ cells containing the pREP-NTAP vector versus in taz1Δ cells transformed with pREP-NTAP-ccq1+ were found to be highly statistically significant (P < 0.001, pairwise chi-square analysis). (B, left) Representative MFP131 (taz1Δ) cells transformed with the pREP-NTAP empty vector, exhibiting dramatically increased cell length and chromosomal bridges at mid-logarithmic phase during culture at 20°C as previously reported (31). (Right) Representative MFP131 (taz1Δ) cells transformed with pREP-NTAP-ccq1+ exhibit reduced cell length at division and segregating DNA masses. DNA “tails” (asterisks) possibly indicate elongated telomeres trailing main chromosomal masses. White cell outlines based on corresponding phase images are added for reference. Bar, 5 μm (applies to both images of panel B). (C) Analysis of telomere length by Southern blotting in haploid strains containing the chromosomal taz1+ (control), NTAP, and N-TAP-ccq1+ expression plasmid in the wild-type (strain 99) and taz1Δ (MFP 131) backgrounds.
TABLE 2. Proteins identified by affinity purification with MS/MS*

| Protein       | No. of peptides | % coverage | Score  |
|---------------|-----------------|------------|--------|
| Ccq1p         | 13              | 18.23      | 260.3  |
| Cut3p         | 28              | 17.38      | 268.4  |
| Cut14p        | 30              | 23.21      | 168.2  |

* Proteomic identifications from an RP-ESI-MS/MS analysis of fission yeast proteins affinity enriched with 3×HA-Ccq1p and Ccq1p-ProA. The “no. of peptides” column indicates the number of peptides identified for each protein, and “% coverage” indicates protein coverage by number of amino acids. “Score” was calculated using the TurboSequest program (ThermoElectron); values greater than 150 were considered significant. Ccq1p, Cut3p, and Cut14p were not identified in control experiments conducted with an untagged strain (strain 99) or in a strain lacking the ccq1+ gene (strain MFP102).

As a first test of the possibility that fission yeast condensins function in telomere segregation, we explored whether condensin overexpression is capable of suppressing the chromosome segregation defects associated with decreased ccq1 expression. Specifically, we tested whether condensin (Cut3p or Cut14p) overexpressed from plasmids pREP-NTAP-cut3+ and pREP-NTAP-cut14+ can reduce cell elongation and/or chromosome bridging in our ccq1 promoter repression background under repressive conditions. We first cloned the cut3+ and cut14+ coding regions in-frame into expression plasmid pREP-NTAP (19), which allows for high-level expression of condensin subunits from the unattenuated nmt1 promoter. The resulting plasmids, pREP-NTAP-cut3+ and pREP-NTAP-cut14+, rescued the temperature-sensitive cut3-477 and cut14-208 strains, respectively, as expected (data not shown), indicating the functionality of these plasmids. Strain MFP122 [nmt1(81)-GFP-ccq1+] was then transformed with the pREP-NTAP-cut3+ and pREP-NTAP-cut14+ overexpression plasmids. The empty vector pREP-NTAP and pREP-NTAP-ccq1+ were used as negative and positive controls, respectively. We analyzed the four resulting transformant lines for the presence of anaphase bridges following nmt1(81)-GFP-ccq1+ promoter repression for 9 h. As expected, repressed nmt1(81)-GFP-ccq1+ cells transformed with the pREP-NTAP empty vector exhibited frequent anaphase bridges (Fig. 4B, left panel). In contrast, cells transformed with pREP-NTAP-cut3+ or pREP-NTAP-cut14+ exhibited an 80% decrease in bridge frequency (Fig. 4A and B; at least 200 cells were analyzed per transformant line). The level of rescue with overexpressed condensins is comparable to that achieved with overexpressed Ccq1p (Fig. 4A and B).

Cut3p and Cut14p regulate Ccq1’s association with telomeres. Ccq1p and the condensin proteins share the ability to resolve telomere entanglements to aid mitotic segregation. Our mass spectrometry data suggest that they may be acting in a complex to achieve this. In support of this possibility, condensins are known to bind to telomere regions in budding yeast (49). However, our attempts to demonstrate protein-protein interactions between Ccq1p and Cut3p or Cut14p via coimmunoprecipitation experiments or two-hybrid assays have thus far yielded negative results (data not shown). To further examine possible functional interactions between Ccq1p, Cut3p, and Cut14p, we tested the dependence of Ccq1p telomeric localization on condensin complex integrity. To achieve this, we used the previously described temperature-sensitive condensin alleles cut3-477 and cut14-208 (38). Specifically, we created strains MFP127 and MFP128, expressing Ccq1p-GFP and Taz1p-mRFP and containing cut3-477 and cut14-208, respectively. At a permissive temperature (25°C), both Ccq1p-GFP and Taz1p-mRFP exhibited wild-type localization in these strains (Fig. 5E and F). However, after a shift to the restrictive temperature (37°C) for 4 h, the Taz1p-mRFP signal remained robust but the Ccq1p-GFP signal became much less intense, with diffuse and in many cases undetectable Ccq1p-GFP signals (Fig. 5G and H). Quantitative analysis of the punctate (telomeric) Ccq1p-GFP signal, normalized to the telomeric Taz1p-mRFP signal intensity, revealed a 90-fold drop in intensity in the cut14-208 background (100 cells) (representative cells are shown in Fig. 5H) and a 30-fold drop for the cut3-477 background (100 cells) (not shown) at 37°C (four hours) versus 25°C. The greater effect in the cut14-208 background is likely
due to the relative severity of this allele at 37°C in comparison to cut3-477 (38). Intensity measurements of similarly marked telomeres in a cut3+/cut14+ control strain, MFP130, showed no reduction in signal intensity for either component upon shift of growth temperature from 25°C (Fig. 5A and B) to 37°C (Fig. 5C and D) for 4 h.

**Ccq1p and condensin are required for normal interphase telomere clustering.** In analyzing Ccq1p-GFP and Taz1p-mRFP spot intensity in conditional condensin backgrounds as described above, we also observed in some cells an increase in the number of detectable Taz1p-mRFP foci in MFP127 (cut3-477) and MFP128 (cut14-208) cells at the 4-hour 37°C time point. This suggested the possibility that condensin disruption causes a defect in the telomeric clustering that is known to occur during vegetative growth of *S. pombe*. In fission yeast, telomeric ends cluster at the nuclear envelope during interphase while remaining apart from centromeres. As a result of telomere clustering, a vast majority of fission yeast cells exhibit only one or two visible telomeric foci during normal vegetative growth (16). In contrast, in condensin loss-of-function backgrounds, we observed a significant increase in cells containing 4 to 5 Taz1p-mRFP foci in single-plane images (7%, 351 cells for cut14-208; 6%, 516 cells for cut3-477) (Fig. 5i, graph) and a notable percentage of cells with three detectable Taz1p-mRFP foci in single-plane images (13%, 351 cells for cut14-208; 11%, 516 cells for cut3-477) (Fig. 5, graph). These cells exhibited faint or undetectable Ccq1p-GFP foci, as described earlier (Fig. 5j; see also Fig. 5k). At the permissive temperature, these same strains showed normal numbers of Taz1p-mRFP telomeric foci (0% with 4 to 5 foci, 1% with 3 foci, 159 cells for cut14-208; 0% with 4 to 5 foci, 5% with 3 foci, 206 cells for cut3-477), results very similar to those with a taz1-mRFP ccq1-GFP control strain (MFP130) in which the ccq1-GFP and taz1-mRFP alleles are expressed from their endogenous gene promoters (0% with 4 to 5 foci, 1% with 3 foci, 346 cells). These data indicated that the condensin subunits Cut3p and Cut14p are required for telomeric clustering.

In light of this result, we also examined the number of detectable Taz1p-mRFP foci in our nmt1(81)-GFP-ccq1+ promoter repression background using strain MFP129. While the effect was less pronounced than that in the condensin backgrounds (no cells observed with 4 to 5 foci), we did see a significant increase in the number of cells exhibiting three Taz1p-mRFP foci at the 9-hour/postrepression time point (8%, 121 cells) (Fig. 5k, graph) versus the 0-hour/prerepression time point (1%, 166 cells). As expected, Ccq1p-GFP foci at 9 h after promoter repression were faint or undetectable (Fig. 5l). Thus, a decrease in Ccq1p or condensin function leads to similar clustering defects.

**DISCUSSION**

*Ccq1p is required for proper resolution of telomeres.* In this study, we present evidence for a telomere segregation system in fission yeast dependent upon the telomere-associated protein Ccq1p. Ccq1p was previously identified as a fission yeast protein that associated with telomeres during both the meiotic and mitotic phases of growth (14). In addition to having a role in recruiting telomerase, Ccq1p has been shown to function in a redundant manner with the Poz1 protein in the Pot1p complex to prevent end-to-end chromosome fusions (32), at least in part by suppressing signaling to checkpoint pathways (33, 46). By study of telomere length in strains lacking Ccq1p and the recombination factors Rhp51p and Rhp55p, it was suggested that Ccq1p had an additional, unique function in suppressing homologous recombination (32). Here we extend previous analyses of the mitotic ccq1Δ gene deletion phenotypes by demonstrating high rates of minichromosome loss in *S. pombe* cells lacking Ccq1p. The relatively higher rate of minichromosome loss for ccq1Δ than the rate resulting from deletion of other key telomere component genes, such as *taz1* and *pku70*+, suggests a specific function for Ccq1p in promoting chromosome segregation.

Given Ccq1’s role in recruiting telomerase (32, 46), this segregation defect could have been an indirect effect of telomere attrition. However, using an *nmt1(81)-GFP-ccq1*+ promoter repression assay, we demonstrate that Ccq1p-deficient cells exhibit mitotic defects within one cell cycle; these defects...
include cell elongation and the formation of anaphase bridges and occur prior to any measurable changes in telomere length. The rapid appearance of these defects indicates a function distinct from that of classical telomeric capping components, whose removal generally requires multiple cell cycles of telomeric attrition and failed recombination-dependent repair to exhibit chromosome segregation defects. A telomere resolution activity for Ccq1p is further suggested by our finding that increased Ccq1p levels suppress telomeric bridging in cold-sensitive taz1/H9004 cells, known to possess abnormally elongated telomeres that become entangled at the restrictive temperature (31). Again, this resolution is manifested without altering telomere length in these cells.

Ccq1p and condensin subunits promote proper intertelomeric clustering and telomere resolution. The predicted Ccq1p protein structure, which includes a coiled-coil sequence highly homologous to that found in SMC (structural maintenance of chromosome) domain proteins (24), suggested that Ccq1p participates in protein-protein interactions (14). We therefore employed affinity pulldown assays and mass spectrometry to identify Ccq1p binding partners. This effort yielded the condensin subunits Cut3p and Cut14p, two SMC domain proteins. These condensin subunits promote chromosome compaction and segregation (38) but have not previously been associated with telomere function. As with the phenotypes we observed with the Ccq1 protein, we find that elevated expres-

FIG. 5. Disruption of condensin function causes loss of Ccq1p from telomeric foci and declustering of Taz1p-marked telomeres. (A to D) Control (MFP130) cells containing integrated taz1-mRFP and ccq1-GFP alleles were cultured at a permissive temperature (25°C) and for 4 h at a nonpermissive temperature (37°C), when both Taz1p-mRFP and Ccq1p-GFP foci were clearly visible. (E, F) Temperature-sensitive cut14-208 (MFP128) cells containing integrated taz1-mRFP and ccq1-GFP alleles were cultured at a permissive temperature (25°C), when both Taz1p-mRFP and Ccq1p-GFP foci were clearly visible. (G, H) Following 4 h at 37°C, Taz1p-mRFP spots remained bright and focused, whereas Ccq1p-GFP foci became less distinct, diffuse, and in some cases undetectable. Similar results were obtained with the cut3-477 background (MFP127 [data not shown]). White cell outlines based on corresponding phase images were added for reference. Bar, 5 μm (applies to images in panels A to H). (I, J) An increase in the number of visible Taz1p-mRFP foci was detected in some MFP127 (cut3-477) and MFP128 (cut14-208) cells cultured at 37°C (cut14-208 background shown). (K, L) A similar, although less dramatic, increase in the number of Taz1p-mRFP foci was observed in strain MFP129 [nmt1 (81)-GFP-ccq1+] following promoter repression at the 9-h time point. Bar, 2.5 μm (applies to panels I to L). A graph of the frequency of Taz1p-mRFP focus numbers is shown. More than 100 cells were counted for each strain.
sion of Cut3p and Cut4p promotes resolution of telomeres in mitosis but that loss of condensin function delocalizes Ccq1p from telomeres and causes a defect in telomere clustering in interphase. As condensins have been shown to be involved in the resolution of repetitive sequences in other organisms (re-viewed in the introduction), our results are consistent with a model in which Ccq1p and condensins play an active and direct role in resolving telomere sequences in S. pombe. Such a function for Ccq1p and condensins at telomeres may be similar to the “clamped enzyme-DNA complex” telomere resolution activity associated with the cold-sensitive S. pombe topoisomerase II allele top2-191 (18). Similar to the results of Ccq1p and condensin overexpression (this report), top2-191 activation promotes removal of telomere entanglements associated with taz1Δ cells (31). This activity of the Top2-191 protein occurs independently of the classical catalytic decatenation mecha-nism associated with topoisomerases (18). Thus, telomere-as-soociated Ccq1p and condensins, like the unusual variant of Top2p encoded by top2-191, may similarly stabilize telomere structure in a way that permits telomeric entanglements to be removed.

Prior experiments conducted with S. pombe suggest that chromosome segregation and cytokinesis are not tightly coor-dinated, thus leading in specific cases to “cut” phenotypes, in which cell septation severs unsegregated DNA masses. Our preliminary observations suggest that the long-cell phenotype caused by loss of Ccq1 protein can be suppressed by elimination of the Rad1 checkpoint protein but not by elimination of Rad22p, suggesting that loss of Ccq1p triggers a Rad1p-mediated checkpoint signal (R. Doris and M. Flory, unpublished data). It will be interesting to test in further studies whether Ccq1p plays a role in coordinating chromosome segregation with cytokinesis.

In addition to its role in protecting telomeres from dele-tious recombination events, Ccq1p has a role in establishing the formation of heterochromatin at telomeres, at least in part due to its role in recruiting the SHREC gene-silencing complex (42). Could the functions of Ccq1p and condensins in resolving telomeres be connected to heterochromatin function? Interest-ingly, mutations in the RNAi pathway that disrupt hetero-chromatin at telomeres also have defects in telomere clustering but do not affect telomere length (21).

The challenge of faithfully replicating and segregating a long, highly repetitive telomere sequence is exacerbated by the organization of telomeres into clusters. Our studies of Ccq1p function indicate that the formation of entanglements is a frequent event that must be actively inhibited or resolved in most mitotic divisions. In accord with studies performed with other eukaryotes, we provide evidence that condensin proteins are required to mediate telomere resolution and that the Ccq1 protein has a central role in coordinating these activities.

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