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Cdc14 and Condensin Control the Dissolution of Cohesin-Independent Chromosome Linkages at Repeated DNA

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Summary

Chromosome segregation is triggered by the cleavage of cohesins by separase. Here we show that in budding yeast separation of the ribosomal DNA (rDNA) and telomeres also requires Cdc14, a protein phosphatase known for its role in mitotic exit. Cdc14 shares this role with the FEAR network, which activates Cdc14 during early anaphase, but not the mitotic exit network, which promotes Cdc14 activity during late anaphase. We further show that *CDC14* is necessary and sufficient to promote condensin enrichment at the rDNA locus and to trigger rDNA segregation in a condensin-dependent manner. We propose that Cdc14 released by the FEAR network mediates the partitioning of rDNA by facilitating the localization of condensin thereto. This dual role of the FEAR network in initiating mitotic exit and promoting chromosome segregation ensures that exit from mitosis is coupled to the completion of chromosome segregation.

Introduction

The mechanisms controlling chromosome segregation are conserved among eukaryotes (reviewed in Nasmyth, 2001). Chromosomes duplicated during DNA replication (sister chromatids) remain linked through protein complexes called cohesins. The dissolution of these linkages at the onset of anaphase is brought about by a protease known as separase (Esp1 in budding yeast), which cleaves a component in the cohesin complex (Scc1/Mcd1 in yeast). Owing to the irreversibility of this event, separase activity is tightly controlled. During S phase, G2, and metaphase, the protease is kept inactive by an inhibitory subunit securin (Pds1 in budding yeast). At the onset of anaphase, a ubiquitin ligase known as the anaphase promoting complex or cyclosome (APC/C-Cdc20) ubiquitinates securin thereby targeting it for degradation by the 26S proteasome (Nasmyth, 2001).

Cohesin deposition onto and removal from chromosomes are not the only events controlling chromosome segregation. A protein complex known as condensin is also required. Condensin consists of Smc2, Smc4, Ycs4, Ycg1, and Brn1 and promotes chromosome condensation in vitro and in vivo (reviewed in Hagstrom and Meyer, 2003; Haering and Nasmyth, 2003; Swedlow and Hirano, 2003). Interestingly, condensin localization is dynamic during the cell cycle. In budding yeast for example, the condensin subunits Ycs4 and Smc4 are uniformly distributed throughout the nucleus from G1 to metaphase (Freeman et al., 2000; Bhalla et al., 2002). As cells enter anaphase, condensin becomes highly enriched in the nucleolus (where the rDNA array resides) and at telomeres, both regions of repetitive DNA. Why and how condensin localization changes during the cell cycle is not understood.

Several proteins have been implicated in regulating chromosome condensation. In budding yeast and Sordaria, establishment and maintenance of chromosome condensation during prophase and metaphase require cohesin (Guacci et al., 1997; van Heemst et al., 1999; Lavoie et al., 2002). During anaphase, the Aurora B kinase Ipl1 is required for chromosome condensation and phosphorylation of the condensin subunit Ycg1 (Lavoie et al., 2004). The protein phosphatase Cdc14 is also important for chromosome condensation as rDNA condensation defects have been observed in *cdc14-1* mutants (Guacci et al., 1994).

After chromosome segregation has been completed, cells exit from mitosis and enter G1. In budding yeast, this transition is promoted by the protein phosphatase Cdc14. Cdc14 has many targets in the cell and promotes rapid exit from mitosis by dephosphorylating proteins phosphorylated by cyclin-dependent protein kinases (CDKs; reviewed in Bardin and Amon, 2001; Geymonat et al., 2002). Cdc14 itself is regulated by an inhibitory subunit Cfi1/Net1. Cfi1/Net1 sequesters and keeps Cdc14 inactive in the nucleolus during G1, S phase, and early mitosis but is released from the nucleolus during nuclear division. Two regulatory networks, the Cdc14 early anaphase release network (FEAR network) and the mitotic exit network (MEN) are required for the release of Cdc14 from its inhibitor during nuclear division (reviewed in Bardin and Amon, 2001; Geymonat et al., 2002). The FEAR network promotes Cdc14 release from the nucleolus during early anaphase, whereas the MEN promotes Cdc14 release and maintains Cdc14 in a released state during late stages of anaphase.

Here we show that Cdc14 released by the FEAR network during early anaphase is required for the completion of chromosome segregation. We find that the efficient segregation of telomeres and rDNA requires *CDC14*. Our data further suggest that Cdc14 released by the FEAR network mediates the segregation of the rDNA at least in part by facilitating condensin enrichment at this locus. These findings not only show that specialized chromosomal domains require mechanisms other than cohesin removal for their faithful segregation but also indicate that the FEAR network is a central regulator of anaphase. It promotes the segregation of late segregating genomic regions and simultaneously initiates exit from mitosis. In doing so, the FEAR network ensures that chromosome partitioning is completed before cells exit from mitosis.

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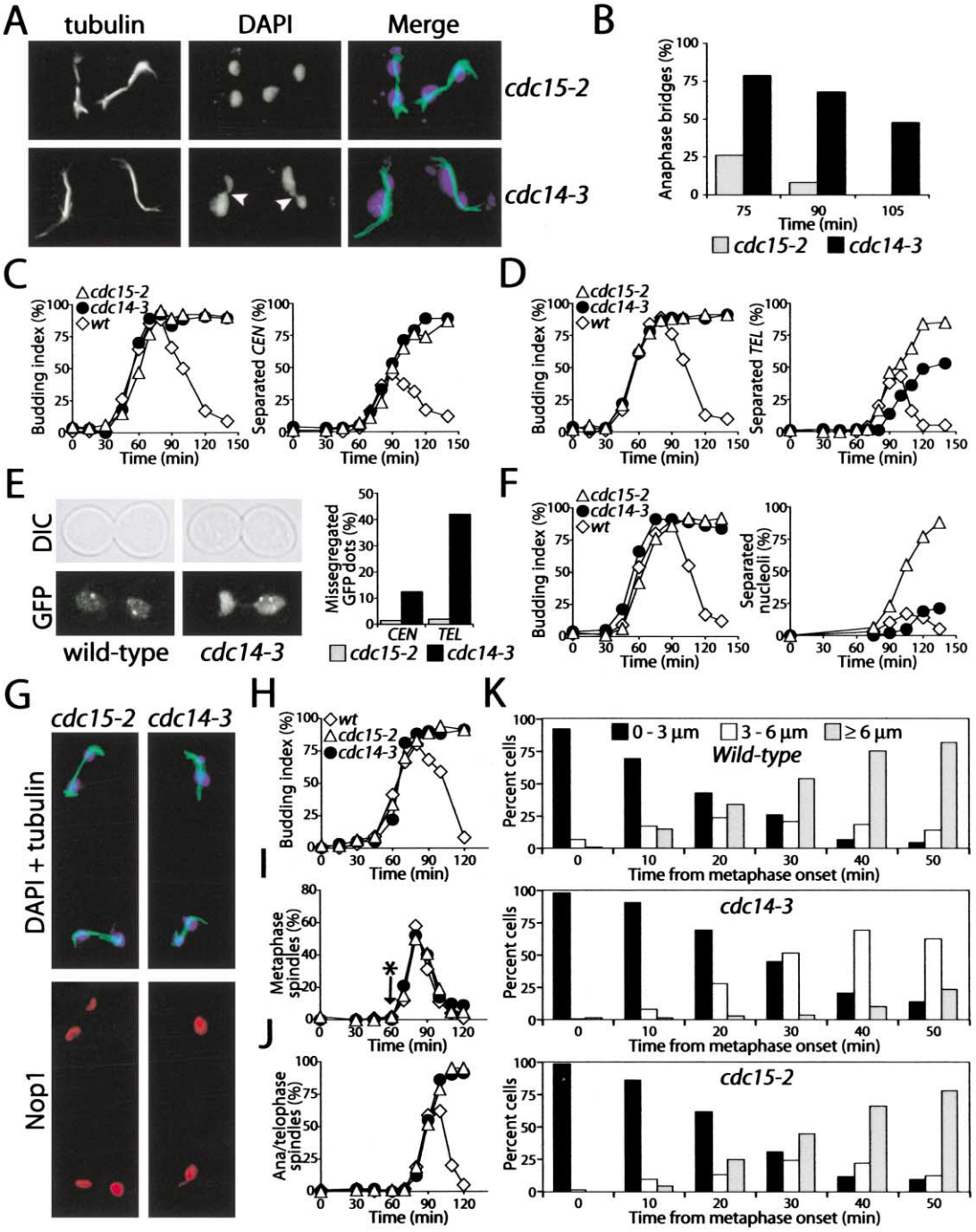

Figure 1. *CDC14* Is Required for the Efficient Segregation of Telomeres and the Nucleolus

(A and B) A794 (*cdc14-3*) and A340 (*cdc15-2*) cells were arrested in G1 in YEPD medium with α-factor (5 μg/ml) followed by release into fresh YEPD medium at 37°C. The percentage of cells with anaphase bridges (defined as DAPI-stained material residing between the nuclear lobes) was scored in anaphase and telophase cells (B, 75 min to 105 min past α-factor release). Micrographs showing representative fields of *cdc15-2* (top) and *cdc14-3* (bottom) cells progressing through anaphase are shown in (A) (75 min after release). Arrowheads show anaphase bridges in *cdc14-3* mutants.

(C) A5244 (wild-type), A5246 (*cdc14-3*) and A5248 (*cdc15-2*) cells carrying a tetO array at the centromere of chromosome IV were grown as described in (A) and the percentage of budded cells (left image) and of cells with two distinct tetR-GFP dots (right image) was determined at the indicated times.

(D) A5236 (wild-type), A5238 (*cdc14-3*), and A5240 (*cdc15-2*) cells carrying a tetO array at the telomere of chromosome V were released synchronously from a G1 arrest as described in (A). The percentage of budded cells (left image) and of cells with two distinct tetR-GFP dots (right image) were determined at the indicated times.

(E) The percentage of cells with separated CEN-GFP and TEL-GFP dots was scored for normal segregation (one dot per nuclear lobe; as

Results

cdc14-3 Mutants Are Defective in the Segregation of Telomeres and the Nucleolus

Cells carrying temperature-sensitive alleles in the MEN genes or *CDC14* arrest in anaphase with the bulk of DNA segregated. In contrast to *cdc15-2* mutants, however, chromosome bridges were observed during early anaphase in *cdc14-3* cells (Figures 1A and 1B). To determine whether specific regions of the genome were trailing during chromosome segregation in *cdc14-3* mutants, we integrated an array of tet operator (tetO) sequences 1.3 kb away from the centromere of chromosome IV (CEN-GFP dots; He et al., 2000) or 30 kb away from the telomere of chromosome V (TEL-GFP dots; Alexandru et al., 2001) and expressed a tet-repressor GFP (tetR-GFP) fusion allowing the detection of these chromosomal regions by fluorescence microscopy. In three independent experiments we observed that the segregation of TEL-GFP dots was delayed and occurred with a decreased efficiency in *cdc14-3* mutants, whereas separation of CEN-GFP dots occurred with wild-type kinetics (Figures 1C and 1D). Furthermore, instead of being present in each of the two nuclear lobes, 40 percent of separated TEL-GFP dots were found in the same nuclear lobe (Figure 1E), indicating that even after TEL-GFP dots separated they were not partitioned efficiently in *cdc14-3* mutants. Interestingly, this trailing of telomere V was not observed in the MEN mutant *cdc15-2*, which like *cdc14-3* mutants arrest in late anaphase (Figures 1C–1E).

cdc14-3 mutants were not only defective in the segregation of telomeric regions but also in partitioning of the rDNA array, as judged by staining of cells with antibodies against the nucleolar protein Nop1. Although the rDNA is located ~600 kb away from the telomere of chromosome XII, this genomic region segregates late during mitosis in wild-type cells, just prior to cytokinesis, causing only a small fraction of cells to show two distinct nucleolar masses during anaphase (Figure 1F). *cdc15-2* mutants arrested in late anaphase, however, exhibited two distinctly separated nucleoli (Figures 1F and 1G). In contrast, *cdc14-3* mutants rarely segregated the nucleolus (Figures 1F and 1G). This defect in nucleolar segregation has been observed previously (Granot and Snyder, 1991; Buonomo et al., 2003). A tetO array integrated adjacent to the rDNA locus also did not segregate in *cdc14-3* mutants but did segregate in *cdc15-2* mutants (Figure 3E), indicating that not only nucleolar but also rDNA partitioning is defective in *cdc14-3* mutants. This defect in nucleolar segregation is not due to a general disorganization of the nucleolus as loss of *CDC14* function neither alters the rate of rRNA synthesis nor disrupts the localization of nucleolar antigens such as Nop1 and Sir2 (Shou et al., 2001). Our results show that *cdc14-3*, but not *cdc15-2*, mutants have difficulties in segregating telomeres and the nucleolus.

We also observed *cdc14-3*, but not *cdc15-2*, mutants to be delayed in anaphase spindle elongation. In three independent experiments we found that 50 min after the onset of metaphase, approximately 80 percent of wild-type cells and *cdc15-2* mutants that were in mitosis (defined as cells with metaphase or anaphase spindles) contained mitotic spindles that were 6 μm in length or longer (Figures 1H–1K). Most mitotic *cdc14-3* cells (63 percent) contained mitotic spindles of intermediate length (3–6 μm; Figure 1K). This defect in spindle elongation could be due to *CDC14* being required for this process or could be a consequence of defects in chromosome segregation, resulting in chromosome linkages resisting spindle elongation. Importantly, however, we could exclude the possibility that the rDNA segregation defect was a consequence of defects in spindle elongation. Elimination of the spindle elongation defect did not restore nucleolar segregation to *cdc14-3* mutants (Figure 4).

The FEAR Network but Not the MEN Is Required for the Efficient Segregation of Telomeric and Nucleolar Regions

The finding that *cdc14-3* mutants, but not *cdc15-2* mutants exhibit difficulties in segregating telomeric and nucleolar regions raised the possibility that Cdc14 released from its inhibitor during early anaphase by the FEAR network was more important for the efficient partitioning of repetitive DNA elements than Cdc14 released by the MEN during late anaphase. Consistent with this idea we find that another MEN mutant, *tem1-3,* did not exhibit defects in segregating TEL-GFP dots or the nucleolus (Figure 2A). Cells lacking the partially redundant FEAR network components *SPO12* and *BNS1* did, however, exhibit telomere and nucleolar segregation defects in three independent experiments, which were most apparent at the onset of chromosome segregation and paralleled the delay in Cdc14 release seen in this mutant (Figure 2B, Visintin et al., 2003). As *spo12Δ bns1Δ* mutants do not exhibit a cell cycle arrest in telophase, it is difficult to compare their chromosome segregation behavior with that of *cdc14-3* and MEN mutants. To circumvent this problem, we analyzed the kinetics of TEL-GFP dot and nucleolar segregation under conditions where *spo12Δ bns1Δ* mutants arrest in telophase, in a MEN mutant background. Whereas *cdc15-2* mutants segregated TEL-GFP dots and nucleolar regions efficiently, *spo12Δ bns1Δ cdc15-2* cells exhibited segregation defects (Figure 2C). Our data suggest that Cdc14

illustrated in left hand side micrograph), or aberrant segregation (two dots per nuclear lobe; as illustrated in right hand side micrograph) 120 min after release from the pheromone-induced G1 arrest.

(F) A2587 (wild-type), A794 (*cdc14-3*), and A2597 (*cdc15-2*) cells were released from a G1 arrest to determine the percentage of budded cells (left image), and of cells with fully separated nucleoli (right image).

(G) Examples of cells that have segregated their nucleolus normally (*cdc15-2* [left] 120 min postrelease) and abnormally (*cdc14-3* [right] 120 min postrelease). Nucleoli were visualized using an antibody against the nucleolar protein Nop1.

(H–K) A2587 (wild-type), A794 (*cdc14-3*), and A340 (*cdc15-2*) cells were release from a G1 arrest at 37°C. The percentage of budded cells (H), cells with metaphase (I), and anaphase/telophase (J) spindles was determined at the indicated times.

(K) Mitotic spindles were measured from the time of metaphase onset (indicated with an asterix in [I]).

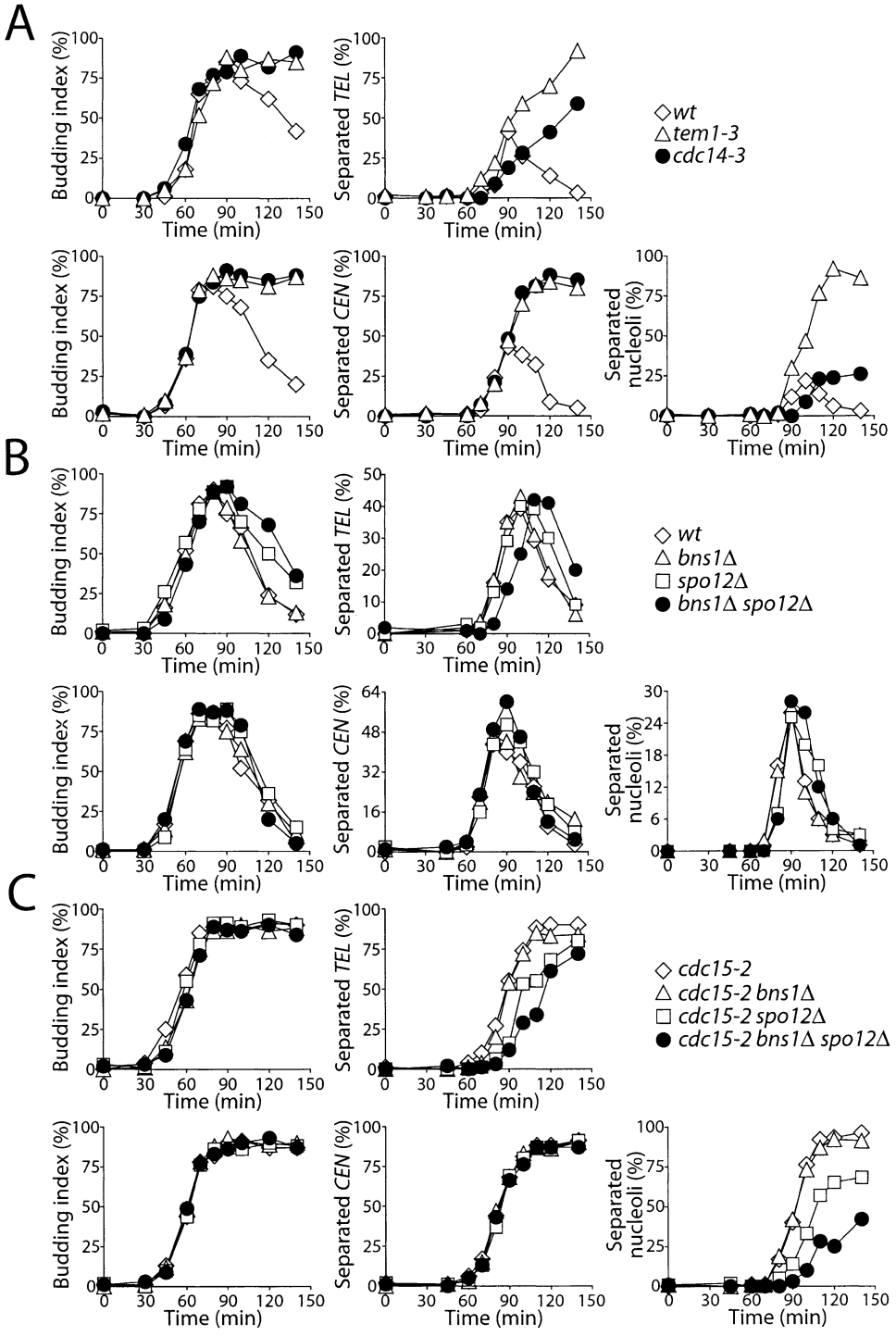

Figure 2. FEAR Network but Not MEN Mutants Exhibit Nucleolar and Telomere Segregation Defects

(A) A5236 (wild-type), A7245 (*tem1-3*), and A5238 (*cdc14-3*) cells were processed for telomere V segregation analysis (top images) and A5244 (wild-type), A7799 (*tem1-3*) and A5246 (*cdc14-3*) cells were processed for centromere IV segregation and nucleolar segregation analyses (bottom images) as described in the legend to Figure 1.

(B) A5236 (wild-type), A6333 (*bns1*Δ), A6331 (*spo12*Δ), and A6329 (*bns1*Δ *spo12*Δ) cells were processed for telomere V segregation analysis at the indicated time points (top images). A5244 (wild-type), A7250 (*bns1*Δ), A7251 (*spo12*Δ), and A7252 (*bns1*Δ *spo12*Δ) cells were processed for centromere IV segregation and nucleolar segregation analyses at the indicated time points (bottom images).

(C) A5240 (*cdc15-2*), A6327 (*cdc15-2 bns1*Δ), A6325 (*cdc15-2 spo12*Δ), and A6323 (*cdc15-2 bns1*Δ *spo12*Δ) cells were processed for telomere V segregation analysis at the indicated time points (top images). A5248 (*cdc15-2*), A7254 (*cdc15-2 bns1*Δ), A7255 (*cdc15-2 spo12*Δ), and A7256 (*cdc15-2 bns1*Δ *spo12*Δ) cells were processed for centromere IV segregation and nucleolar segregation analyses at the indicated time points (bottom images).

released from the nucleolus by the FEAR network during early anaphase is primarily required for the segregation of telomeres and the nucleolus.

Inactivation of *CDC14* or FEAR Network Components Leads to Loss of Viability during Anaphase

The defect in chromosome segregation observed in *cdc14-3* and *spo12*Δ *bns1*Δ mutants is subtle compared to that observed in cohesin or kinetochore mutants. This raises the question of whether *CDC14* and the FEAR network are important for chromosome segregation and, more generally, for cell viability. We found that, in contrast to wild-type and *cdc15-2* cells, only 82% (±2.9%) of *spo12*Δ *bns1*Δ and 55% (±6.6%) of *cdc15-2 spo12*Δ *bns1*Δ cells in exponentially growing cultures formed colonies ($n = 4$; standard errors for wild-type and *cdc15-2* strains were of 4.2% and 5.9%, respectively; [t test $P < 0.05$]). To test whether mutants lose viability as they undergo chromosome segregation, we released cells from a nocodazole-induced metaphase arrest into anaphase under restrictive conditions (37°C). Whereas the MEN mutants *cdc15-2, tem1-3* and *dbf2-2* progressed through anaphase with little loss in viability, *cdc14-3* and *spo12*Δ *bns1*Δ *cdc15-2* mutants exhibited a 4–5-fold increase in cell death (Figures 3A and 3B). All mutants exhibited a first cycle arrest in telophase (data not shown) demonstrating that cell death occurred during anaphase. We further excluded the possibility that the loss of viability in *cdc14-3* mutants was due to a failure to recover from the nocodazole arrest. *cdc14-3* or *cdc15-2* cells kept in nocodazole throughout the time of the experiment (without release into anaphase) exhibited similar levels of viability (Figure 3C). Our results show that *cdc14-3* mutants and FEAR network but not MEN mutants lose viability during anaphase. This loss in viability during anaphase may be a consequence of chromosome loss as *cdc14-1* mutants exhibit a 200- and 20-fold increase in chromosome loss compared to wild-type and *cdc15-2* cells, respectively (Hartwell and Smith, 1985).

Deletion of the rDNA Locus Allows the Segregation of Flanking Regions in *cdc14-3* Mutants

Telomeric and the rDNA loci consist of repetitive DNA elements and are silenced for RNA pol II expression (reviewed in Grunstein, 1998). To determine whether silencing was responsible for the delayed segregation of telomeres and the rDNA in *cdc14-3* mutants, we deleted *SIR2*, which significantly reduces silencing at all known silent loci. Upon release from a nocodazole-induced metaphase arrest, *cdc14-3 sir2*Δ cells segregated their telomeres and nucleolus with poor efficiency compared to *cdc15-2 sir2*Δ mutants (Figure 3D), indicating that silencing is not likely to impose a need for Cdc14 during the segregation of these genomic regions.

To determine whether the rDNA locus per se was responsible for the nucleolar segregation defect observed in *cdc14-3* and FEAR network mutants, we examined the segregation behavior of a tetO array integrated adjacent to the rDNA locus (rDNA-GFP dots; on the telomeric side of the locus) in *cdc14-3* mutants and *cdc14-3* mutants lacking the entire rDNA array (Wai et al., 2000). rDNA-GFP dots separated normally in wild-type and *cdc15-2* cells but not in *cdc14-3* mutants (Figure 3E). Deletion of the rDNA locus largely rescued the segregation defects of this chromosomal region in *cdc14-3* mutants (Figure 3E). The separation did not completely reach the levels observed in *cdc15-2* mutants, suggesting that perhaps other regions of chromosome XII (i.e., telomeres) are still hampered in their segregation.

Consistent with the idea that repetitiveness at telomeres imposes a need for mechanisms in addition to separase for their segregation was the observation that lengthening of telomeric TG_{1-3} repeats by ~5–10-fold over wild-type levels impaired TEL-GFP dot segregation in *cdc15-2* mutants (Figure 3F). This was achieved using a *rap1-17* mutant background, where telomeric TG_{1-3} repeats are 2–4 kb longer than in wild-type cells (Kyrion et al.,1992). *cdc15-2 rap1-17* mutants exhibited defects in TEL-GFP dot segregation (Figure 3F), which is consistent with the finding that *rap1-17* mutants exhibit a significant increase in chromosome nondisjunction during mitosis (Kyrion et al., 1992). This finding also suggests that the delay in telomere segregation is not merely due to a general inefficiency of chromosome segregation that is evident at loci most distant from centromeres. The *rap1-17* allele, however, did not decrease the efficiency with which TEL-GFP dots were segregated in *cdc14-3* mutants (Figure 3F), suggesting that the severity of the telomere segregation defect of *cdc14-3* mutants is at a level where it cannot be further enhanced by lengthening of telomeric repeats. Our results indicate that the presence of the rDNA repeats imposes a need for Cdc14 released by the FEAR network for the completion of chromosome XII segregation. The finding that telomere repeat length influences the segregation of telomeres is consistent with, though not proves the idea that a similar requirement exists for telomere segregation.

Removal of Cohesin Does Not Promote the Segregation of the Nucleolus in *cdc14-3* Mutants

How do *CDC14* and the FEAR network promote the segregation of telomeres and the rDNA? One possibility we considered was that *CDC14* and the FEAR network were necessary for some aspect of cohesin removal. A key regulatory step in the initiation of sister-chromatid separation is the destruction of securin/Pds1 by the APC/C-dependent proteolysis machinery. Inactivation of *PDS1* reduced the segregation efficiency of TEL-GFP dots and the nucleolus in both *cdc14-3* and *cdc15-2* mutants, which is likely to be due to lower separase activity in *pds1*Δ cells (Agarwal and Cohen-Fix, 2002). However, segregation of TEL-GFP dots and the nucleolus as well as mitotic spindle elongation was significantly more impaired in *cdc14-3* than in *cdc15-2* mutants in the absence of Pds1 (Figure 4A, data not shown). Similar results were obtained in *cdc14-3* mutants, in which the mitotic spindle checkpoint was inactivated due to the deletion of *MAD1* (Figure 4B, data not shown; reviewed in Gardner and Burke, 2000). Furthermore, degradation of Pds1 was not delayed in *cdc14-3* mutants (Supplemental Figure 1 available at http://www.cell.com/cgi/

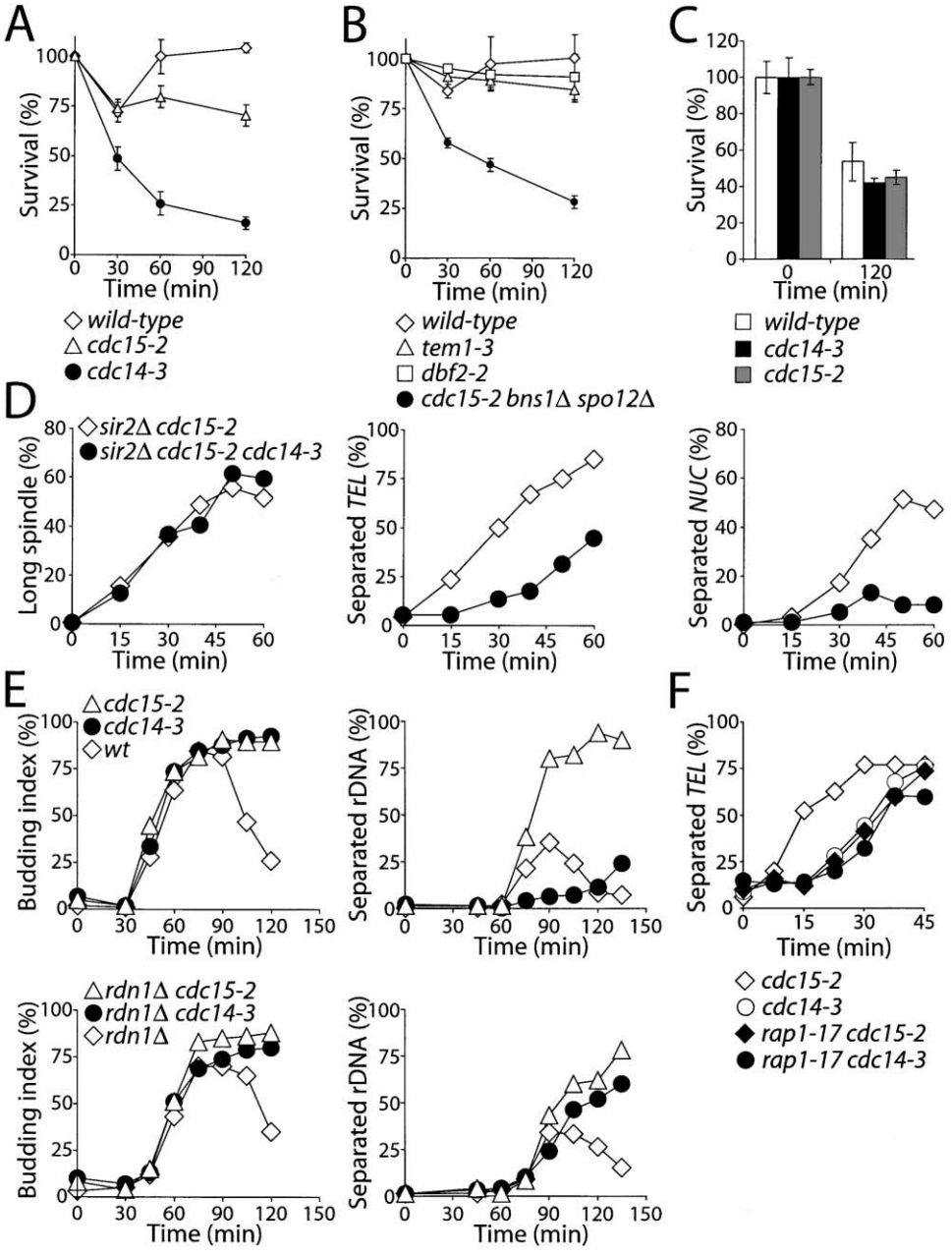

Figure 3. Cdc14 and the FEAR Network Are Essential for Anaphase Events and Their Nucleolar Segregation Defects Are Suppressed by Loss of rDNA Repeats

(A) A2587 (wild-type), A794 (*cdc14-3*), and A2597 (*cdc15-2*) cells were arrested with nocodazole (15 μg/ml) for 2.5 hr followed by release at 37°C. Aliquots were taken at the indicated times, washed, and plated at permissive temperature for survival analysis. α-factor was added 45 min after release to prevent wild-type cells from entering the subsequent cell cycle. Error bars represent standard errors.

(B) A2587 (wild-type), A1740 (*tem1-3*), A851 (*dbf2-2*), and A6335 (*cdc15-2 bns1Δ spo12Δ*) cells were processed for survival analysis, as described in (A).

(C) A2587 (wild-type), A794 (*cdc14-3*), and A2597 (*cdc15-2*) cells were arrested with nocodazole (15 μg/ml) for 2.5 hr and resuspended into fresh medium containing (15 μg/ml) nocodazole at 37°C. Survival was determined at the time of transfer into fresh medium containing nocodazole (0 time point) and 120 min thereafter.

(D) A8785 (*sir2Δ cdc15-2)* and A8786 (*sir2Δ cdc15-2 cdc14-3*) cells were released from a nocodazole-induced arrest. The percentage of cells with long (anaphase) spindles (left image), of cells with two distinct TEL-GFP dots (middle image), and of cells with fully separated nucleoli (right image) was determined at the indicated times.

(E) A9970 (wild-type), A9971 (*cdc14-3*), A9972 (*cdc15-2*), A9711 (*rdn1Δ*), A9714 (*rdn1Δ cdc14-3*), and A9715 (*rdn1Δ cdc15-2*) cells carrying a tetO array adjacent to the rDNA locus were released for a G1 arrest. The percentage of budded cells (left images) and of cells with two distinct tetR-GFP dots (right images) was determined at the indicated times. Top images show cells with a normal *RDN1* locus and bottom images show cells with a deleted *RDN1* locus.

(F) A5238 (*cdc14-3)*, A5240 (*cdc15-2*), A9468 (*rap1-17 cdc14-3)*, and A9469 (*rap1-17 cdc15-2)* cells carrying a tetO array at the telomere of chromosome V were released from a nocodazole-induced arrest into fresh YEPD medium at 37°C and the percentage of cells with two distinct TEL-GFP dots was determined at the indicated time points.

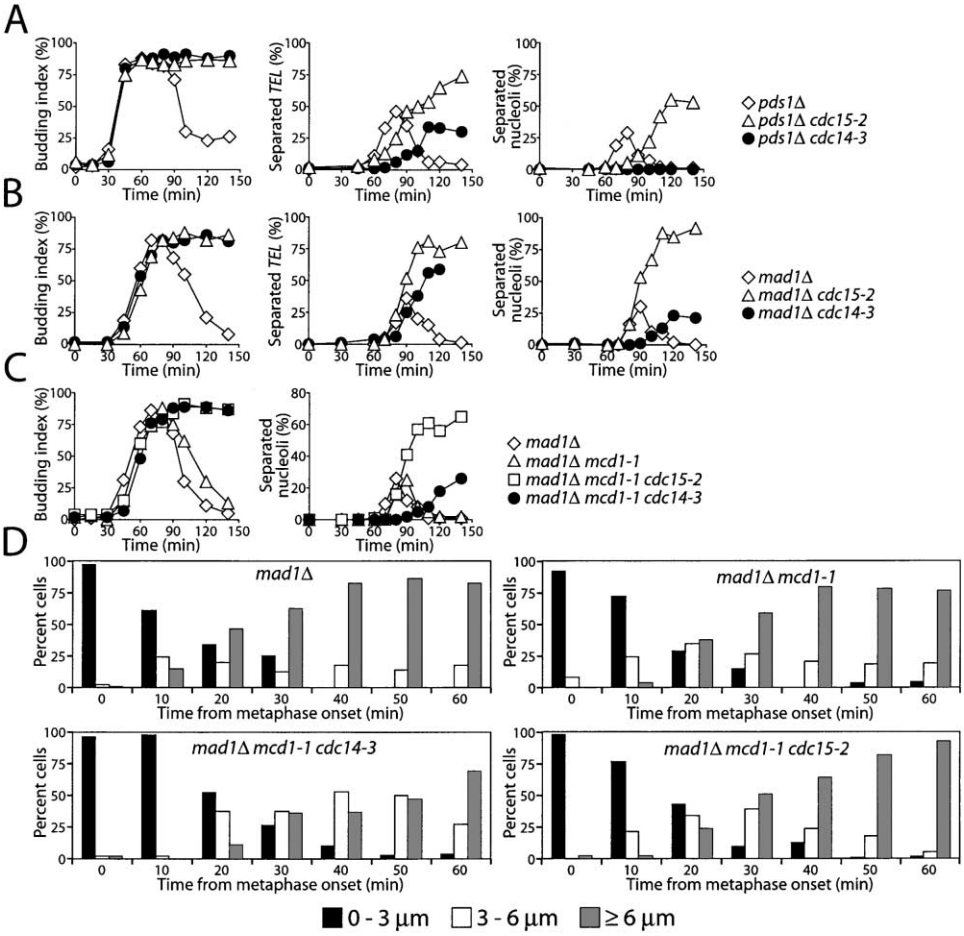

Figure 4. The Chromosome Segregation Defects of *cdc14-3* Mutants Are Independent of Securin and Cohesin Function

(A) A7044 (*pdsΔ*), A7441 (*pds1Δ cdc15-2*), and A7048 (*pds1Δ cdc15-2*) cells were released from a G1 arrest first at 30°C for 45 min (to bypass Pds1's S phase function [Yamamoto et al., 1996]) before being incubated at 37°C for the remainder of the experiment.

(B) A5943 (*mad1Δ*), A6480 (*mad1Δ cdc14-3*), and A6482 (*mad1Δ cdc15-2*) cells were processed for telomere V segregation and nucleolar segregation analyses at the indicated time points.

(C and D) A5943 (*mad1Δ*), A5945 (*mad1Δ mcd1-1*), A5947 (*mad1Δ mcd1-1 cdc14-3*), and A6246 (*mad1Δ mcd1-1 cdc15-2*) cells were processed for nucleolar segregation analysis (C) and spindle length measurement (D).

content/full/117/4/455/DC1). Our results indicate that the defect in partitioning of repetitive DNA elements in *cdc14-3* mutants is not due to activation of checkpoints or a delay in APC/C-mediated degradation of Pds1.

Next, we examined whether inactivation of cohesin restored efficient segregation of nucleolar regions in *cdc14-3* mutants. The defect in nucleolar fission was only slightly, if at all, rescued in cells carrying a temperature sensitive *mcd1-1* allele (compare Figures 4B and 4C). In contrast, inactivation of Scc1/Mcd1 largely rescued the mitotic spindle elongation defect in *cdc14-3* mutants (Figure 4D). The reason(s) why inactivation of *SCC1/MCD1* rescues the spindle elongation defect of *cdc14-3* mutants is unclear at present. Irrespective of the cause, this finding indicates that defects in mitotic spindle elongation are not the main reason for the delay in nucleolar segregation in *cdc14-3* mutants. Similar results were obtained when *SCC1/MCD1* was depleted from cells (Supplemental Figure 2 available on *Cell* website) or when cleavage of Scc1/Mcd1 was induced using the TEV protease in the absence of separase and hence FEAR network function (Sullivan and Uhlmann, 2003). Furthermore, Scc1/Mcd1 cleavage was not delayed in *cdc14-3* mutants (data not shown). Our data indicate that the need for Cdc14 released by the FEAR network during early anaphase in nucleolar segregation is largely independent of cohesin-mediated chromosome linkages.

Ycs4 Enrichment in the Nucleolus during Anaphase Depends on *CDC14* and the FEAR Network

Mutants defective in the condensin complex exhibit nucleolar segregation defects reminiscent of that of *cdc14-3* mutants (Freeman et al., 2000; Bhalla et al., 2002). We therefore investigated whether *CDC14* was necessary for the anaphase-specific enrichment of the condensin subunit Ycs4 in the nucleolus. Whereas Ysc4 was found enriched in the nucleolus during anaphase in wild-type and *cdc15-2* cells (Bhalla et al., 2002; Figures 5A and 5C), no such enrichment occurred in *cdc14-3* mutants (Figures 5A and 5C). The defect in Ycs4 localization was not likely due to a general disorganiza-

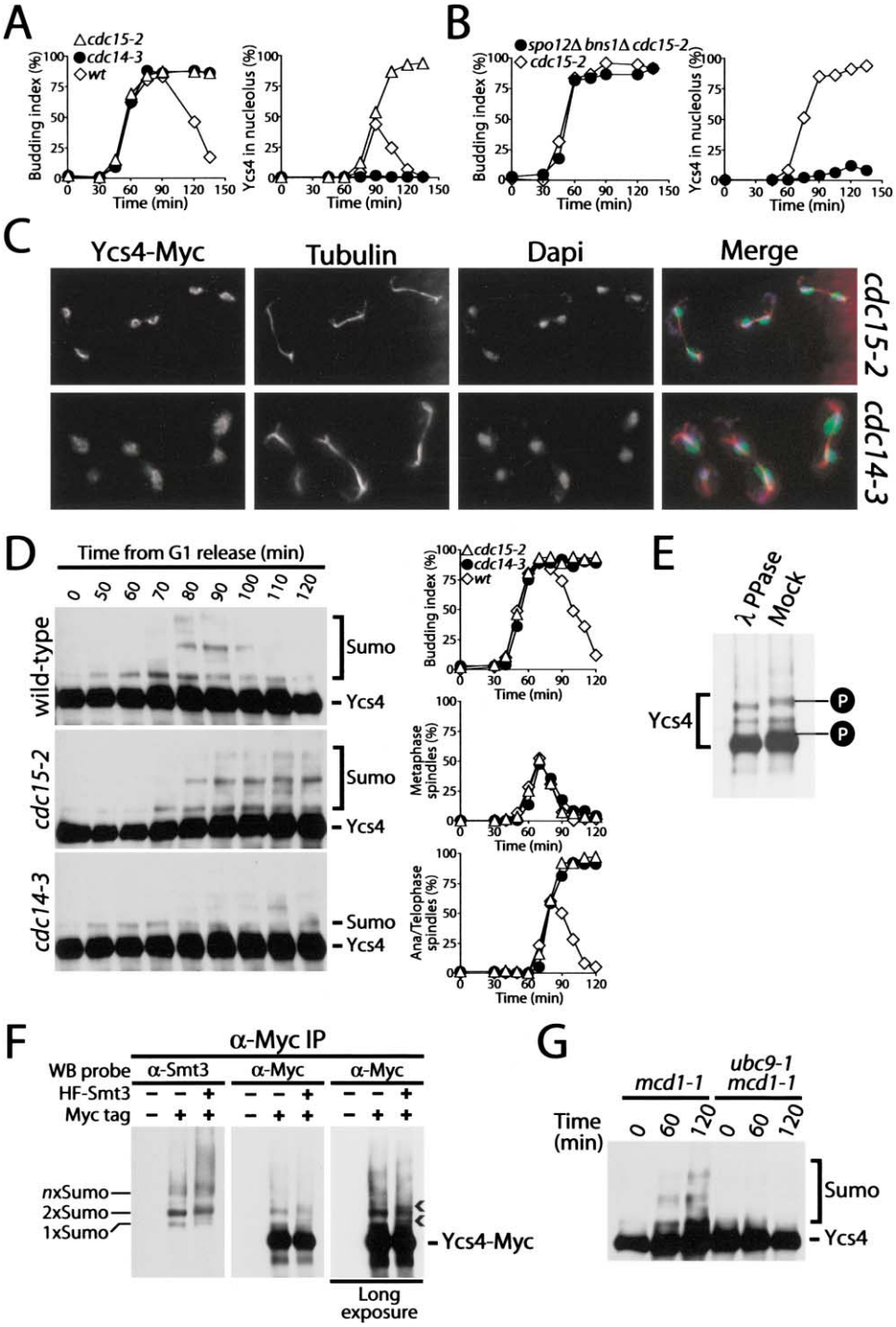

Figure 5. *CDC14* Is Required for the Anaphase-Specific Enrichment of Ycs4 in the Nucleolus

(A) A8854 (wild-type), A8855 (*cdc14-3*), and A9201 (*cdc15-2*) cells expressing Ycs4-Myc were released from a G1 arrest at 37°C. The percentage of budded cells (left image) and of cells with nucleolar Ycs4 localization (right image) was determined at the indicated times. Cells with either one or two nucleolar-specific Ycs4 signal were considered positive.

(B) A9201 (*cdc15-2*) and A9106 (*cdc15-2 bns1Δ spo12Δ*) cells expressing Ycs4-Myc were released from a G1 arrest at 37°C and Ycs4 localization was analyzed.

(C) Micrographs showing representative fields of *cdc15-2* (top) and *cdc14-3* (bottom) cells 135 min after release from the G1 arrest from the experiment shown in (A). Ycs4 is shown in green, microtubules in red and DNA in blue. The localization of Ycs4 in *cdc15-2* cells is essentially identical to that of wild-type cells in anaphase (Bhalla et al., 2002). Identical exposure conditions were used for both series of pictures.

(D) A8854 (*YCS4-MYC*), A8855 (*YCS4-MYC, cdc14-3*), and A9201 (*YCS4-MYC, cdc15-2*) cells were arrested in G1 with α-factor, released at 37°C and samples were taken at indicated time points to determine the percentage of budded cells, metaphase and anaphase spindles, and Ycs4-Myc protein by Western blot analysis.

tion of the nucleolus as loss of *CDC14* function does not disrupts the localization of other nucleolar proteins such as Sir2 and Nop1 (Shou et al., 2001). The anaphase-specific enrichment of Ycs4 in the nucleolus was also delayed and reduced in *spo12Δ bns1Δ cdc15-2* mutants (Figure 5B). Our results indicate that Cdc14 released by the FEAR network is necessary for the anaphase-specific enrichment of Ycs4 in the nucleolus.

CDC14 Is Required for Efficient Sumoylation of Ycs4 during Anaphase

As wild-type and *cdc15-2* cells progressed through the cell cycle a faint slower migrating form of Ycs4 was apparent prior to entry into mitosis. As cells entered anaphase, the intensity of this slower migrating species increased and two additional slower migrating forms became apparent (Figure 5D). No increase in slower migrating species of Ycs4 was observed in metaphase-arrested cell (Figure 7C). The slower migrating forms of Ycs4 are largely resistant to phosphatase treatment (Figure 5E) and resembled ubiquitination or sumoylation owing to the ladder-like appearance of this modification. Anti-Smt3 (Sumo in yeast) antibodies recognized the slower migrating forms of Ycs4 but not the fastest migrating form (Figure 5F), indicating that Ycs4 was sumoylated. Consistent with this idea, overexpression of *HIS-FLAG-SMT3* (Johnson and Blobel, 1999) led to a further retardation of the slower migrating forms of Ycs4 (Figure 5F; arrowheads). Moreover, whereas *mcd1-1* mutants after 2 hr at the restrictive temperature had accumulated slower migrating forms of Ycs4, presumably because these cells are delayed in anaphase (Guacci et al., 1997; Figure 5G), these slower migrating species were not present in *mcd1-1* mutants lacking the sumo-conjugating enzyme Ubc9 (Figure 5G; *mcd1-1 ubc9-1* mutants do not arrest in metaphase, like *ubc9-1* mutants [Seufert et al., 1995], excluding the possibility that the absence of Ycs4 sumoylation was due to cell cycle arrest, data not shown). Treatment of immunoprecipitated Ycs4 with λ phosphatase showed that both the sumoylated and nonsumoylated forms of Ycs4 were phosphorylated (Figure 5E). Strikingly, when compared to *cdc15-2* cells, the anaphase-specific sumoylated form of Ycs4 was strongly reduced and occurred with a delay in *cdc14-3* mutants (Figure 5D). Our results suggest that Ycs4 is monosumoylated prior to entry into mitosis but becomes at least disumoylated during anaphase. This anaphase-specific sumoylation is at least in part dependent on *CDC14*.

Inactivation of *SMT4* Causes Ycs4 Localization and Nucleolar Segregation Defects

If sumoylation were important for nucleolar segregation, cells impaired in sumoylation should also exhibit Ycs4 localization and rDNA segregation defects. *ubc9-1 mcd1-1* double mutants did not segregate the nucleolus (data not shown) but cells with fully elongated spindles were not or only very rarely observed. We, therefore, cannot exclude the possibility that the defect in nucleolar segregation observed in these mutants is a consequence of a failure to elongate the mitotic spindle. To gain more insight into the role that sumoylation plays in rDNA segregation, we analyzed the consequences of inactivating the desumoylation enzyme *SMT4*. In most cases analyzed to date, inactivation of desumoylating enzymes affects the processes regulated by Ubc9 (Li and Hochstrasser, 2000; Bachant et al., 2002), perhaps because a balance between sumoylation and desumoylation is necessary to mediate these processes. Furthermore, because there are two partially redundant desumoylating enzymes in yeast, we reasoned that inactivating one of them should lead to a less severe anaphase phenotype than that of *ubc9-1* mutants. Strikingly, inactivation of *SMT4* in *cdc15-2* mutants resulted in a strong defect in the anaphase-specific localization of Ycs4 in the nucleolus as has been observed previously for Smc4 (Strunnikov et al., 2001) and in an inefficiency in rDNA segregation (Figure 6A). This defect was not due to the delay in early anaphase observed in *smt4Δ* cells (Bachant et al., 2002) as Ycs4 enrichment in the nucleolus and nucleolar segregation was reduced even in cells with long anaphase spindle (Figure 6B). Our results suggest that *SMT4* is required for Ycs4 enrichment in the nucleolus and rDNA segregation.

Overexpression of *CDC14* Is Sufficient to Induce Ycs4 Sumoylation, Ycs4 Recruitment to the Nucleolus, and rDNA Segregation

To determine whether *CDC14* might be a limiting factor for Ycs4 sumoylation and accumulation in the nucleolus, we examined whether overexpression of *CDC14* was sufficient to induce these events in stages of the cell cycle when they normally do not occur. *cdc14-3* cells carrying three copies of the *CDC14* gene under the control of the galactose-inducible *GAL1-10* promoter were arrested in metaphase using nocodazole at the permissive temperature. As soon as 30 min after galactose addition, slower migrating forms of Ycs4 appeared (Figure 7C) and Ycs4 started to accumulate in the nucleolus (Figures 7A and 7B), indicating that overexpression of *CDC14* was sufficient to induce Ycs4 sumoylation and to target the protein to the nucleolus.

Next, we determined whether overexpression of *CDC14* was also sufficient to induce segregation of a tetO array integrated next to the rDNA locus (rDNA-GFP dots). In nocodazole-arrested cells carrying a *mcd1-1* allele (to inactivate cohesin-dependent chromosome

(E) Ycs4-Myc was immunoprecipitated from a protein extracts of A9201 (*YCS4-MYC, cdc15-2*) cells arrested in late anaphase (100 min after G1 block release at 37°C). Immunoprecipitated material was split and treated with either λ phosphatase or buffer alone for one hour.
(F) Ycs4-Myc was immunoprecipitated from *cdc15-2* arrested A2597 (*cdc15-2*), A9201 (*YCS4-MYC, cdc15-2*), or A9201 cells carrying pGAL-HF-SMT3 (an episomal plasmid expressing an hexahistidine-FLAG-tagged variant of Smt3; Johnson and Blobel, 1999) and processed for western analysis using either anti-MYC or anti-Smt3 antibodies. The image on the right is a longer exposure of the Western blot shown in the middle image. The arrowheads on the image on the right show the additional bands that correspond to the HF-Smt3-Ycs4 adducts which run with a slightly reduced mobility compared to the endogenous Smt3 adducts in SDS-PAGE.
(G) A10904 (*YCS4-MYC mcd1-1*) and A10905 (*YCS4-MYC mcd1-1 ubc9-1*) cells were grown to exponential phase (t = 0) and transferred to 37°C for 2 hr. Samples were taken 1 hr and 2 hr after transfer to 37°C to determine Ycs4-Myc protein sumoylation by Western blot analysis.

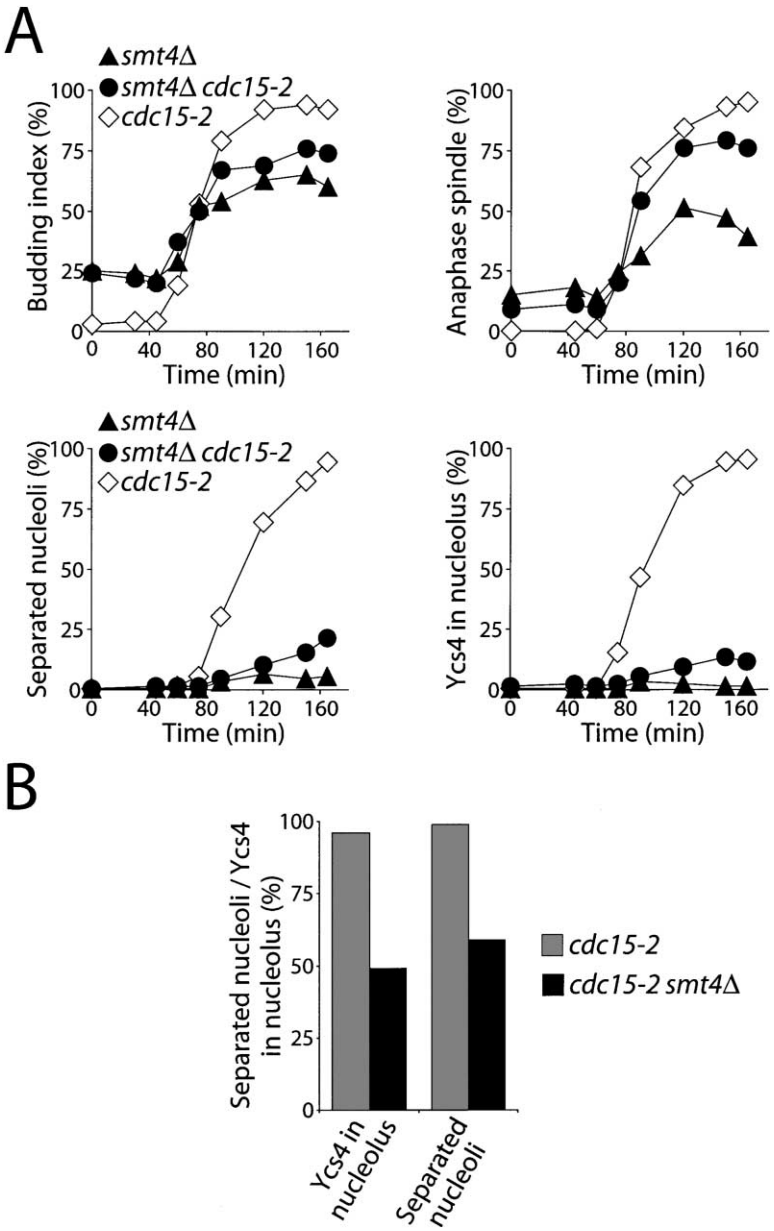

Figure 6. *SMT4* Is Required for the Anaphase-Specific Enrichment of Ycs4 in the Nucleolus and for the Segregation of the rDNA Array

(A) A9201 (*YCS4-MYC, cdc15-2*), A9470 (*YCS4-MYC, smt4Δ*), and A9775 (*YCS4-MYC, smt4Δ, cdc15-2*) cells were released from a G1 arrest at 37°C and the percentage of budded cells, cells with anaphase spindles, separated nucleoli, and Ycs4 localized in the nucleolus was determined.

(B) Nucleolar segregation and Ycs4 localization in cells with extended spindle at the ultimate and penultimate time points in (A).

linkages) centromeric and chromosomal arm sequences separate due to the absence of cohesin (Guacci et al., 1997) but the rDNA-GFP dots were tightly associated (Figures 7D and 7E), indicating the presence of cohesin-independent chromosomal linkages at this locus. Overexpression of *CDC14* in such cells led to the separation of rDNA GFP dots (Figure 7E). Our results suggest that overexpression of *CDC14* is sufficient to induce the dissolution of cohesin-independent sister-chromatid linkages at the rDNA.

A Functional Condensin Complex Is Required for *CDC14*-Induced Nucleolar Segregation

To determine whether the separation of rDNA-GFP dots observed in cells overexpressing *CDC14* could be due to *CDC14* promoting condensin function at the rDNA, we examined whether *CDC14*-induced separation of rDNA-GFP dots occurred in a *brn1-60* mutant (Ouspenski et al., 2000). *CDC14* failed to promote rDNA segregation in nocodazole-arrested *mcd1-1 brn1-60* cells (Figure 7E), indicating that *CDC14*-induced rDNA-GFP dot segregation required condensin function.

Topoisomerase II has also been shown to be required for the partition of late-segregating sequences (Bhalla et al., 2002). We therefore examined whether *CDC14*-induced rDNA GFP dot segregation required *TOP2* function. Surprisingly, overexpression of *CDC14* in nocodazole-arrested *top2-4* mutants led to efficient separation of rDNA-GFP dots (Figure 7F). The top2-4 protein was being inactivated under these experimental conditions because when *top2-4* mutants were released from a nocodazole block into anaphase at 37°C, nuclear segregation and rDNA-GFP dot segregation was impaired (Supplemental Figure 3 available on *Cell* website). Our

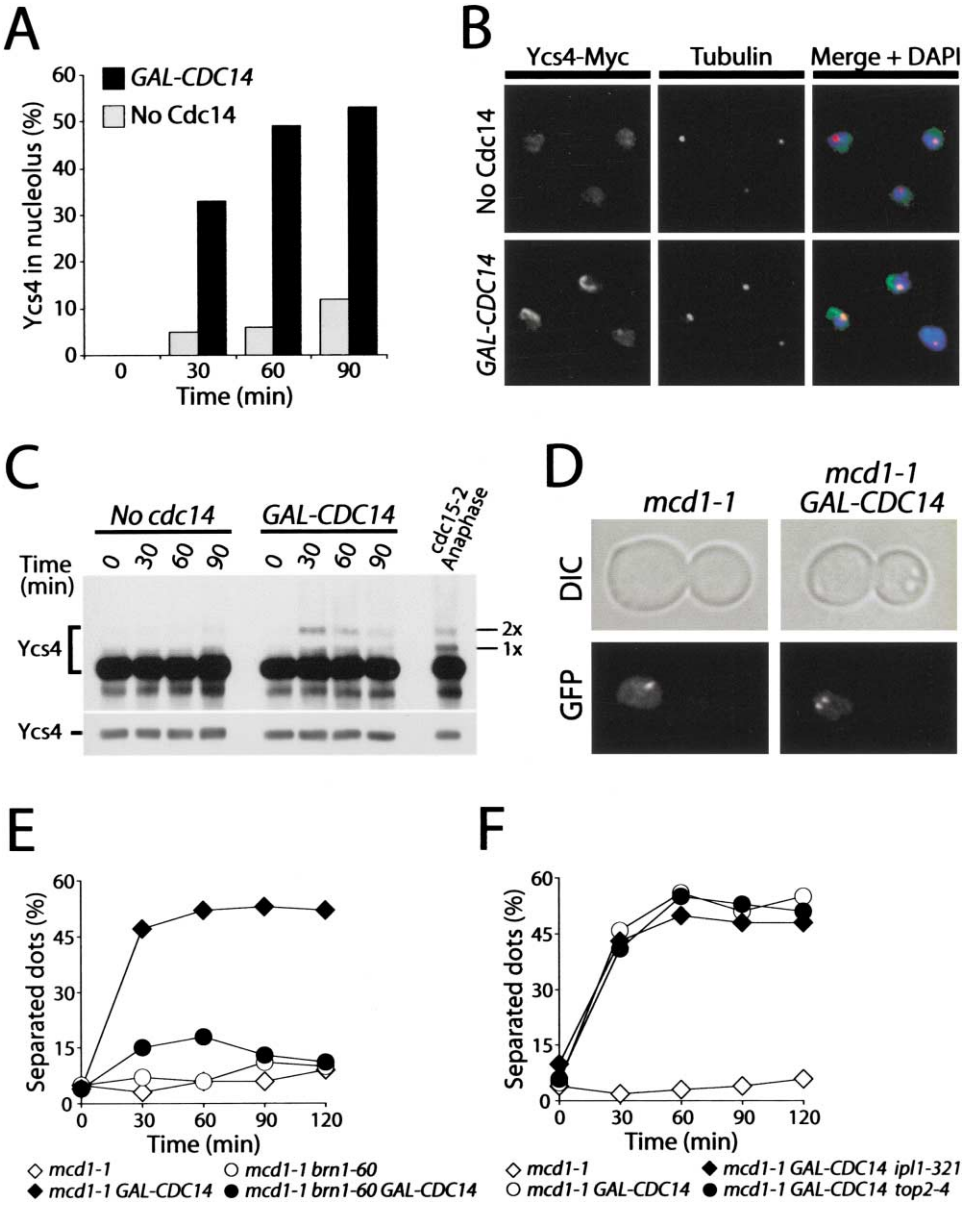

Figure 7. Overexpression of *CDC14* Is Sufficient to Induce Ycs4 Enrichment in the Nucleolus and rDNA Separation in a Condensin-Dependent Manner

(A) A8855 (*YCS4-MYC, cdc14-3*) and A9105 (*YCS4-MYC, cdc14-3, GAL-CDC14*) cells were arrested with nocodazole in YEP-Raf at room temperature for 2 hr followed by an hour preinactivation step at 37°C (in nocodazole). Galactose was then added to induce the expression of wild-type *CDC14* and samples were collected at indicated time points for Ycs4-Myc localization analysis.

(B) Micrographs showing control nocodazole-arrested cells and cells overexpressing wild-type *CDC14* for 1 hr. Ycs4 is shown in green, microtubules in red and DNA in blue.

(C) Protein samples from cells treated as in (A) were processed for western analysis. A shorter exposure of the blot is presented below the main image to show equal loading of the nonsumoylated form of Ycs4 in all lanes.

(D) Micrographs representing examples of large-budded mononucleated cells having maintained (left) or lost (right) cohesin-independent cohesion at the rDNA locus following Cdc14 overexpression.

(E) A9881 (*mcd1-1*), A9996 (*mcd1-1, GAL-CDC14*), A9997 (*mcd1-1, brn1-60, GAL-CDC14*), and A9999 (*mcd1-1, brn1-60*) cells carrying a tetO array adjacent to the rDNA locus on chromosome XII were arrested with nocodazole for 3 hr. Cells were then transferred to 37°C and induced with galactose. Samples were collected at indicated time points to analyze rDNA-GFP dot separation. Only large-budded cells without extended nuclei were counted as a small fraction of cells escaped the nocodazole arrest.

(F) A9881 (*mcd1-1*), A9996 (*mcd1-1, GAL-CDC14*), A10733 (*mcd1-1, ipl1-321, GAL-CDC14*), and A10775 (*mcd1-1, top2-4, GAL-CDC14*) cells carrying a tetO array adjacent to the rDNA locus on chromosome XII were processed as above to determine rDNA-GFP dot separation in the presence of overexpressed *CDC14*.

results suggest that high levels of Cdc14 suppress the need for *TOP2* in rDNA-GFP dot separation raising the possibility that Cdc14-induced rDNA segregation does not require *TOP2* function.

A Condensation Independent Role for Condensins in rDNA Segregation?

Condensins have been identified based on their role in chromosome condensation (reviewed in Swedlow and Hirano, 2003). To examine whether condensins' condensation function was required for Cdc14-induced rDNA-GFP dot separation, we asked whether inactivation of chromosome condensation by means other than inactivating condensins would prohibit Cdc14-induced rDNA-GFP dot segregation in metaphase. Chromosome condensation requires cohesins during prophase and metaphase and *IPL1* during anaphase (Lavoie et al., 2004). Inactivation of both *IPL1* and cohesin (using a *mcd1-1* allele) did not prevent rDNA-GFP dot segregation in cells overexpressing *CDC14* (Figure 7F). Furthermore, inactivation of *IPL1* did not affect Ycs4 sumoylation, Ycs4 enrichment in the nucleolus or nucleolar segregation (Supplemental Figure 4A available on *Cell* website). Our results indicate that Cdc14 induces rDNA segregation by promoting the enrichment of condensin at this locus and raise the intriguing possibility that the function of condensin in segregating the rDNA is independent of its condensation function.

Discussion

It has long been known that the nucleolus and telomeres in yeast segregate late during mitosis (Guilliermond, 1917; Alexandru et al., 2001). Whether this reflects specific requirements for the segregation of these chromosomal regions was unknown. We now show that these repetitive, heterochromatin-like regions are special in that they require Cdc14 and the FEAR network for their segregation. Our results show that Cdc14 released by the FEAR network during early anaphase is required for the efficient segregation of telomeres and the rDNA. Furthermore, *cdc14-3* and FEAR network mutants lose viability during anaphase raising the possibility that this function of Cdc14 and the FEAR network is essential for the maintenance of genomic stability. Our data also provide insight into the mechanism whereby Cdc14 promotes the segregation of these specialized genomic regions. The condensin subunit Ycs4 is not fully sumoylated and does not accumulate in the nucleolus during anaphase in *cdc14-3* and FEAR network mutants. Conversely, overexpression of *CDC14* is sufficient to induce Ycs4 sumoylation, to recruit condensin to the rDNA, and to induce rDNA separation in stages of the cell cycle when these events are normally not occurring. Importantly, the ability of Cdc14 to induce rDNA separation depends on a functional condensin complex. Our results indicate that Cdc14 released by the FEAR network promotes the segregation of repetitive sequences by promoting condensin enrichment at the rDNA locus.

Telomeres and the rDNA Locus Require Mechanisms Other Than Separase's Proteolytic Activity for Their Segregation

Our studies show that mechanisms in addition to separase's protease activity are required to promote the segregation of the rDNA and telomeres and we have identified Cdc14 released by the FEAR network as being required for the dissolution of these cohesin-independent linkages. We do not yet know whether the rDNA and telomeres are the only genomic regions that require *CDC14* and the FEAR network for their segregation during anaphase. Given that the bulk of DNA is segregated in *cdc14-3* mutants, such other regions would constitute only a minor part of the genome and could perhaps be identified as chromosomal areas rich in condensins.

Why do telomeres and the rDNA need mechanisms other than separase activity for their segregation? The repetitive nature of these genomic regions appears to be the reason. It is however important to note that not all repetitive DNA elements require Cdc14 released by the FEAR network for their segregation. The tetO arrays, which consist of $\sim$11.2 kb of repeated sequence segregate efficiently when integrated near the centromere. So what is it about the repetitive DNA elements at the rDNA and telomeres that impose a need for *CDC14* during their segregation? The silenced nature of these loci appears not to be the reason and neither does chromatid catenation. Although topoisomerase II is essential for the partition of late-segregating sequences (Bhalla et al., 2002), Cdc14 can efficiently induce rDNA-GFP dot separation in a *top2-4* mutant. Thus, it appears that high levels of Cdc14 actually suppress the need for *TOP2* in rDNA-GFP dot segregation. Perhaps repetitive regions are more prone to mitotic recombination or potential nuclear envelope tethers need to be removed for efficient segregation to occur.

Condensin's Role in Segregating the rDNA

A phenotype shared by most if not all organisms lacking condensin function are anaphase bridges (reviewed in Hagstrom and Meyer, 2003; Haering and Nasmyth, 2003; Swedlow and Hirano, 2003). We propose that in *S. cerevisiae* one role of condensin is to promote the segregation of repetitive DNA. Condensin could compact the rDNA thereby facilitating individualization of sister chromatids in these genomic regions and in this function allow for more efficient segregation. However, it is also possible that yet to be identified compaction-independent functions of condensin are needed for the segregation of repetitive regions. This idea is supported by several observations. First, in a nocodazole arrest, overexpression of *CDC14* allows the rDNA to separate in a condensin-dependent manner yet elimination of condensin's condensation function (by inactivating cohesins and *IPL1*; Lavoie et al., 2004) does not interfere with Cdc14-induced rDNA-GFP dot separation. Second, *IPL1* is required to maintain anaphase condensation at the rDNA locus (Lavoie et al., 2004) but is not necessary for rDNA segregation (F.S., unpublished observation). Third, the rDNA is highly condensed in metaphase-arrested cells (Guacci et al., 1994; Lavoie et al., 2004) but anaphase-like condensin enrichment in the nucleolus is not observed (Figure 7A, Bhalla et al., 2002). These results not only indicate that the increase in condensin signal in the nucleolus of anaphase cells is unlikely to be a simple consequence of the condensation of the rDNA locus (i.e., a reflection of the reduction in volume of the nucleolus), but they raise the possibility that pre-anaphase levels of condensin are sufficient to initiate

rDNA condensation whereas the anaphase-specific enrichment performs a segregation-specific function.

Irrespective of whether compaction or other aspects of condensin function are necessary for the segregation of the rDNA and telomeres, the question arises why condensin is needed for the segregation of these genomic regions. It seems unlikely that the segregation defect of yeast condensin mutants, however similar to that of *top2* mutants, could be explained by a general reduction in topoisomerase II activity. Overexpression of *TOP2* does not rescue the chromosome segregation defect of condensin mutants (Bhalla et al., 2002). Furthermore, neither condensin mutants nor *cdc14* mutants show obvious defects in topoisomerase activity in vivo (Koshland and Hartwell, 1987; Lavoie et al., 2000). Finally, *CDC14*-induced rDNA segregation does not require *TOP2* activity. Ultimately, it is possible that the Cdc14-condensin segregation pathway and Top2 contribute to the same process independently of each other. For instance, the structural role of Top2 in the nuclear scaffold of chromosomes could contribute to chromosome segregation. This idea would reconcile the findings that Top2 is required for rDNA segregation during anaphase when a rigid chromosome structure is required (Sullivan et al., 2004) and that Cdc14-mediated rDNA-GFP dot separation in metaphase-arrested cells is *TOP2*-independent. Condensin enrichment in the nucleolus may, however, be important to promote the dissociation of telomeres and the nucleolus from the nuclear envelope, which does not break down during mitosis in yeast. It is also possible that condensin localization to repetitive DNA during anaphase may be essential for preventing mitotic recombination (Freeman et al., 2000, Bhalla et al., 2002).

Cdc14 and the FEAR Network Control rDNA Segregation through Regulating Condensin Function

Several lines of evidence suggest that Cdc14 released by the FEAR network through inducing anaphase-specific targeting of condensin to the rDNA locus promotes rDNA condensation and segregation. (1) Guacci et al. (1994) showed that the rDNA remains condensed throughout anaphase whereas other genomic regions decondense during early anaphase. This anaphase-specific condensation of the rDNA depends on *CDC14* but not on *CDC15*. Our data show that *CDC14* but not *CDC15* is required for the anaphase specific enrichment of the condensin subunit Ycs4 at the rDNA providing a molecular basis for this observation. (2) *cdc14-3* and FEAR network mutants, like condensin mutants, are defective in the segregation of the rDNA. (3) Overexpression of *CDC14* is sufficient to induce enrichment of Ycs4 in the nucleolus in stages of the cell cycle when the protein is normally not found concentrated at this locus. (4) Overexpression of *CDC14* is sufficient to induce segregation of the nucleolus under conditions when the structure is not segregated. Importantly, this segregation depends on a functional condensin complex. Whether Cdc14 and the FEAR network regulate telomere segregation by similar mechanisms remains to be determined but it is interesting to note that condensin is also present at telomeres during anaphase (Freeman et al., 2000) and condensin mutants have defects in telomere segregation in both budding yeast and *Drosophila* (Steffensen et al., 2001; Bhalla et al., 2002).

How does Cdc14 target condensin to the rDNA? Our data show that *CDC14* is required for sumoylation of Ycs4. Furthermore, overexpression of *CDC14* is sufficient to induce sumoylation of Ycs4 in stages of the cell cycle when the protein is normally not sumoylated. Is it then possible that *CDC14* targets condensin to the rDNA by inducing sumoylation of one of its subunits? The findings that inactivation of the sumo-deconjugating enzyme Smt4 causes condensin localization defects (Strunnikov et al., 2001) and rDNA segregation defects (Figure 6) support though not prove, this idea. Given that Cdc14 is also required for Ycg1 phosphorylation (Supplemental Figure 4B available on *Cell* website) it is likely that Cdc14 targets condensins to the rDNA in multiple ways.

The FEAR Network Is a Central Regulator of Anaphase—It Finishes Chromosome Segregation and Induces Exit from Mitosis

A long-standing question in the field of chromosome segregation is that of how cells ensure that exit from mitosis occurs only after chromosome segregation has been completed. Surveillance mechanisms could exist that halt cell cycle progression in response to partially segregated chromosomes. Our results suggest that, rather than employing sensors that monitor completion of chromosome segregation, the cell couples exit from mitosis and the completion of chromosome segregation by employing the same regulatory pathway, the FEAR network, for both processes. The FEAR network promotes the segregation of the genomic regions that segregate last during mitosis, the telomeres and the rDNA, and initiates exit from mitosis. Furthermore, as the FEAR network itself cannot promote exit from mitosis but functions as a "starter" of mitotic exit through its role in activating the MEN (Stegmeier et al., 2002), a temporal order is established. Whether, in addition to temporal coupling, feedback mechanisms exit that inhibit FEAR network activity when the segregation of early and/or late segregating sequences is aberrant is unknown.

It has long been known that in metazoans heterochromatic or repeated DNA regions of the genome such as the Y chromosome in *Drosophila melanogaster* and chromosomes with a high density of pericentric heterochromatin/repeated DNA in many mammals segregate late during mitosis (Vig, 1987; Gonzalez et al., 1991). Interestingly, vertebrate cells deficient for the Scc1/Mcd1 homolog have been shown to maintain cohesion at centromeres or telomeres, two sites enriched for repeated DNA (Sonoda et al., 2001). The parallels between these observations and our results are striking and suggest the existence of a cohesin-independent cohesion mechanism in metazoans.

Experimental Procedures

Strains and Growth Conditions

All yeast strains are isogenic with strain W303 (K699). Conditions for growth and release are as described in Amon (2002). α-factor was readded to all cultures 75 min after release from the G1 arrest to prevent wild-type cells from entering the next cell cycle. *cdc15-2* mutants were routinely 10 min delayed in the onset of budding and hence subsequent cell cycle events. To compare the kinetics of

spindle elongation and segregation of CEN-GFP dots, TEL-GFP dots, and the nucleolus we corrected the *cdc15-2* time course by 10 min in all experiments so that onset of budding and spindle formation could be compared with wild-type and *cdc14-3* cultures. For viability measurements, cells were grown to exponential phase at 23°C, synchronized in G2/M with nocodazole at 23°C, and released from the arrest in fresh YEPD at 37°C. Cells were then mildly sonicated and plated onto YEPD plates. Colony formation was determined after three days.

To construct a tetO array adjacent to the rDNA locus, we subcloned a 1 kb DNA fragment located between *YLR162W* and *MAS1* into an integrative vector containing a tetO array (Alexandru et al., 2001). The construct was linearized using XbaI and was integrated 3.4 kb away from the last *ASP3* repeat on the telomeric side of the *RDN1* locus.

Other Techniques

Detection of chromosomal GFP dots was performed as described by Alexandru et al. (2001) and He et al. (2000), that of tubulin, Ycs4-Myc and Nop1 as described by Visintin et al. (2003) and references therein. Separated nucleoli were defined as two distinct and unconnected Nop1 masses in cells with anaphase spindles. In the case of *cdc14-3* mutants, the nucleolus was often one elongated structure with a constriction of variable thickness. Mitotic spindle length was measured as described in Stegmeier et al. (2002). 100 cells were scored at each time point for each strain, unless noted otherwise. Dephosphorylation of immunoprecipitated proteins and Western blot analyses were performed as described in D'Amours and Jackson (2001) for phosphorylated proteins. To specifically detect sumoylated forms of Ycs4, the protein was immunoprecipitated from a denatured protein extract and probed with an α-Smt3 antibody (gift from C. Lima).

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