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Report

Condensin-Dependent rDNA Decatenation Introduces a Temporal Pattern to Chromosome Segregation

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Summary

The chromosomal condensin complex gives metaphase chromosomes structural stability. In addition, condensin is required for sister-chromatid resolution during their segregation in anaphase [1–7]. How condensin promotes chromosome resolution is poorly understood. Chromosome segregation during anaphase also fails after inactivation of topoisomerase II (topo II), the enzyme that removes catenation between sister chromatids left behind after completion of DNA replication [8, 9]. This has led to the proposal that condensin promotes DNA decatenation [3, 10, 11], but direct evidence for this is missing and alternative roles for condensin in chromosome resolution have been suggested [12–14]. Using the budding-yeast rDNA as a model, we now show that anaphase bridges in a condensin mutant are resolved by ectopic expression of a foreign (*Chlorella* virus) but not endogenous topo II. This suggests that catenation prevents sister-rDNA segregation but that yeast topo II is ineffective in decatenating the locus without condensin. Condensin and topo II colocalize along both rDNA and euchromatin, consistent with coordination of their activities. We investigate the physiological consequences of condensin-dependent rDNA decatenation and find that late decatenation determines the late segregation timing of this locus during anaphase. Regulation of decatenation therefore provides a means to fine tune the segregation timing of chromosomes in mitosis.

Results and Discussion

rDNA Anaphase Bridges Resolved by *Chlorella* Virus Topo II

Mutation of condensin in budding yeast results in approximately 1.5-fold undercompaction of chromosome arms in mitosis [15]. This would prevent only the longest chromosome arms from fully segregating during anaphase but, as in other organisms, resolution of most of the genome is impeded [2, 16, 17]. To facilitate our analysis of chromosome segregation, we have studied one specific locus, the rDNA repeats on the long arm of chromosome 12. Unlike most of the genome, the rDNA locus reaches full compaction only during anaphase, promoted by Cdc14 phosphatase-dependent condensin recruitment at this time [11, 12, 18]. By allowing condensation of much of the genome in metaphase, and inactivating condensin specifically at the onset of anaphase, we can demonstrate condensin's role in rDNA resolution [11].

To address whether the rDNA segregation failure in the absence of condensin can be explained by persisting catenation of the locus, we first tested whether increased dosage of yeast topo II could overcome the defect. Cells with the temperature-sensitive condensin mutation *ycg1-10* were arrested in metaphase at a permissive condition to allow condensation and resolution of most of the genome, except the rDNA. Then, yeast topo II was overexpressed under control of the galactose-inducible *GAL1* promoter. Condensin was inactivated by temperature shift, and cells were released from the metaphase block into anaphase. We monitored rDNA segregation by visualizing the rDNA-binding protein Net1 fused to GFP. Inactivation of condensin impeded nucleolar segregation, but this could not, or could only marginally, be improved by increased topo II dosage (Figure S1 available online). Topo II binding to yeast chromosomes is somewhat reduced in the absence of condensin, but overexpression restored chromosome binding to equal or greater levels than endogenous topo II (Figure S1 and [17]). This suggests that a reduction of topo II levels on chromosomes cannot by itself explain rDNA segregation defects in the absence of condensin.

The inability of increased topo II levels to restore rDNA segregation could mean that linkages other than catenation prevent rDNA segregation in the condensin mutant. Alternatively, it is possible that catenation could link the rDNA but that endogenous topo II is unable to resolve it without condensin. If the latter is true, supplying a condensin-independent decatenation activity might be able to resolve the rDNA. We therefore ectopically expressed the small topo II enzyme encoded in the *Paramecium bursaria chlorella* virus PBCV-1 genome (cv-topo II [19]). Because of its evolutionarily distinct origin, it might evade possible condensin-dependent regulation in the yeast host. Cv-topo II shows 43% sequence identity to budding-yeast topo II over the catalytic domain but lacks the C-terminal extension that has been implicated in the regulation of eukaryotic topo II. When we expressed cv-topo II in metaphase-arrested yeast cells, rDNA segregation in the condensin mutant was fully restored upon release into anaphase (Figure 1A). This suggests that decatenation failure was responsible for the rDNA segregation defect.

Cv-topo II possesses strong DNA cleavage activity [20], so DNA breakage, rather than decatenation, could have allowed rDNA segregation. Analysis of chromosome 12 by pulsed-field gel electrophoresis showed that it remained intact during segregation, even when DNA double-strand-break repair pathways were inactivated (Figure S2). This indicates that cv-topo II did not cause DNA cleavage under the conditions of our experiment. It has also been shown that suppression of rDNA transcription facilitates condensin recruitment to, and segregation of, the locus [13, 14]. We therefore measured

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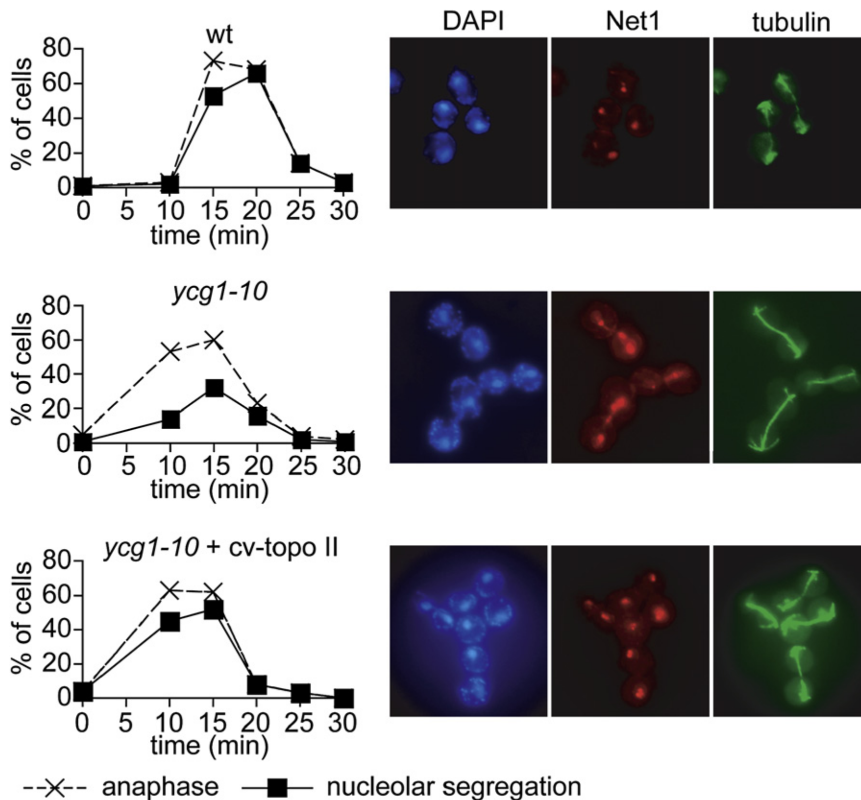

Figure 1. Chlorella Virus Topo II, cv-topo II, Rescues rDNA Segregation in a Condensin Mutant

Cells of strains Y2748 (*MAT*a *MET3-CDC20 NET1-GFP*), Y2749 (*MAT*α *ycg1-10 MET3-CDC20 NET1-GFP*), and Y2793 (*MAT*α *ycg1-10 MET3-CDC20 GAL1-cvTOP2 NET1-GFP*) were arrested in metaphase, condensin inactivated by shift to 37°C, and expression of topo II induced before cells were released into synchronous anaphase. rDNA segregation was monitored by staining the rDNA-binding protein Net1 fused to GFP with an α-GFP antibody. At least 100 cells were analyzed at each time point.

whether cv-topo II expression caused reduced rDNA transcription, but found that levels of the nascent 35S rRNA transcript remained constant throughout the course of the experiment (Figure S3). Together, these results suggest that cv-topo II most likely acts to resolve catenation of the rDNA locus, a function that yeast topo II performs inefficiently in the absence of condensin.

Colocalization of Topo II and Condensin on Chromosomes

Condensin could interact with, and activate, topo II, as has been observed for *Drosophila* condensin [3, 10]. Studies in other model organisms, including budding yeast, have failed to confirm such an interaction [16, 17, 21]. We therefore took a complementary approach to address possible cooperation between condensin and topo II by analyzing their localization. We mapped the distribution of the two proteins along the rDNA, as well as euchromatic regions of chromosome 12, by chromatin immunoprecipitation followed by hybridization to high-resolution oligonucleotide microarrays [22]. The patterns of the two proteins were not identical, but the peaks of association showed a statistically significant degree of coincidence, both at the rDNA locus and at euchromatic sequences (Figure 2A and Figure S4). Colocalization of topo II and condensin was further confirmed by immunostaining on spread chromosomes (Figure 2B), consistent with a functional interaction between the two proteins. The fact that colocalization is not restricted to the rDNA opens the possibility that condensin promotes decatenation of other parts of the genome as well.

Late Decatenation Determines Late rDNA Segregation

During wild-type mitosis, the rDNA segregates late, trailing behind most other chromosomes during anaphase [11, 12]. We therefore asked whether late segregation was the consequence of late decatenation of the locus. As an internal standard for segregation timing, we measured the anaphase spindle length at which the rDNA-associated Net1 signal separated. In wild-type cells, rDNA segregation occurred when the spindle reached 6–7 μm in length (Figure 3A), consistent with previous observations [11]. After condensin inactivation, even at a spindle length of 10 μm only half of the cells showed segregated rDNA. In rare instances, when spindles reached a greater length, rDNA segregation occurred with higher frequency (see below). Expression of cv-topo II advanced rDNA segregation in condensin mutant cells to a spindle length of about 6 μm, close to what is observed in wild-type cells. Notably, expression of cv-topo II in wild-type cells led to a further small advance of rDNA segregation. This became most apparent in analysis of rDNA relative to bulk chromosomes visualized by DAPI staining. In wild-type early anaphase cells, displaying a dumbbell-shaped nucleus, the rDNA was inevitably found unsegregated, bridging the middle of the dividing nucleus (Figure 3B). Later in anaphase, the rDNA trailed behind the rest of the genome on its way toward the cell poles. After cv-topo II expression, the rDNA split in most early anaphase nuclei, rDNA bridges were never seen, and in many cells the rDNA migrated close to the leading edge of the chromosome mass. This suggests that anaphase bridging, and consequent late segregation of the rDNA, is due to persistent catenation.

Condensin Promotes, but Is Not Essential for, Decatenation

We noticed that even in the *ycg1-10* condensin mutant, the rDNA eventually segregated in cells with very long anaphase spindles. Condensin was undetectable on chromosomes after inactivation of the *ycg1-10* allele, suggesting that delayed rDNA segregation occurs in the absence of condensin (Figure S5). Condensin-independent rDNA segregation could be achieved if the elongating spindle pushes sites of catenation along the chromosome until sister chromatids are released as catenation slips off chromosomes ends. To test this possibility, we analyzed rDNA segregation when decatenation was prevented by inactivating topo II at anaphase onset. We monitored two loci, on both sides of the rDNA locus, by integrating *lacO* repeats visualized by a lacI-GFP fusion protein [11]. In metaphase, all cells showed two GFP dots representing the two loci. After release into anaphase, wild-type cells transiently displayed one strong and two weaker signals,

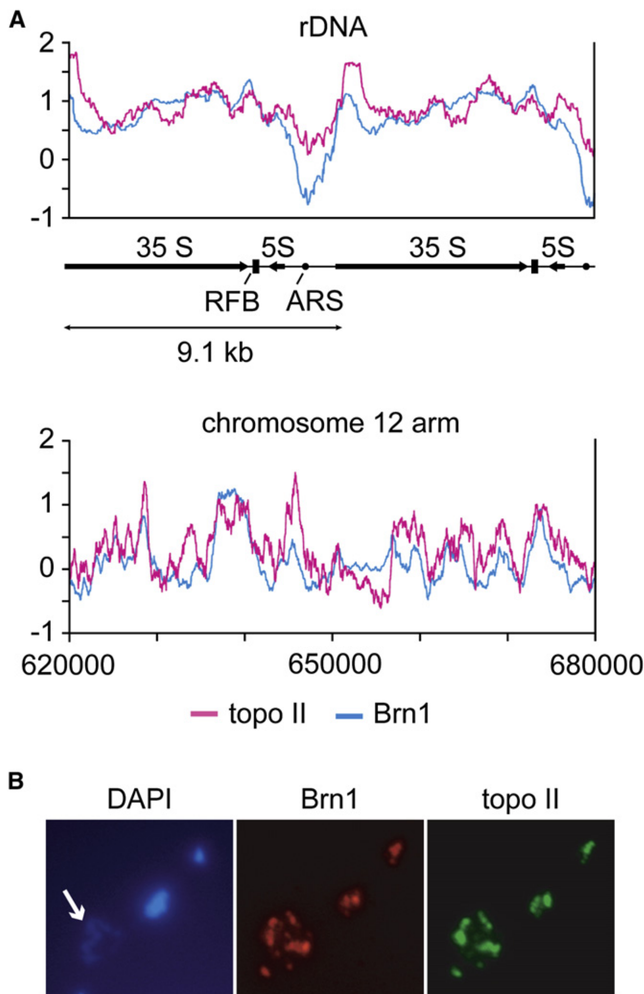

Figure 2. Colocalization of Condensin and Yeast Topo II

(A) Cells of strains Y2200 (*MAT*a *BRN1-Pk$_9$*) and Y2280 (*MAT*a *TOP2-Pk$_9$*) were arrested in metaphase by nocodazole treatment. Cells were processed for chromatin immunoprecipitation against the epitope-tagged Brn1 condensin subunit and against topo II. Binding along the rDNA and chromosome 12 between nucleotide coordinates 620,000 and 680,000 is shown as the ratio between chromatin immunoprecipitate and a whole-genome DNA sample. The y axis scale is $\log_2$. Comparison with the distinct association pattern of cohesin, and statistical analysis of the colocalization, is presented in Figure S4.

(B) Chromosome spreads were prepared from metaphase-arrested cells of strain Y3278 (*MAT*a *BRN1-HA$_6$ TOP2-Pk$_3$*). Colocalization of barren and topo II can be seen on bulk chromatin as well as the poorly DAPI-stained rDNA loop (arrow).

indicating that one of the loci had split whereas the other was still joined (Figure 4A). The split locus was almost always found closer to the spindle than the unsplit locus, suggesting that it was the centromere proximal locus that separated first. After this, all cells accumulated four GFP dots, two in each cell half, indicative of complete rDNA segregation. When condensin was inactivated, cells accumulated with one split and one unresolved locus for a longer time, before eventually cells appeared with fully separated rDNA. After inactivation of topo II, with the temperature-sensitive *top2-4* mutation, both pairs of loci failed to split throughout the course of the experiment. This suggests that persistent catenation cannot be pushed along the rDNA and, therefore, that eventual rDNA resolution in the absence of condensin is probably due to residual decatenation. Condensin may not be indispensable for decatenation of other chromosomes either. Resolution of the chromosome 4 arm is less strongly impeded by inactivation of condensin as compared to topo II inactivation [17].

We next tested whether pulling by the elongating mitotic spindle can compensate for condensin in promoting decatenation. Cells were synchronized in G1 by mating pheromone α-factor treatment and released into the cell cycle in the presence or absence of nocodazole, a microtubule poison that abolishes mitotic spindle function. The spindle checkpoint was inactivated in these cells by deletion of *MAD2*, so that cell-cycle progression into anaphase occurred despite the absence of a spindle. In wild-type cells, the presence of nocodazole caused only a slight delay to rDNA resolution, visualized by splitting of the GFP marks at both ends of the locus (Figure 4B). In the condensin mutant, rDNA separation was almost completely abolished by nocodazole. This confirms that the *ycg1-10* allele largely eliminates condensin function in rDNA resolution, but that the mitotic spindle restores slow segregation of the rDNA. Pulling by the spindle may promote rDNA decatenation by a basal level of condensin-independent topo II activity. Anaphase bridges, and their slow resolution, have been observed in human cells depleted of condensin [7]. Our findings open the possibility that these bridges are due to persisting catenation that, in the absence of condensin, are only slowly resolved by topo II.

Sister-Chromatid Decatenation and Chromosome Segregation

DNA catenation between sister chromatids most likely arises as the consequence of replication termination. Studies of simian virus 40 replication suggest that convergent replication forks are unable to completely resolve topological linkage between the unwound single strands, such that after completion of DNA synthesis the two replication products are left catenated [23]. Little is known about termination mechanisms of cellular replication and whether, where, and for how long catenation persists after S phase. Topo II activity is required for chromosome segregation in anaphase [8, 9], but it was not known whether chromosome compaction in metaphase, and segregation of sister chromatids away from each other during anaphase, is sufficient to give directionality to decatenation by constitutively active topo II, or whether topo II is subject to cellular regulation that has an impact on chromosome segregation. Our findings suggest that at least the budding-yeast rDNA locus awaits condensin-dependent activation of decatenation during anaphase. Topo II inherently favors decatenation over catenation, and condensin could promote intramolecular DNA looping that forms the basis for this directionality [24, 25]. Although we cannot exclude it, this scenario would not explain how ectopic cv-topo II, but not the overexpressed yeast enzyme, efficiently decatenates rDNA without condensin. An alternative scenario therefore is that condensin directly or indirectly stimulates endogenous topo II activity on chromosomes [3, 10]. Although the mechanism underlying condensin-dependent topo II activation remains to be investigated, the colocalization of the two proteins along yeast chromosomes is consistent with this possibility.

DNA catenation has been suggested as a means to provide sister-chromatid cohesion after DNA replication. Meanwhile, it has become clear that the chromosomal cohesin complex plays the major role in this process [26, 27]. Cohesin provides the essential counterforce to microtubule attachment in mitosis that allows chromosome biorientation on the mitotic

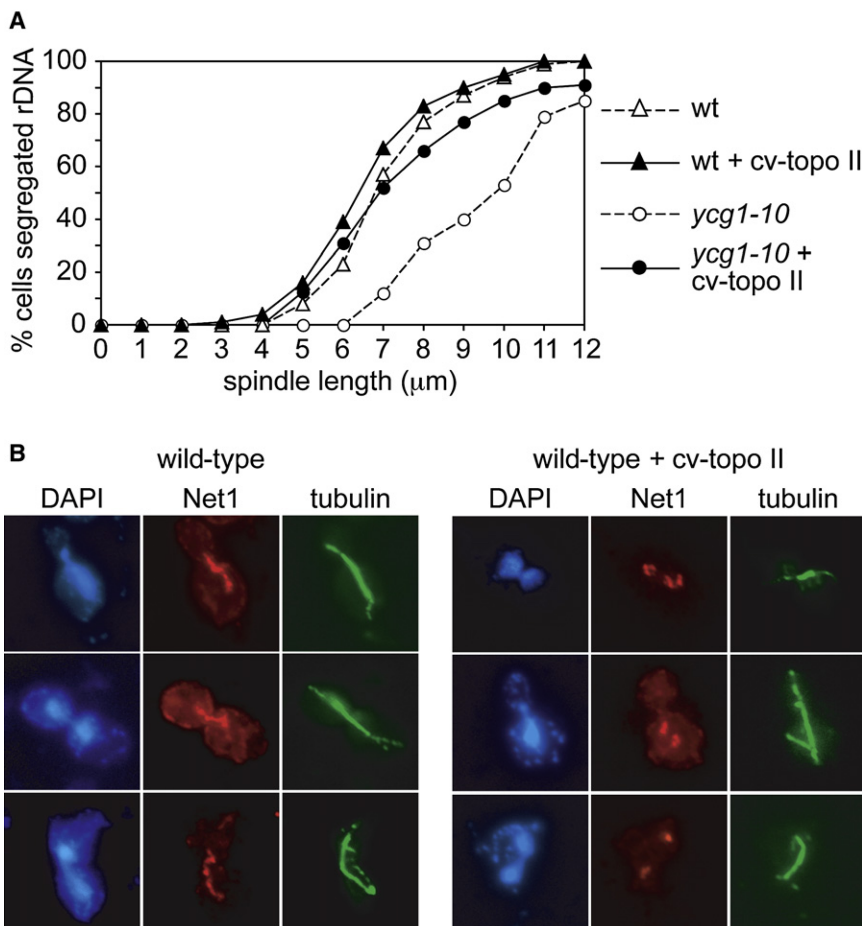

Figure 3. cv-topo II Expression Advances rDNA Segregation

(A) rDNA segregation as a function of spindle length is depicted during the experiment shown in Figure 1. At least 30 cells at each length were scored for rDNA segregation.

(B) Examples of wild-type cells displaying typical late rDNA segregation, and of cells expressing cv-topo II that show complete rDNA segregation already at early stages of anaphase.

spindle. Increased DNA catenation can only partly compensate for cohesin in this process [28]. Instead, we find that persisting catenation during anaphase is able to delay segregation at least of the budding-yeast rDNA locus. Differential segregation timing of individual chromosomes has also been observed in human mitosis [29, 30], and DNA bridges that require topo II for their resolution persist between centromeres of human chromosomes during anaphase [31]. Whether resolution of these DNA bridges influences the segregation timing of the affected chromosomes is not known. It has been observed that differential segregation timing determines the positioning of chromosomes with respect to each other in daughter nuclei [30]. In budding yeast, the rDNA is inevitably found in the nuclear periphery, consistent with its late segregation. It is conceivable that regulation of sister-chromatid decatenation provides the possibility to fine tune the segregation timing of complex genomes during anaphase.

Accession Numbers

The microarray data contained in this manuscript can be obtained from Gene Expression Omnibus with accession number GSE11988.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and five figures and can be found with this article online at http://www.current-biology.com/cgi/content/full/18/14/1084/DC1/.

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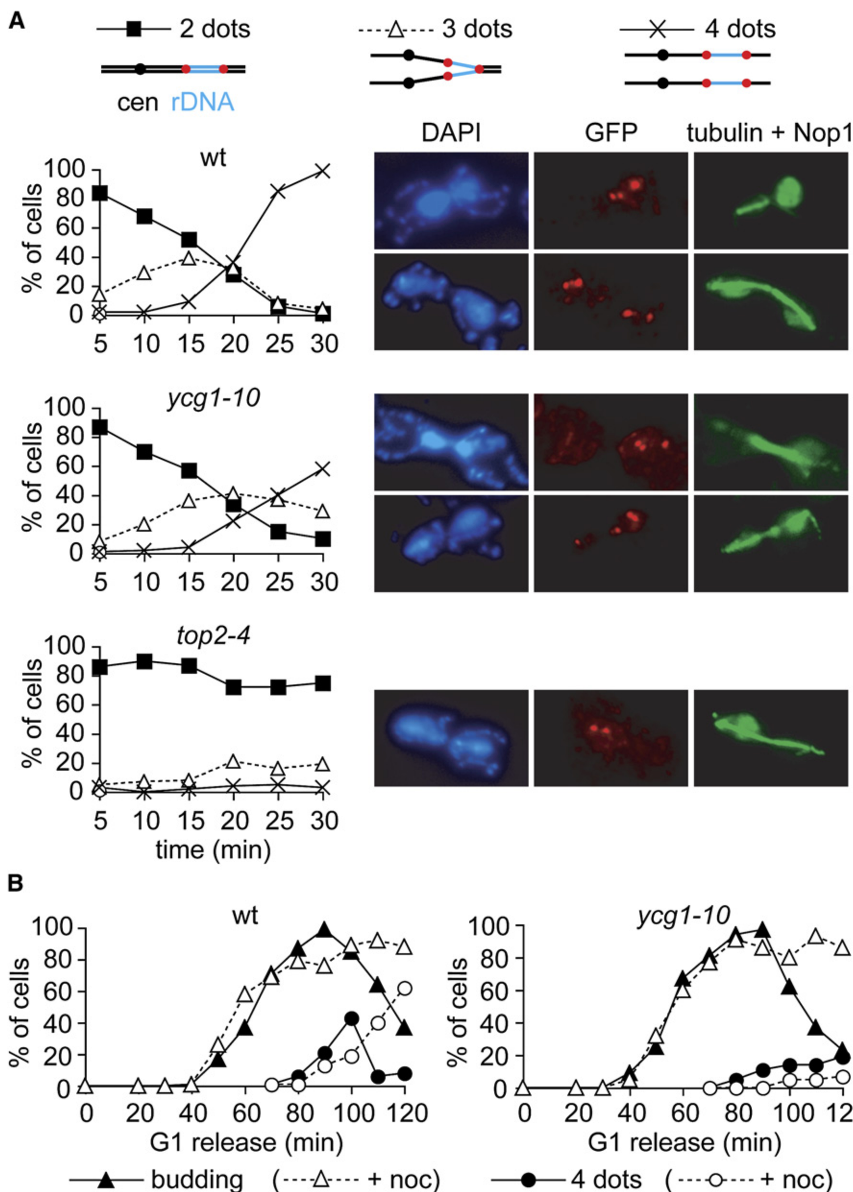

Figure 4. Slow rDNA Decatenation in the Absence of Condensin

(A) Delayed rDNA resolution after condensin but not topoisomerase II inactivation. Cells of strains Y2945 (*MAT*a *MET3-CDC20 lacI-GFP LacOs::MAS1 LacOs::ACS2*), Y2944 (as Y2945, but *MAT*α *ycg1-10*), and Y3152 (as Y2945, but *top2-4*) were arrested in metaphase and released into synchronous anaphase after temperature shift. Resolution of the rDNA proximal (*MAS1*) and rDNA distal (*ACS2*) locus was analyzed by splitting of the GFP dots. Staining of the mitotic spindle and the nucleolar marker Nop1 provided spatial clues as to the orientation of the rDNA array. At least 100 cells were analyzed at each time point.

(B) Strains Y3042 (*MAT*a *lacI-GFP LacOs::MAS1 LacOs::ACS2 mad2Δ*) and Y3245 (as Y3024, but *ycg1-10*) were synchronized in G1 with α-factor and released into the cell cycle at 37°C in the presence or absence of nocodazole. The budding index was analyzed to monitor cell-cycle progression, and rDNA resolution was scored by the appearance of four distinct GFP signals.

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