In Vivo Analysis of Chromosome Condensation in *Saccharomyces cerevisiae*[□]

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Although chromosome condensation in the yeast *Saccharomyces cerevisiae* has been widely studied, visualization of this process in vivo has not been achieved. Using Lac operator sequences integrated at two loci on the right arm of chromosome IV and a Lac repressor-GFP fusion protein, we were able to visualize linear condensation of this chromosome arm during G2/M phase. As previously determined in fixed cells, condensation in yeast required the condensin complex. Not seen after fixation of cells, we found that topoisomerase II is required for linear condensation. Further analysis of perturbed mitoses unexpectedly revealed that condensation is a transient state that occurs before anaphase in budding yeast. Blocking anaphase progression by activation of the spindle assembly checkpoint caused a loss of condensation that was dependent on Mad2, followed by a delayed loss of cohesion between sister chromatids. Release of cells from spindle checkpoint arrest resulted in recondensation before anaphase onset. The loss of condensation in preanaphase-arrested cells was abrogated by overproduction of the aurora B kinase, Ipl1, whereas in *ipl1-321* mutant cells condensation was prematurely lost in anaphase/telophase. In vivo analysis of chromosome condensation has therefore revealed unsuspected relationships between higher order chromatin structure and cell cycle control.

INTRODUCTION

Accurate chromosome segregation during mitosis is facilitated by the resolution of chromosomes into compact structures. From the level of the nucleosome, chromatin undergoes several changes in organization (Earnshaw, 1988; Filipinski *et al.*, 1990). A scaffold of proteins form an axis along which solenoidal chromatin loops are folded into rosettes, which then coalesce to form a sausage-shaped chromonema (Paulson and Laemmli, 1977; Marsden and Laemmli, 1979; Mullinger and Johnson, 1979, 1980; Earnshaw and Laemmli, 1983; Earnshaw, 1988). The metaphase chromosome consists of a folded or coiled chromonema (Manton, 1950). In a more recent model of chromosome organization, chromatin is folded into fibers around the central axis, which acts as a “glue” (Kireeva *et al.*, 2004). In both of these models, the protein scaffold is proposed to condense linearly during mitosis.

The mitotic scaffold was first observed in histone-depleted mammalian chromosomes and components of this protein scaffold have been of considerable interest (Adolphs *et al.*, 1977; Paulson and Laemmli, 1977). In higher eukaryotes, the process of condensing chromosomes before anaphase requires a protein scaffold comprised of at least condensin and topoisomerase II (Earnshaw *et al.*, 1985; Gasser *et al.*, 1986;

Adachi *et al.*, 1991; Saitoh *et al.*, 1994; Gimenez-Abian *et al.*, 1995; Hirano *et al.*, 1997; Maeshima and Laemmli, 2003). Condensin is a five-subunit complex that was first identified in *Xenopus* egg extracts (Hirano, 2005). Homologues of mitotic scaffold proteins are present in all eukaryotes (reviewed in Hirano, 2005). In the budding yeast *Saccharomyces cerevisiae*, homologues of the condensin complex, encoded by *SMC2*, *SMC4*, *YCG1*, *YCS4*, and *BRN1*, were deemed essential for chromosome condensation based primarily on analysis of the structure of the rDNA locus (Strunnikov *et al.*, 1995; Ouspenski *et al.*, 2000; Biggins *et al.*, 2001; Bhalla *et al.*, 2002; Lavoie *et al.*, 2002). However, *top2* mutants did not exhibit defects in condensation of the rDNA locus, suggesting that unlike the case in mammalian cells, budding yeast Top2 does not play an essential role in condensation (Lavoie *et al.*, 2002). Other studies have reported that Top2 activity is required for the proper resolution of the rDNA locus in anaphase (Sullivan *et al.*, 2004) and in fission yeast, Top2 is required for chromosome condensation (Uemura *et al.*, 1987).

All eukaryotic chromosomes condense in a cell cycle-regulated manner. Two pathways are required to establish and maintain chromosomes in the condensed state through mitosis. In fission yeast and *Xenopus*, the establishment of condensation is dependent on phosphorylation of the Smc4 subunit of condensin by the mitotic Cdk (Kimura *et al.*, 1998; Sutani *et al.*, 1999). However, in budding yeast the cyclin-dependent kinase, Cdc28, has not been identified as a key factor that regulates condensation. The first pathway, required to establish condensation in budding yeast, is dependent on condensin and cohesin complexes (Lavoie *et al.*, 2004). The second pathway, which maintains condensation, is thought to require the Aurora B kinase (Ipl1 in *S. cerevisiae*; Giet and Glover, 2001; Petersen *et al.*, 2001; Hagstrom *et al.*,

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Table 1. Strain genotypes

Strain	Genotype
AVY88	<i>MATa bar1Δ trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY89	<i>MATα bar1Δ trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3 his3::LacR-GFP::HIS</i>
AVY101	<i>MATa bar1Δ smc2-8 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY106	<i>MATa bar1Δ brn1-9 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY99	<i>MATa bar1Δ top2-4 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY147	<i>MATa bar1Δ trp1::TetO::TRP1 lys4::LacO::LEU2,TRP1 his3::LacR-GFP::HIS3 TetR-mRFP</i>
AVY153	<i>MATa bar1Δ smc2-8 trp1::TetO::TRP1 lys4::LacO::LEU2 his3::LacR-GFP::HIS3 TetR-mRFP</i>
AVY120	<i>MATa bar1Δ apc2::KAN pCEN-apc2-4(TRP1) trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY91	<i>MATa bar1Δ leu2::GAL1:pds1-Δdb::LEU2 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY179	<i>MATa bar1Δ ura3::GAL1:MPS1::URA3 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY94	<i>MATa bar1Δ leu2::GAL1:pds1-Δdb::LEU2 trp1::LacO::TRP1,LEU2 his3::LacR-GFP::HIS3</i>
AVY132	<i>MATa bar1Δ leu2::GAL1:pds1-Δdb::LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY127	<i>MATa bar1Δ leu2::GAL1:pds1-Δdb::LEU2 CEN4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY175	<i>MATa bar1Δ ura3::GAL1:MPS1::URA3 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY177	<i>MATa bar1Δ ura3::GAL1:MPS1::URA3 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY193	<i>MATa bar1Δ ura3::GAL1:IPL1(URA3) trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY164	<i>MATa bar1Δ trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3 mad2::KAN^R</i>
AVY235	<i>MATa bar1Δ ipl1-321 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY154	<i>MATa bar1Δ cdc28-1N trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY232	<i>MATa bar1Δ mcd1-1 trp1::LacO::TRP1,LEU2 lys4::LacO::LEU2 his3::LacR-GFP::HIS3</i>
AVY243	<i>MATa bar1Δ MMP1::LacO::URA3 CMS1::LacO::URA3 his3::LacR-GFP::HIS3</i>

2002; Kaitna *et al.*, 2002; Lavoie *et al.*, 2004; Gadea and Ruderman, 2005). After cleavage of the Mcd1/Scc1 subunit of cohesin, Ipl1 activity appears to be required to maintain chromosomes in the condensed state, possibly through phosphorylation of the condensin complex and histone H3. Loss of cohesin from chromosomes coincides with the switch in the requirements for condensation from the initial cohesin-dependent condensed state to an Ipl1-dependent state of maintained condensation (Lavoie *et al.*, 2004).

Chromosomes of *S. cerevisiae* do not form visibly discrete structures in mitosis, making direct observation of mitotic chromosomes by light microscopy unfeasible. However, several indirect methods to visualize chromosomes have been developed (reviewed in Loidl, 2003). Visualization of chromosome condensation using fluorescent *in situ* hybridization (FISH) in budding yeast revealed that highly repetitive rDNA sequences become compact during mitosis (Guacci *et al.*, 1994) and hence this locus became the paradigm to study the process of condensation in yeast. FISH analysis of the rDNA locus revealed that this region of the genome undergoes dramatic structural changes through the cell cycle. In G1 and S, the rDNA appears as large or small puffs that become highly condensed into a cluster in G2/M. On nocodazole arrest, the cluster is then resolved into lines and loops. This reorganization of the rDNA from clusters into lines and loops during mitosis is dependent on the condensin complex (Lavoie *et al.*, 2004). Although analysis of the rDNA locus has proven to be a valuable approach, other regions of the genome have been largely neglected. Only a few studies have correlated rDNA condensation to condensation of chromosome regions that are not highly repetitive (Guacci *et al.*, 1994; Lavoie *et al.*, 2000). These studies observed condensation of chromosome XVI using FISH probes 145 kb and/or 255 kb apart. Another study used chromosome painting to visualize condensation of chromosome VII (Freeman *et al.*, 2000). However, the major focus in all these studies was the rDNA locus. Although condensin is presumably essential for condensation of other regions of the genome in *S. cerevisiae*, as is evident from the many binding sites for condensin on every chromosome (Wang *et al.*, 2005), this has not been tested directly *in vivo*. In strains in which

the rDNA repeats have been excised from their native genomic locus, the condensin complex remains essential (Freeman *et al.*, 2000), indicating that condensin has roles in organizing other regions of the genome. Therefore, it is important to study condensation of non-rDNA regions of chromosomes in order to fully understand faithful chromosome segregation during mitosis.

Using the LacO/LacR-GFP system to mark chromosomal loci (Straight *et al.*, 1996), we devised a method to study condensation in live yeast. Using two sets of LacO repeats spaced at ~450 kb apart on the right arm of chromosome IV, we directly observed condensation immediately before anaphase. The condensation observed was dependent on the condensin complex and Topo II. In depth analysis using this system revealed an unexpected finding: in strains that were arrested before the metaphase-to-anaphase transition we found that the condensed state of the right arm of chromosome IV was only temporarily maintained. Further investigation of this phenomenon revealed that elevated Ipl1 kinase activity could maintain condensation in arrested cells and that, in an unperturbed cell cycle, Ipl1 functions to maintain chromosomes in the compact state during anaphase/telophase.

MATERIALS AND METHODS

Yeast Strains

List of strain genotypes are in Table 1. All strains are derivatives of BF264-15 15DU: *MATa ura3Δns ade1 his2 leu2-3112 trp1-1^a* (Richardson *et al.*, 1989). Strains were grown at 30°C except for temperature-sensitive mutants that were grown at 24°C in overnight cultures and at the nonpermissive temperatures during time-course experiments.

Cell Cycle Experiments

Overnight cultures were grown to stationary phase in rich medium containing extra adenine. Cells were diluted to OD 0.2 in rich medium with α -factor (concentration ranging from 0.2 to 1 μ g/ml) to synchronize cells in G1. Synchrony was monitored under the microscope after 2–3 h. Cells were washed three times with water and then released into fresh medium at 30°C. For arresting cells with nocodazole (15 μ g/ml), the nocodazole was added 10 min after the release. For expression of *GAL1-MPS1*, *GAL1-pds1-Δdb*, or *GAL1-IPL1*, the strains were grown overnight with raffinose as the carbon source and then upon G1 release, galactose was added to a final concentration of 4%.

Figure 1. Chromosome condensation in *S. cerevisiae*. (A) Schematic diagram (not drawn to scale) showing the positions of integrated LacO repeats at loci on the right arm of chromosome IV. (B) Cartoon showing the possible organization of the chromosome IV right arm, based on the radial loop model of chromosome organization and the data presented herein. Predicted locations of the *TRP1* and *LYS4* loci in interphase and condensed chromosomes are shown. (C) Wild-type cells at different cell cycle stages with LacO repeats integrated at *TRP1* and *LYS4* and expressing LacR-GFP (i.e., the *TRP1-LYS4* strain). Two fluorescent dots are typically seen in interphase (leftmost two panels), and a single dot is often seen before mitosis (third panel from the left), followed by segregation of the dots to daughter cells (rightmost panel). Each panel represents the populations of cells that were scored in the time-course experiment in D.

Time points were collected as shown for each experiment. A total of 100–200 cells were counted per time point.

Microscopy and Imaging

Cells were visualized for fluorescence and DIC images using an Zeiss Axio Plan II microscope with Alpha Plan Fluor 100 $\times$ /1.45 or Plan Apo 63 $\times$ /1.4 objectives (Thornwood, NY). All images of live cells were captured using the AxioCam camera and Axiovision software v4.0. Images were then adjusted using Axiovision software or Axiovision LE v.4.4/4.5. Calculation of compaction ratios within the *TRP1-LYS4* region is described in Supplementary Figure 5.

RESULTS

Visualizing Condensation of the Right Arm of Chromosome IV In Vivo

Chromosome condensation in *S. cerevisiae* has primarily been studied by analysis of the large rDNA locus. Study of condensation within non rDNA regions of the yeast genome has been limited to a few loci using fixed chromatin, spread on glass slides (Guacci *et al.*, 1994). Indeed, the 16 yeast chromosomes are of varying sizes, some of which could potentially be too small to require condensation before metaphase. Moreover, mechanisms involved in condensation of the highly repetitive rDNA might differ from those that result in compaction of other chromosomal regions.

We addressed whether condensation occurs in vivo in yeast at regions other than the rDNA. We modified the LacO/LacR-GFP system (Straight *et al.*, 1996), constructing strains carrying combinations of LacO sequences placed pairwise at loci along the right arm of chromosome IV. These regions included *CEN4*, *TRP1*, *LYS4*, and *TEL4* loci (Figure 1, A and B, and Supplementary Figure 1). We used the rationale that two loci separated by a large linear distance along a chromosome arm would be brought into close proximity during mitosis (linear condensation). Thus, two separate and distinct fluorescent signals in interphase cells would, in theory, coalesce to form a single fluorescent dot in mitotic cells. We found that *TRP1*- and *LYS4*-tagged loci display this behavior (Figure 1, A–D). In asynchronously growing populations of this strain (referred to as the *TRP1-LYS4* strain), most cells had two dots, whereas a small percentage of large-budded cells contained a single dot (Figure 1C). To investigate the possibility that the large-budded cells with one dot represented a population of cells in G2/M with condensed chromosomes, the *TRP1-LYS4* strain was synchronized with α factor for 2 h and then released into the cell cycle. We monitored the number of dots per cell through the cell cycle by collecting cells at 5-min intervals after release (Figure 1D). In a representative experiment shown in Figure 1D, we found that as the cells progressed out of G1

The colored outline of each panel corresponds to the color scheme used in D. Bar, 5 μ m. DIC and the green fluorescence channel (eGFP) are overlaid. (D) Time-course experiment after an α -factor arrest and release at 30°C. Cells with LacO sequences at *TRP1* and *LYS4* were scored according to the photos in C. Anaphase/telophase was defined as when two fluorescent signals were greater than 4 μ m apart and were positioned along the division plane (i.e., the chromosomes were in the process of segregating). (E) Analysis of condensation/decondensation in the *TRP1-LYS4* region in telophase cells. The number of fluorescent dots in the mother cell and bud were scored and cells categorized as having one dot in each (i.e., both *TRP1-LYS4* regions were condensed), two dots in each (i.e., both *TRP1-LYS4* regions were decondensed), or two dots in either the mother or the bud and one dot in the other (i.e., one *TRP1-LYS4* region was condensed and the other was decondensed). (F) Representative images of the categories of telophase cells quantified in E. DIC and the green fluorescence channel (eGFP) are overlaid.

(shmoo cells decreased; blue line, Figure 1D), the percentage of budded cells with two dots (green line, Figure 1D) increased, peaking ~45 min after release. As this category decreased with time (green line, Figure 1D), budded cells with a single dot appeared in the population (red line, Figure 1D), their number peaking ~60 min after release from G1. At this time ~50% of the budded cells contained a single dot. Thereafter, a decrease in the number of budded cells with a single dot mirrored the appearance of cells in anaphase (black line, Figure 1D). These data suggest that the two LacO-tagged loci coalesce before anaphase. The presence of a single dot in budded cells could be due to a process through which the LacO sequences are brought into proximity through the looping out of the intervening DNA. Alternately, the LacO sequences could have been brought together through a process of regulated condensation.

After the completion of anaphase, chromosomes are thought to decondense during the formation of the daughter cells. We observed this process of decondensation in telophase cells in time-course experiments performed as described above (Figure 1, E and F). When the first cells reached telophase, ~60 min after release from G1, most of these contained either a single dot in the mother and daughter cell or a single dot in one and two dots in the other. Between 65 and 85 min, the number of telophase cells with two dots in both the mother and the daughter increased (Figure 1E).

Not Every Locus on the Chromosome IV Right Arm Condenses

Surprisingly, the above analysis did not hold true for all locus pairs tested on the right arm of chromosome IV. The distance between the two LacO-tagged regions at *TRP1* and *LYS4* is ~450 kb and on average, in unbudded cells and cells with small buds, the two fluorescent signals corresponding to these loci are spaced ~1.06 μm apart ($n = 120$, $SD = 0.24$). Before mitosis, the two dots coalesce and the distance between the dots is reduced to ~0.33 μm ($n = 150$, $SD = 0.2$). The distance between the dots was measured from the center of one dot to the center of the second dot. In contrast, although the LacO-tagged regions at *LYS4* and *TEL4* are ~500 kb apart, their corresponding fluorescent signals are spaced no more than 0.1 μm apart (Supplementary Figure S1). Thus the proper placement of the LacO repeats along a chromosome is essential to allow visualization of condensation. These data lead us to speculate that the *TRP1* and *LYS4* loci lie in separate rosettes and that the *LYS4* and *TEL4* loci lie in the same rosette (Figure 1B). From the distances between the *TRP1* and *LYS4* dots, we calculated the compaction ratio to be ~120 $\times$ for cells in interphase, compared with ~270 $\times$ in G2/M cells (see *Materials and Methods* for calculations). Thus the *TRP1*-*LYS4* region condenses about twofold before anaphase, which is similar to previous estimates based on FISH analysis of fixed chromatin.

Condensation of the MMP1-CMS1 Region of Chromosome XII

Because two regions of chromosome IV behaved differently in our assay, we sought to establish if we could observe coalescence of another chromosome region during G2/M phase. We integrated LacO-repeats at several loci on chromosome XII and after expression of LacR-GFP, we observed separation of the *MMP1*-*CMS1* interval in unbudded and small-budded cells, but coalescence of the two fluorescent dots in some large-budded cells (Supplementary Figure 2A). The *MMP1*-*CMS1* region encompasses most of the left arm of chromosome XII. Using synchronized cultures of these cells, we performed the same analysis as described in Figure

1D and found that the behavior of the *MMP1*-*CMS1* region is consistent with condensation in G2/M phase (Supplementary Figure 2B).

Mutations in Condensin and Topoisomerase II Disrupt Condensation

In most eukaryotes, condensation of chromosomes requires the condensin complex and Topo II (reviewed in Hirano, 2005). To substantiate our claim that chromosome IV does condense before anaphase (as seen by the coalescence of the two fluorescent dots at *TRP1* and *LYS4*), we constructed strains carrying mutations in *SMC2* and *BRN1*, two components of the condensin complex (Strunnikov *et al.*, 1995; Ouspenski *et al.*, 2000). If, indeed, the LacO sequences were brought into close proximity through condensation, mutations that affect the condensin complex would prevent the dots from coalescing. Hence, we expected to observe a decrease in the number of cells with a single dot in G2/M-phase. After constructing strains with temperature-sensitive *smc2-8* or *brn1-9* alleles and possessing LacO sequences integrated at *TRP1* and *LYS4*, we observed large-budded cells with two fluorescent dots (Figure 2A). In these strains, condensation might have occurred initially, followed by a failure to maintain condensation, or alternatively condensation may have failed altogether. To distinguish these possibilities, the *smc2-8* and the *brn1-9* strains were synchronized in G1 as described above and then observed through a single cell cycle (Figure 2B). Unlike wild-type cells, we found that in both mutant strains the population of budded cells with a single dot never exceeded 15% of the total cells. These data suggest that the process by which the LacO sequences are brought together before mitosis is dependent on Smc2 and Brn1. It is therefore reasonable to equate coalescence of the *TRP1* and *LYS4* signals with condensation of this region of the chromosome IV right arm.

Topo II, another component of the mitotic scaffold, is an essential enzyme required for decatenation of chromosomes before mitosis. Although numerous studies have reported a requirement of Topo II for condensation in most eukaryotes, there is no evidence that Top2 is required for condensation of budding yeast chromosomes (based on analysis of the rDNA locus; Lavoie *et al.*, 2004; Sullivan *et al.*, 2004). Therefore, we used our *in vivo* system to determine if Top2 is required for condensation of the chromosome IV right arm. The *top2-4* allele was crossed to the strain with LacO repeats at *TRP1* and *LYS4* and we analyzed these cells after release from G1 synchrony. As seen in Figure 2, A and B, the two LacO dots failed to coalesce during G2/M-phase, indicating that Top2 is required for condensation of chromosome IV in budding yeast.

Loss of Cohesion Can Be Distinguished from a Loss of Condensation

A large body of data in yeast links the process of condensation to the process of cohesion, which holds sister chromatids together before anaphase (Guacci *et al.*, 1994; Hartman *et al.*, 2000; Biggins *et al.*, 2001; Bhalla *et al.*, 2002; Lavoie *et al.*, 2004). A limitation with our strategy to study condensation is that we are unable to easily distinguish between loss of condensation and loss of cohesion between sister chromatids. However, the *TRP1* dot is larger than the *LYS4* dot in our strains because twice as many LacO repeats are integrated at *TRP1* than at *LYS4*. Thus, the two loci can be distinguished based on the size of the fluorescent dot. In most cells we are able to distinguish a loss of cohesion from a lack of condensation using these criteria. Still, in the condensin and *top2* mutants that we analyzed, some cells with two dots might have possessed a condensed chromosome IV

Figure 2. Chromosome condensation requires condensin and Topo II. (A) Images of wild-type and mutant cells with LacO repeats integrated at *TRP1* and *LYS4* and expressing LacR-GFP, captured after growth at 30°C (semipermissive for the mutant strains) for 2 h. Large-budded cells of similar size were compared for the four strains. Bar, 5 μ m. DIC and the green fluorescence channel (eGFP) are overlaid. (B) Analysis of *TRP1-LYS4* region compaction in synchronized wild-type and mutant cells. Cells were arrested in G1 and released at the semipermissive temperature for the mutant strains. Cells were scored into the categories described in Figure 1, C and D, and in addition, preanaphase cells with more than two fluorescent signals were counted (Multiple dots). (C and D) Photomicrographs of wild-type (C) and *smc2-8* mutant cells (D) with TetO sequences at *TRP1* and LacO at *LYS4* and expressing LacR-GFP and TetR-mRFP. DIC and the red and green fluorescence channels are overlaid. The *TRP1* locus is visualized as a single red dot and the *LYS4* locus is the green dot. Cells were arrested with α -factor, washed, and released into fresh medium. Cells were collected at different intervals and photographed. In D, the cartoon shows the expected pattern of dots resulting from a loss of cohesion and a loss of condensation.

right arm and have lost cohesion at either *TRP1* or *LYS4*. We sought to rule out this possibility by visualizing the LacO sequences at single loci, either *TRP1* or *LYS4*, in the mutant strains, but this was complicated because these analyses revealed that each of the strains contained cells that had lost cohesion at either *TRP1* or *LYS4* (data not shown). Therefore, to unequivocally determine if condensation can be observed to fail using the *TRP1-LYS4* system, we utilized strains with two different fluorescent colors (using LacO repeats at *LYS4* and TetO repeats at *TRP1*, combined with expression of LacR-GFP and TetR-RFP) to distinguish between cohesion and condensation (Figure 2C). α -factor-arrested cells of this strain contained G1 cells with distinct red and green dots. As cells were followed through the cell cycle after release from the α -factor block, the red and green signals coalesced in large-budded cells when compared with small-budded cells in the population (Figure 2C and data not shown). The distance between these dots was on average

1.5 μ m (SD = 0.3, n = 37) in interphase (unbudded or small-budded cells) and was 0.8 μ m (SD = 0.2, n = 45) just before nuclear division (in large-budded cells). Importantly, the red and green dots failed to come close together in large-budded *smc2-8* cells (Figure 2D), indicating a lack of condensation. These data confirm that coalescence of the *TRP1* and *LYS4* loci can be used to monitor condensation of a chromosome IV region in live cells. Interestingly, some *smc2-8* cells also displayed loss of cohesion at the nonpermissive temperature (Figure 2D and see below), congruent with previous studies that linked the processes of condensation and cohesion (Guacci *et al.*, 1997; Hartman *et al.*, 2000; Biggins *et al.*, 2001; Bhalla *et al.*, 2002; Lavoie *et al.*, 2004).

Budding Yeast Chromosomes Only Transiently Condense before Anaphase

In most eukaryotes studied, chromosome condensation is completed in prometaphase. Prolonged mitotic arrest, for

Figure 3. Chromosomes decondense in response to a preanaphase arrest. (A) Images of cells captured 1.5 h after release from G1 synchrony (preanaphase arrest induced by nocodazole treatment, expression of *GAL1-MPS1*, or *GAL1-pds1-Δdb* or by growth of *apc2-4* cells at the nonpermissive temperature). Galactose was added to the culture upon release from G1, and nocodazole was added 10 min later. Each strain expressed LacR-GFP and possessed LacO repeats at *TRP1* and *LYS4*. DIC and the green fluorescence channel (eGFP) are overlaid. (B) Images of cells expressing *GAL1-pds1-Δdb* after release from G1 as described in A. LacO repeats are integrated at *CEN4*, *TRP1*, or *LYS4*. (C) Analysis of the timing of condensation and decondensation of the *TRP1-LYS4* region in preanaphase-arrested cells. Strains were first synchronized in G1 and released as described in A. (D) Condensation and decondensation of the *TRP1-LYS4* region upon nocodazole arrest visualized with the combination of TetO repeats at *TRP1* and LacO repeats at *LYS4* (cells expressing TetR-mRFP and LacR-GFP). Nocodazole was added 10 min after release from G1 arrest, and photos were taken after 1.5 h. Panels shown represent cells with condensed (single yellow spot) or decondensed (one red and one green spot) chromosomes. DIC and the red and green fluorescence channels are overlaid.

example, in the presence of microtubule destabilizing drugs, results in hypercondensation. We reasoned that we would be most successful in visualizing condensed chromosomes (presumably chromosomes would be hypercondensed) if cells were arrested just before the metaphase-to-anaphase transition. To our surprise, we found that most cells that were arrested just before anaphase possessed two dots (Figure 3A). We used four different methods of arresting cells in mitosis before anaphase. Cells were arrested with nocodazole (a microtubule-destabilizing drug), by using a temperature-sensitive APC/C mutation (*apc2-4*), by overexpression of a destruction box-minus allele of *PDS1* (*pds1-Δdb*), or by overproduction of the Mps1 kinase (Figure 3A). Irrespective of the method used to block anaphase progression, most large-budded cells in the population contained two fluorescent dots. (This was also the case when we analyzed the MMP1-CMS1 region of chromosome XII after cell cycle arrest in the presence of nocodazole; see Supplementary Figure 2, A and C.) Using *pds1-Δdb*, an inhibitor of sister chromatid separation, we arrested cells carrying single LacO repeats at *CEN4*, *TRP1*, or *LYS4*. As expected, the *CEN4* region possessed separated dots, as has been previously observed to reveal tension at the centromere that results from biorientation of the chromosome on the spindle (Figure 3B; Goshima and Yanagida, 2001; Pearson et al., 2001). However, most of the *TRP1* and almost all of the *LYS4* cells arrested with a single dot (Figure 3B and data not shown). Thus, observation of two dots in the *TRP1-LYS4* strain expressing *pds1-Δdb* suggested that the chromosomes were decondensed in the preanaphase arrest. This raised the question as to whether the chromosomes had failed to condense or whether the chromosomes had initially condensed but then decondensed at the arrest point. To address this issue,

we characterized all four preanaphase arrests in time-course experiments (Figure 3C). Using this assay we found that in each case, the two dots did coalesce before ultimately separating in cells arrested before anaphase (Figure 3C). This result suggests that the establishment of chromosome condensation occurred before metaphase but could not be maintained. Once more, to confirm our findings, we used the red-green system of dots. It was apparent that nocodazole-arrested cells lost condensation during the preanaphase block (Figure 3D). Interestingly, we also noticed that some cells that had been arrested in nocodazole for a prolonged time (3.5 h) possessed three or four fluorescent dots, indicating that in these cells loss of cohesion between sister chromatids had also occurred (explored further below).

Decondensation in Preanaphase Arrested Cells Depends on the Spindle Checkpoint

The above experiments raised the interesting possibility that cells arrested before anaphase only transiently condense their chromosomes. Decondensation could have occurred because *S. cerevisiae* cells only transiently maintain the condensed state upon cell cycle arrest, or decondensation could have been due to either checkpoint adaptation or leakiness of the cell cycle arrest. Typically, cell cycle arrests are transiently enforced; cell cycle progression can be actively reinitiated through checkpoint adaptation, or the cell cycle might resume if the cause of the arrest abates (for example, in these experiments, if the nocodazole were unstable). To test if decondensation was a result of checkpoint adaptation, or due to a leaky arrest, we first asked if the timing of decondensation was nocodazole concentration dependent (Figure 4A). The *TRP1-LYS4* strain was arrested in G1 with α -factor then released into medium containing 5, 10, or 15 μ g/ml

nocodazole. After cell cycle arrest before anaphase (between 1.5 and 3.5 h after release from G1), 70–80% of the cells had decondensed the *TRP1-LYS4* region and decondensation was largely nocodazole concentration independent.

We next examined the timing of adaptation, based on cell cycle progression (measured as rebudding), relative to the timing of decondensation. After G1 arrest and release, either nocodazole was added to the medium, *GAL1-MPS1* expression was induced or *GAL1-pds1-Δdb* expression was induced. On the basis of the experiments described above, we knew that under these conditions most cells have reached the preanaphase arrest point and have decondensed the *TRP1-LYS4* region by 1.5 h after G1 release. Therefore, we took samples from this time point onward and determined when rebudding occurred (the appearance of cells with small buds that had adapted and had advanced into a new cell cycle). Several conclusions can be drawn from the results of this experiment (Figure 4B) that are inconsistent with decondensation being a result of checkpoint adaptation. First, decondensation occurred with a similar frequency (~80% of the cells) by the 1.5-h time point in each of the three conditions: c-mitosis arrest in nocodazole and metaphase arrests induced by *Mps1* or *Pds1-Δdb* overproduction. However, the timing of rebudding differed, occurring

later in the *MPS1* and *PDS1-Δdb*-expressing cells than in the cells grown with nocodazole. This suggests that *Mps1* and *Pds1-Δdb* might strengthen the spindle checkpoint, resulting in delayed adaptation, but because decondensation occurred with similar timing in each condition, this argues that decondensation is not closely related to adaptation as measured by cell cycle progression. Furthermore, the timing of decondensation versus rebudding (under each condition) was inconsistent with decondensation resulting from adaptation, because rebudding occurred at least 3 h after decondensation. Taking into account the time that cells would normally require to progress from G2 to the next S phase, we estimate that decondensation ought to have occurred no more than 60 min before rebudding if decondensation were a result of adaptation. In similar experiments we determined that *Pds1* was stable in cells that had decondensed the *TRP1-LYS4* region and that *Pds1* decay began only 2 h later, consistent with the timing of rebudding. This was the case in nocodazole-arrested cells and in *apc2-4* mutants (Supplementary Figure 2, D and E). Therefore, decondensation occurs well before the cell cycle continues past the preanaphase arrests. There remains a possibility that distinct adaptive mechanisms exist that control decondensation and cell cycle progression.

In the experiments shown in Figure 4B, we also estimated the frequencies of cells that had lost cohesion between sister chromatids (scored by counting cells with more than two fluorescent signals). Similar to rebudding, the extent of loss of cohesion was higher in the presence of nocodazole than with Mps1 or Pds1- Δdb overproduction, and in agreement with this, the timing of loss of cohesion under each condition was more consistent with an adaptive process. We therefore conclude that decondensation is unlikely to result from an adaptive process, whereas our data indicate that the timing of loss of cohesion does approximately correlate with adaptation.

Although in the above experiment the timing of decondensation was inconsistent with adaptation, we wanted to exclude a role of adaptation in decondensation using a genetic approach. The only known mutations that compromise the ability of *S. cerevisiae* cells to adapt to the spindle checkpoint are hypomorphic *cdc28* alleles. Unfortunately, however, we found that such *cdc28* mutants are unable to perform condensation, and thus we were unable to assess a possible role of adaptation in decondensation using this strategy (Supplementary Figure 2F). As an alternative, we explored whether the spindle checkpoint is required for decondensation by comparing the timing of decondensation in wild-type and *mad2 Δ* cells after release from G1 synchrony. We added nocodazole to rule out possible effects on the chromatin that might be due to tension at the kinetochore produced by the spindle. On release from G1, both strains condensed the *TRP1-LYS4* region with similar timing, but strikingly, decondensation was perturbed in most *mad2 Δ* cells (Figure 4C). This difference was not due to cell cycle differences between the strains because we observed the same trend when both strains expressed *GAL1-pds1- Δdb* to arrest the cells before anaphase (data not shown). One explanation of these data is that decondensation is dependent on the spindle checkpoint, contrary to the possibility that decondensation is an adaptive response.

If decondensation is dependent on the spindle checkpoint and not an adaptive response, then upon inactivation of the spindle checkpoint, chromosomes might be expected to recondense before anaphase, for example, after removal of nocodazole from the growth medium. To test this, we harvested cells at time points of 1.5, 2.5, and 3.5 h after treatment with nocodazole, washed out the drug, and released the cells into fresh medium. For each time point, we examined chromosome IV signals at *TRP1* and *LYS4* at 5-min intervals after the release. These experiments demonstrated that chromosomes did transiently recondense before anaphase after release from nocodazole (Figure 4D and data not shown). Together these studies indicate that condensation occurs transiently in *S. cerevisiae* and that the spindle checkpoint might actively promote decondensation.

A Prolonged Preanaphase Arrest Results in Loss of Cohesion

Arresting *S. cerevisiae* cells at the metaphase to anaphase transition yielded surprising results. First, we observed that a region of the right arm of chromosome IV decondensed in response to the cell cycle block. Second, we observed loss of cohesion at either the *TRP1* locus, the *LYS4* locus, or both loci, after prolonged nocodazole-induced arrest. This latter finding merited further study. We used two of the different conditions described above to prevent anaphase entry (nocodazole or overexpression of *pds1- Δdb*) and we performed this analysis in strains possessing integrated LacO sequences at either *TRP1*, *LYS4*, or both loci (Figure 5). Cells were collected 1.5, 2.5, and 3.5 h after addition of nocodazole or

Figure 5. Loss of cohesion and condensation upon preanaphase arrest. Wild-type and *GAL1-pds1- Δdb* strains were synchronized in G1 and released into the cell cycle either in the presence of nocodazole (wild-type cells) or with galactose to induce expression from the *GAL1* promoter. The strains contained LacO sequences at the *TRP1* and the *LYS4* loci in A, the *TRP1* locus only in B, and the *LYS4* locus only in C. The number of fluorescent signals per cell was scored in large-budded cells after arresting in a preanaphase state. (A) 1 dot, condensed *TRP1-LYS4* region; 2 dots, decondensed *TRP1-LYS4* region; and ≥ 3 dots, loss of cohesion. (B and C) 1 dot, cohered locus (either *TRP1* or *LYS4*); 2 dots, loss of cohesion.

induction of *pds1- Δdb* from the *GAL1* promoter and were characterized under the microscope. Nocodazole treatment (Figure 5A) resulted in a time-dependent loss of cohesion at either the *TRP1* or the *LYS4* locus. Under these conditions, examination of strains possessing either *TRP1* or *LYS4* locus tags confirmed that both of these loci could undergo inappropriate loss of cohesion (Figure 5, B and C). We also assayed Pds1 decay in cells tagged at the *TRP1* locus (Supplementary Figure 3A). Most cells that lost cohesion at *TRP1* had degraded Pds1, though some cells lost cohesion in the presence of Pds1. In strains expressing *GAL1-pds1- Δdb* , however, few cells appeared to lose cohesion at either *TRP1* or *LYS4* (Figure 5, B and C). These data indicate that the budding yeast spindle assembly checkpoint is not capable of robustly maintaining the cohered state of sister chromatids in the presence of nocodazole. To determine how the degree of loss of cohesion seen in wild-type cells arrested in nocodazole compared with the cohesion defect seen in cohesin mutants, we examined loss of cohesion at *TRP1* after release from α -factor arrest in *mcd1-1* mutant cells (Supplementary Figure 3B). In wild-type cells, 3.5 h after release from G1, about 20% of the cells had lost cohesion at *TRP1* compared with more than 70% of the *mcd1-1* cells. We also examined *smc2-8* mutants in the same experiments, as we had previously noticed some loss of cohesion in these cells (see Figure 2), indicating that condensin may participate in maintenance of cohesion. The *smc2-8* cells had an intermediate cohesion

Figure 6. Ipl1 promotes maintenance of condensation during anaphase/tephase. (A and B) The *TRP1-LYS4* strain and an identical strain but harboring an integrated *GAL1-IPL1* construct were synchronized in G1 and released into the cell cycle in the presence of nocodazole and galactose (to induce *IPL1* overexpression). Cells in A are examples of *IPL1*-overexpressing cells after arrest before anaphase (1.5 h after release from G1). (B) 1 dot, condensed *TRP1-LYS4* region; 2 dots, decondensed *TRP1-LYS4* region. (C) Timing of condensation and decondensation in the wild-type *TRP1-LYS4* strain compared with an identical strain but with an *ipl1-321* mutant allele. Cells were arrested in G1, released into rich medium $\pm$ nocodazole and at a semipermissive temperature for the *ipl1* mutant (30°C), and then condensation status in the *TRP1-LYS4* region was scored as described in Figure 1. (D) Analysis of decondensation of the *TRP1-LYS4* region during anaphase/tephase in an unperturbed cell cycle. Wild-type and *ipl1-321* strains were handled as described in C, and the percentage of anaphase/tephase cells in which both *TRP1-LYS4* regions had decondensed was determined. (E) Photomicrographs of anaphase/tephase *ipl1-321* cells in which both *TRP1-LYS4* regions have decondensed (i.e., the category of cells scored in D) in anaphase (right panel) or late anaphase/tephase (left cell).

defect at *TRP1* compared with wild-type cells and *mcd1-1* cells (Supplementary Figure 3).

Condensation of Chromosome IV Can Be Restored by Overexpressing *IPL1*

Several explanations could account for our observation that chromosomes decondense when cells are arrested just before anaphase. That decondensation was perturbed in *mad2* null cells suggests that an active mechanism promotes decondensation should cells arrest before anaphase. The Aurora B kinase, Ipl1, is the only activity that had previously been linked to maintenance of condensation in *S. cerevisiae*, based on analysis of the rDNA locus in telophase-arrested cells (Biggins *et al.*, 2001; Lavoie *et al.*, 2004). Therefore, we tested the hypothesis that cells arrested preanaphase lack sufficient Ipl1 activity to maintain condensation of the chromosome IV right arm. We introduced a *GAL1-IPL1* allele into a strain with LacO sequences at *TRP1* and *LYS4*. Overexpression of *IPL1* has no obvious detrimental effect on cell growth (Biggins *et al.*, 1999; data not shown), but we observed that ~50% of cells with large buds contained a condensed chromosome IV compared with ~20% in control cells not overexpressing *IPL1*. We therefore released cells from a G1 synchrony and asked if overproduction of the Ipl1 kinase could promote maintenance of chromosome IV condensation in the presence of nocodazole. *GAL1-IPL1* and wild-type cells were arrested with α factor in medium con-

taining galactose to induce expression of *IPL1*. The cells were then washed and released into fresh medium containing nocodazole. Although wild-type cells exhibited condensation and subsequently decondensed the chromosome IV right arm in nocodazole, *GAL1-IPL1* cells accumulated with a single dot (Figure 6, A and B). After accumulation at the preanaphase arrest point, ~65–70% of the cells in which Ipl1 was overproduced contained a condensed chromosome IV, compared with ~20–30% of cells without *GAL1-IPL1* (Figure 6, A and B). Thus, these data suggest that Ipl1 kinase can maintain chromosomes in the condensed state, but that the kinase activity may become limited in cells arrested immediately before the metaphase-to-anaphase transition.

The above experiment, and those of Lavoie *et al.* (2004), implicated Ipl1 in the maintenance of condensation in the *TRP1-LYS4* region and at the rDNA locus, respectively, rather than a role in the initial establishment of condensation. To test more directly how Ipl1 contributes to condensation of the *TRP1-LYS4* region, we determined the kinetics of condensation and decondensation in wild-type cells versus *ipl1-321* mutant cells, grown at the semipermissive temperature (30°C) for the *ipl1-321* mutant. After release from G1 synchrony, both strains condensed the *TRP1-LYS4* region with similar timing (Figure 6C), and this was the case whether or not nocodazole was present in the medium. Thus, Ipl1 is not required for the initial establishment of condensation in the *TRP1-LYS4* region. In the absence of

Figure 7. Model for chromosome condensation in yeast. Based on the data herein and other studies (Lavoie *et al.*, 2004) chromosome condensation in *S. cerevisiae* appears to require establishment, dependent on condensin and Topoisomerase II, and maintenance, dependent on Ipl1 (Aurora B) kinase. Establishment of condensation occurs in G2/M phase and is maintained until telophase. Ipl1 presumably helps to avoid chromosome loss or missegregation by delaying decondensation until the chromosomes have migrated to the cell poles. When cells are arrested in metaphase or in c-metaphase, condensation is not maintained.

nocodazole, ~70 min after release from G1, we scored a decreased percentage of budded cells with condensed chromosomes because from this time point onward, anaphase cells began to appear (Anaphase cells, black line in Figure 6C, are plotted as a separate category, not discriminating between condensed or decondensed). When anaphase and telophase cells were categorized into condensed or decondensed at *TRP1-LYS4*, we noticed the clear trend that decondensation occurred in *ipl1-321* mutant cells earlier than in wild-type cells (Figure 6, D and E). At the 65-min time point, when few cells had initiated anaphase, only ~18% of wild-type anaphase/telophase cells had decondensed the *TRP1-LYS4* region of both sister chromatids. Only at the 80- and 85-min time points had most wild-type anaphase/telophase cells decondensed both of the sisters. *ipl1-321* cells seemed to decondense the *TRP1-LYS4* region soon after anaphase initiation (or earlier in telophase than wild-type cells) because as soon as anaphase cells appeared (65 min after release from G1), ~50% of the anaphase/telophase cells had already decondensed both *TRP1-LYS4* regions. These data are consistent with the model that Ipl1 is not required for chromosome condensation that occurs in G2 or early in mitosis, but that Ipl1 helps maintain the condensed state during anaphase and early telophase.

DISCUSSION

In this study we have designed a method to visualize chromosome condensation in the yeast *S. cerevisiae*. Using the LacO system in yeast first described by Straight *et al.* (1996), with Lac operator sequences at *TRP1* and *LYS4*, we were able to visualize that the two fluorescent signals come close together or coalesce in G2/M of the cell cycle. Further, the coalescence seen was dependent on components of the condensin complex, Smc2 and Brn1 as well as Topo II. A more surprising finding was that cells decondensed this chromosome IV region when arrested after G2 but before anaphase, a phenomenon that was alleviated by overproduction of the Ipl1 kinase, suggesting that Ipl1 activity is required to maintain the condensed state (Figure 7).

We developed this *in vivo* condensation assay for several reasons. First, the *TRP1-LYS4* strains will allow us to observe condensation and analyze the effects of mutations on this process in live cells. Second, condensation can be visualized quickly without the need to fix cells and perform FISH (a time-consuming procedure, making this assay system ame-

nable to genetic screens. Third, we are able to observe condensation of a chromosome arm in comparison to the rDNA, which is the current paradigm to study condensation. Although study of condensation of the rDNA locus has yielded important information, the locus has a specialized chromatin structure that may overshadow effects of some mutations on condensation of other regions of the genome (Guarente, 1999; Rusche *et al.*, 2003).

A previous study that used LacO sequences integrated at loci on chromosome V that were separated by 250 kb observed no coalescence of the two fluorescent signals in G2/M cells (Freeman *et al.*, 2000). This suggested that a greater distance between the two loci containing LacO arrays might be required. We based this logic on the radial-loop model of mammalian chromosome organization, making the assumption that in order to visualize condensation the LacO sequences would have to be placed in adjacent rosettes. The radial-loop model predicts that adjacent rosettes of chromatin are brought into proximity (coalesce linearly) in mitosis. Loci within the same rosette may or may not come into proximity during condensation because compaction of the chromatin within a rosette is proposed to involve coiling of chromatin loops. With many loops per rosette, whether or not two loci coalesce during rosette compaction would depend on the specific position of each locus in the loop-structure of the rosette (see Figure 1). To maximize our chances of placing LacO sequences in adjacent rosettes, we targeted three loci on the right arm of the largest chromosome (chromosome IV), near the centromere, half way down the arm and near the telomere. Interestingly, merely separating loci by a large distance on this chromosome arm was not sufficient to allow observation of separated fluorescent signals in interphase cells (see Supplementary Figure 1). LacO sequences integrated at *LYS4* and near *TEL4* (~550 kb apart), were invariably very close to each other, or overlapping, in interphase nuclei. This could be due to the chromatin state and organization of the chromatin in the subtelomeric region of the chromosome (reviewed in Loidl, 2003). Alternately, the LacO arrays at these two loci may be positioned within the same rosette such that they are situated in close proximity even in interphase. Following this logic, it seems likely that the *TRP1* locus and the *LYS4* locus (separated by ~450 kb) are situated in separate rosettes, thereby allowing us to observe two fluorescent signals in interphase as well as coalescence of these signals in preparation for nuclear division. Also of interest, separation of the LacO repeats by a large distance was not necessary for allowing observation of two signals in interphase cells and coalescence in G2/M phase. This behavior of LacO-tagged loci was seen in the *MMP1-CMS1* region that spans 138 kb. A comprehensive analysis of yeast chromosomes using this method of LacO pairs will likely reveal a more detailed description of chromosome structure and organization in *S. cerevisiae*.

Temperature-sensitive alleles of components of the condensin complex and Topo II prevented the *TRP1-LYS4* fluorescent dots from coalescing in G2/M, thereby allowing us to confirm that we are able to visualize compaction of this chromosome arm before mitosis. Although the experiments with *smc2-8* and *brn1-9* agree with all previous studies, our finding that Topo II is required for linear chromosome compaction in budding yeast is surprising in light of studies in which condensation of the rDNA locus was examined (Lavoie *et al.*, 2002; Sullivan *et al.*, 2004). The rDNA locus is organized into a heterochromatic-like structure by the RENT complex (Straight *et al.*, 1999), perhaps giving this locus a unique chromatin arrangement. It is therefore quite possible

that mutant alleles of *TOP2* result in a general chromosomal condensation defect, with the rDNA locus being a notable exception. Indeed, in higher eukaryotes, Topoisomerase II is an essential component of the mitotic scaffold and is clearly required for linear chromosome compaction. However, in the absence of Topo II activity, nucleoli are able to reorganize into discrete nucleolar organizer regions that are not different in appearance from those in properly condensed chromosomes (Gimenez-Abian *et al.*, 1995). Thus we propose that Topo II is required for linear chromosome compaction in budding yeast as it is in other eukaryotes.

A surprising finding was that the right arm of chromosome IV decondensed in response to perturbing the cell cycle by blocking cells at the metaphase-to-anaphase transition. This unexpected finding is contrary to the normal biology of most eukaryotes. Decondensation of chromosomes during preanaphase arrests could have been due to an active process that targeted chromosomes for decondensation. The timing of decondensation relative to cell cycle progression was not consistent with decondensation being an adaptation response, although it is possible that decondensation and cell cycle progression are distinct adaptive events. However, the apparent dependence of decondensation on Mad2 indicates that the spindle checkpoint is required for decondensation. In metaphase-arrested cells, with intact spindles, the dynamic movement of the spindle (Pearson *et al.*, 2001) could account for stretching of the chromatin between the *TRP1* and the *LYS4* loci, resulting in dot separation. And in anaphase, linear chromosome stretching has been previously described, a result of telomere cohesion that is not resolved until late anaphase (Straight *et al.*, 1997; Pearson *et al.*, 2001). We similarly observed separation of the *TRP1* and *LYS4* loci in some anaphase cells as the sister chromatids moved to opposite poles (Figure 6D and Supplementary Figure 4, A and B). However, in the absence of a spindle, when cells were treated with nocodazole, we also observed chromosome decondensation in vivo. Therefore tension on the chromosomes cannot account for preanaphase decondensation. Other studies had reported that Ipl1 kinase promotes compaction of the rDNA locus at the metaphase-to-anaphase transition and is required to maintain this compaction from anaphase to telophase (Lavoie *et al.*, 2004). In agreement, we found that decondensation of the *TRP1-LYS4* region was partially restored by overexpression of *IPL1*. This was the case when cells were arrested before anaphase either by overexpression of *pds1-Δdb* or in the presence of nocodazole (Figure 6B and data not shown). Therefore, during a preanaphase arrest, Ipl1 may become a limiting factor causing the chromosomes to decondense linearly. More importantly, under more physiological conditions, we found that Ipl1 helps to delay decondensation of the *TRP1-LYS4* region in anaphase and telophase cells. These data indicate that Ipl1 may act transiently during an unperturbed cell cycle to prevent premature decondensation during chromosome segregation, which otherwise could lead to chromosome loss or missegregation.

In previous studies it was assumed that nocodazole-induced preanaphase arrest would result in maximal chromosome condensation. Condensation was assayed at the rDNA locus in these studies and was determined to undergo a series of structural changes as cells passed through the cell cycle to become arrested before anaphase (Guacci *et al.*, 1994, 1997; Lavoie *et al.*, 2004). These states were designated as “rDNA puffs” in interphase, a “highly compact” structure seen around the time of G2, and finally “rDNA loops” that are seen upon arrest in nocodazole. The rDNA loops have a much more open structure than the highly compact spherical rDNA arrangement seen after S phase but before pro-

longed arrest in nocodazole. Therefore, the extremely compact rDNA structure seen before the nocodazole arrest point could be the true condensed state of this region (Lavoie *et al.*, 2004). Our observation of condensed chromosome IV may be equivalent to the highly compact rDNA structure, whereas decondensation of chromosome IV upon nocodazole arrest may be a phenomenon similar to the opening up of the rDNA locus into loops.

In summary, here we have described direct observation of condensation of the right arm of chromosome IV in vivo in budding yeast. Condensation was not maintained under conditions of preanaphase checkpoint arrest, but was dependent on condensin and topoisomerase II. In addition, we have generated and characterized an invaluable tool to study the process of chromosome condensation in *S. cerevisiae*. Because of the genetic amenability of yeast, this tool would prove useful to isolate and study new proteins that contribute to and regulate the process of chromosome condensation. Identification of these factors would then hopefully provide insights into mechanisms required to maintain genome stability in eukaryotes.

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