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Condensin function at centromere chromatin facilitates proper kinetochore tension and ensures correct mitotic segregation of sister chromatids

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Abstract

The condensin complex is essential for sister chromatid segregation in eukaryotic mitosis. Nevertheless, in budding yeast, condensin mutations result in massive mis-segregation of chromosomes containing the nucleolar organizer, while other chromosomes, which also contain condensin binding sites, remain genetically stable. To investigate this phenomenon we analyzed the mechanism of the cell-cycle arrest elicited by condensin mutations. Under restrictive conditions, the majority of condensin-deficient cells arrest in metaphase. This metaphase arrest is mediated by the spindle checkpoint, particularly by the spindle-kinetochore tension-controlling pathway. Inactivation of the spindle checkpoint in condensin mutants resulted in frequent chromosome non-disjunction, eliminating the bias in chromosome mis-segregation towards rDNA-containing chromosomes. The spindle tension defect in condensin-impaired cells is likely mediated by structural defects in centromere chromatin reflected by the partial loss of the centromere histone Cse4p. These findings show that, in addition to its essential role in rDNA segregation, condensin mediates segregation of the whole genome by maintaining the centromere structure in *Saccharomyces cerevisiae*.

Introduction

The genome segregation during cell division requires concerted functions of different chromatin proteins, which ensure faithful transmission of sister chromatids to daughter cells. One of these factors, the condensin complex, is highly conserved and is essential in all eukaryotes. Condensin is believed to resolve the tangles between sister chromatids, allowing their timely and synchronous segregation in anaphase (Hirano 2005). While most higher eukaryotes have two cooperating condensins: condensin I and II, which differ in their localization in the cell and on chromosomes (Ono *et al.* 2003), yeast have a single condensin complex (Sutani *et al.* 1999; Freeman *et al.* 2000). Genetic studies in yeast showed that condensin mutations disrupt chromosome structure, resulting in segregation failure of the centromere-distal regions of sister chromatids, producing the “cut” phenotype in fission yeast (Saka *et al.* 1994) and “stretched” chromatin in budding yeast (Strunnikov *et al.* 1995). Studies on budding yeast, in particular, have pointed out that the essential *in vivo* function of condensin is not in chromatin-compacting *per se*, but rather in separating sister chromatids in metaphase and possibly through anaphase (Freeman *et al.* 2000; Machin *et al.* 2005). In budding yeast, chromosomes containing the nucleolar organizer (NOR or rDNA) are especially sensitive to condensin deficiency. Therefore, presence of rDNA on a chromosome results in its mis-segregation and loss in condensin ts-mutants subjected to transient temperature shift, while

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non-rDNA chromosomes segregate properly upon return to permissive conditions (Freeman *et al.* 2000). This surprising resistance of non-rDNA chromosomes to condensin dysfunction has not been explained.

The failure of sister chromosomal sites to separate in condensin mutants has traditionally been attributed to topological tangles between sister chromatids (Strunnikov 2003), inferred by analogy with the topoisomerase II mutant phenotype (Uemura *et al.* 1987). The occurrence of anaphase chromosomal bridges in condensin-deficient cells in Metazoa, however, is not as frequent as one would expect, taking into account the gross disarray of chromosome structure in prometaphase (Hagstrom *et al.* 2002; Hudson *et al.* 2003; Ono *et al.* 2003). Similarly, in budding yeast, while condensin mutants arrest at non-permissive conditions with partially separated sister chromatids (Freeman *et al.* 2000) (widely accepted to represent an anaphase delay), these cells do maintain high viability throughout the period of arrest (Freeman *et al.* 2000; Lavoie *et al.* 2002) (Fig. 1A). The nature of this pathway, which largely rescues segregation defects upon condensin dysfunction or depletion in eukaryotes, remains unknown.

An answer to questions about the nature of the bias towards rDNA mis-segregation in condensin mutants and their high viability could be in condensin's function unrelated to rDNA segregation. The preference of *Saccharomyces cerevisiae* condensin for nucleolar localization in mitosis and its essential function in segregating rDNA (Freeman *et al.* 2000; D'Amours *et al.* 2004; Sullivan *et al.* 2004; Wang *et al.* 2004; Machin *et al.* 2005) has been overshadowing condensin binding to other chromatin sites, which have been characterized only recently (Wang *et al.* 2005). Among them are the pericentromeric regions that are enriched in condensin binding, especially in mitotic cells (Wang *et al.* 2005), indicating that a subset of condensin's activities could be related to centromere function. Data from other model systems have also revealed a consistent pattern of condensin co-localization with centromere chromatin: in *Schizosaccharomyces pombe* (Nakaseko *et al.* 2001), mammalian cells (Ono *et al.* 2004), *Caenorhabditis elegans* (Hagstrom *et al.* 2002) and *Drosophila* (Jager *et al.* 2005). Moreover, condensin inactivation in mammalian cells and *Drosophila* exhibits certain morphological defects in centromere and/or kinetochore structure (Ono *et al.* 2004; Oliveira *et al.* 2005).

We undertook a detailed investigation of the mechanism mediating the cell-cycle arrest in condensin mutants. This analysis allowed us to reconcile the severity of condensation defects in *S. cerevisiae* condensin mutants (Freeman *et al.* 2000; Lavoie *et al.* 2000; Ouspenski *et al.* 2000; Bhalla *et al.* 2002) with their prolonged viability and high stability of non-rDNA chromosomes, upon return to permissive conditions (Freeman *et al.* 2000; Lavoie *et al.* 2002). It also elucidated the role of non-rDNA condensin sites in budding yeast. We showed that the spindle assembly checkpoint monitors the mitotic activity of condensin, thereby securing its function in genome segregation. Inactivation of spindle checkpoint genes has demonstrated that the essential condensin function in *S. cerevisiae* mitosis is not circumscribed to its role in rDNA segregation, but is required for the correct transmission of all chromosomes. The centromeric role of condensin is a part of the tension generator at the kinetochore and involves functional interaction with the Cse4 centromere histone. Thus, we have uncovered an interface between the condensin complex and kinetochore function essential for partitioning of the entire genome.

Results

After exposure to non-permissive conditions most conditional condensin mutants display a mitotic delay and are reported to enter anaphase with signs of chromosome mis-segregation. Most of the prior analysis of *S. cerevisiae* condensin was focused on these defects (Strunnikov *et al.* 1995; Freeman *et al.* 2000; Lavoie *et al.* 2000, 2002; Ouspenski *et al.* 2000). However, the *smc2-8* allele in budding yeast leads to a tighter mitotic arrest in the first cell cycle, retains

high cell viability and shows no dramatic loss of non-rDNA chromosomes (Freeman *et al.* 2000). Thus, we re-examined the nature of the *smc2-8* mutant arrest in order to understand the mechanism of high cell viability and chromosome stability in this mutant.

We confirmed that *smc2-8* population arrests as two different cell types (Fig. 1A). The majority are mid-anaphase-like cells with partially separated sister chromatids and unsegregated nucleoli (Freeman *et al.* 2000) (Fig. 1A, top panel). These cells were shown to retain high viability (by elutriation and plating, data not shown). A smaller fraction is inviable cells (Strunnikov *et al.* 1995; Freeman *et al.* 2000), either unbudded or with a small bud (Fig. 1A, middle panel). The fraction of unbudded cells (and the corresponding cell viability) varies, dependent on the experimental conditions, but generally falls within 20%–50% range for this particular condensin allele. It has been suggested that these inviable cells are aneuploids (Strunnikov *et al.* 1995). Our analysis of nucleolar and/or chromosome XII segregation in *smc2-8* supports this assessment, by showing that they either lack nucleoli or have very small nucleoli, probably a result of chromosome XII segregation errors and breaks (Fig. 1A, lower panel). Interestingly, a minor subpopulation composed of small-budded cells evidently initiated (but not finished) S-phase (Fig. 1A, right panel), a conclusion supported by budding, Pds1p presence and FACS profile of the arrested *smc2-8* population (Fig. 1A). These cells, unlike the unbudded cells that lack visible nucleoli, have reduced-size nucleoli (Fig. 1A, right panel arrow). Diminution of nucleoli, accompanying S-phase block and loss of viability in these cells are likely related and probably can explain an indirect requirement of condensin (through its role in nucleolar maintenance) for robust DNA replication, which is not addressed in this work.

For the rest of the study we focused on the large-budded cells resulting from condensin inactivation, representing the fraction of the cell population arrested in mitosis. First, we tested the arrested condensin mutants for a biochemical marker of anaphase: cleavage and degradation of the cohesin subunit Mcd1p/Scc1p (Uhlmann *et al.* 1999; Rao *et al.* 2001). In nine independent condensin mutants the arrest was not consistent with uniform anaphase: the full-length Mcd1p levels remained high as compared to the *cdc15* anaphase arrest (Fig. 1B), and the cleavage products were undetectable (not shown). Therefore, at least some condensin mutants must have a high proportion of metaphase cells.

Next, we undertook time course analysis of the synchronized *smc2-8* cell population, in comparison to the wild-type. While wild-type cells released from G1 arrest proceeded synchronously through anaphase after 90 min (according to nuclear division kinetics, Pds1p and Mcd1p dynamics) and exited mitosis between 120 and 135 min (Clb2p degradation) (Fig. 1C,D), the condensin mutants did not indicate significant anaphase entry or exit from mitosis. To the contrary, mutant cells accumulated Pds1p, Mcd1p and Clb2p, consistent with prolonged metaphase arrest. This suggests that condensin mutants arrest in metaphase and that the partial sister chromatid separation in the arrested cells (Freeman *et al.* 2000; Bhalla *et al.* 2002) is largely a result of metaphase, not anaphase, spindle forces (Goshima & Yanagida 2000; He *et al.* 2000; Tanaka *et al.* 2000).

A metaphase arrest and high viability suggest that a certain checkpoint is activated in condensin mutants. As some specific mutant alleles of condensin subunits lead to DNA damage (Aono *et al.* 2002; Huang & Koshland 2003), we tested the possibility that condensin mutants arrest is a result of DNA damage checkpoint (Weinert *et al.* 1994). We constructed a triple *smc2-8 chk1 rad53* mutant defective in condensin function, as well as in two branches of the DNA integrity checkpoint pathway (Sanchez *et al.* 1999; Early *et al.* 2004). The resulting strain displayed unaltered arrest morphology at 37 °C, did not form microcolonies at 37 °C (not shown), had viability at the same level as single mutants (Fig. 2A) and its growth recovery after the exposure to 37 °C was similar to the single *smc2-8* mutants (Fig. 2B). The *smc4-1*

allele displayed similar properties (not shown). This result demonstrates that the mitotic arrest caused by these highly penetrant condensin alleles is not mediated by a DNA damage checkpoint.

Another checkpoint pathway that can arrest cells in metaphase is the spindle assembly checkpoint (Hoyt *et al.* 1991; Li & Murray 1991). Thus, we tested genetic interaction between the *smc2-8* allele and the *bub1*, *mad3*, *mad2* and *mad1* deletion alleles. In stark contrast to the single *smc2-8* mutants, the double mutants did display microcolony formation at 37 °C (not shown), rapid loss of viability upon exposure to non-permissive temperature (Fig. 2C) and absence of growth recovery after exposure to 37 °C (Fig. 2D). Two other condensin alleles: *ycs4-2* and *brn1-9*, which are very different in their survival rate after exposure to non-permissive conditions, nevertheless showed similar dependence of viability on spindle checkpoint (Fig. 2E). Interaction between individual checkpoint genes and the *smc2-8* mutation was also indicated by an additional cell division cycle in double mutants at 37 °C (see Fig. 3B for *bub1* example). *smc2-8* also showed strong interaction with *bub2-Δ*, resulting in synthetic lethality at 23 °C (Fig. 2F), suggesting that condensin mutants also require a mitotic exit delay for viability, even at the permissive temperature.

While the possibility of engaging the spindle checkpoint was previously suggested for the *ycs4-1* allele (Bhalla *et al.* 2002), data in Fig. 2 suggests that dependence of condensin mutant arrest on the spindle checkpoint is not allele-specific. This finding provides an opportunity to elucidate the mitotic condensin function unobscured by checkpoint controls and to resolve the apparent paradox of the relative stability of non-rDNA chromosomes in condensin mutants (Freeman *et al.* 2000). The fact that the double mutants (in condensin subunits and in the spindle checkpoint) have very low viability (Fig. 2) may indicate that such cells undergo massive chromosome mis-segregation. If it is the case, bypass of the spindle checkpoint should not allow multiple cell divisions, but instead should be limited to one or very few divisions. Indeed, the *bub1Δ smc2-8* cells exit the first mitosis (Fig. 3A), but undergo no more than two cell divisions after exposure to 37 °C (Fig. 3B). Moreover, comparison of the FACS profiles for the *smc2-8* single and the *bub1Δ smc2-8* double mutants (Fig. 3C) suggests substantial mis-segregation of individual chromosomes (indicated by the broadening peaks and appearance of the peak with less than G1 DNA content).

In order to compare the rates of mis-segregation for individual chromosomes we employed their *in vivo* labeling by RFP or GFP markers in the wild-type, *bub1Δ*, *smc2-8* and *bub1Δ smc2-8* cells. For rDNA/Chr. XII segregation we simultaneously monitored nucleoli (Sik1p-mRFP) and spindle microtubules (Tub1p-GFP). The mis-segregation event was scored when the mitotic spindle appeared fully elongated but discontinuous, and the nucleolus was in only one of the dividing cells (Fig. 3D). For non-rDNA chromosomes IV and IX we combined DAPI staining with LacO/LacI-GFP tags. In this case the mis-segregation event was scored when the DAPI signal had separated to opposite polar positions in mother and daughter cells, but two LacI-GFP signals were detected in only one of the DAPI masses (Fig. 3E). While wild-type cells had no mis-segregation and *bub1Δ* displayed no more than 8% mis-segregation events, regardless of the chromosomal tag position, analysis of the *smc2-8* mutant confirmed that the rDNA-containing chromosome XII was much more prone to mis-segregation than chromosomes IV or IX (Fig. 3F). In contrast, *bub1Δ smc2-8* cells had equally high mis-segregation rates for chromosomes XII, IV and IX (Fig. 3F).

Thus, condensin in budding yeast is essential for segregation of the whole genome (not just the rDNA chromosome), accounting for its presence on every *S. cerevisiae* chromosome (Wang *et al.* 2005). As it is well-established that the spindle checkpoint signal is generated at the kinetochore (Tan *et al.* 2005), it is conceivable that condensin is required for some aspect of kinetochore function. As condensin is a chromatin protein, it can potentially affect kinetochores

through its *cis*-activity at *CEN*-neighboring sequences. Therefore, condensin mutations may lead to altered pericentromeric chromatin, resulting in spindle defects due to disruption of spindle-kinetochore attachment and/or metaphase tension. Such defects are indirectly suggested by experimental data: *smc2-8* and *smc4-1* alleles display notable sensitivity to low doses of a microtubule polymerization inhibitor (Fig. 4A), a sign of diminished spindle stability. Thus, we employed a more sensitive genetic assay to assess the spindle-kinetochore tension defects in condensin mutants: interaction with the *sgo1Δ* (Kitajima *et al.* 2004; Indjeian *et al.* 2005) and *skp1-AA* alleles (Kitagawa *et al.* 2003), which are unable to transmit a signal of inadequate tension. Double *sgo1Δ smc2-8* and *skp1-AA smc2-8* mutants were indeed unable to recover from the 37 °C shift, due to a progressive and rapid decrease in cell viability (Fig. 4B,C). These double mutants also bypassed the metaphase arrest, as assayed by nuclear division and Western-blotting (not shown). Further analysis was performed for the *skp1-AA* mutant, as this allele, apparently, confers a very targeted disruption of the tension sensory mechanism, with no effect on the essential kinetochore functions of Skp1p (Kitagawa *et al.* 2003). Using a colony color-sectoring assay, we observed a significant increase in the loss rate of a non-essential chromosome fragment in the double mutant in comparison to the wild-type or single mutants (Fig. 4D).

We directly confirmed the existence of tension defect in condensin mutants by using the kinetochore GFP marker Slk19p-GFP and a control pericentromeric site marker (*TRP1::LacO*). In order to be able to compare the condensin mutant to wild-type cells, we utilized an experimental approach involving synchronous release of cells from G1 at the non-permissive temperature in the presence of Cdc20p depletion (Tanaka *et al.* 2000). Under proper tension conditions (in wild-type cells depleted for Cdc20p), individual sister centromeres are able to separate but remain close to each other (Tanaka *et al.* 2000; Collins *et al.* 2007). Such a short-distance separation was largely masked in wild-type Cdc20⁻ cells (Fig. 4E), due to the converging Slk19p-GFP signals from all 32 kinetochores. However, the *smc2-8* cells analyzed in the same experiment showed a significant fraction of cells with clear separation of kinetochore clusters (Fig. 4E). In contrast, the *TRP1::LacO* pericentromeric tag (12 kb from *CEN4*) showed virtually no separation of sister chromatids in both wild-type and *Smc2⁻* Cdc20⁻ cells (Fig. 4E). The Slk19p-GFP clusters observed in the arrested mutant likely represent sister kinetochores, as distance between them remains stable and cells maintain metaphase arrest. Thus, both genetic (Fig. 3) and cytological (Fig. 4E) data confirm that a sister kinetochore tension defect exists in condensin mutants.

The nature of the spindle-kinetochore tension defect in condensin mutants is probably related to the centromere structure, as condensin is a chromatin component. In the absence of comprehensive morphological tests for centromere and kinetochore structure in *S. cerevisiae* it is difficult to identify the underlying molecular defect, unless there is a substantial loss of kinetochore proteins. Our Slk19p-GFP analysis (Fig. 4E) did not suggest such a significant defect. It is also evident that disruption of the kinetochore structure in *smc2-8* is unlikely to be dramatic, because some cells do go through anaphase (Fig. 1) and there is no indication of gross chromosome loss in the single mutant (Freeman *et al.* 2000). However, as kinetochore structure is extremely complex, it possible that some factors required for kinetochore assembly and bipolar attachment are not equally required for maintenance (during metaphase arrest), and therefore their loss can be detected. Thus, we monitored the localization of GFP fusions (Huh *et al.* 2003) of other representative subunits of kinetochore subcomplexes (Kline-Smith *et al.* 2005) in *smc2-8*. As condensin-deficient yeast cells do maintain centromere clustering (data not shown and Figs 4E and 5A), it was feasible to visualize these low-abundance proteins as fluorescent dots in the nuclei of metaphase-arrested cells. After 3-h exposure of *smc2-8* cells to 37 °C no dramatic localization changes were observed for Dad1p (DDD complex) (Fig. A), Ame1p (COMA complex), Nuf2p, Spc24p, Spc25p (all Ndc80 complex) or inner kinetochore proteins (Ndc10p, Cbf2p, Ctf3p and Mif2p), yet some un-clustering of kinetochores was

observed in a fraction of cells (data similar to Fig. 5A, not shown). Limited dissipation of the GFP signal was also observed for MIND complex components Dsn1p and Mtw1p (confirmed by ChIP, not shown).

In contrast, localization of Cse4p, the centromere histone, was significantly altered in *smc2-8* at 37 °C, so that Cse4p-GFP, in addition to a weakened signal at the centromere cluster, displayed a diffuse signal throughout the nucleus (Fig. 5B). No such delocalization of Cse4p was observed in the wild-type, even upon metaphase delay due to expression of the dominant non-cleavable cohesin subunit Mcd1p (Fig. 5B, upper panel), ruling out the possibility that Cse4p delocalization in *smc2-8* is an indirect consequence of the prolonged mitotic arrest itself. The diffuse nuclear distribution of Cse4p-GFP is attributable to either its over-expression (Collins *et al.* 2004,2007) or its loss from centromeric chromatin (Crotti & Basrai 2004). The former was ruled out by showing that the amount of full-length Cse4p-GFP is unchanged in *smc2-8* cells compared to wild-type (Fig. 5C). Conversely, partial Cse4p-GFP depletion from centromeric chromatin in *smc2-8* was readily demonstrated by ChIP analysis (Fig. 5E). The qPCR/ChIP data show that centromere chromatin (at *CEN4*) at 37 °C is 50% less enriched for Cse4p-GFP in *smc2-8* than in wild-type cells. The result was even more pronounced for *CEN12* (Fig. 5F). Such a significant Cse4p depletion from the centromere (Fig. 5E,F) would generally exert a detrimental effect on the kinetochore assembly (Pinsky *et al.* 2003;Crotti & Basrai 2004;Collins *et al.* 2005), but has never been demonstrated or characterized in metaphase (fully assembled) centromere. General localization stability of GFP-fused kinetochore proteins in our analysis suggests that once sister kinetochores are already assembled and properly attached to the spindle (in *smc2* metaphase arrest), most kinetochore proteins are able to sustain the observed twofold reduction in Cse4p levels. Moreover, it is likely that the observed loss of Cse4 is limited to exchangeable nucleosomes, which are not in the centromere core (Fig. 6).

Thus, our data show that condensin inactivation induces partial depletion of Cse4p, probably resulting from the underlying defect—decondensation of centromeric chromatin, and leads to altered kinetochore tension (Fig. 6). The ensuing triggering of the spindle checkpoint, in turn, is responsible for preserving viability and chromosome stability in condensin-deficient cells until most of the damage caused by the condensin malfunction is repaired.

Discussion

Mitotic checkpoint pathways monitor condensin function

Condensin activity *in vivo* has been shown to be essential for chromosome compaction and segregation (Sutani *et al.* 1999; Freeman *et al.* 2000; Lavoie *et al.* 2000; Ouspenski *et al.* 2000; Hagstrom *et al.* 2002; Coelho *et al.* 2003; Hudson *et al.* 2003; Ono *et al.* 2003; Savvidou *et al.* 2005). In all of these studies the disruption of condensin function manifested in cytological defects in the prometaphase and anaphase: abnormal chromosome condensation and anaphase bridging, respectively. Importantly, some of the studies in higher eukaryotes have demonstrated that condensin-depleted cells exhibit a notable mitotic delay (Hudson *et al.* 2003; Ono *et al.* 2003; Hirano 2005), the mechanism of which was unknown. It was proposed that condensin defects in these systems generate merotelic attachment (Salmon *et al.* 2005) of microtubules to the kinetochore, which, despite being aberrant, nevertheless does not activate the spindle checkpoint (Hirano 2005). Our preliminary results show, however, that condensin deficiency does trigger a spindle checkpoint-dependent mitotic delay in cultured human cells (Ilia Ouspenski, unpublished). On the other hand, since the discovery of the first chromosomal condensin binding site, the rDNA locus in budding yeast, it was perplexing to see the apparent bias in condensin function towards rDNA segregation (Freeman *et al.* 2000). The present study apparently resolves both of these issues: the nature of mitotic delay/arrest and the bias in rDNA mis-segregation upon condensin dysfunction.

The demonstration that mutations in condensin, when combined with mutations in spindle checkpoint genes, bring about bypass of metaphase arrest, chromosome loss and lethality, proves that condensin function is monitored by a mitotic surveillance mechanism. Most notably, we found strong genetic interaction of the *smc2-8* allele with the *bub1*, *mad1*, *mad2* and *mad3* null mutations, defective in monitoring bipolar orientation of chromosomes (Shonn *et al.* 2003; Gillett *et al.* 2004; Howell *et al.* 2004). The unveiled genetic interactions of *smc2-8* with *skp1-AA* or *sgo1Δ* alleles, which impair the detection of tension defects (Kitagawa *et al.* 2003; Indjeian *et al.* 2005), and the increased separation of sister kinetochores in metaphase in *smc2-8* (Fig. 4), both suggest that condensin is required for proper tension at the kinetochore. At the same time, the high viability of condensin mutants indicates that spindle-kinetochore attachment and sister kinetochore orientation are maintained at nearly wild-type fidelity in checkpoint-proficient condensin mutants.

The synthetic lethality between the *smc2-8* allele and the *BUB2* pathway could suggest that condensin activity is also monitored at the late stages of mitosis. The Bub2 protein, in complex with Bfa1p, is a regulator of the mitotic exit network (MEN) (Wang *et al.* 2000; Pereira *et al.* 2002). As condensin was shown to be required not only for establishment but also for maintenance of condensation in metaphase (Freeman *et al.* 2000) and anaphase (Machin *et al.* 2005), it is possible that the *BUB2* pathway is required to monitor condensin function in chromosomal regions that segregate late in anaphase, such as telomeres and the nucleolus. Further investigation is required to elucidate the mechanism of interaction between the condensin pathway and the *BUB2* checkpoint.

The results of the present study offer a plausible explanation for the noticeable mis-segregation of an rDNA chromosome in checkpoint-proficient condensin mutants, while non-rDNA chromosomes maintain a high fidelity of segregation (Freeman *et al.* 2000). As the ultimate function of a checkpoint is to provide an unscheduled or extended cell cycle delay to repair specific damage (Elledge 1996), we can hypothesize that relatively mild condensin-mediated defect at the centromeres (i.e. altered tension) triggers such a delay. Our work suggests that some condensin function (probably at the sites proximal to natural centromeres) is required for proper kinetochore structure and thus such sites could serve as “circuit breakers” engaging spindle checkpoint upon condensin failure (Fig. 6). The mitotic delay generated this way should allow correction of the negative effect of condensin mutations on chromosome arm segregation. However, the nucleolar segregation block is not efficiently repaired during such a delay, probably due to constant regeneration of segregation impediment as a result of active rDNA transcription (Tomson *et al.* 2006; Wang *et al.* 2006). Such ineffectiveness of a metaphase delay for repair of condensin dysfunction in the transcriptionally active nucleolus in *S. cerevisiae* may be an evolutionary inducement for developing a mitotic nucleoli-reorganization pathway in multicellular organisms (Dimario 2004).

Condensin activity is required for robust centromere function

In several higher systems condensin either co-localizes with the centromere (Ono *et al.* 2004) or facilitates proper chromosome arrangement on the spindle (Hagstrom *et al.* 2002; Wignall *et al.* 2003; Ono *et al.* 2004). However, in all these systems centromeres are immense structures and the specificity of condensin defects for centromeric chromatin vs. overall chromosomal defects, is difficult to isolate. Our recent findings that *S. cerevisiae* condensin is enriched at centromeres in general, localizes in close proximity to particular centromeres and that pericentromeric sites display a mitotic increase in condensin binding (Wang *et al.* 2005) suggested that condensin activity can be an important supplement to mitotic centromere functionality. The present work supports this hypothesis by showing that the spindle checkpoint is activated in condensin mutants, these mutants have a kinetochore tension defect, chromosome non-disjunction in condensin/checkpoint double mutants is chromosome-size-

independent and centromere proteins are lost in condensin mutation-mediated arrest. Data showing that the mitotic spindle checkpoint is also activated by condensin depletion in human cells (Ilia Ouspenski, unpublished) indicates that the role for condensin in centromere maintenance and proper kinetochore tension may be conserved in evolution.

The exact molecular function of the centromere-proximal condensin binding sites in wild-type yeast chromosomes remains to be uncovered. However, it is unlikely that in *S. cerevisiae* condensin is an essential centromere component *per se*, as damage to kinetochore appears to be quite limited (Fig. 5A,D) in the *smc2-8* mutant and there are no obvious spindle-attachment defects. Nevertheless, the interface between the condensin activity and the kinetochore appears to be in centromeric chromatin, that is, chromatin enriched in Cse4p, a CENP-A homologue. While the exact stoichiometry of Cse4p per each centromere is still controversial, the recent study (Riedel *et al.* 2006) shows that, unlike commonly assumed (Meluh *et al.* 1998; Joglekar *et al.* 2006) based largely on the small size of centromere footprint and low-resolution ChIP data, Cse4p is not limited to one nucleosome, but instead can occupy a more extensive region with a prominent peak centered at the *CEN* DNA, probably corresponding to the positioned Cse4 nucleosome at the centromere core. Our ChIP data show quite a narrow window of Cse4p enrichment at the *CEN4* (Fig. 5E), but much broader at *CEN12* (Fig. 5F), in both cases being consistent with rather extended Cse4p-bound region (i.e. several nucleosomes). Therefore, based on the most comprehensive analysis of Cse4p occupancy to date (Riedel *et al.* 2006) (and partially on our ChIP data) we can assume, that in addition to its core centromeric position, Cse4p is also incorporated in the broader pericentromeric region, commonly referred to as centromeric chromatin in other species. This places Cse4p-enriched chromatin in close proximity to the pericentromeric condensin binding peaks (Wang *et al.* 2005). Therefore, it is plausible that condensin activity affects kinetochore tension not through a direct condensin-kinetochore protein contact, but is mediated by centromeric chromatin. In support of this hypothesis, a recent *Drosophila* study shows the possibility of direct interaction between the CAP-G condensin subunit and CID (Cse4p ortholog) (Jager *et al.* 2005), while no reliable interaction of condensin with kinetochore proteins has been reported. Condensin's involvement in the generation of proper tension at native kinetochores could be in establishing localized condensation of Cse4p-enriched chromatin (Fig. 6A).

Another interesting phenomenon discovered in the course of our study is that complete saturation of centromeric chromatin by Cse4p may not be necessary for the maintenance of the spindle-kinetochore attachment and orientation once they are established. It is known that Cse4p is required for formation of functional kinetochore (Collins *et al.* 2005); however, the *smc2* mutant apparently has robust maintenance of proper orientation of sister kinetochores (as well as stable localization of kinetochore proteins), while up to 50% of Cse4p is lost from the centromeres. This result could be related to the fact that only a small subpopulation of Cse4p (and CENP-A) has direct interaction with kinetochore proteins (Fig. 6). This is suggested by the results of several yeast proteomic screens that failed to uncover robust physical interactions between kinetochore proteins and Cse4p, as well as by the recent comprehensive analysis of CENP-A interactions in mammalian cells, which shows no biochemical evidence of complexes with known kinetochore components (Foltz *et al.* 2006). The model in Fig. 6B adequately explains the induction of spindle checkpoint, extended sister kinetochore separation while more distant regions of sister chromatids remain associated by cohesion, partial loss of Cse4p from centromeres and stability of kinetochore structure in condensin mutants. It could also give a direction to future analysis of the interface between Cse4p-containing chromatin and pericentromeric condensin binding (Wang *et al.* 2005), as well as between Cse4p and the kinetochore itself.

Experimental procedures

Yeast methods and genetic assays

All the strains used are isogenic to S288c unless otherwise indicated (Table 1). To analyze the genetic interaction between condensin mutations and the spindle checkpoint pathway, the deletions in *BUB1*, *BUB2*, *MAD1* and *MAD3* genes were first complemented with the respective wild-types genes on a CEN plasmid. The null mutants strains were then transformed with the linearized vector pLF733 (Wang *et al.* 2005) replacing the wild-type *SMC2* gene with the *smc2-8::LEU2*. The minichromosomes bearing the wild-type checkpoint genes were then counter-selected. A similar approach was used to introduce the *bub1* deletion into the *ycs4-2* and *brn1-9* mutants, except the multi-copy plasmids pAS1024 and pAS1062, expressing *YCS4* and *BRN1* respectively, were introduced before the *BUB1* gene was disrupted as described (Brachmann *et al.* 1998). Similarly, the *smc2-8* mutation (pLF733) was introduced into *bub2Δ* background after complementing this deletion in BY4741 with a *BUB2* plasmid (pMA1183, A. Hoyt). The combined mutants *rad53-Δ chk1-Δ smc2-8*, *skp1-AA smc2-8* and *sgo1-Δ smc2-8* were obtained through genetic crosses. *sgo1Δ* handling was according to Kitajima *et al.* (2004). Chromosome III fragment loss assay was performed as described by Freeman *et al.* (2000), after a 3 h incubation at 37 °C. For time-course cell cycle analysis cells were pre-synchronized by treating Bar1⁻ or Bar1⁺ strains with α factor (50 ng/mL or 250 ng/mL, respectively) for 2 h at 23 °C, followed by 1 h at 37 °C.

Biochemical methods

Protein analysis of crude cell extracts (prepared in 2% SDS) or immunoprecipitates was performed by Western blotting. Anti-Mcd1p antibody was from Guacci *et al.* (1997), anti-Clb2p and Cdc28p were from Santa Cruz Biotechnology (Santa Cruz, CA). Cse4p-GFP immunoprecipitation was performed according to an established method (Kagansky *et al.* 2004) with polyclonal anti-GFP antibodies (BD Biosciences). The qPCR/ChIP procedure was performed as described (Wang *et al.* 2004), with extracts from *smc2-8* cells expressing Cse4p-GFP or Dad1p-GFP that were incubated at 37 °C for 3 h after synchronous release from G1 arrest. The ChIP controls included isogenic untagged strains, as well as the Smc2⁺ strain with the pAK727 plasmid expressing non-cleavable *MCD1-9* allele (deletion of codons 177–271) under the *MET15* promoter control. Ectopic induction of the *MCD1-9* for 3 h results in prolonged mitotic arrest, unlike the *SCC1-RRDD* mutant described in Uhlmann *et al.* (1999), which exits mitosis after a transient delay (O. Cohen-Fix, personal communications). All controls were also exposed to 37 °C for 3 h.

Microscopy

The fluorescent markers for the nucleolus (Sik1p-mRFP) (Huh *et al.* 2003), mitotic spindle (Tub1p-GFP) and the LacI-GFP/LacO tags (Straight *et al.* 1996) were described previously. Pds1p-GFP was introduced using the pAC256 construct (Quimby *et al.* 2005). To evaluate segregation of different chromosomal loci (Fig. 3), cells were fixed and stained with DAPI as in Indjeian *et al.* (2005). The fluorescence was visualized using a Zeiss AxioVert epifluorescence microscope with a cooled CCD camera and OpenLab image analysis software. Twenty Z-axis stacked images of each sample were taken. At least 100 cells were analyzed to quantify the nucleolar and chromosomal morphology. The GFP screen for kinetochore protein localization was performed in a set of strains that express different kinetochore proteins tagged with the GFP (Huh *et al.* 2003). Cultures of 15-mL were incubated at 37 °C for 3 h and then analyzed by fluorescent microscopy. For the subsequent analysis of Cse4p and Dad1p fusions, cells were synchronously released from α -factor arrest at 37 °C and monitored for GFP localization every 30 min. The control Smc2⁺ strain was subjected to the same protocol, except it expressed non-cleavable *MCD1-9* allele (pAK727 plasmid).

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Figure 1.

Condensin mutants arrest in metaphase. (A) Schematic of condensin mutants morphology after exposure to non-permissive conditions. A large fraction of the cell population (up to 85%) has short spindles and unsegregated nucleoli (upper panel). Cell division is stalled in these cells, and they remain viable (Strunnikov *et al.* 1995). For this analysis, *smc2-8* cells (733-BY4741ST) containing Tub1p-GFP (spindle) and Sik1p-RFP (the nucleolus) markers were arrested for 4 h at 37 °C. A smaller fraction (minimum 15%) of unbudded and small-budded cells is also produced (right panel). For this panel, *smc2-8* cells (733-BY4741SP) expressing Pds1p-GFP and Sik1p-RFP were arrested for 4 h at 37 °C. These cells are inviable, probably largely due to chromosome non-disjunction in defective anaphase (lower panel, 733-

BY4741ST exposed to 37 °C for 2 h). Arrows denote nucleolar mis-segregation events. FACS analysis shows *smc2-8* cells (733-BY4733b) after 4-h exposure to 37 °C. (B) Condensin mutants arrest with high levels of Mcd1p. The strains listed in rows 1 through 10 in Table 1 were arrested at 37 °C for 3 h and analyzed by Western-blotting. The *cdc15-2* strain was used as a control for Mcd1p degradation in anaphase. *—the background band, which can be used as loading control. (C) Time-course analysis of nuclear division kinetics in the wild-type and *smc2-8*. Cells synchronously released from α factor arrest were analyzed by DAPI staining and bud morphology at each time point along the cell cycle. The BY4733b (WT) and 733-BY4733b (*smc2-8* condensin mutant) are indicated. (D) Time-course analysis of cell cycle markers in the wild-type and *smc2-8* strains at 37 °C. The extracts of cell cultures shown in (C) were probed with antibodies against Mcd1p and Clb2p. For Pds1p-HA analysis an identical time-course was performed with the same strains containing the *PDS1:HA* integration. Cdc28p levels were used as an internal loading control.

Figure 2.

Condensin mutant viability is maintained by spindle checkpoint. (A) *smc2-8* mutant viability is not dependent on DNA integrity checkpoints. Wild-type (RDK3615), *chk1 rad53* (Chk⁻, RDK3751), *chk1 rad53 smc2-8* (Chk⁻, 733-RDK3751) and *smc2-8* (733-RDK3615) cells were grown at 23 °C and then shifted to 37 °C (time 0). The aliquots were tested for viability by plating every 2 h. (B) *smc2-8* recovery after prolonged arrest is not dependent on DNA integrity checkpoints. The strains (same as in A) were grown for 4 days at 23, 37 or at 23 °C after 18-h exposure to 37 °C. The serial dilutions are fivefold. (C) Spindle checkpoint is required for *smc2-8* viability at 37 °C. Wild-type (BY4733), *bub1Δ* (bub1-BY4741), *bub1Δ smc2-8* (733-bub1-BY4741), *smc2-8* (733-BY4733), *mad3Δ* (mad3-BY4741) and *mad3Δ smc2-8* (733-mad3-BY4741) cells were analyzed as in (A). (D) Spindle checkpoint is required for *smc2-8* recovery after prolonged arrest at 37 °C. The wild-type and mutant strains (same as in C) cells were processed as in (B). (E) Non-SMC condensin mutant viability depends on spindle checkpoint. Wild-type (YPH499), *ycs4-2* (ZW204), *brn1-9* (CH2524), *ycs4-2 bub1* (bub1-ZW204) and *brn1-9 bub1* (bub1-CH2524) strains were manipulated as in (B) and (D). (F) *smc2-8* is synthetically lethal with *bub2* deletion. Wild-type strain BY4733 (WT), *bub2Δ* (bub2-BY4741) as well as *smc2-8* (733-BY4733) and *bub2Δ smc2-8* cells 8 (733-bub2-BY4741), all with *URA3 BUB2* minichromosome, were incubated on YPD (23 and 37 °C) and FOA plates. Serial dilutions are fivefold.

Figure 3.

Spindle checkpoint prevents chromosome mis-segregation in condensin mutants. (A) Double *bub1Δ smc2-8* mutants exit mitosis at 37 °C. The *bub1Δ smc2-8* strain (733-*bub1*-BY4741b) was arrested in G1 with α factor and then released at 37 °C. Time-course aliquots were analyzed for Mcd1p and Clb2p levels by Western-blotting, similarly to 1D (Cdc28p—loading control). (B) Double *bub1Δ smc2-8* mutants halt proliferation after an extra division at 37 °C. Cultures of wild-type (BY4733b), *smc2-8* (733-BY4733b) and *bub1Δ smc2-8* (733-*bub1*-BY4741b) cells were arrested in G1 with α factor and then released at 37 °C. Eight-hour growth curves were compiled from cell counts. (C) Double *bub1Δ smc2-8* mutants lead to aneuploidy at 37 °C. Cultures of wild-type (BY4733b), *bub1Δ* (*bub1*-BY4741b), *smc2-8* (733-BY4733b) and

bub1Δ smc2-8 (733-bub1-BY4741b) cells were arrested in G1 with α factor and then released at 37 °C, similarly to (B). Cell samples were taken periodically and processed for flow cytometry. (D) Nucleolus mis-segregation in the double *bub1Δ smc2-8* mutant. The micrograph shows an example of a cell with mis-segregated nucleolus at 37 °C in *bub1Δ smc2-8* cells (733-bub1-BY4741ST). Red—nucleolar marker Sik1p-RFP, green—spindle marker Tub1p-GFP, *a*—anucleolar cells, *m*—mis-segregated nucleolus. (E) Chromosome IV mis-segregation in double *bub1Δ smc2-8* mutant. The micrograph shows an example of cells with normal segregation (*n*) and mis-segregation (*m*) of chromosome IV (*TRP1::lacO/lacI:GFP* marker) at 37 °C in *bub1Δ smc2-8* cells (733-bub1-BY4741bIV strain). Blue—DAPI-stained nuclear DNA, green—LacI-GFP (chromosomal marker). (F) Spindle checkpoint is responsible for non-rDNA chromosomal stability and nucleolar mis-segregation bias in *smc2-8*. Time course analysis of nucleolar mis-segregation (Sik1p-RFP marker) was performed in synchronized cells (G1 release into 37 °C) in comparison to chromosome IV (*TRP1::lacO/lacI:GFP* marker) and chromosome IX (*BAR1::lacO/lacI:GFP* marker) mis-segregation. The strains are BY4741 and BY4733 derivatives listed in Table 1. The numbers in the legend indicate the chromosome and the distance of fluorescent markers from the centromere (kb). The apparent transient increase in mis-segregation around 90 min is probably due to a segregation delay, not actual mis-segregation.

Figure 4.

Impaired condensin function interferes with proper kinetochore tension. (A) Condensin mutants *smc2-8* and *smc4-1* are hypersensitive to nocodazole. The wild-type (YPH499), *smc2-8* (1bAS330) and *smc4-1* (1aAS342) cells were spotted onto YPD plates with increasing concentration of nocodazole (fivefold dilutions). (B, C) Proper spindle tension control is required for condensin mutant arrest. Genetic interaction between the *smc2-8* mutation and the *sgo1Δ* or *skp1-aa* allele suggests that condensin mutants are deficient in kinetochore tension. For the 37–23 °C (right panels) plates were pre-incubated at 37 °C for 18 h and then transferred back to 23 °C. The strains are: 6dAS593 (WT), 1cAS594 (*skp1-AA*), 7aAS596 (*smc2-8*), 7bAS595 (*smc2-8 skp1-AA*), 6dAS598 (*sgo1Δ*) and 7aAS597 (*sgo1Δ smc2-8*). (D) Uncoupling

of tension control mechanism results in chromosome loss in condensin mutants. Chromosome III fragment loss assay was performed by a colony half-sector assay (Koshland & Hieter 1987) after transient 3-h shift to 37 °C. The red sectors result from chromosome fragment loss. The strains are as in (C). (E) Condensin mutant shows increased separation of sister kinetochores in Cdc20p-depleted cells. The *pGAL:CDC20* (*Smc2⁺* or *smc2-8*) strains marked either with *lacO/LacI::GFP* at the *TRP1* locus (6dAS606 and 5bAS606, respectively) or with *SLK19::GFP* (BY4733galCDC20/pJK145 and 733-BY4733galCDC20/pJK145, respectively) were released from α -factor arrest into dextrose containing media at 37 °C. Sister kinetochore (*Slk19p-GFP*) and sister chromatid (*TRP1::LacO*) separation was scored microscopically (*Slk19p-GFP* example is shown in the left panel) and quantified as a fraction of total mitotic cell population (right graph).

Figure 5.

Condensin is required for the Cse4p localization to centromeres. (A) Dad1p-GFP localization is not visibly affected by condensin dysfunction. Micrographs of isogenic wild-type (DAD1GFP-EY0896) and *smc2-8* mutant cells (733-DAD1GFP-EY0896), both expressing Dad1p-GFP, after 3 h at 37 °C. (B) Clustered Cse4p-GFP localization in the nucleus is disrupted by condensin dysfunction. Micrographs of isogenic wild-type (CSE4GFP-EY0896) and *smc2-8* mutant cells (733-CSE4GFP-EY0896), both expressing Cse4p-GFP, at 37 °C (3 h). Cse4p-GFP dissipation becomes visible after 2 h at 37 °C. To arrest wild-type cells in mitosis (upper panel), similarly to condensin mutant arrest, wild-type cells were transformed with a *pMET:MCD1-9* construct. Inserts show individual cells at higher magnification. (C) Delocalization of Cse4p is not a result of over-expression. The levels of Cse4p-GFP were determined by comparing the protein concentration in supernatants (*Sup.*) and chromatin pellets (*Chr.*) of wild-type and *smc2-8* cells, after treatment as in (B). The immunoprecipitate (*IP*) of the Cse4p-GFP protein was used as a protein standard. (D) Dad1p level at kinetochores is unchanged in the *smc2-8* mutant. The amount of Dad1p-GFP present at *CEN4* was quantified by ChIP/qPCR. ChIP for *CEN4* flanking regions was used as a control. Strains are as in (A), except both strains were transformed with a plasmid containing *pMET:MCD1-9* (see Methods). (E, F) Cse4p level at centromeres is reduced in the *smc2-8* mutant. The amounts of Cse4p-GFP present at *CEN4* (E) and *CEN12* (F) were quantified by ChIP/qPCR. ChIP for *CEN*-flanking regions was also reduced. Strains are as in (B), both transformed with a plasmid containing *pMET:MCD1-9*.

Figure 6.

Model for condensin's role at the centromere. Hypothetical stretching of centromeric chromatin as a result of condensin dysfunction at the centromere. (A) Putative organization of condensed metaphase centromeric chromatin. Position of condensin binding sites and the Cse4-enriched region is based on the ChIP-chip data from Wang *et al.* (2005) and Riedel *et al.* (2006), respectively. A single Cse4 nucleosome is shown at the centromere core itself, in accordance with traditional views (Joglekar *et al.* 2006). DNA is shown as black line, MT-spindle microtubules. (B) Putative decondensation of the centromeric chromatin domain in condensin mutants, resulting in extended distance between sister kinetochores, while their proper attachment to the spindle and orientation are unperturbed. (C) Cse4p loss/relocalization upon prolonged stretching of the centromeric chromatin. The loss of the whole Cse4 nucleosomes is hypothesized based on Riedel *et al.* (2006) data. It is also possible that a fraction of Cse4p histone itself is lost/exchanged.

Table 1

Saccharomyces cerevisiae strains used

Strain	Relevant genotype	Origin
SLJ127	<i>MATa bar1 ade2 trp1 his3 leu2 ura3 can1 cdc15-2</i>	D. Morgan
CH2524	<i>MATa leu2 trp1 ura3 brn1-9::TRP1</i>	B. Lavoie
YIL178-20	<i>MATa ade2 can1 his3 leu2 trp1 ura3 brn1-20</i>	(Ouspenski <i>et al.</i> 2000)
YIL178-34	<i>MATa ade2 can1 his3 leu2 trp1 ura3 brn1-34</i>	(Ouspenski <i>et al.</i> 2000)
YIL178-60	<i>MATa ade2 can1 his3 leu2 trp1 ura3 brn1-60</i>	(Ouspenski <i>et al.</i> 2000)
YIL310	<i>MATa ade2 can1 his3 leu2 trp1 ura3 ycs5-1</i>	(Ouspenski <i>et al.</i> 2000)
SBY747	<i>MATa ade2 can1 HIS3::pCUP1::GFP12::LacI leu2 TRP1::lacO</i> <i>LYS2::pGal-J176-Clb2 ura3 bar1 ycs4-1</i>	S. Biggins
ZW204	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 ycs4-2::MYC12::HIS3</i>	B. Lavoie
1bAS330	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 smc2-8</i>	(Freeman <i>et al.</i> 2000)
1bAS344	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 smc4-1</i>	(Freeman <i>et al.</i> 2000)
BY4733b	<i>MATa his3 leu2 met15 trp1 ura3 bar1A</i>	This work
733-BY4733b	<i>MATa his3 leu2 met15 trp1 ura3 smc2-8::LEU2 bar1::URA3</i>	This work
RDK3615	<i>MATa ade2 ade8 his3 hom3 leu2 lys2 ura3 trp1</i>	R. Kolodner
RDK3751	<i>MATa ade2 ade8 his3 hom3 leu2 lys2 ura3 trp1 sml1A::kanMX</i> <i>rad53A::HIS3 chk1A::TRP1 smc2-8::LEU2</i>	R. Kolodner
733-RDK3751	<i>MATa ade2 ade8 his3 hom3 leu2 lys2 ura3 trp1 sml1A::kanMX</i> <i>rad53A::HIS3 chk1A::TRP1 smc2-8::LEU2</i>	This work
733-RDK3615	<i>MATa ade2 ade8 his3 hom3 leu2 lys2 ura3 trp1 smc2-8::LEU2</i>	This work
BY4733	<i>MATa his3 leu2 met15 trp1 ura3</i>	ATCC
bub1-BY4741	<i>MATa his3 leu2 ura3 met15 bub1A::KanMX</i>	This work
bub1-BY4741b	<i>MATa his3 leu2 ura3 met15 bar1::URA3 bub1A::KanMX</i>	This work
733-bub1-BY4741	<i>MATa his3 leu2 ura3 met15 bub1A::KanMX smc2-8::LEU2</i>	This work
733-bub1-BY4741b	<i>MATa his3 leu2 ura3 met15 bar1::URA3 bub1A::KanMX smc2-8::LEU2</i>	This work
YPH499	<i>MATa ade2 his3 leu2 lys2 trp1 ura3</i>	Ph. Hieter
bub1-ZW204	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 bub1A::LEU2 ycs4-2::MYC12::HIS3</i>	This work
bub1-CH2524	<i>MATa leu2 trp1 ura3 bub1A::LEU2 brn1-9::TRP1</i>	This work
733-mad1-BY4741	<i>MATa his3 leu2 met15 ura3 mad1A::KanMX smc2-8::LEU2</i>	This work
mad1-BY4741	<i>MATa his3 leu2 met15 ura3 mad1A::KanMX</i>	ATCC
733-mad2-BY4741	<i>MATa his3 leu2 met15 ura3 mad2::URA3 smc2-8::LEU2</i>	This work
mad2-BY4741	<i>MATa his3 leu2 met15 ura3 mad2::URA3</i>	This work
733-mad3-BY4741	<i>MATa his3 leu2 met15 ura3 mad3A::KanMX smc2-8::LEU2</i>	This work
mad3-BY4741	<i>MATa his3 leu2 met15 ura3 mad3A::KanMX</i>	ATCC
bub2-BY4741/pMA1183	<i>MATa his3 leu2 met15 ura3 bub2A::KanMX/CEN BUB2 URA3</i>	This work
733-bub2-BY4741/pMA1183	<i>MATa his3 leu2 met15 ura3 bub2A::KanMX</i> <i>smc2-8::LEU2/CEN BUB2 URA3</i>	This work
BY4733ST	<i>MATa his3 leu2 met15 trp1 bar1::HIS3</i>	This work

Strain	Relevant genotype	Origin
BY4733bIV	<i>URA3::TUB3:GFP SIK1:mRFP::KanMX SMC2::LEU2</i> <i>MATa leu2 met15 ura3 bar1::URA3</i>	This work
BY4733bIX	<i>HIS3::LacI-GFPTRP1::LacO::MET15 SMC2::LEU2</i> <i>MATa leu2 met15 ura3 HIS3::LacI-GFP</i>	This work
bub1-BY4741ST	<i>bar1::URA3::LacO::MET15 SMC2::LEU2</i> <i>MATa his3 leu2 met15 bar1::HIS3 URA3::TUB3:GFP</i>	This work
bub1-BY4741bIV	<i>SIK1:mRFP::KanMX bub1Δ::MET15</i> <i>MATa leu2 met15 ura3 bar1::URA3</i>	This work
bub1-BY4741bIX	<i>HIS3::LacI-GFPTRP1::LacO::MET15 bub1Δ::KanMx</i> <i>MATa bub1Δ::KanMx his3::LacI-GFP::HIS3</i>	This work
733-BY4733ST	<i>leu2 met15 ura3 bar1::URA3:lacO::MET15 bub1Δ::KanMx</i> <i>MATa his3 leu2 met15 trp1 bar1::HIS3</i>	This work
733-BY4733bIV	<i>URA3::TUB3:GFP SIK1:mRFP::KanMX smc2-8::LEU2</i> <i>MATa leu2 met15 ura3 bar1::URA3</i>	This work
733-BY4733bIX	<i>HIS3::LacI-GFPTRP1::LacO::MET15 smc2-8::LEU2</i> <i>MATa leu2 met15 ura3 HIS3::LacI-GFP</i>	This work
733-bub1-BY4741ST	<i>bar1::URA3::LacO::MET15 smc2-8::LEU2</i> <i>MATa his3 leu2 met15 bar1::HIS3 URA3::TUB3:GFP</i>	This work
733-bub1-BY4741bIV	<i>SIK1:mRFP::KanMX smc2-8::LEU2 bub1Δ::MET15</i> <i>MATa leu2 met15 ura3 bar1::URA3 HIS3::LacI-</i>	This work
733-bub1-BY4741bIX	<i>GFP TRP1::LacO::MET15 smc2-8::LEU2 bub1Δ::KanMx</i> <i>MATa bub1Δ::KanMx his3::LacI-GFP::HIS3 leu2 met15</i>	This work
1aAS342	<i>ura3 smc2-8:LEU2 bar1::URA3:lacO::MET15 bub1Δ::KanMx</i> <i>MATa ade2 his3 leu2 lys2 trp1 smc4-1</i>	(Freeman <i>et al.</i> 2000)
6dAS593	<i>MATa ade2 his3 leu2 lys2 ura3/CFIII(CEN3.L.YPH983) HIS3 SUP11</i>	This work
7bAS595	<i>MATa ade2 his3 leu2 lys2 ura3 skp1-AA::LEU2</i> <i>smc2-8/CFIII(CEN3.L.YPH983) HIS3 SUP11</i>	This work
1cAS594	<i>MATa ade2 his3 leu2 lys2 ura3 skp1-</i> <i>AA:LEU2/CFIII(CEN3.L.YPH983) HIS3 SUP11</i>	This work
7aAS596	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 smc2-</i> <i>8/CFIII(CEN3.L.YPH983) HIS3 SUP11</i>	This work
7aAS597	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 sgo1::LEU2</i> <i>smc2-8/CFIII(CEN3.L.YPH983) HIS3 SUP11</i>	This work
6dAS598	<i>MATa ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1</i> <i>sgo1::URA3/CFIII(CEN3.L.YPH983) HIS3 SUP11</i>	This work
CSE4GFP-EY0896	<i>MATa his3 leu2 met15 ura3 CSE4:GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-CSE4GFP-EY0896	<i>MATa his3 leu2 met15 ura3 CSE4:GFP::HIS3Mx6 smc2-8:LEU2</i>	This work
DAD1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 DAD1:GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-DAD1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 DAD1:GFP::HIS3Mx6 smc2-8:LEU2</i>	This work
DSN1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 DSN1:GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-DSN1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 DSN1:GFP::HIS3Mx6 smc2-8:LEU2</i>	This work
AME1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 AME1:GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)

Strain	Relevant genotype	Origin
733-AME1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 AME1::GFP::HIS3Mx6 smc2-8::LEU2</i>	This work
MTW1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 MTW1::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-MTW1GFP-EY0896	<i>MATa his3 leu2 met15 ura3 MTW1::GFP::HIS3MX6 smc2-8::LEU2</i>	This work
NUF2GFP-EY0896	<i>MATa his3 leu2 met15 ura3 NUF2::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-NUF2GFP-EY0896	<i>MATa his3 leu2 met15 ura3 NUF2::GFP::HIS3Mx6 smc2-8::LEU2</i>	This work
SPC24GFP-EY0896	<i>MATa his3 leu2 met15 ura3 SPC24::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-SPC24GFP-EY0896	<i>MATa his3 leu2 met15 ura3 SPC24::GFP::HIS3Mx6 smc2-8::LEU2</i>	This work
SPC25GFP-EY0896	<i>MATa his3 leu2 met15 ura3 SPC25::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-SPC25GFP-EY0896	<i>MATa his3 leu2 met15 ura3 SPC25::GFP::HIS3MX6 smc2-8::LEU2</i>	This work
NDC10GFP-EY0896	<i>MATa his3 leu2 met15 ura3 NDC10::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-NDC10GFP-EY0896	<i>MATa his3 leu2 met15 ura3 NDC10::GFP::HIS3Mx6 smc2-8::LEU2</i>	This work
CBF2GFP-EY0896	<i>MATa his3 leu2 met15 ura3 CBF2::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-CBF2GFP-EY0896	<i>MATa his3 leu2 met15 ura3 CBF2::GFP::HIS3Mx6 smc2-8::LEU2</i>	This work
CTF3GFP-EY0896	<i>MATa his3 leu2 met15 ura3 CTF3::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-CTF3GFP-EY0896	<i>MATa his3 leu2 met15 ura3 CTF3::GFP::HIS3MX6 smc2-8::LEU2</i>	This work
MIF2GFP-EY0896	<i>MATa his3 leu2 met15 ura3 MIF2::GFP::HIS3Mx6</i>	(Huh <i>et al.</i> 2003)
733-MIF2GFP-EY0896	<i>MATa his3 leu2 met15 ura3 MIF2::GFP::HIS3Mx6 smc2-8::LEU2</i>	This work
6dAS606	<i>MATa leu2 met15 ura3 HIS3::LacI-GFP TRP1::LacO::MET15</i> <i>pGAL::CDC20::TRP1</i>	This work
5bAS606	<i>MATa leu2 met15 ura3 HIS3::LacI-GFP TRP1::LacO::MET15</i> <i>smc2-8::LEU2 pGAL::CDC20::TRP1</i>	This work
BY4733galCDC20/pJK145	<i>MATa his3 leu2 met15 trp1 ura3 pGAL::CDC20::TRP1</i> <i>SLK19::GFP::URA3</i>	This work
733-BY4733galCDC20/pJK145	<i>MATa his3 leu2 met15 trp1 ura3 smc2-8::LEU2</i> <i>pGAL::CDC20::TRP1 SLK19::GFP::URA3</i>	This work