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Chromosome Condensation Factor Brn1p Is Required for Chromatid Separation in Mitosis

Ilia I. Ouspenski,* Olga A. Cabello, and B. R. Brinkley

Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030

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This work describes *BRN1*, the budding yeast homologue of *Drosophila* Barren and *Xenopus* condensin subunit XCAP-H. The *Drosophila* protein is required for proper chromosome segregation in mitosis, and *Xenopus* protein functions in mitotic chromosome condensation. Mutant *brn1* cells show a defect in mitotic chromosome condensation and sister chromatid separation and segregation in anaphase. Chromatid cohesion before anaphase is properly maintained in the mutants. Some *brn1* mutant cells apparently arrest in S-phase, pointing to a possible function for Brn1p at this stage of the cell cycle. Brn1p is a nuclear protein with a nonuniform distribution pattern, and its level is up-regulated at mitosis. Temperature-sensitive mutations of *BRN1* can be suppressed by overexpression of a novel gene *YCG1*, which is homologous to another *Xenopus* condensin subunit, XCAP-G. Overexpression of *SMC2*, a gene necessary for chromosome condensation, and a homologue of the XCAP-E condensin, does not suppress *brn1*, pointing to functional specialization of components of the condensin complex.

INTRODUCTION

Equal distribution of genetic material during eukaryotic cell division requires reorganization of chromosome structure in mitosis, known as mitotic chromosome condensation. Condensation results in compaction of chromosomes, such that the average distance between points along the chromosome is reduced approximately fivefold in higher eukaryotes and twofold in budding yeast (reviewed by Koshland and Strunnikov, 1996; Hirano, 1999). Condensation is thought to serve several functions. These include the reduction of the length of chromosome arms such that they are shorter than half the length of the mitotic spindle and thus can be completely segregated into daughter cells during cytokinesis. Condensation may also help to resolve entangled chromatin fibers and increase mechanical resistance of the chromosomes to the forces of the mitotic spindle.

Several factors involved in this process have been identified in various organisms. They are evolutionarily related, as judged by their sequences, pointing to conservation of the basic mechanisms of mitotic chromosome condensation. These factors include a so-called condensin complex, topoisomerases, histone H3, and a number of additional proteins identified by yeast mutations.

The best biochemically characterized chromosome condensation factors are the 8S and 13S "condensin" complexes, identified in the *Xenopus* egg extract system (Hirano *et al.*, 1997). The 8S complex is important, but not sufficient for mitotic chromosome condensation. It consists of two SMC-type (structural maintenance of chromosomes) proteins, XCAP-C and XCAP-E. Their budding yeast homologues, Smc2p and Smc4p, have also been implicated in chromosome condensation (Strunnikov *et al.*, 1995). The 13S condensin complex is necessary and sufficient to perform *Xenopus* mitotic chromosome condensation in vitro. It consists of five subunits, which in addition to XCAP-C and XCAP-E include three unrelated proteins: XCAP-D2, XCAP-G, and XCAP-H (Hirano *et al.*, 1997). The 13S condensin complex is capable of binding DNA and using ATP to induce a global change in DNA configuration (Kimura and Hirano, 1997; Kimura *et al.*, 1999).

In mitosis, XCAP-H, and to a lesser extent XCAP-G and XCAP-D2, subunits are hyperphosphorylated, and the complex is targeted to the chromosomes (Hirano *et al.*, 1997). Cdc2 protein kinase is at least partly responsible for this phosphorylation, which is accompanied by a shift in electrophoretic mobility of these proteins (Kimura *et al.*, 1998). This phosphorylation is necessary to activate the DNA reconfiguring activity of the condensin complex. It was hypothesized that this activity provides the driving force for mitotic DNA condensation (Hirano *et al.*, 1997; Kimura *et al.*, 1999). From the biochemical studies in *Xenopus*, it appears that the function of condensins is limited to mitotic compaction of chromatin.

Mutations in fission yeast *Schizosaccharomyces pombe* genes homologous to *Xenopus* condensins cause defective chromosome condensation in mitosis (Sutani *et al.*, 1999). In this organism, mitotic phosphorylation of Cut3/SMC4 subunit, which is homologous to *Xenopus* XCAP-C, is required for mitotic relocation of condensins from cytoplasm to the nucleus (Sutani *et al.*, 1999).

* Corresponding author. E-mail address: iliao@bcm.tmc.edu.

A mutation in the homologue of condensin subunit XCAP-H has been described in *Drosophila* (Bhat *et al.*, 1996). It results in a mitotic chromosome segregation defect, in which the centromeres separate but chromosome arms do not get resolved. In contrast to the situation in *Xenopus* egg extracts depleted of condensins, no detectable defect in chromosome condensation could be observed in the *barren* mutant. The Barren protein was reported to interact with topoisomerase II and to activate its decatenating activity. It was hypothesized that the defect in topoisomerase II activation is responsible for the failure of chromosome resolution in mitosis in *barren* mutant embryos (Bhat *et al.*, 1996).

Although chromosome condensation cannot be directly observed in budding yeast, it can be detected using fluorescence in situ hybridization (FISH), using either cosmid-size probes or probes that hybridize to the ribosomal DNA array (Guacci *et al.*, 1994). Ribosomal DNA encompasses a region of ~500 kb on chromosome XII, and its condensation state can be visually assessed after hybridization of a fluorescent probe. In interphase, the rDNA appears as a diffuse area, whereas in mitotic cells it has a defined string-like or bead-like shape (Guacci *et al.*, 1994).

A mutation in *SMC2*, the budding yeast homologue of XCAP-E, leads to a defect in mitotic chromosome condensation and segregation (Strunnikov *et al.*, 1995). Mutant cells accumulate in mitosis while retaining relatively high viability. Some cells eventually undergo an abnormal division and arrest as unbudded cells (i.e., in the G1 phase of the cell cycle). When grown at permissive temperatures, the cells do not show a significant increase in the rate of chromosome loss. This set of characteristics is different in some respects from the phenotype of the yeast *top2* mutants, which affect topoisomerase II (DiNardo *et al.*, 1984; Holm *et al.*, 1989). These cells attempt to segregate their chromosomes, which results in lethality. Unlike *smc2*, the *top2* mutant also has an increased chromosome loss rate.

Condensation defect was also detected in a double mutant *trf4 top1* (Castano *et al.*, 1996). *TRF4* was identified in a screen for mutations that are inviable in combination with topoisomerase I null mutation. Trf4p physically interacts with Smc1p and Smc2p. Its biochemical activities or cellular functions are unknown.

All five known *Xenopus* condensin subunits have highly similar homologues in the budding yeast genome. In addition to *SMC2* and *SMC4*, there is *BRN1*, the homologue of the XCAP-H and *Drosophila* Barren, which is the focus of this work. We have also identified the yeast homologue of XCAP-G, *YCG1*, as a dosage suppressor of *brn1* mutation. The homologue of XCAP-D2, named *LOC7*, was identified in a screen for genes necessary for sister chromatid separation and segregation (N. Bhalla and A. Murray [University of California, San Francisco, CA] *Saccharomyces* Genome Database entry). Here we explore the properties of *BRN1* as a step to dissect the molecular mechanisms of mitotic chromosome condensation.

MATERIALS AND METHODS

Deletion of *BRN1* was accomplished by replacing the complete ORF of the gene with the KanMX4 marker, which confers resistance to G418 (Wach *et al.*, 1994). This was done by PCR amplification of the KanMX4 module from the pFA6a-kanMX4 plasmid (Wach *et al.*, 1994), using the primers containing 18–19 bp identity to the regions flanking the KanMX4 gene at their 3′ ends, and 45 bp identity to the sequences flanking the *BRN1* ORF at the 5′ ends. The PCR product was transformed into a diploid yeast strain (W303 derivative), and G418-resistant colonies were tested for correct replacement of *BRN1* using PCR, encompassing both 5′ and 3′ junctions.

Temperature-sensitive mutations of *BRN1* were created by PCR-based mutagenesis or by chemical mutagenesis of the cloned gene. In the PCR experiment, we have separately mutagenized the regions approximately corresponding to the N-terminal, middle, and C-terminal one-third of the protein. The *BRN1* gene in a *TRP1 CEN* plasmid was cut ("gapped") with BsrGI+*Nco*I, *Sph*I, or Eco47III+*Sal*I, respectively. The gapped plasmids were cotransformed with the corresponding PCR products into a *brn1*-Δ1 + p(*BRN1 URA3*) strain, followed by eviction of the *BRN1 URA3* plasmid on 5FOA-containing plates. Temperature-sensitive strains were selected and verified by plasmid rescue in *Escherichia coli* and retransformation into yeast. We have recovered one mutant resulting from the mutagenesis of the middle part of the gene (*brn1–20*), and several mutants in the C-terminal part of the gene. Mutants of the latter group had multiple substitutions, two of which were common to all alleles; we chose the *brn1–34* allele, which has only two substitutions, for further analysis. Chemical mutagenesis with hydroxylamine, which produced the *brn1–60* mutation, was performed as described (Sikorski and Boeke, 1991).

Chromosome condensation was assayed by FISH of the ribosomal DNA region, as described (Guacci *et al.*, 1994). The probe was generated by PCR amplification of a fragment of rDNA repeat unit and labeled with biotin using the BioNick nick-translation system (Life Technologies, Grand Island, NY). Blind scoring of at least 100 cells in each preparation was used to determine the percentage of condensed chromosomes.

Sister chromatid cohesion and segregation were analyzed in a strain containing an array of Lac operator sequence repeats integrated at the *LEU2* locus, close to the centromere of chromosome IV, and expressing a LacI::GFP fusion protein (Straight *et al.*, 1996, 1997). The strain was crossed to *brn1*-60 mutant, and ts^-, green fluorescent protein–positive (GFP^+) segregants were selected. Cells were fixed with 4% formaldehyde for 15 min, placed onto polylysine-coated slides, stained with 0.1 μg/ml DAPI, and mounted in Vectashield (Vector Laboratories, Burlingame, CA) for microscopy.

Flow cytometry was performed as described earlier (Ouspenski *et al.*, 1995). Cells were fixed in 80% ethanol, treated with RNase A (1 mg/ml, 2 h at 37°C), and stained with 10 μg/ml propidium iodide. The samples were briefly sonicated just before analysis to disperse clumps.

Pulse-field gel electrophoresis was performed using the CHEF-DR II system (Bio-Rad, Hercules, CA) according to manufacturer's recommendations. Samples were run at 200 V with 120 s pulse time for 36 h.

Anti-*Brn1p* antibody was raised in a rabbit against the synthetic peptide IDMPIKNRKNDTHYL, corresponding to amino acids 457–471 of the predicted sequence. Affinity purification, immunoblotting, and immunofluorescence were done according to conventional procedures (Harlow and Lane, 1988; Pringle *et al.*, 1989).

Immunofluorescent staining of yeast cells was done as described (Kilmartin and Adams, 1984), except that cells were fixed with formaldehyde for 30 min.

Immunoprecipitation was performed from cells containing pAS443 (2 mm SMC2::MYC6, a gift from A. Strunnikov) and pIL114 (CEN GAL->3HA::BRN1, this study), induced with galactose overnight. Cells (~10^9) were broken with glass beads in 2 ml IP buffer (20 mM HEPES, pH 7.9, 150 mM KCl, 2 mM $MgCl_2$, 0.1 mM DTT, 10% glycerol, supplemented with protease and phosphatase inhibitors [Harlow and Lane, 1988]), and insoluble matter was removed by centrifugation (20,000 × *g* for 20 min). Extracts were supplemented with Triton X-100 to 0.1% and BSA to 1 mg/ml. After preclearing with protein G Sepharose, the extract was split in four, and each portion was incubated overnight with protein G beads preloaded with monoclonal antibodies to Myc (9E10), hemagglutinin (HA) (12CA5), tubulin (negative control, YOL1/34), or an affinity-purified rabbit polyclonal anti-Brn1p antibody described above.

Beads were washed six times with IP buffer, boiled in SDS-containing sample buffer, and analyzed by immunoblotting.

RESULTS

BRN1 mutations

The yeast gene corresponding to the ORF YBL097W, for which we use the name *BRN1*, has been pointed out as the possible homologue of the *Drosophila* Barren gene, on the basis of sequence homology (Bhat *et al.*, 1996). It also has high sequence similarity to *Xenopus* condensin subunit XCAP-H and human BRRN1 (Hirano *et al.*, 1997; Cabello *et al.*, 1997). We cloned the *BRN1* gene from a W300-derived strain and found that its sequence differs from the corresponding *Saccharomyces* Genome Database entry by one amino acid: glycine-495 rather than alanine. The difference may be due to strain polymorphism.

To explore the function of *BRN1* in yeast, we deleted the ORF of the gene in a diploid strain, replacing it with the KanMX4 kanamycine resistance module (Wach *et al.*, 1994). Sporulation and spore dissection of this strain demonstrated that *BRN1* is essential for viability.

We created three independent temperature-sensitive alleles of the *BRN1* gene. *brn1*-20 (K489E) and *brn1*-34 (K592E + E638G) alleles were obtained by error-prone PCR mutagenesis of the middle one-third and C-terminal one-third of the *BRN1* ORF, respectively. *brn1*-60 mutation was generated by chemical mutagenesis of the plasmid containing *BRN1* gene. This allele has the same mutation as *brn1*-20 (K489E), plus an additional substitution P490S. This second substitution can be viewed as a partial reversion, because it improves the growth of cells at subrestrictive temperatures (although they exhibit a tight arrest at 37°C). The mutant alleles were substituted for *BRN1* in the genome by "pop in, pop out" gene replacement. The resulting *brn1*-20 mutant cells grow slower than wild type at all temperatures; *brn1*-34 cells grow at a wild-type rate at temperatures up to 35°C and stop growth at 37°C, but frequently give rise to spontaneous "revertant" colonies; and the *brn1*-60 mutant exhibits normal growth up to 35°C and a tight growth arrest at 37°C. Unless indicated otherwise, all experiments described here were performed with *brn1–34* and *brn1–60* alleles, and only the results obtained with *brn1–60* are shown, because no significant differences in the phenotypes between these two alleles were detected.

BRN1 Is Necessary for Chromosome Condensation and Segregation, but Not for Sister Chromatid Cohesion

Chromosome Condensation. Because the *BRN1* homologue in *Xenopus*, XCAP-H, is necessary for mitotic chromosome condensation, we tested *brn1* mutant cells for a condensation defect. As a marker of mitotic condensation, we have assessed the state of the ribosomal DNA region of chromosome XII, which encompasses ~500 kb of DNA sequence. When visualized by FISH, the rDNA array appears as a diffuse mass in interphase, whereas in mitotic cells it forms defined string-like structures (Guacci *et al.*, 1994) (Figure 1). In this experiment, exponentially growing cells were shifted to the restrictive temperature, and at the same time, nocodazole was added to arrest cells in mitosis. After incubation for 3.5 h, the cells were processed for FISH and analyzed by fluorescence microscopy. Mutant cells proceed though the cell cycle and arrest at mitosis under these conditions, as evidenced by the accumulation of large-budded cells. In *brn1* mutants, the ribosomal DNA region is uncondensed in most cells at the restrictive temperature (Figure 1). The observed rDNA morphology in *brn1* cells is indistinguishable from that of *smc2* mutant (Strunnikov *et al.*, 1995) (Figure 1), indicating that *BRN1* is also necessary for proper mitotic condensation.

To test whether *BRN1* is required for maintenance of the condensed state after it is established, we arrested the cells with nocodazole for 2.5 h at the permissive temperature and then shifted them to 37°C for 1 h. Decondensation of rDNA was observed under these conditions (Figure 1), indicating that continued *BRN1* activity is required to maintain chromatin in the condensed state.

Sister Chromatid Cohesion. Some aspects of mitotic chromosome condensation are linked to sister chromatid cohesion, as illustrated by *mcd1/scc1* mutations, in which sister chromatids separate prematurely and fail to condense properly in mitosis (Guacci *et al.*, 1997; Michaelis *et al.*, 1997). To determine whether *BRN1* is necessary for chromatid cohesion, we tagged the centromeric region of chromosome IV in *brn1* mutant cells with an array of Lac operator repeats (Straight *et al.*, 1996). Expression of a fusion of LacI repressor with the GFP allows visualization of the centromeric region. In wild-type cells, sister chromatids remain attached until the onset of anaphase, and GFP fluorescence appears as a single spot. We tested *brn1* mutants for maintenance of sister chromatid cohesion, when the cells were prevented from progression into anaphase by nocodazole. Midlog phase cultures were split into two halves, and one half was shifted to the restrictive temperature. At the same time, cell cycle progression was blocked by addition of nocodazole, and cells were examined by fluorescence microscopy 3 h later. Almost all cells produced a single fluorescent spot at both permissive and restrictive temperatures: 90–95% in wild-type as well as mutant cells (200 cells of each genotype scored). This indicates that Brn1p is not required to establish or maintain sister chromatid cohesion after DNA replication (Figure 2).

Chromosome Segregation in Mitosis. Chromosome segregation in *brn1* mutants was followed in cells with chromosome IV centromere tagged with GFP, as in the above experiment. Shifting exponentially growing mutant cells to the restrictive temperature for 3 h resulted in accumulation of large-budded cells with a single nucleus. In some of these, the nuclei were elongated and traversed the bud neck (Figure 3A). In contrast to wild-type cells, where the centromeres in elongated nuclei were always separated, centromeric GFP signal appeared as a single dot in most mutant cells (70–85% depending on the allele; 200 cells scored). After 5 h at restrictive temperature, some mutant cells (30–60%) separate their centromeres, whereas the chromatin mass remains stretched through the bud neck, a morphology never observed in wild-type cells (Figure 3A, compare the rightmost cells). This likely reflects the failure of chromo-

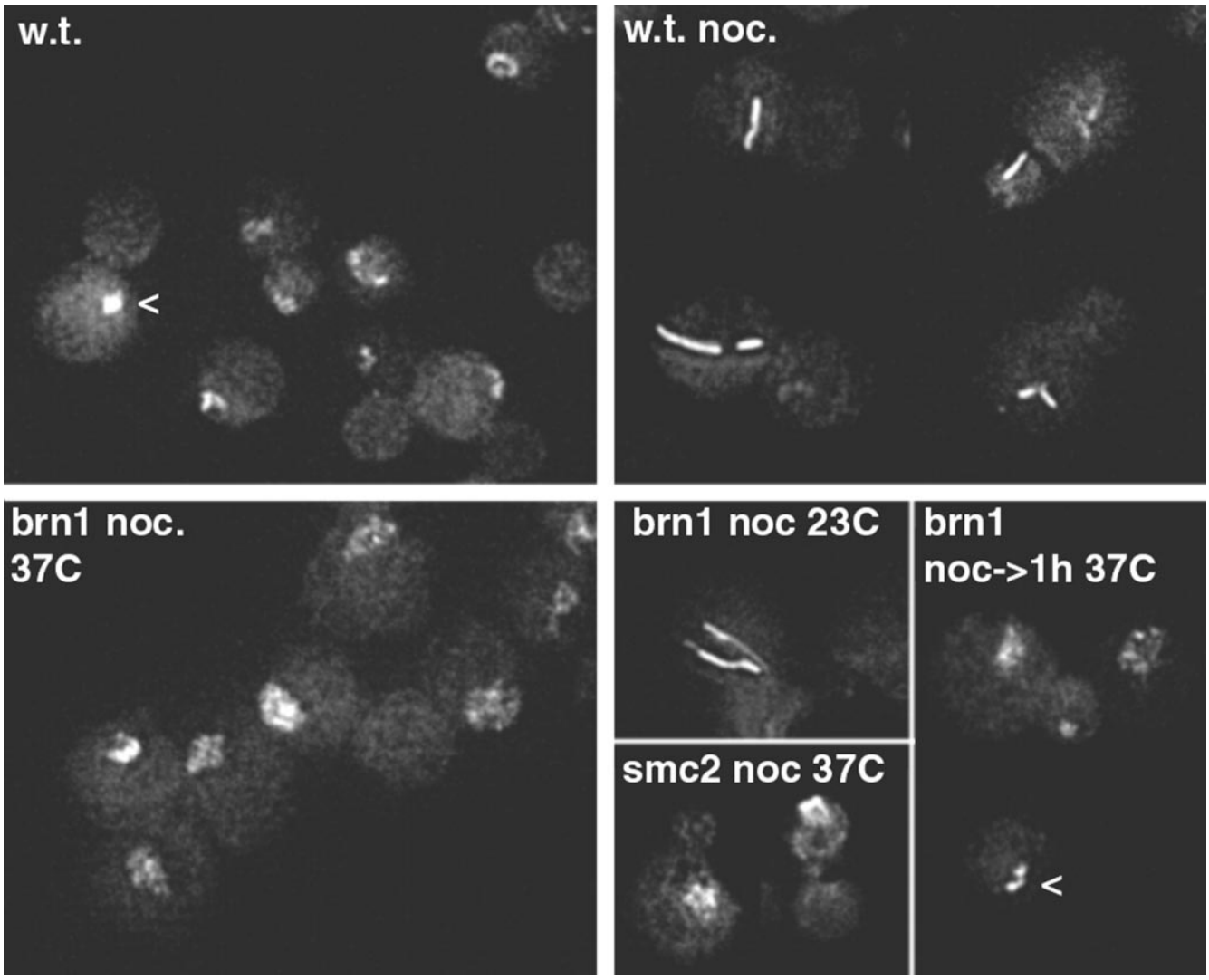

Figure 1. Mitotic chromosome condensation defect in *brn1* mutant cells. Examples of rDNA array morphologies, visualized by fluorescence in situ hybridization, are shown. *BRN1* (w.t.) or *brn1–60* cells were incubated at indicated temperatures for 3.5 h with or without nocodazole (noc). rDNA morphology in *smc2–8* cells is shown for comparison. The bottom right panel (noc–>1h 37C) shows *brn1–60* cells blocked in nocodazole at permissive temperature for 2.5 h, followed by a shift to 37°C in the continued presence of nocodazole. Arrowheads point to examples of rDNA morphology scored as "intermediate." The graph below shows percentages of cells with the indicated rDNA morphologies after 3.5 h at the restrictive temperature in the presence of nocodazole. Blind scoring of at least 100 cells in each preparation was performed. Cells that could not be unequivocally assigned to one of the two classes were scored as intermediate (see arrowheads above).

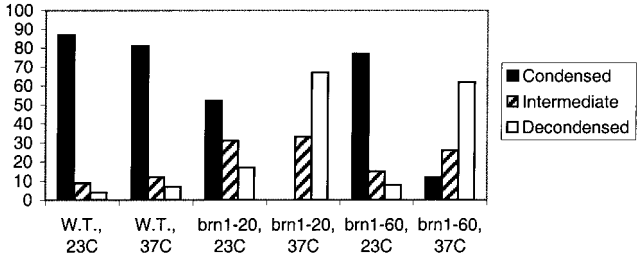

Figure 1 (Cont).

some arms to compact properly, so that their length remains greater than half the mitotic spindle length.

The observed defect in centromere separation may indicate that chromosome condensation or another *BRN1*-dependent function is directly required to release cohesion of sister chromatids. Alternatively, chromosome condensation may be necessary for the formation of functional mitotic kinetochores. The latter scenario would result in inefficient attachment of microtubules to the centromeres. Antitubulin immunofluorescence revealed that this is likely to be the case. Mutant *brn1* cells accumulated mitotic spindles that were comparable in length to normal metaphase spindles

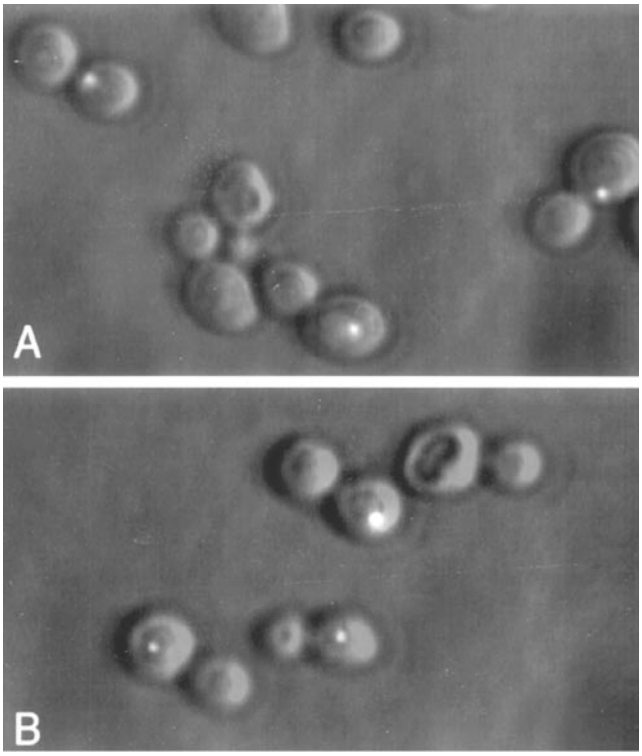

Figure 2. Sister chromatid cohesion is normal in *brn1* mutant cells. The centromeric region of chromosome IV in these strains is tagged with an array of Lac operator repeats and visualized by expressing LacI::GFP fusion protein. GFP fluorescence (bright dots) is shown overlaid onto DIC images of cells. Wild-type (A) and *brn1*-60 mutant (B) cells were incubated at 37°C for 3 h in the presence of nocodazole. The two sister centromeres appear as a single dot in >90% of wild-type and mutant cells under these conditions (200 cells of each type scored).

but appeared discontinuous in the middle (Figure 3B). Such morphology is expected if the pole-to-pole microtubules function normally, but the pole-to-centromere microtubules are not efficiently stabilized by attachment to the kinetochores (Page and Snyder, 1993). Dot-like tubulin staining characteristic of unduplicated spindle pole bodies in normal G1 cells or typical elongated anaphase spindles were rarely observed in mutant cells (Figure 3B; our unpublished data).

Cell Cycle Progression. To determine how the absence of *BRN1* function affects cell cycle progression, we followed the mutant cell morphology by microscopy and the DNA content by flow cytometry. Because of the relatively high viability of mutant cells (Figure 4A), we could not assign the essential function of *BRN1* to a defined cell cycle stage. When asynchronously growing cells were shifted to the restrictive temperature for 3 h, they accumulated at the large-budded stage, indicating a delay or arrest in G2 or mitosis (Figure 4B). The large-budded cells contain a single nucleus that either has not migrated to the bud or has traversed the bud neck and is abnormally stretched (Figures 3 and 4B).

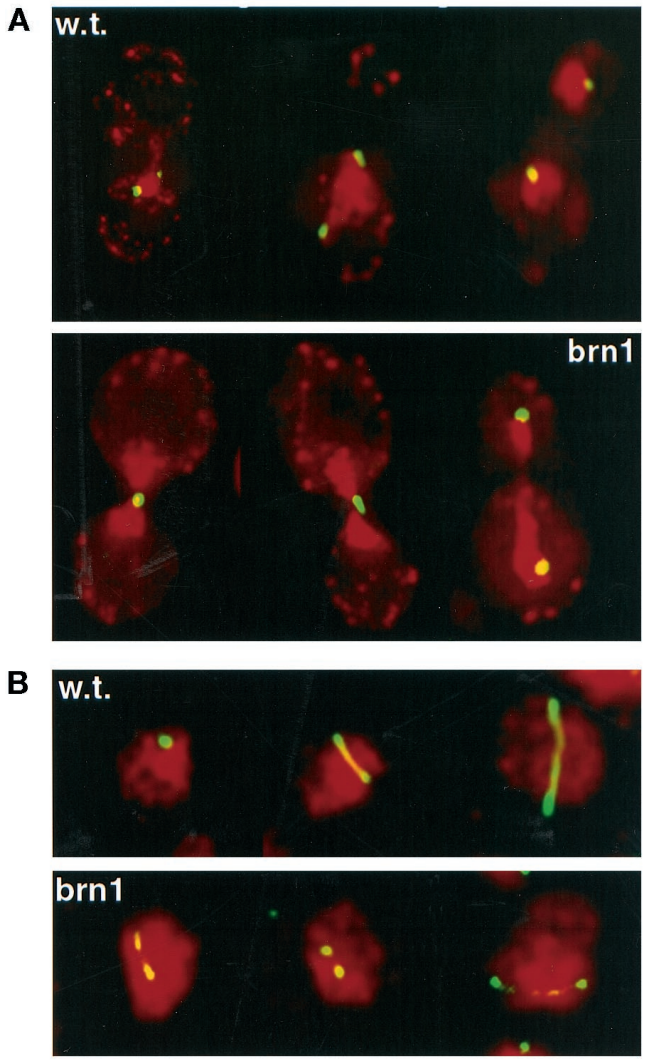

Figure 3. (A) Chromatid separation and segregation in *BRN1* (w.t.) and *brn1–60* mutant cells. Centromere of chromosome IV, visualized with LacI::GFP, is shown in green; cell nuclei, stained with DAPI (pseudocolored), are bright red; the outline of the cells is visible as red background. Exponentially growing cultures were shifted to 37°C for 3 h and processed for microscopy. (B) Examples of mitotic spindle morphology in *BRN1* (w.t.) and *brn1–60* cells incubated at 37°C for 3 h. Antitubulin immunofluorescence is shown in green; DNA stained with DAPI is pseudocolored red.

To characterize the cell cycle progression of the mutants, we arrested the cells in G1 with α-factor at the permissive temperature, released them from the block at the restrictive temperature, and followed their DNA content by flow cytometry. At 1.5 h after the release, *BRN1* cells reached mitosis and started proceeding to G1, whereas most mutant cells remained with G2/M DNA content (Figure 4C). During continued incubation at the restrictive temperature, a significant fraction of mutant cells proceeded through cell division, as evidenced by the reappearance of the 1C DNA peak (Figure 4C) and unbudded morphology, while other cells

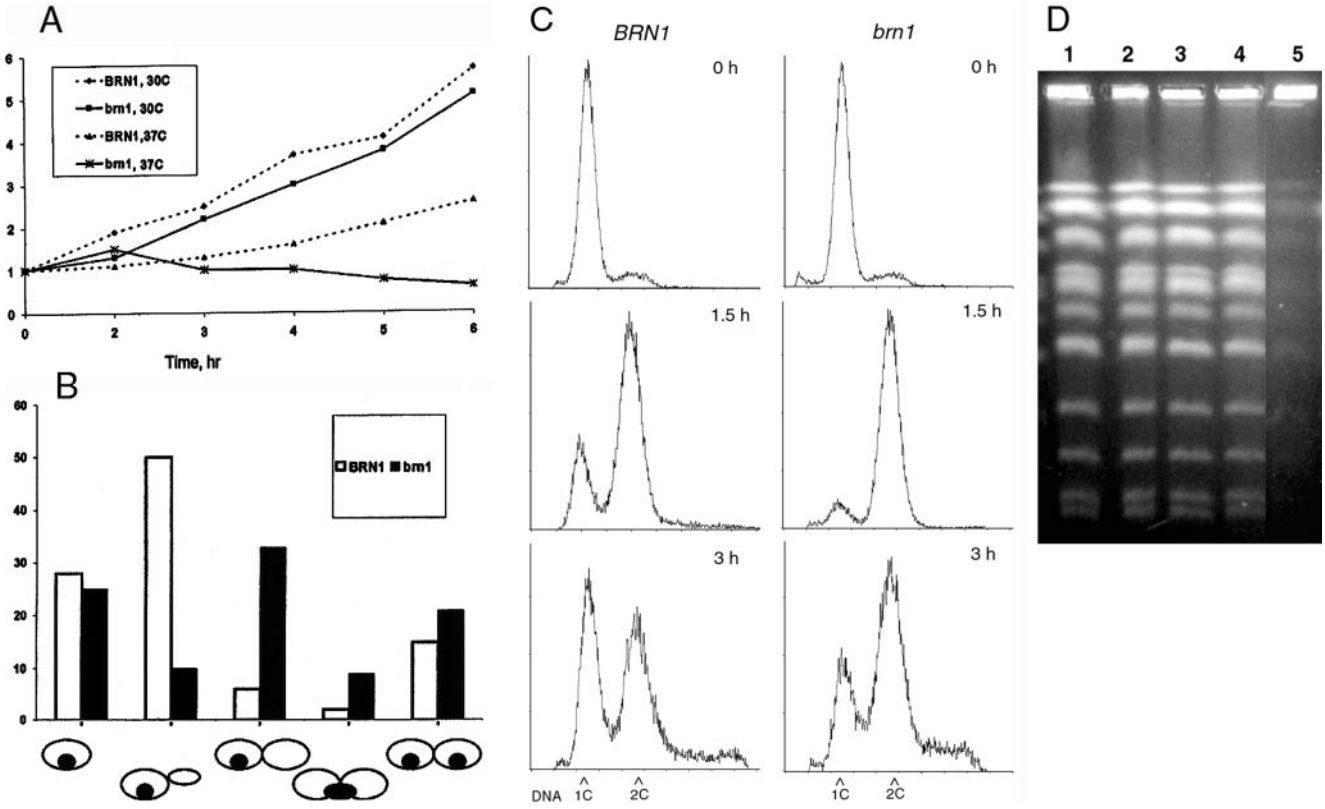

Figure 4. The effect of *brn1* mutation on cell cycle progression. (A) Relative viability of *brn1*-60 cells. Midlog phase cultures were split into two halves, and one half was shifted to the restrictive temperature (37°C). At indicated time points, portions of each culture were plated out at a density of 50–500 cells per plate, and the numbers of resulting colonies, divided by the corresponding number of colonies at time zero, were plotted on the graph. (B) Cell cycle stage distribution (% of total; 200 cells scored) of *brn1–60* cells at the restrictive temperature, compared with wild-type (*BRN1*) cells. Asynchronous cultures were incubated at 37°C for 3 h. (C) DNA content of *BRN1* and *brn1*-60 cells arrested at the G1 phase with α-factor and released at the restrictive temperature. Samples of cells were fixed at the indicated time points, stained with propidium iodide, and analyzed by flow cytometry. (D) Pulse-field electrophoresis of chromosomal DNA of *BRN1* (lanes 1, 2) and *brn1–60* mutant cells (lanes 3, 4) grown at 23°C (lanes 1, 3) or incubated at 37°C for 3 h. Lane 5, *BRN1* cells treated with 100 mM hydroxyurea for 3 h.

remained large-budded. Most large-budded cells had slightly elongated nuclei, whereas a small fraction had a fully stretched DNA mass. Of the mutant cells that divided, some remained unbudded, whereas others initiated growth of a new bud, which is indicative of the next round of DNA synthesis; however, these "second cycle" cells never reached the next mitotic stage, arresting with a final morphology characteristic of early to mid-S phase. This suggests that cell division without *BRN1* function damages the chromosomes and leads to cell death in the next cell cycle or that *BRN1* may also be necessary for progression through the S phase.

To test whether cell cycle progression without *BRN1* function results in DNA damage, we performed the analysis of chromosomal DNA in the mutants by pulse-field gel electrophoresis. Intact yeast chromosomes are resolved into distinct bands by this method, whereas DNA replication intermediates in hydroxyurea-treated cells do not enter the gel under the conditions used (Figure 4D). We could not detect any DNA damage by this method. The fact that the mutants do not show a significant increase in chromosome loss rate at subrestrictive temperatures, or after transient incubation at the restrictive temperature, also argues against substantial DNA damage in these cells.

Brn1p Interacts with Smc2p In Vivo

Because the *Xenopus* homologue of Brn1p, XCAP-H, is a part of the 13S condensin complex (Hirano *et al.*, 1997), we sought to determine whether it interacts with other prospective condensin subunits in yeast cells. Brn1p tagged with HA epitope was coexpressed with Smc2p tagged with Myc (Strunnikov *et al.*, 1995), and proteins were immunoprecipitated with anti-Myc antibody. Immunoblotting with anti-HA antibody revealed specific coimmunoprecipitation of Brn1p with Smc2p only when Myc-tagged Smc2p was present (Figure 5). Thus *BRN1* and *SMC2* encode components of the same molecular complex in yeast.

Genetic Interactions of BRN1 with Other Chromosome Condensation Factors

To identify proteins that functionally interact with *BRN1*, we performed a screen for dosage suppressors of *brn1–60* mu-

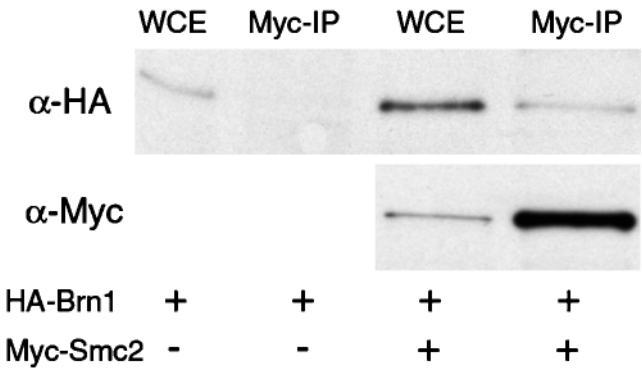

Figure 5. Physical association between Brn1p and Smc2p. Total protein extracts from cells expressing HA-Brn1p (from *GAL1* promoter on a *CEN* plasmid) and Myc-Smc2p (from endogenous promoter on a 2-μm plasmid) were immunoprecipitated with anti-Myc antibody (9E10) and analyzed by immunoblotting with anti-HA and anti-Myc antibodies, as indicated. WCE, Whole cell extract (input), 20 μl; Myc-IP, immunoprecipitation from 400 μl of WCE.

tation. Two overlapping sets of clones have been recovered multiple times (more than 50 independent clones in each set), one set encoding *BRN1* itself and the other containing an uncharacterized ORF YDR325W. This gene is homologous to XCAP-G, a component of *Xenopus* 13S condensin complex, and likely encodes the corresponding condensin subunit in yeast. We name this gene *YCG1* (*Yeast CAP G*). Overexpression of *YCG1* restores growth of *brn1–60* cells at 37°C to a nearly wild-type rate (Table 1). The suppression is allele-specific, because *YCG1* suppresses the *brn1–20* mutation only to a limited extent and does not suppress the *brn1–34* allele.

To explore the functional relations of *BRN1* with other condensins, we tested the gene for dosage interactions with *SMC2*. Overexpression of *SMC2* from a high-copy plasmid failed to suppress temperature sensitivity of *brn1* mutants (Table 1). The reciprocal experiment produced a similar outcome: overproduction of Brn1p did not rescue the *smc2* temperature-sensitive mutation. Overexpression of *YCG1* in *smc2* mutant cells resulted in only marginal suppression, possibly reflecting a closer interaction of *YCG1* with *BRN1*, as compared with *SMC2*.

Table 1. Dosage interactions of *YCG1*, *BRN1*, and *SMC2*

Gene overexpressed	Mutation			
	brn1-20	*brn1-34*	*brn1-60*	*smc2-8*
BRN1	+	+	+	−
YCG1	±	−	+	−*
SMC2	−	−	−	+

The indicated genes were expressed from 2-μm plasmids under the control of their respective endogenous promoters. Cells were grown on SD plates for 3 d at 37°C (*brn1*) or 34°C (*smc2*).

* Prolonged incubation of *smc2-8* cells overexpressing *YCG1* resulted in marginal growth at 34°C.

Brn1p Is a Cell Cycle-regulated Nuclear Protein

We raised and affinity-purified an antibody to a peptide derived from the predicted Brn1p sequence. When this antibody was used for immunofluorescence, we were unable to detect the endogenous protein, presumably because of its low abundance. Overexpression of Brn1p from *GAL1* promoter resulted in an uneven pattern of nuclear staining in some cells, possibly reflecting the subnuclear distribution of the protein (Figure 6). Specificity of immunofluorescence staining was confirmed by preincubating the antibody with the antigenic peptide, which abolished the staining.

The genome-wide survey of cell cycle regulation of gene expression in yeast showed that the level of *BRN1* transcript is increased at G2/M (Cho *et al.*, 1998), suggesting that the protein level may be regulated as well. Using the anti-Brn1p antibody, we compared endogenous Brn1p levels in wild-type cells arrested in G1 with α-factor and in mitosis with nocodazole. The level of protein in mitosis is significantly higher than in G1 (Figure 7A). The dynamics of Brn1p level in the cell cycle was followed in cells synchronized at the G1/S boundary with hydroxyurea (Figure 7B). Protein abundance drops 45–60 min after release from the block, as the cells complete mitosis, followed by accumulation as the cells approach mitosis in the next cell cycle.

DISCUSSION

Yeast Brn1p protein is similar in sequence to *Xenopus* condensin subunit XCAP-H, human and mouse BRRN1, *S. pombe* Cnd2, and a predicted protein from *Arabidopsis thaliana* (pairwise BLAST E values in the range of e^{-23} to e^{-34}). This sequence homology reflects functional conservation, because the human protein can substitute for *BRN1* function in yeast (our unpublished observation). Homology between these proteins is significantly higher than between Brn1p and *Drosophila* Barren (Blast E value = 0.004). This may account for some of the specific features of the *barren* mutant phenotype in *Drosophila*, as compared with the yeast mutant phenotype and functional data in the *Xenopus* system.

The sequence of *BRN1*, as well as its homologues in other species, contains several potential PEST sequences, which are characteristic of unstable proteins and may serve as signals for regulated degradation (Rechsteiner and Rogers, 1996). This is in agreement with our data that Brn1p level is regulated in the cell cycle. Perhaps the protein must be degraded for chromosome decondensation after completion of mitosis.

The lack of electrophoretic mobility change of Brn1p in the cell cycle is surprising, because XCAP-H exhibits a substantial shift attributable to hyperphosphorylation in mitosis (Kimura *et al.*, 1998). It should be noted that although Brn1p, as well as XCAP-H and other homologues, has numerous consensus phosphorylation sites for several protein kinases, few of these sites are located within regions that are highly conserved between species. This raises the possibility that the mechanisms of regulation of the protein vary in different species.

Although some *brn1* mutant cells permanently arrest in mitosis, a significant proportion of cells arrest with a terminal morphology characteristic of S-phase. This raises the possibility that Brn1p may have a function in interphase, possibly in DNA replication. Alternatively, mitotic *BRN1*

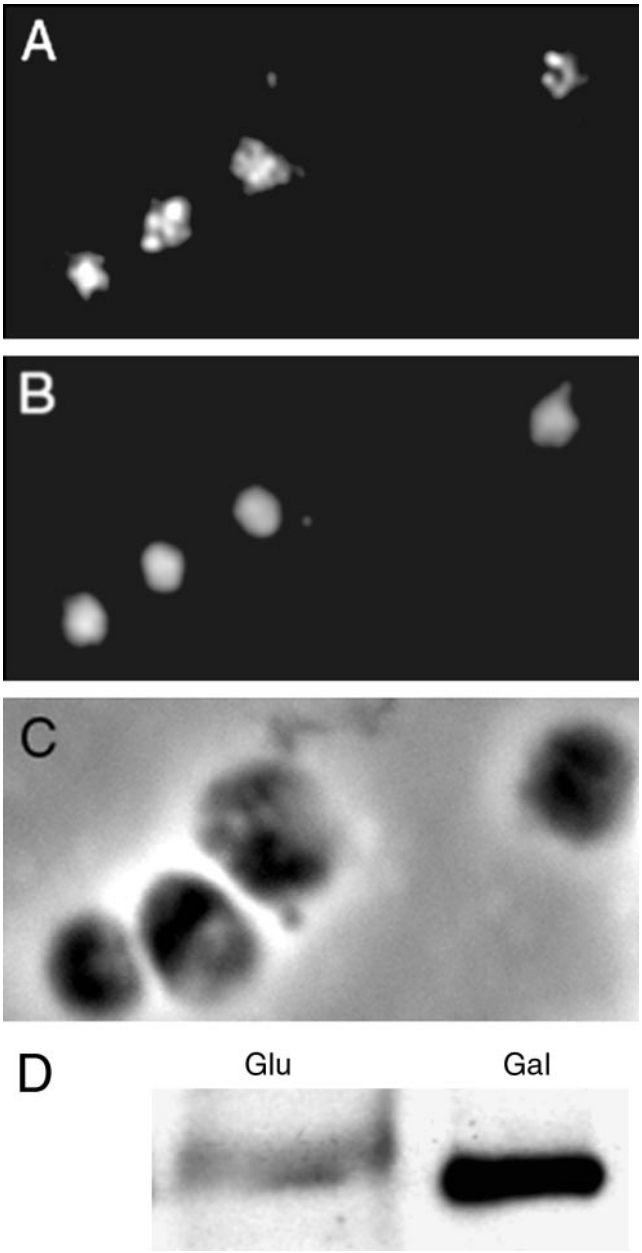

Figure 6. Intracellular localization of the Brn1p protein. (A) Immunofluorescent staining of cells overexpressing Brn1p from the *GAL1* promoter on a *CEN* plasmid, using an affinity-purified anti-Brn1p antibody. (B) Nuclear DNA of the same cells stained with DAPI. (C) Phase-contrast image of the same cells. (D) Levels of Brn1p under induced (Gal) and uninduced (Glu) conditions.

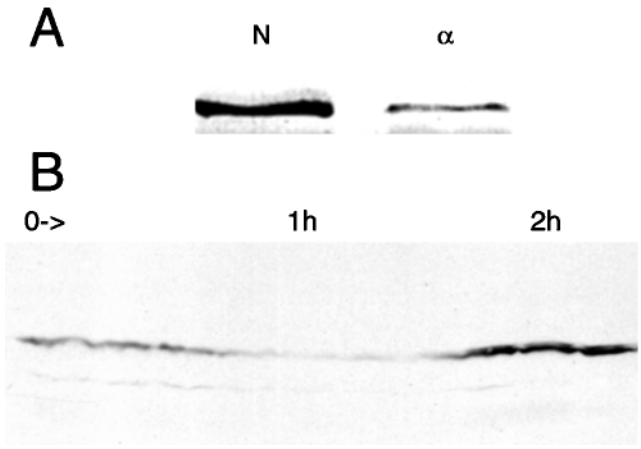

Figure 7. Cell cycle regulation of Brn1p. (A) Levels of endogenous Brn1p in wild-type cells blocked in mitosis with nocodazole (N), or in G1 with α-factor (α), detected by immunoblotting with anti-Brn1p antibody. Equal amounts of total protein were loaded in the two lanes. (B) Brn1p in wild-type cells released from hydroxyurea arrest. Cells were collected every 15 min, and total protein extracts obtained from identical culture volumes were loaded onto each lane. Most cells have completed mitosis (78% unbudded cells, n = 200) 1 h after release, whereas at the 2 h point most cells were in G2 or M (73% cells with bud size more than one-half of the mother cell, n = 200).

function may be necessary to "reset" chromatin for the next round of replication.

The mitotic defects of *brn1* cells are similar to those described for the *smc2* mutant (Strunnikov *et al*., 1995). In addition to chromosome condensation defect, this includes the arrest of some cells in mitosis, stretched nuclei, and terminal arrest in interphase of the second cell cycle. This indicates that the two genes are involved in a common cellular function, as suggested by their homology to subunits of the same molecular complex in *Xenopus*. There are some differences, however, which include the initiation of bud development by some *brn1* cells before arrest. It remains to be determined whether this difference reflects functional specialization of the two proteins or is specific to alleles of the respective mutations. Specialization of condensin subunits is further illustrated by the fact that overexpression of *YCG1*, but not *SMC2*, can suppress *brn1* lethality.

Like XCAP-H in *Xenopus*, yeast Brn1p is required for chromosome condensation in mitosis. This is in contrast to the phenotype of *barren* mutation in *Drosophila*, in which no condensation defect could be detected (Bhat *et al*., 1996). An additional difference is the centromere separation defect in our mutants. In *Drosophila* mutant, centromere separation occurs normally in mitosis, whereas chromosome arms remain interlocked and form chromatin bridges. These *Drosophila* phenotypes are observed in a null mutation of *barren*, so the differences cannot be explained by allele-specific defects.

According to the "superhelical tension" model of mitotic chromosome condensation (Hirano, 1999), XCAP-H functions in mitosis as a component of condensin complex. The activity of the complex is to introduce positive supercoils into DNA, leading to compaction of the chromatin fiber. A defect in this function should result in mitotic chromosomes that are less compact, but it does not predict a chromatid separation defect like the one observed in *brn1* mutants in yeast. This may indicate that chromatin compaction is mechanistically necessary for resolution of sister chromatids. If this is the case, further development of the model is required to account for interdependence of chromatid condensation

and separation. Alternatively, Brn1p may have a role in chromatid separation, which is separate from its function in condensation. It will be of interest to use the yeast homologues of other condensin subunits to dissect the molecular details of chromatin rearrangements during cell division.

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