The Rabl chromosome configuration masks a kinetochore reassembly mechanism in yeast mitosis

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ABSTRACT During cell cycle progression in metazoans, the kinetochore is assembled at mitotic onset and disassembled during mitotic exit. Once assembled, the kinetochore complex attached to centromeres interacts directly with the spindle microtubules, the vehicle of chromosome segregation. This reassembly program is assumed to be absent in budding and fission yeast, because most kinetochore proteins are stably maintained at the centromeres throughout the entire cell cycle. Here, we show that the reassembly program of the outer kinetochore at mitotic onset is unexpectedly conserved in the fission yeast Schizosaccharomyces pombe. We identified this behavior by removing the Rabl chromosome configuration, in which centromeres are permanently associated with the nuclear envelope beneath the spindle pole body during interphase. In addition to having evolutionary implications for kinetochore reassembly, our results aid the understanding of the molecular processes responsible for kinetochore disassembly and assembly during mitotic entry.

INTRODUCTION

The three-dimensional architecture of the yeast genome is characterized by the evolutionarily conserved Rabl chromosome configuration, which is defined by the stable association of centromeres and telomeres with the nuclear envelope (NE; Jin et al., 1998, 2000; Gerlich and Ellenberg, 2003; Berger et al., 2008; Duan et al., 2010; Taddei and Gasser, 2012; Mizuguchi et al., 2014). The NE comprises the inner nuclear membrane (INM) and the outer nuclear membrane (ONM). INM proteins play key roles in the interaction of the NE with chromatin (Czapiewski et al., 2016; Fernandez-Alvarez and Cooper, 2017a). In particular, in fission yeast, centromeres are clustered together in a kinetochore-dependent manner at the INM, beneath the spindle pole body (SPB, the centrosome equivalent in yeast) and opposite the nucleolus (Funabiki et al., 1993; Ding et al., 1997). The linkage between centromeres and the INM occurs via the SPB-associated linker of nucleoskeleton and cytoskeleton (LINC) complex, which comprises the KASH-domain ONM proteins (Kms1 and Kms2) and the SUN-domain INM protein (Sad1; Hagan and Yanagida, 1995; Shimanuki et al., 1997; Hiraoka and Dernburg, 2009; Unruh et al., 2018). Sad1 plays an essential role in supporting the associations between centromeres and the SPB. These associations are also strengthened by the protein Csi1, which bridges Sad1 and outer kinetochore proteins, and by the conserved LEM-domain INM protein Lem2, which localizes at the nuclear periphery and the SPB (Hou et al., 2012; Barrales et al., 2016; Fernandez-Alvarez and Cooper, 2017a). The Rabl configuration is thought to reflect the positioning of the chromosomes during their segregation from the preceding mitosis; in metazoans, the Rabl configuration is dismantled at the mitotic exit, but in yeast, it is maintained throughout the subsequent interphase (Cremer and Cremer, 2001;...
Taddei and Gasser, 2012; Mizuguchi et al., 2014, 2015). Why the Rabl configuration in yeast is not disassembled at mitotic exit but is maintained throughout interphase is not known. Recently, it has been observed in fission yeast that the interaction of at least one centromere with the SPB during interphase is required to trigger SPB insertion into the NE, a crucial event for nucleating the spindle microtubules. Hence, disassembly of the Rabl configuration abolishes SPB insertion and spindle formation, leading to cellular lethality (Fernandez-Alvarez et al., 2016).

Attachment of centromeres to the SPB, and thus the maintenance of the Rabl configuration, is supported by the kinetochore, which is built on the centromeres (Cheeseman, 2014). This large complex comprises around 80 proteins identified in humans, and its major components are conserved throughout eukaryotes. The kinetochore can be subdivided into two distinct regions: the inner kinetochore, which interacts with chromatin, and the outer kinetochore, which constitutes the platform for interacting with spindle microtubules. Therefore, the kinetochore establishes the chromosomal attachment place for spindle microtubules, the motors that drive chromosome distribution to daughter cells (Cheeseman and Desai, 2008).

Kinetochore composition is dynamically regulated during the cell cycle in metazoans (Hara and Fukagawa, 2018). Some kinetochore proteins are constitutively present at centromeres, establishing the centromere-associated network (CCAN), but most are recruited to the kinetochore during late G2, prophase, or mitosis. In this manner, proteins of the outer kinetochore, such as Mis12 and Ndc80, are recruited precisely to the centromeres in late interphase and prophase, respectively. Once the chromosomes have segregated, Ndc80 and Mis12 are depleted in an orderly way following the onset of anaphase and the end of mitosis, respectively (Cheeseman and Desai, 2008; Nagpal and Fukagawa, 2016; Dhattchamoorthy et al., 2018). This well-regulated recruitment of kinetochore components to the centromeres is assumed to be absent in Schizosaccharomyces pombe. In the fission yeast, most of the outer kinetochore components, such as Ndc80 and Nuf2, are constitutively present at centromeric regions throughout the cell cycle (Saitoh et al., 1997; Nabetani et al., 2001; Wigge and Kilmartin, 2001; Hayashi et al., 2004; Biggins, 2013), and only the components of the DASH complex, an essential element of the kinetochore that is required for the orientation of sister chromatids, are recruited during mitosis (Cheeseman et al., 2001; Janke et al., 2002; Liu et al., 2005).

Two hypotheses aim to explain the absence of the outer kinetochore disassembly/assembly program at mitotic onset in fission yeast. The first hypothesis posits that the disassembly/assembly program is coordinated with NE breakdown in metazoans (Guttinger et al., 2009; Smoyer and Jaspersen, 2014; Hattersley et al., 2016). Thus, the fact that the NE is not disassembled before mitosis in S. pombe suggests that a kinetochore disassembly/assembly program might not be an efficient mechanism of outer kinetochore formation, as it involves the active transit of proteins from the cytoplasm to the nucleus. The second hypothesis suggests that the preservation of the outer kinetochore structure during interphase might be justified by its crucial role in maintaining the Rabl configuration (Takahashi et al., 2000; Asakawa et al., 2005). Thus, in this case, the absence of an assembly program in fission yeast mitosis would be, in principle, independent of the absence of proper NE breakdown.

It is challenging to determine which hypothesis explains the absence of the outer kinetochore disassembly/assembly program in fission yeast. This challenge could be addressed by studying the behavior of the kinetochore in cells without the interphase Rabl configuration. However, it has been difficult to generate Rabl configuration–deficient cells without compromising either kinetochore structure or cell viability. For instance, mutations to Nuf2 or Ndc80 partially remove the Rabl configuration but also alter kinetochore structure (Nabetani et al., 2001; Asakawa et al., 2005; Hsu and Toda, 2011); in contrast, the presence of a thermosensitive allele of sad1 (sad1.2) at a restrictive growth temperature (36°C) abolishes all centromere–SPB associations but immediately leads to cell death (Fernandez-Alvarez et al., 2016). Hence, the identification of a new genetic background in which the intact kinetochore is completely disconnected from the SPB at 32°C (the standard growth temperature of fission yeast) without dramatically impairing cell viability would help to reveal the behavior of kinetochore proteins in the absence of the Rabl configuration during yeast interphase. With that goal in mind, we found that the combination of the sad1.2 allele and the deletion of csi1 at the semi-permissive temperature of 32°C generates severe centromere dissociation defects. However, most of the cells are still viable due to occasional centromere interactions with the SPB, which are sufficient to trigger spindle formation. Thus, sad1.2 csi1A represents a new scenario in which it is possible to characterize the behavior of kinetochores dissociated from the SPB independently of their essential function in maintaining the Rabl configuration.

Here, we show that key elements of the outer kinetochore structure are lost in interphase sad1.2 csi1A cells. More unexpectedly, we show that similar to the situation in metazoans, the outer kinetochore in yeast is reassembled in late G2. These results suggest that the outer kinetochore assembly program at mitotic onset is conserved in fission yeast but has not been observed thus far, because it is masked by the Rabl configuration. Our observations establish S. pombe as a model organism for studying the mechanisms behind kinetochore assembly, which is highly conserved in metazoans and has enormous relevance to faithful chromosome segregation during cell cycle progression.

RESULTS AND DISCUSSION

The Rabl configuration in fission yeast is not supported by the cytoskeleton

We explored different approaches to removing interphase centromere-SPB associations at 32°C without altering the stability of either the kinetochore or the SPB. In budding yeast, centromere–SPB associations require nuclear microtubules (Jin et al., 2000; Bystricky et al., 2004), but studies in fission yeast have shown by electron microscopy an apparent absence of microtubules in the nuclear microenvironment between centromeres and the SPB (Ding et al., 1997; Appelgren et al., 2003). To rule out a role for microtubules in supporting centromere–SPB associations, we evaluated the state of these associations on addition of the microtubule-depolymerizing drug carbendazim (also known as methyl-2-benzimidazole carbamate, or MBC). These experiments confirmed that, in contrast to the situation in Saccharomyces cerevisiae, the maintenance of the Rabl configuration in S. pombe is independent of nuclear microtubules (Supplemental Figure 1, A–D).

We also considered whether actin is involved in maintaining the Rabl configuration, as actin has a well-characterized role in promoting telomere positioning at the NE during budding yeast meiotic prophase (Treles-Sticken et al., 2005). We disrupted actin by adding latrunculin A (LatA), an actin polymerization inhibitor (Riedl et al., 2008; Huang et al., 2012). Although LatA treatment led to major structural defects in actin, centromere–SPB associations persisted (Supplemental Figure 1, B–E). Hence, we discount...
a major role for cytoskeleton motors in maintaining the Rabl configuration.

**Phosphomutants and phosphomimetics of the sad1.2 allele do not show defects in centromere dissociation from the spindle pole body**

Another approach to addressing the complete disruption of the Rabl configuration in *S. pombe* is to use the thermosensitive Sad1 allele sad1.2. The Sad1.2 protein harbors two single substitutions, Thr-3-Ser and Ser-52-Pro, the latter of which disrupts Ser-52, a validated phosphorylation site for the cyclin-dependent protein kinase Cdc2/CDK-1 (Carpy et al., 2014; Fernandez-Alvarez et al., 2016; Swaffer et al., 2016). All three centromeres dissociate from the SPB when sad1.2 cells are grown at 36°C (Supplemental Figure 1F). However, this scenario leads to cell lethality as a result of failures in SPB insertion into the NE and in spindle formation (Fernandez-Alvarez et al., 2016). In contrast, the growth of sad1.2 cells at a semipermissive temperature (32°C) produces only partial dissociation of the centromeres from the SPB and does not completely disrupt the Rabl configuration (Fernandez-Alvarez and Cooper, 2017b).

To improve the penetrance of centromere declustering in sad1.2 cells at 32°C, we tested whether any other phosphomutants or phosphomimetics at Thr-3 and Ser-52 increases the percentage of cells showing centromere declustering during interphase. However, this analysis showed that only the combination of Thr-3-Ser and Ser-52-Pro leads to centromere declustering from SPB; no other combinations produce centromere dissociation defects (Supplemental Figure 1G). In addition, analysis of cellular growth on MBC-containing media showed that hypersensitivity to MBC was higher for sad1.2 cells than for the other sad1 mutant allele combinations (Supplemental Figure 1H). Previous studies have shown that hypersensitivity to microtubule-depolymerizing drugs (MBC or TBZ) in mutants showing centromere–SPB dissociation might be linked to problems in chromosome recapture for the spindle during mitosis; the fact that centromeres are dissociated and located far from the SPB, the major microtubule nucleator center, would complicate their capture (Hou et al., 2012). These observations argue against the possibility that centromere–SPB associations are regulated only by phosphorylation of the Sad1 residues Thr-3 and Ser-52. Therefore, the association of centromeres with Sad1 must be controlled by other complementary mechanisms, which are probably altered by the Thr-3-Ser and Ser-52-Pro substitutions but not by cytoskeleton motors.

**Loss of Csi1 in sad1.2 cells leads to a higher rate of total centromere–spindle pole body dissociation**

The aforementioned observations indicate that the Rabl configuration in *S. pombe* is independent of microtubules and actin. Our analysis of the sad1.2 phosphomutant and phosphomimetic alleles also suggests that only the combination of Thr-3-Ser and Ser-52-Pro at sad1.2 leads to centromere dissociation from the SPB. Hence, to increase the penetrance of centromere–SPB dissociations in sad1.2 cells, we constructed strains combining mutations in sad1, csi1, and lem2, since the Sad1-1-interacting factor Csi1 and the LEM-domain INM protein Lem2 could be the only major regulators in maintaining the centromere–SPB associations (Hou et al., 2012; Barrales et al., 2016; Fernandez-Alvarez and Cooper, 2017b). We found a strong negative interaction in the triple mutant sad1.2 lem2Δ csi1Δ (Figure 1A); in 69.3% of asci dissected (*n* = 62), spores were unable to germinate or produced very small colonies. The remaining sad1.2 lem2Δ csi1Δ spores were able to generate colonies. This rate of suppression might be explained by the compensatory increase in Inf1 gene expression, which frequently suppresses the loss of lem2, as has been observed previously (Tange et al., 2016). Due to these severe viability defects, we ceased working with the triple mutant. Analysis of the behavior of all possible double mutant combinations showed that double loss of Csi1 and Lem2 also severely hindered cell viability (Figure 1B). These defects have been associated with defective pericentromeric heterochromatin identity, which impairs kinetochore proteins’ association with centromeres. This leads to chromosome loss and subsequent growing defects on MBC-containing media, as has been reported previously (Hou et al., 2012; Barrales et al., 2016; Figure 1C). The other double mutant showing cellular growth defects was sad1.2 csi1Δ, although these defects were weaker than those in lem2Δ csi1Δ; sad1.2 csi1Δ also showed increased sensitivity to MBC (Figure 1, B and C).

Due to the correlation between centromere dissociation from the SPB and MBC sensitivity, these experiments pointed to the double mutants sad1.2 csi1Δ and lem2Δ csi1Δ as possibly having greater centromere–SPB dissociation than the single mutants csi1Δ and sad1.2. Around 10–15% of lem2Δ csi1Δ cells show all centromeres transiently disconnected from the SPB (Barrales et al., 2016; Fernandez-Alvarez and Cooper, 2017b), but no information has been obtained yet about sad1.2 csi1Δ cells. For this reason, we investigated centromere–SPB associations in sad1.2 csi1Δ cells. For comparative purposes, we included all single and double mutant combinations and allocated centromere dissociation phenotypes into two categories: 1) partial centromere–SPB dissociation, where at least one centromere is detached from the SPB during the analysis (example in −30’ frame in Figure 1E); and 2) total centromere–SPB dissociation, where all three centromeres are dissociated from the SPB. In the latter category, we established two subtypes: transient, where at least one frame in interphase showed total centromere–SPB dissociation during our time-lapse analysis (example in −20’ frame in Figure 1E); or persistent, where centromeres did not interact at all with the SPB at any time during the analysis in interphase (Figure 1F). In the case of transient total centromere–SPB dissociation, cells are still able to divide because one centromere–SPB interaction is sufficient to trigger SPB insertion into the NE, which allows spindle formation (Fernandez-Alvarez et al., 2016; Figure 1E). In contrast, in persistent total centromere–SPB dissociation, SPB insertion and thus spindle formation are abolished (Fernandez-Alvarez et al., 2016; Figure 1F). Notably, we found the highest penetrance of centromere–SPB dissociation in sad1.2 csi1Δ cells: around 80% of sad1.2 csi1Δ cells showed centromere clustering defects (Figure 1G). Most importantly, ~25% of these mutant cells showed transient total centromere–SPB dissociation, which was never seen in the csi1Δ, lem2Δ or sad1.2 single mutants (Figure 1H). In contrast, ~9% of sad1.2 csi1Δ cells displayed persistent total centromere–SPB dissociation (Figure 1I). This population of cells might explain the slight reduction in cellular viability, because, as described above, persistent total centromere–SPB dissociation leads to cell death. Intriguingly, although the cell growth defects and MBC sensitivity of the lem2Δ csi1Δ strain are more severe than those of the sad1.2 csi1Δ genotype (Figure 1C), the penetrance of centromere–SPB dissociation is lower in lem2Δ csi1Δ than in sad1.2 csi1Δ (Figures 1, G–I). This suggests that some of the growth defects could be unrelated to the loss of centromere–SPB contacts. For instance, the role of Lem2 in the maintenance of centromeric heterochromatin and nuclear envelope integrity might be behind the strong growth defects in lem2Δ csi1Δ (Barrales et al., 2016; Tange et al., 2016; Kume et al., 2019). In conclusion, we identified sad1.2 csi1Δ cells as an optimal strain for exploring the behavior of the kinetochore in Rabl configuration-deficient cells, because they present higher penetrance of total
centromere–SPB dissociation and weaker defects in cell viability than lem2Δ csi1Δ cells.

**Inner kinetochore components are stably maintained at the centromeres during interphase in sad1.2 csi1Δ cells**

To address the behavior of kinetochore proteins during cell cycle progression in fission yeast with (wt) and without the Rabl configuration (sad1.2 csi1Δ), we followed the focal intensity of endogenously GFP-tagged outer and inner kinetochore proteins on exponentially growing cells by live fluorescence microscopy. We analyzed Mis6 and Cnp20 (CENP-I and CENP-T orthologues, respectively) as representative members of the inner kinetochore (Takahashi et al., 2000; Hou et al., 2012). Protein intensity levels of all images acquired from living cells were quantified (see Methods for details), and we delineated the focal intensity of both kinetochore proteins normalized per SPB signal (visualized via Sid4-mCherry). A comparison of the sum of Mis6-GFP signals in wt and sad1.2 csi1Δ cells confirmed the presence of a stable Mis6-GFP signal throughout interphase in both strains (Figure 2, A and B). By analyzing the sum of the centromeres dissociated from the SPB and the sum of the centromeres associated with the SPB independently, we found that both centromere locations show a stable presence of Mis6-GFP during mitotic interphase (Figure 2C).

Consistent with the previous results, our analysis of the inner kinetochore protein Cnp20 showed that it has behavior similar to that of Mis6-GFP: wt and sad1.2 csi1Δ cells showed a stable signal in interphase independent of whether or not centromeres are

![Image of graphs and diagrams]

**FIGURE 1:** Loss of Csi1 in sad1.2 cells leads to a higher rate of Rabl configuration-deficient cells. (A) Tetrad analysis of h- sad1.2 csi1Δ crossed with h+ lem2Δ csi1Δ shows a strong negative genetic interaction between sad1.2, lem2Δ, and csi1Δ when spores harbor the three mutations. Spores were grown at 32°C for 5 d. (B) Cell viability relative to wt cells was evaluated by colony formation assays. Cells were cultured in liquid medium to 10⁷ cells/mL, 300 cells spotted onto YE4S plates, and incubated at 32°C for 5 d (n > 500 colonies per genotype were scored in four independent experiments). Data were subjected to Fisher’s exact test; ****, p-value < 0.0001. (C) Serial dilutions (fivefold) of log-phase cultures were spotted and grown on rich media with DMSO (control) and rich media containing MBC. Plates were incubated at 32°C for 48 h. (D–F) Frames from films of proliferating cells carrying Sid4-mCherry (endogenously tagged; SPB), Mis6-GFP (endogenously tagged; centromeres), and ectopically expressed mCherry-Atb2 (tubulin). Scale bar represents 5 µm. (G–I) Centromere–SPB association patterns. >30 cells were scored for each genotype in at least three independent experiments. p-values were determined by Fisher’s exact test; **, 0.001 < p < 0.01; *, 0.01 < p < 0.05.
FIGURE 2: Mis6 and Cnp20 are stably associated with the centromeres in Rabl configuration-deficient cells.
(A–F) Frames from films of mitotic cells carrying Sid4-mCherry (SPB), ectopically expressed mCherry-Atb2 (tubulin), and endogenously tagged Mis6-GFP, A–C, or Cnp20-GFP, D–F. Bars, 5 µm. Mean of total Mis6-GFP, A, B, and Cnp20-GFP, D, E intensities through interphase and mitosis were quantified. Ten cells during more than three independent experiments were monitored for focal intensity of Mis6-GFP and Cnp20-GFP. Error bars represent standard deviations; t = 0 min is just before SPB separation. (C, F) Quantification of Mis6-GFP and Cnp20-GFP focus intensity separately in centromeres with and without SPB association in sad1.2 csi1Δ cells. (A, D) In wt cells, all Mis6-GFP and Cnp20-GFP signals localize to the SPB during interphase. (B, C, E, F) sad1.2 csi1Δ cells showing centromere dissociation from the SPB also show stable signals through interphase.
associated with the SPB (Figure 2, D–F). Together, these observations indicate that the inner kinetochore, at least Mis6 and Cnp20, is stably associated with the centromeres in Rabl configuration-deficient cells.

We also explored the behavior of Mis12, a component of the NMS (Ndc80-MIND-Spc7) complex (Obuse et al., 2004; see Supplemental Figure 2A for a schematic representation of the main elements of the fission yeast outer kinetochore). Mis12 is also assembled and disassembled at mitotic onset and exit, respectively, during the meiocyte cell cycle, but is constantly attached to centromeres in yeast (Biggins, 2013). Analysis of Mis12-GFP in wt and Rabl configuration-deficient cells (sad1.2 cs1Δ) showed a stable signal across interphase (Supplemental Figure 2, B and C), similar to the behavior of the inner kinetochore proteins Mis6 and Cnp20. However, the endogenous GFP-tagging of Mis12 greatly reduced the penetrance of centromere–SPB dissociation defects in sad1.2 cs1Δ cells (Supplemental Figure 2D). Hence, the tagging of Mis12 is able to stabilize the interaction between the kinetochore and Sad1, reducing the impact of sad1.2 and cs1 mutations on centromere dissociation from the SPB.

Ndc80 and Nuf2 are dissociated from centromeres during interphase in the Rabl configuration-deficient cells sad1.2 cs1Δ

We studied two representative members of the outer kinetochore, Ndc80 and Nuf2 (part of the Ndc80 complex; see Supplemental Figure 2A), which are absent in interphase in metazoans but localize to centromeres throughout the cell cycle in budding and fission yeast (Liu et al., 2005; Biggins, 2013). Contrary to our observations for the inner kinetochore, we could not detect a clear signal for Ndc80-GFP at the centromeres in the vast majority of sad1.2 cs1Δ interphase cells (Figure 3, A–B; Supplemental Figure 3A). This loss of signal at the centromeres during interphase was also observed for Nuf2, which indicates that the absence of a normal signal is a general defect in the Ndc80 complex (Figure 3, D–E). To confirm this observation, we analyzed the inner and outer kinetochore together using Mis6-mCherry and Ndc80-GFP, respectively. In agreement with our previous observations, we found that the deletion of Csl1 in sad1.2 cells leads to the loss of the Ndc80-GFP signal but not of Mis6-mCherry in interphase, and in cases where sad1.2 cs1Δ cells displayed the Ndc80-GFP signal (about 20% of cells; Supplemental Figure 3A), most of these centromeres (>90%) colocalized with the SPB (Supplemental Figure 3B). This suggests that the loss of Csl1 or/and Sad1.2 might destabilize the outer kinetochore, Ndc80 and Nuf2, and thus debilitate the centromere–SPB association, which has been observed using thermosensitive alleles of Ndc80 and Nuf2 (Asakawa et al., 2005). Western blot analysis of cultures enriched in G1, using the thermosensitive allele cdc10-129 (Tormos-Perez et al., 2016), indicated that Ndc80 protein levels are reduced in sad1.2 cs1Δ cells from those in wt settings (Figure 4A). Furthermore, ChiP-qPCR analysis showed lower enrichment of Ndc80 at sad1.2 cs1Δ centromeres (1 and 3) than in wt (Figure 4B).

To test the hypothesis that the absence of the normal Ndc80 signal is the cause, and not the consequence, of the centromere–SPB dissociation in sad1.2 cs1Δ cells, we ectopically targeted Ndc80-GFP to Sad1.2 using the GFP binding protein GBP to recruit GFP-tagged proteins (Rothbauer et al., 2006; Fernandez-Alvarez et al., 2016; Supplemental Figure 3C). Using this system, we confirmed that the Ndc80-GFP signal is constant throughout interphase in all sad1.2 cs1Δ cells analyzed (Figure 3C). Moreover, we found that these cells did not show centromere–SPB dissociation and consequently restored the sad1.2 cs1Δ growth defects on TBZ-containing media (Supplemental Figure 3D). Together, these results indicate that the phenotype of centromere–SPB dissociation in sad1.2, cs1Δ, and sad1.2 cs1Δ cells might be explained by the loss of Ndc80 at centromeres, which would weaken the interaction of the kinetochore with the SPB. Outer kinetochore instability and, consequently, centromere–SPB dissociation could also be caused by loss of proximity of the centromeres to the NE region below the SPB, which could provide the necessary nuclear microenvironment to maintain kinetochore and centromere identity, as has recently been suggested by the Allshire lab (Wu et al., 2021).

Although the outer kinetochore is crucial for maintaining the interaction of the centromeres with the SPB in fission yeast (Nabetani et al., 2001; Asakawa et al., 2005), interactions in the absence of the outer kinetochore have been characterized. For instance, during meiotic prophase, the outer kinetochore is disassembled and reassembled to prepare the kinetochore for the segregation of homologous chromosomes in meiosis I (Hayashi et al., 2006). At the beginning of meiotic prophase, the centromeres are dissociated from the SPB while the telomeres are moved by cytoskeleton motors to the SPB to form the telomere bouquet (Yoshida et al., 2013), the phase-specific chromosomal configuration where the telomeres cluster together at the SPB (Chikashige et al., 1994; Chikashige et al., 2006). In the case of telomere bouquet mutants, such as bqt1Δ, the absence of a telomere–SPB interaction allows sporadic and short-lived centromere–SPB interactions (lasting around 30 min) during meiotic prophase, even in the absence of Ndc80 and Nuf2 at the centromeres (Fennell et al., 2015). Transient total centromere–SPB dissociation, one of the most common centromere dissociation phenotypes in sad1.2 cs1Δ cells, might reflect a situation similar to meiotic prophase in bouquet-deficient meiocytes, in which centromeres are able to interact with the SPB for short periods of times when little of Ndc80 is present. Current studies aim to decipher the molecular basis of centromere anchoring to the SPB.

The outer kinetochore is reassembled at mitotic onset

Although we did not observe normal Ndc80 and Nuf2 signals in interphase sad1.2 cs1Δ cells, these proteins are clearly visible at the centromeres in mitotic cells. In fact, western blot analysis confirmed that Ndc80 protein levels were similar in G2/M phase–cultured cells with and without the Rabl configuration (Figure 4A). This observation indicates that the outer kinetochore can be rebuilt at mitotic onset to prepare cells for chromosome segregation. To establish the dynamics of outer kinetochore reassembly, we quantified the Ndc80-GFP and Nuf2-GFP signals using the same methodology used for Mis6, Cnp20, and Mis12. We found that Ndc80-GFP and Nuf2-GFP signals accumulate during late interphase, around 40–20 min before separation of the duplicated SPBs (Figure 3, B and E). This accumulation of Ndc80 at the centromeres is never seen in a wt setting, and it precedes the increment of the protein levels observed during anaphase (Dhatchinamoorthy et al., 2017). Hence, the active accumulation of the outer kinetochore, or at least its core proteins Ndc80 and Nuf2, at mitotic onset in fission yeast is similar in timing to that seen in metazoans.

Next, to understand the behavior of other kinetochore proteins, we analyzed Spc7 (KNL1 ortholog in humans), a member of the NMS complex (Supplemental Figure 2A). Analysis of endogenously GFP-tagged Spc7 showed that while the signal is stably maintained throughout interphase in wt settings, it is dramatically reduced at the centromeres in interphase and recovered at mitotic onset in sad1.2 cs1Δ cells, similar to the behavior observed for Ndc80 and Nuf2 (Figure 4, C and D). In other words, the stability of Spc7 at the centromeres is compromised in sad1.2 cs1Δ cells, and cells with
FIGURE 3: Ndc80 and Nuf2 are dissociated from centromeres during interphase and reassembled at mitotic onset in sad1.2 csi1Δ cells. (A–E) Frames from films of mitotic cells carrying SPB and tubulin markers as in Figure 2, and endogenously tagged Ndc80-GFP, A–C, or Nuf2-GFP, D, E. Bars, 5 µm. Means of total Ndc80-GFP and Nuf2-GFP intensities were quantified as in Figure 2. Ten cells during more than three independent experiments were monitored. Error bars represent standard deviations; $t = 0$ min is just before SPB separation. (C) The GBP-GFP system was used to force centromere–SPB interactions (see Supplemental Figure 3C). Association with centromeres and levels of Ndc80 protein in interphase are recovered in sad1.2-GBP csi1Δ settings compared with wt settings.
FIGURE 4: Rabl configuration-deficient cells efficiently complete outer kinetochore assembly. (A) Western blot analysis of protein samples from cells synchronized in G2/M (left) or G1 (right). Labels to the left indicate the antibodies used to probe the blots. The data shown are from a single representative experiment out of two repeats and quantifications from below are the averages of the HA signal relative to the tubulin control from two independent experiments. (B) ChIP analysis of Ndc80 levels at centromeres 1 and 3 in cells synchronized in G1. Error bars represent standard deviations of three biological replicates. p-values were determined by t test, **** p < 0.0001, ** p < 0.005. (C–F) Frames from films of mitotic cells carrying SPB and tubulin markers as in Figure 2, and endogenously tagged Spc7-GFP, C, D, or Dad2-GFP, E, F. Bars, 5 µm. Means of total Spc7-GFP and Dad2-GFP intensities were quantified as in Figure 2. Ten cells during more than three independent experiments were quantified. Error bars represent standard deviations; t = 0 min is just before the separation of the SPBs.
single mutations to Sad1 and Csi1 did not show any clear loss of Spc7 signal (100% of 50 cells analyzed). However, unlike Ndc80 and Nuf2, which are recruited around 40–20 min before mitosis, Spc7 is recruited around 10–5 min before mitosis (Figure 4D). Hence, our data indicate that Ndc80 and Nuf2 are recruited to the centromere before Spc7, which suggests that centromere dissociation from the SPB in sad1.2 csi1A cells is due to the loss of Ndc80 and/or Nuf2 rather than the loss of Spc7.

To confirm the ability of Rabl configuration–deficient cells (sad1.2 csi1A) to complete outer kinetochore assembly efficiently, we studied the behavior of the recruitment of Dad2, a member of the DASH complex in S. pombe. Wt and sad1.2 csi1A cells showed similar recruitment profiles at the beginning of mitosis (Figure 4, E and F), confirming that one of the final stages of the outer kinetochore assembly is performed correctly in sad1.2 csi1A cells, which ensures chromosome segregation. Our results indicate the existence of a program in fission yeast to actively recruit the outer kinetochore that is triggered at the beginning of mitosis in a manner reminiscent of the assembly program in metazoans.

The high conservation of the outer kinetochore disassembly/assembly program during cell cycle progression appears to have a remarkable exception in yeast, where this program is assumed to be absent. Here, we present evidence that the program might actually be present in fission yeast mitosis but masked by the Rabl configuration. A plausible explanation is that the maintenance of the Rabl configuration during interphase appears in evolution later than the outer kinetochore disassembly/assembly program. According to this hypothesis, the function of the Rabl configuration in controlling SPB insertion into the NE, a yeast-specific mechanism, favors Ndc80 and Nuf2 remaining stable at the centromeres to preserve centromere–SPB interactions. Using the double mutant sad1.2 csi1A, we found that Ndc80 and Nuf2 are lost from the centromere during interphase, which likely destroys the Rabl configuration. Unexpectedly, we found that Ndc80 and Nuf2 are recruited to the centromeres with a timing reminiscent of that seen in metazoans. We also found that Spc7 is recruited to the centromeres before mitosis but slightly later than Ndc80 and Nuf2 recruitment. Hence, we establish four groups of kinetochore proteins based on their pattern of location across interphase in Rabl configuration-deficient cells (sad1.2 csi1A): 1) persistent signal throughout interphase: Mis6 and Cnp20; 2) early recruitment in the transition from G2 to M: Ndc80 and Nuf2; 3) late recruitment in the transition from G2 to M: Spc7; 4) assembly at mitosis onset: Dad2. In contrast, we identified only two groups in wt settings: 1) persistent signal throughout interphase: Mis6, Cnp20, Ndc80, Nuf2, and Spc7; 2) assembly at mitosis onset: Dad2 (Figure 5).
controlling the disassembly and reassembly of the outer kinetochore might also be conserved in fission yeast. Discovering the existence of this mechanism in S. pombe opens up the possibility of future studies using this yeast model to explore the metazoan outer kinetochore reconstruction program in mitosis.

METHODS

Request a protocol through Bio-protocol.

Strains and growth conditions

Strains used are listed in Supplementary Table 1. Growth conditions and molecular biology approaches were used as described previously (Moreno et al., 1991). Gene deletion and C-terminal tagging were performed as described by Bahler et al. (1998) and Fennell et al. (2015). Insertions of mCherry-Atb2 at the aur1 locus (Hashida-Okado et al., 1998) utilized pYC19-mCherryAtb2 (Nakamura et al., 2011) provided by T. Toda (Hiroshima University). Haploid cells were usually grown at 32°C in YE4S or EMM2 medium. Final concentrations of aureobasidin A (0.5 µg/ml), nourseothricin (100 µg/ml cloning-NAT), G418 (150 µg/ml geneticin), and hygromycin B (300 µg/ml) were added for selection purpose.

MBC and TBZ sensitivity test

Strains were grown to exponential phase (1 × 10^7–1.4 × 10^7 cells/mL) at 32°C and normalized to 10^7 cells/mL, and fivefold serial dilutions were spotted onto YE4S plates containing different concentrations of MBC (carbendazim, Sigma-Aldrich) or TBZ (tiabendazole, Sigma-Aldrich). The plates were incubated at 32°C for 48–72 h.

Colony formation assays

Strains were grown to exponential phase (1 × 10^7–1.4 × 10^7 cells/mL) at 32°C and normalized to 10^7 cells/mL, and cell viability was determined by plating 300 cells in triplicate onto YE4S plates and counting colony-forming units after five days incubation at 32°C. Percentage of viable cells from each genotype is normalized respect to the colony number of wt cells.

Fluorescence microscopy, live analysis, and quantification

Fluorescence microscopy images were generated using the DeltaVision microscope system (Applied Precision, Seattle, WA). Cells were adhered to 35 mm glass culture dishes (MatTek) using 0.2 mg/ml soybean lectin (Sigma-Aldrich) and immersed in EMM (with required supplements). Time-lapse imaging was carried out at 32°C in an Environmental Chamber with a DeltaVision Spectris (Applied Precision) comprising an Olympus IX70 widefield inverted epifluorescence microscope, an Olympus UPlanSapo 100× NA 1.4 oil immersion objective, and a Photometrics CCD CoolSnap HQ camera. Images were acquired over 15 focal planes at a 0.4-µm step size. For the quantification of protein fluorescence intensity, sum-projected raw microscopy data were used. Foci intensity time series were obtained after detection with a Laplacian of Gaussian filter and tracking with the LAP algorithm (TrackMate plugin in ImageJ). Tracks were time aligned according to the SPB duplication events; time zero was set as the last frame before the SPB duplication. Intensities were normalized to background mean intensity. Background was measured by taking the mean of four regions of interest (circle area of 18 pixels each), in this case fluorescence intensity of the cell, excluding the SPBs.

Spot selection of the SPB-associated/dissociated centromeres was performed in a semiautomated manner with a custom-written ImageJ macro and the Trackmate plugin in ImageJ. The correspondence of the SPB-associated centromere is established by colocalization with the SPB signal. Satellite foci are all those outside the SPBs colocalization area. Under our image conditions, SPBs have a size of 3 × 3 pixels, so we designate the signal as the mean of the nine pixels with the most intensity inside the designated area, and nine more pixels for each satellite focus. The mean intensity is set as the signal and is normalized with respect to background mean intensity. Image processing for representation was performed by deconvolving and combining each color channel into a 2D image using the maximum-intensity projection setting in softWoRx (Applied Precision) from raw microscopy data. Combined maximum Z-projection images were treated using ImageJ and Adobe Photoshop CS5 Extended.

Carbendazim and latrunculin treatments

For carbendazim treatment, a working solution of YE4S +MBC (15 µg/ml) was used. Strains were grown to OD (600 nm) = 0.3–0.4 in YE4S. Lectin (50 µl; 0.2 µg/ml; Sigma-Aldrich, L1395) was used for cell immobilization on a µ-Slide 8-well uncoated (Ibidi GmbH). YE4S+MBC (experiment) or YE4S (control) medium was used for filming cells. On the other hand, for latrunculin A treatment, exponentially growing cells were incubated for 10 min in 3 ml of YE4S rich medium with a total concentration of latrunculin A of 5 µM (15 µl of a 1 mM stock). After incubation, cells were immobilized with lectin as in MBC treatment for image acquisition. Images were taken with 100 ms and 50 ms exposure time for fluorescent and brightfield channels, respectively, and 13 focal planes with a 0.5-µm step size, using a spinning disk confocal microscopy system (Photometrics Evolve camera; Olympus 100 × 1.4 NA oil immersion objective; Roper Scientific). For the colocalization analysis, maximum Z-projection images of interphase cells, those with one single Sid4-mCherry dot (SPB), were subjected to colocalization analysis. For each cell, an axis containing the centers of both Sid4-mCherry and Mis6-GFP (centromeres) dots was drawn, and the intensity of the pixels from both channels was measured, normalized, and plotted along this axis. The final intensity profiles were used to measure the distance between the dots, defined as the micrometers between the x-coordinates of the maximum intensity of both profiles, considered to correspond with the center of the dots.

Cell cycle synchronizations in G1 and G2/M

Cell enrichment in G1 or G2/M phases, using strains harboring the cdc10-129 or cdc25-22 thermosensitive allele, respectively, was performed following a protocol from the Moreno lab (Tormos-Perez et al., 2016). Cells were grown in rich medium at 25°C to 10^7 cells/ml and transferred at 36°C for 4 h to arrest. G1 or G2/M enrichment was checked by quantifying the percentage of binucleated cells and septum formation.

Western blot analysis

Samples of cdc10-129 or cdc25-22 cells were obtained from 10 ml of exponentially growing cultures (10^7 cells/ml) at 25°C (asynchronous) or grown at 36°C for 4 h (enriched in G1 or G2/M phases, respectively). Cells were collected by centrifugation at 4°C, and cell pellets were flash-frozen in liquid nitrogen and stored at −80°C until use.

Protein extracts were prepared from trichloroacetic acid-treated cells as described (Grallert and Hagan, 2017). Briefly, cell pellets corresponding to 5 × 10^7 cells per condition and strain were removed from storage at −80°C and mixed with ice-cold 20% TCA and cold acid-washed glass beads (Sigma-Aldrich). Cell integrity was disrupted by FastPrep-24 (MP Biomedicals) for four cycles of 20 s at 4 m/s. Then ice-cold 5% TCA was added, and cell lysate was recovered. Next, samples were centrifuged, and supernatant was
discarded. The protein pellet was washed with ice-cold 100% ace-
tone and centrifuged. All previous manipulations were performed at
4°C. Supernatant was discarded and the protein pellet was resus-
pended at room temperature in SDS loading buffer and 1 M Tris-HCl
pH 8 to raise the pH of the sample to neutral. Samples were dena-
tured by heating, centrifuged, and loaded in a 10% SDS–PAGE gel
(Biorad) to separate proteins. HA-tagged proteins were detected
with an anti-HA antibody (Biolegend) and tubulin, with an anti-tubu-
lin antibody (Sigma-Aldrich). The secondary antibody was horserad-
ish peroxidase (HRP)-coupled anti-mouse IgG (Sigma-Aldrich). Visu-
alization was performed using the SuperSignal WestFemto
Maximum Sensitivity Substrate (ThermoFisher) in a Chemidoc MP
imaging system (Biorad). Image processing for representation was
performed using Adobe Photoshop CS5 Extended and signal quan-
tifications were performed in ImageJ using the gel analysis tool.

ChIP-qPCR experiments
Based on protocols from Cam and Whitehall (2016) and Migeot and
Hermard (2018), we performed ChIP-qPCR experiments as follows:
samples of 2 × 10^9 cdc10-129 synchronized cells at 36°C for 4 h in
YE4S were cross-linked with 1% formaldehyde (Sigma-Aldrich).
Cross-linking was quenched by adding 150 mM glycin. All the next
manipulations were performed at 4°C. Cell pellets were obtained by
centrifugation, washed two times with prechilled PBS 1X, flash-frozen
in liquid nitrogen, and stored at −80°C until use. The frozen cell pel-
lets were thawed in ice, resuspended in ice-cold ChIP buffer I (50 mM
HEPES-KOH, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA
pH 8, 0.1% Na deoxycholate, 0.1% SDS, 1 mM PMSF, proteinase
inhibitor cocktail [Roche Complete, EDTA-free]) and mixed with cold
acid-washed glass beads (Sigma-Aldrich) and cell integrity was dis-
rupted by FastPrep-24 (MP Biomedicals) for six cycles of 20 s at
6 m/s. After cell lysis was checked under the microscope, cell lysate
was recovered. Next, samples were centrifuged and the cross-linked
chromatin appeared as a transparent layer around the pellet of cell
debris. This pellet was resuspended with a Pasteur pipet in ice-cold
ChIP buffer I and incubated on a rocking platform for 1 h. Next,
samples were centrifuged and the pellet was resuspended in ice-
cold ChIP buffer I before sonication in milliTUBE 1 ml AFA Fiber
(Covaris) using a M220 Focused ultrasonicator (Covaris; 20% duty,
PIP75w, 200 cycles/burst for 30 min) to obtain an average DNA frag-
ment size of ~200–400 bp. The sonicated samples were incubated in
a rotating wheel for 30 min and centrifuged to clarify chromatin su-
pernatant. This solution was precleared with pan mouse IgG protein
G magnetic dynabeads (ThermoFisher) for 3 h in a rocking platform.
Then the chromatin solution was incubated overnight with anti-HA
antibody (Biolegend) in a rocking platform. The next day, the solu-
tion was centrifuged and the supernatant was incubated with protein
G magnetic beads for 3 h in a rocking platform. Beads were se-
parated from chromatin solution and washed three times with ice-cold
ChIP buffer I, two times with ice-cold ChIP buffer II (50 mM HEPES-
KOH, pH 7.5, 500 mM NaCl, 1% Triton X-100, 1 mM EDTA pH 8,
0.1% Na deoxycholate, 0.1% SDS), and two times with ice-cold ChIP
buffer III (10 mM Tris HCl, pH 8, 250 mM LiCl, 0.5% NP40, 0.5% Na
deoxycholate, 1 mM EDTA pH 8). Then beads were washed with TE
buffer and DNA was eluted with elution buffer (50 mM Tris HCl, pH 8,
10 mM EDTA pH 8, 1% SDS) at 65°C for 20 min. Both input and
ChIP samples were treated with proteinase K to de–crosslink proteins
dNA. The next day, samples were treated with RNase A and DNA
was purified using a QIAquick PCR purification kit (Qiaegen).

Quantitative PCRs were performed as Migeot and Hermard
(2018) described and the percentage of input from ctn1/3 frag-
ments was calculated for each sample relative to act1 as housekeep-
ing control, using the ΔΔCt method. Oligonucleotides sequences
used in this study are shown in Supplemental Table 2.

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REFERENCES
Appelgren H, Niola B, Ekwall K (2003). Distinct centromere domain struc-
tures with separate functions demonstrated in live fission yeast cells. J
Cell Sci 116, 4035–4042.
Asakawa H, Hayashi A, Haraguichi T, Hiiraka Y (2005). Dissociation of
the NuDNcnd complex releases centromeres from the spindel-pole body
during meiotic prophase in fission yeast. Mol Biol Cell 16, 2325–2338.
Bajler J, Wu JQ, Longtie MS, Shah NG, McKenzie A 3rd, Steever AB,
Wach A, Philippson F, Pringle JR (1998). Heterologous modules for ef-
icient and versatile PCR-based gene targeting in Schizosaccharomyces
pombe. Yeast 14, 943–951.
Barrales RR, Forn M, Gorgescu PR, Sarkadi Z, Braun S (2016). Control of
heterochromatin location and silencing by the nuclear membrane
protein Lem2. Genes Dev 30, 133–148.
Berger AB, Cabal GG, Fabre E, Duong T, Buc H, Nehrbass U, Olivo-Marin
JC, Gadal O, Zimmer C (2008). High-resolution statistical mapping
reveals gene territories in live yeast. Nat Methods 5, 1031–1037.
Biggins S (2013). The composition, functions, and regulation of the budding
yeast kinetochore. Genetics 194, 817–846.
Bystricky K, Heun P, Gehlen L, Langowski J, Gasser SM (2004). Long-range
compaction and flexibility of interphase chromatin in budding yeast
defined by high-resolution imaging techniques. Proc Natl Acad Sci
USA 101, 1495–1500.
Cam HP, Whitehall S (2016). Chromatin Immunoprecipitation (ChIP) in
Schizosaccharomyces pombe. Cold Spring Harb Protoc, https://doi.org/
10.1101/pdb.prot091546.
Carpy A, Krug K, Graf S, Koch A, Popic S, Hauf S, Macek B (2014). Absolute
proteome and phosphoproteome dynamics during the cell cycle of
Schizosaccharomyces pombe (Fission Yeast). Mol Cell Proteomics. 13,
1925–1936.
Cheeseman IM (2014). The kinetochore. Cold Spring Harb Perspect Biol
6, a015826.
Cheeseman IM, Brew C, Wolyniak M, Desai A, Anderson S, Mustor N, Yates
JR, Hufkacker TC, Drubin DG, Barnes G (2001). Implication of a novel
multiprotein Dam1p complex in outer kinetochore function. J Cell Biol
155, 1137–1145.
Cheeseman IM, Desai A (2008). Molecular architecture of the kinetochore–
microtubule interface. Nat Rev Mol Cell Biol 9, 33–46.
Chikashige Y, Ding DQ, Funakibi H, Haraguichi T, Mashiko S, Yanagida M,
Hiiraka Y (1994). Telomere-led premeiotic chromosome movement in
fission yeast. Science 264, 270–273.
Chikashige Y, Tsutsui C, Yamane M, Okamasa K, Haraguchi T, Hiiraka Y
(2006). Meiotic proteins Bqt1 and Bqt2 tether telomeres to form the
bouquet arrangement of chromosomes. Cell 125, 59–69.
Cremer T, Cremer C (2001). Chromosome territories, nuclear architecture
and gene regulation in mammalian cells. Nat Rev Genet 2, 292–301.
Czapirowski R, Robson MJ, Schirmer EC (2016). Anchoring a levita-
thon: how the nuclear membrane tethers the genome. Front Cell Sci 7, 82.
Dhatchinamoorthi K, Mattingly M, Gerton JL (2018). Regulation of kineto- 
chore configuration during mitosis. Curr Genet 64, 1197–1203.

Dhatchinamoorthi K, Shiravaro M, Lange JJ, Rubinstein B, Unruh JR, 
Slaughter BD, Gerton JL (2017). Structural plasticity of the living kineto- 
chore. J Cell Biol 216, 3551–3570.

Ding R, West RR, Morphew M, Oakley BR, McIntosh JR (1997). The spindle 
pole body of Schizosaccharomyces pombe enters and leaves the 
nuclear envelope as the cell cycle proceeds. Mol Biol Cell 8, 1461–1479.

Duan Z, Andronescu M, Schutz K, Mcllwain S, Kim YJ, Lee C, Shendure J, 
Fields S, Blau CA, Noble WS (2010). A three-dimensional model of the 
yeast genome. Nature 463, 365–3637.

Fennell A, Fernández-alvarez A, Tomita K, Cooper JP (2015). Telomeres and 
centromeres have interchangeable roles in promoting meiotic spindle 
formation. J Cell Biol 208, 415–428.

Fernandez-Alvarez A, Bez C, O’Too ET, Moorjani M, Cooper JP (2016). 
Mitotic nuclear envelope breakdown and spindle nucleation are con- 
trolled by interphase contacts between centromeres and the nuclear 
envelope. Dev Cell 39, 544–559.

Fernandez-Alvarez A, Cooper JP (2017a). Chromosomes orchestrate their 
own organization: nuclear envelope disassembly. Trends Cell Biol 27, 
255–265.

Fernandez-Alvarez A, Cooper JP (2017b). The functionally elusive RabI chro- 
mosome configuration directly regulates nuclear membrane remodeling at 
mitotic onset. Cell Cycle 16, 1392–1396.

Funabiki H, Hagan I, Uzawa S, Yanagida M (1993). Cell cycle-dependent 
spatial positioning and clustering of centromeres and telomeres in 
fission yeast. J Cell Biol 121, 961–976.

Gerlich D, Ellenberg J (2003). Dynamics of chromosome positioning during 
the cell cycle. Curr Opin Cell Biol 15, 664–671.

Grallert A, Hagan IM (2017). Preparation of protein extracts from Schizosac- 
charomyces pombe using trichloroacetic acid precipitation. Cold Spring 
Harb Protoc (2017).

Guttinger S, Laurell E, Kutay U (2009). Orchestrating nuclear envelope 
disassembly and reassembly during mitosis. Nat Rev Mol Cell Biol 10, 
178–191.

Hagan I, Yanagida M (1995). The product of the spindle formation gene 
sad1+ associates with the fission yeast spindle pole body and is essen- 
tial for viability. J Cell Biol 129, 1033–1047.

Har A, Fukagawa T (2018). Kinetochoore assembly and disassembly during 
mitotic entry and exit. Curr Opin Cell Biol 52, 73–81.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Kato I (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.

Hashida-Okado T, Yasumoto R, Endo M, Takesako K, Ito K (1998). Isolation 
and characterization of the aureobasidin A-resistant gene, aur1R, on 
the chromosome during meiosis in fission yeast. Curr Genet 33, 38–45.