

The half-bridge component Kar1 promotes centrosome separation and duplication during budding yeast meiosis

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ABSTRACT The budding yeast centrosome, often called the spindle pole body (SPB), nucleates microtubules for chromosome segregation during cell division. An appendage, called the half bridge, attaches to one side of the SPB and regulates SPB duplication and separation. Like DNA, the SPB is duplicated only once per cell cycle. During meiosis, however, after one round of DNA replication, two rounds of SPB duplication and separation are coupled with homologue segregation in meiosis I and sister-chromatid segregation in meiosis II. How SPB duplication and separation are regulated during meiosis remains to be elucidated, and whether regulation in meiosis differs from that in mitosis is unclear. Here we show that overproduction of the half-bridge component Kar1 leads to premature SPB separation during meiosis. Furthermore, excessive Kar1 induces SPB overduplication to form supernumerary SPBs, leading to chromosome missegregation and erroneous ascospore formation. Kar1-mediated SPB duplication bypasses the requirement of dephosphorylation of Sfi1, another half-bridge component previously identified as a licensing factor. Our results therefore reveal an unexpected role of Kar1 in licensing meiotic SPB duplication and suggest a unique mechanism of SPB regulation during budding yeast meiosis.

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INTRODUCTION

The spindle pole body (SPB) of budding yeast is a microtubule-organizing center, equivalent to the centrosome in animal cells (Adams and Kilmartin, 2000; Jaspersen and Winey, 2004; Cavanaugh and Jaspersen, 2017). Embedded in the nuclear envelope, the SPB is formed by a three-layered structure, with the central plaque directly interacting with the nuclear membranes, whereas the outer and inner plaques nucleate cytoplasmic and nuclear microtubules, respectively (Moens and Rapport, 1971; Byers

and Goetsch, 1974; Winey and Byers, 1993). During meiosis, the outer plaque is modified to nucleate prospore membranes instead of microtubules for ascospore development (Moens and Rapport, 1971; Neiman, 2011). In addition to the three plaques, the SPB has a membrane-associated appendage, called the half bridge, which is asymmetrically attached to one side of the SPB (Byers and Goetsch, 1975). The main function of the half bridge is to provide a platform for SPB duplication and to keep duplicated SPBs engaged before their separation (Jaspersen and Winey, 2004; Seybold *et al.*, 2015; Cavanaugh and Jaspersen, 2017). How exactly SPB duplication and separation are regulated at the half bridge remains to be further elucidated.

The half bridge is made up of four proteins, Cdc31, Kar1, Sfi1, and Mps3 (Spang *et al.*, 1993, 1995; Jaspersen *et al.*, 2002; Kilmartin, 2003). The first three localize to the cytoplasmic side of the nuclear envelope, whereas Mps3 localizes to the nuclear side. Acting as the major structural component of the half bridge, Sfi1 is a filamentous protein that possesses ~20 Cdc31-binding sites at its central alpha-helical domain (Kilmartin, 2003; Li *et al.*, 2006), whereas Kar1 and

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Abbreviations used: EM, electron microscopy; GFP, green fluorescent protein; SPB, spindle pole body.

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Mps3 are integral membrane proteins (Vallen *et al.*, 1992; Jaspersen *et al.*, 2002) and thought to anchor the half bridge to the nuclear envelope (Seybold *et al.*, 2015). Structural analysis has shown that the N-terminus of Sfi1 is attached to the central plaque of the mother SPB (Li *et al.*, 2006; Burns *et al.*, 2015). At the time of SPB duplication, the half bridge is elongated to become a full bridge, owing to the dimerization of Sfi1 molecules at their C-termini (Li *et al.*, 2006; Seybold *et al.*, 2015). Protein–protein interaction assays have shown that Kar1 binds to the C-terminus of Sfi1 and thus tethers Sfi1 and Cdc31 to the outer nuclear membrane (Seybold *et al.*, 2015). These findings suggest that Kar1 plays an important role in maintaining the integrity of the half bridge.

Genetically identified as a key factor that regulates karyogamy during cell mating in budding yeast, Kar1 localizes to the half bridge and is also required for SPB duplication (Rose and Fink, 1987). Protein domain analysis has revealed that the Kar1 region important for karyogamy is separable from that required for SPB duplication (Vallen *et al.*, 1992). To initiate SPB duplication, a precursor of the SPB, the satellite, made up of SPB components primarily of the central plaque, is deposited at the distal end of the bridge (Adams and Kilmartin, 1999; Jaspersen and Winey, 2004). Duplication of the SPB is controlled by the cyclin-dependent kinase Cdk1 and to a lesser extent by the pololike kinase, Cdc5 (Haase *et al.*, 2001; Jaspersen *et al.*, 2004; Crasta *et al.*, 2008; Avena *et al.*, 2014; Elserafy *et al.*, 2014). Like the nuclear genome, the SPB is duplicated only once per cell cycle (Elserafy *et al.*, 2014; Ruthnick and Schiebel, 2016). Recent studies have shown that licensing of SPB duplication is modulated by the state of Sfi1 phosphorylation at its C-terminus (Avena *et al.*, 2014; Elserafy *et al.*, 2014), to which Kar1 binds. Phosphorylation of Sfi1 at six key residues by Cdk1 promotes SPB separation, whereas dephosphorylation of Sfi1 at anaphase by the phosphatase Cdc14 is critical for the next round of SPB duplication (Avena *et al.*, 2014; Elserafy *et al.*, 2014; Fox *et al.*, 2017). Surprisingly, removal of the C-terminus of Sfi1, which eliminates its dimerization domain as well as the six key phosphorylation sites, still maintains yeast cell growth at room temperature (Seybold *et al.*, 2015). Therefore, other components of the half bridge, including Kar1, may play an alternative role for licensing SPB duplication.

We show here that overproduction of Kar1 in meiosis leads to supernumerary SPB formation and chromosome missegregation. During meiosis, after only one round of DNA replication, two continuous cell divisions occur to reduce the genome size by half in gametes. In budding yeast, the SPB is first duplicated in meiosis I to form a bipolar spindle for homologue segregation, and then SPBs reduplicate at interphase II to form two spindles for sister-chromatid segregation in meiosis II (Moens and Rapport, 1971). Therefore, the second round of SPB duplication is decoupled from DNA replication, but before the end of meiosis I, SPBs must be licensed for reduplication (Fox *et al.*, 2017). Furthermore, during the early phase of meiosis I when homologous chromosomes pair and recombine, duplicated SPBs are engaged without separation, forming a side-by-side configuration for an extended period (Li *et al.*, 2015). We have found that ectopically overproduced Kar1 promotes SPB separation at prophase I, but SPB reduplication occurs only after the onset of metaphase I. Our findings thereby are in contrast to those in mitosis, where high dosage of Kar1 arrests yeast cells with a single unduplicated SPB (Rose and Fink, 1987; Seybold *et al.*, 2015). We also show that Kar1-mediated SPB duplication is independent of the phosphorylation status of Sfi1, indicating the existence of an alternative mechanism to license SPB duplication during yeast meiosis.

RESULTS

Kar1 is expressed in yeast meiosis and localizes to the SPB

We seek to understand the function of Kar1 in yeast meiosis. Previously, we have shown that Kar1 is copurified with the SPB component Spc97 (Li *et al.*, 2015), revealing that Kar1 is present at the SPB during budding yeast meiosis. To determine the protein level of Kar1, we generated an N-terminal V5-tagged *KAR1* allele, which is under the control of its endogenous promoter (Figure 1A). By Western blotting, we found that Kar1 was present throughout meiosis, but its protein level increased approximately fourfold 6 h after the induction of meiosis (Figure 1A and Supplemental Figure 1), which approximately corresponded to meiosis I. The level of Kar1 remained high for the rest of meiosis (Figure 1A), supporting the finding of an upregulation of *KAR1* gene expression during mid and late meiosis (Chu *et al.*, 1998). This elevated level of Kar1 during meiosis is in sharp contrast to that of another half-bridge component of the SPB, Mps3, whose protein level decreases dramatically after meiosis I (Li *et al.*, 2017). Of note, Kar1 appeared to be modified posttranslationally, forming multiple upper shifting bands (Figure 1A, $t = 6$ h, and Supplemental Figure 1) that are indicative of protein hyperphosphorylation. Taking these observations together, we conclude that *KAR1* is upregulated and its gene product is present throughout yeast meiosis.

To localize Kar1 in meiotic cells, we generated a functional *GFP-KAR1* allele, which served as the only copy of *KAR1* in the yeast genome (Figure 1B). We used the SPB component, Tub4, which was fused to mApple, to serve as a marker for the SPB (Figure 1B and Supplemental Figure 1). By time-lapse live-cell fluorescence microscopy, we found that GFP-Kar1 was colocalized with Tub4-mApple throughout meiosis (Figure 1B and Figure 2A). Quantitative analysis of SPB separation parameters, including the duration of metaphase I (35 ± 13 min, $n = 11$) and the pole-to-pole distance at metaphase I and anaphase I, showed that cells with GFP-Kar1 appeared normal (Figure 1, B and C; Shirk *et al.*, 2011 and see below). To pinpoint Kar1's localization to the half-bridge area of the SPB, we focused on cell cycle transition from prophase I to metaphase I, when duplicated SPBs separated to form two distinctive Tub4 foci (Figure 2A). Line scan of fluorescence intensity revealed that Kar1 was concentrated at the interspace of the separating Tub4 foci (Figure 2D), indicating that Kar1 is localized to the half-bridge area, consistent with it being a half-bridge protein previously found in mitosis (Vallen *et al.*, 1992; Burns *et al.*, 2015; Seybold *et al.*, 2015). Together, our data demonstrate that Kar1 is an SPB component and likely localizes to the region of the SPB half bridge during yeast meiosis.

Increased level of Kar1 leads to supernumerary SPB formation in yeast meiosis

Because the expression of *KAR1* is up-regulated (Chu *et al.*, 1998) and Kar1 protein level increases during meiosis (Figure 1A), we hypothesized that Kar1 plays a regulatory role in meiotic SPB duplication and separation. To dramatically increase the protein level of Kar1 specifically during yeast meiosis, we used an enhanced *DMC1* promoter that we developed (Fan *et al.*, 2017) to generate the heterologous allele *P_{DMC1}-KAR1* (Figure 1A and Supplemental Figure 1). Compared to the endogenous level of Kar1 in wild-type cells, *P_{DMC1}-KAR1* cells produced ~25-fold more Kar1 during yeast meiosis as determined by Western blotting and fluorescence-intensity-based assay (Figure 1A and Supplemental Figure 1). By Western blotting, we found that overproduced Kar1 also showed retarded migrating bands 6 h after the induction of meiosis, supporting the idea that Kar1 is modified posttranslationally during yeast meiosis.

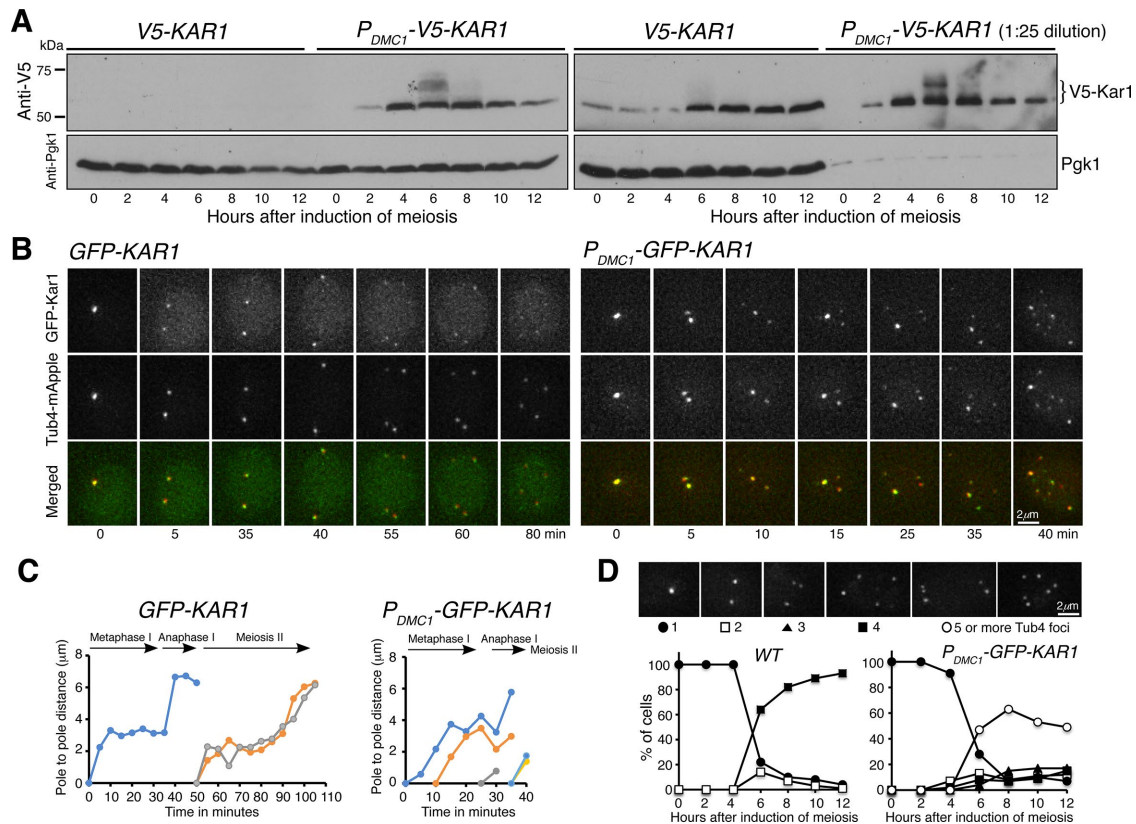


FIGURE 1: Protein level and localization of Kar1 in budding yeast meiosis. (A) Western blots showing the protein level of Kar1. Yeast cells were induced to undergo synchronous meiosis, and aliquots were withdrawn at indicated times and prepared for Western blotting. V5-Kar1 was probed by an anti-V5 antibody. The P_{DMC1} -V5-KAR1 allele specifically overproduces Kar1 in meiosis. Note that in the gel shown to the right, samples from P_{DMC1} -V5-KAR1 were diluted 25-fold. The level of Pgk1 serves as a loading control. (B) Time-lapse live-cell microscopy showing GFP-Kar1 localization in yeast meiosis. Tub4-mApple serves as a marker for the yeast SPB. Projected images from 12 optical sections are shown. Note that GFP-Kar1 colocalized with Tub4-mApple and that supernumerary Kar1 and Tub4 foci were formed in the P_{DMC1} -GFP-KAR1 strain. Time zero refers to the point of SPB separation in meiosis I. (C) Pole-to-pole distance from the GFP-KAR1 and P_{DMC1} -GFP-KAR1 strains shown in B. Note that ~30 min after the formation of supernumerary Tub4 foci, it becomes difficult to track the corresponding spindle poles. (D) Quantification of supernumerary Tub4 foci formation. Yeast cells were induced to undergo synchronous meiosis as in A, and fluorescence microscopy was performed to determine the number of Tub4 foci at indicated time points. At least 100 cells were counted at each time point. Time-course experiments were repeated, and data from one representative experiment are shown.

To determine the consequence of Kar1 overproduction during meiosis, we performed time-lapse fluorescence microscopy to monitor SPB duplication and separation (Figure 1B). In wild-type cells, SPBs, marked by Tub4-mApple and GFP-Kar1, separated at the onset of metaphase I to form a bipolar spindle; this process had a duration of 35 min (Figures 1, B and C, and 3, A and B, and Supplemental Figure 2). At anaphase I, the pole-to-pole distance of the spindle increased to more than 6 μ m (Figures 1, B and C, and 3A and Supplemental Figure 2). On entering into meiosis II, cells underwent a second round of SPB duplication and separation to generate four spores, each inheriting exactly one SPB (Figures 1D and 3, A–D). In contrast, in P_{DMC1} -KAR1 cells, the third Tub4/Kar1 focus started to emerge ~10 min after the first round of SPB separation in meiosis I (Figures 1, B and C, and 3, A–C), indicating an accelerated rate of SPB duplication and/or premature SPB separation when Kar1 is overproduced. Noticeably, 6 h after the induction of meiosis P_{DMC1} -KAR1 cells formed 5 or more Tub4/Kar1 foci (Figures 1, B–D, and 3, A–C). The Tub4 foci formed in mutant cells always colocalized with Kar1, although their fluorescence intensity varied (Figure 1B). Twelve hours after induction of meiosis, more than half of the

P_{DMC1} -KAR1 cells formed five or more Tub4/Kar1 foci (Figures 1D and 3D). Because the wild-type copy of KAR1 was present in these cells, the phenotype caused by P_{DMC1} -KAR1 therefore was hypermorphic. In addition, we found that the core component of the SPB central plaque, Spc42, also colocalized with the supernumerary Kar1 foci during meiosis (Figure 2B). Altogether, these results support the idea that, in the presence of excessive Kar1, meiotic cells overduplicate and form supernumerary SPBs.

Kar1-mediated supernumerary SPBs are functional in nucleating microtubules

To determine whether the supernumerary SPBs formed in P_{DMC1} -KAR1 cells are functional in nucleating microtubules and thus regulate meiotic cell division, we monitored spindle formation and chromosome segregation by time-lapse fluorescence microscopy (Figure 3). To visualize microtubules, one copy of Tub1, the α -tubulin in budding yeast, was tagged by mApple (Figure 3A). In wild-type cells, a bipolar spindle formed at the onset of metaphase I, elongated at anaphase I, and then disassembled before the second round of SPB duplication and separation in meiosis II (Figure 3A).

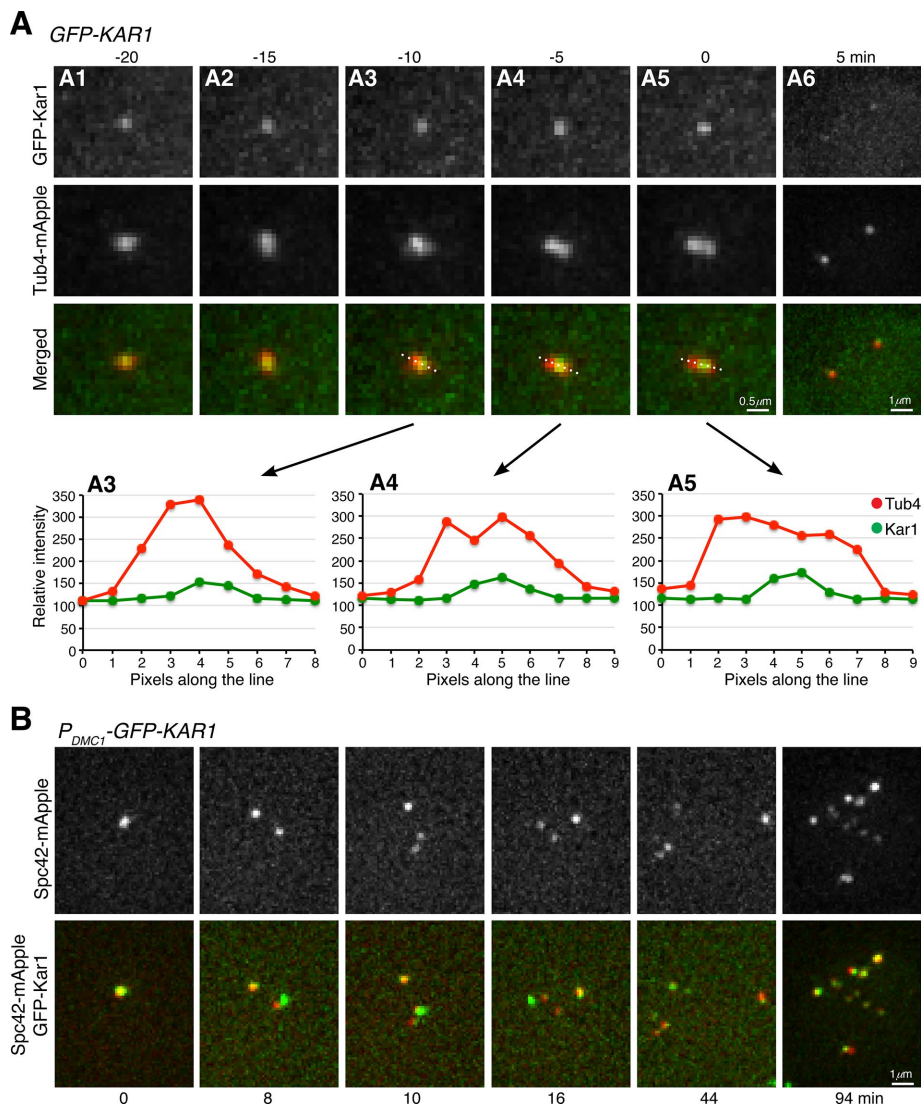


FIGURE 2: Localization of Kar1 to the half-bridge area during yeast meiosis. (A) Time-lapse live-cell microscopy was performed as in Figure 1B. Single optical sections are shown. Graphs of line-scan pixel intensity shown in A3 to A4 correspond to the top images. Time zero refers to the point of SPB separation in meiosis I. Note that GFP-Kar1 localizes to the central region of two separating SPBs, which are marked by Tub4-mApple. (B) Colocalization of Spc42 with Kar1 in yeast meiosis. Time-lapse live-cell microscopy was performed as in A. Time interval was set at 2 min; projected images from 12 optical sections are shown. Time zero refers to the point of the first round of Spc42 separation in meiosis I. Note that all Spc42-mApple foci colocalize with GFP-Kar1.

After SPB separation at the onset of metaphase I, spindles formed in *P_{DMC1}-KAR1* cells and elongated at anaphase I to a length that was comparable to those of the wild-type cells (Figures 1 and 3). Noticeably, the third and sometimes the fourth Kar1 focus emerged well before spindle elongation (Figure 3A, $t = 10$, and Supplemental Figure 2B). Essentially all of the supernumerary Kar1 foci in *P_{DMC1}-KAR1* cells were associated with microtubules, leading to multipolar spindle formation (Figure 3A). These findings demonstrate that these Kar1 foci represent functional microtubule-organizing centers in mutant cells.

To visualize chromosome segregation, we observed the dynamics of histone H2A with an *HTA1-mApple* allele (Figure 3B). Compared to those in wild-type cells, meiotic chromosome segregation appeared on time in *P_{DMC1}-KAR1* cells, of which two distinctive Hta1

masses formed around 35 min after SPB separation in meiosis I (Figure 3B). Wild-type cells formed four separate Hta1 masses at the end of meiosis, whereas ~40% of mutant cells formed five or more Hta1 blobs, each associated with one or more Kar1 foci (Figure 3C). As a consequence, more than four ascospores formed in *P_{DMC1}-KAR1* asci (Figure 3D), and even in the asci with four spores, less than 60% of the spores were viable (Figure 3E), indicating massive chromosome missegregation in mutant cells. The above observations further support the idea that in the presence of excessive Kar1, yeast cells overduplicate the SPB in meiosis.

We used serial-section electron microscopy (EM) to determine the ultrastructure of Kar1-mediated SPBs (Figure 4). Morphologically, SPBs in *P_{DMC1}-KAR1* cells appeared normal, because they were imbedded into the nuclear membranes, formed layered structures, and nucleated microtubules, as those in wild-type cells (Figure 4). At prophase I, duplicated SPBs engaged and formed a side-by-side SPB configuration, but the bridge that linked the SPBs appeared smaller ($p < 0.05$, $n = 15$) with less electron density in *P_{DMC1}-KAR1* cells (Figure 4A). Importantly, at metaphase I before spindle elongation, wild-type cells always formed bipolar spindles (Figures 3A and 4B), but two of the 6 *P_{DMC1}-KAR1* metaphase I cells that we examined by serial-section EM possessed three functional SPBs and appeared to form multipolar spindles (Figure 4C shows one of them). This finding supports our light microscopy observation of premature SPB duplication and separation in cells with excessive Kar1 (Figure 3A and Supplemental Figure 2). Of note, in 14 wild-type and 10 mutant meiosis II cells that were examined by serial-section EM, only three or four SPBs in each cell could be determined with certainty, on the basis of their association with microtubules. Nevertheless, our light and electron microscopy data together show that the supernumerary SPBs formed in the presence of excessive Kar1 are capable of nucleating

microtubules and forming spindles and therefore are functional in mediating chromosome segregation. Because excessive and often multipolar spindles form in these cells, chromosome segregation becomes erroneous, leading to inviable spores (Figure 3E).

SPBs separate prematurely at prophase I in the excess of Kar1

To address the execution point of excessive Kar1-mediated supernumerary SPB formation, we arrested yeast cells at prophase I by deleting *NDT80*, which encodes a transcription factor that regulates the expression of mid- and late meiotic genes (Xu et al., 1995), and observed SPB duplication and separation when Kar1 was overproduced (Figure 5). In *ndt80Δ* cells, SPBs were duplicated but remained cohesive, most of the time forming only one

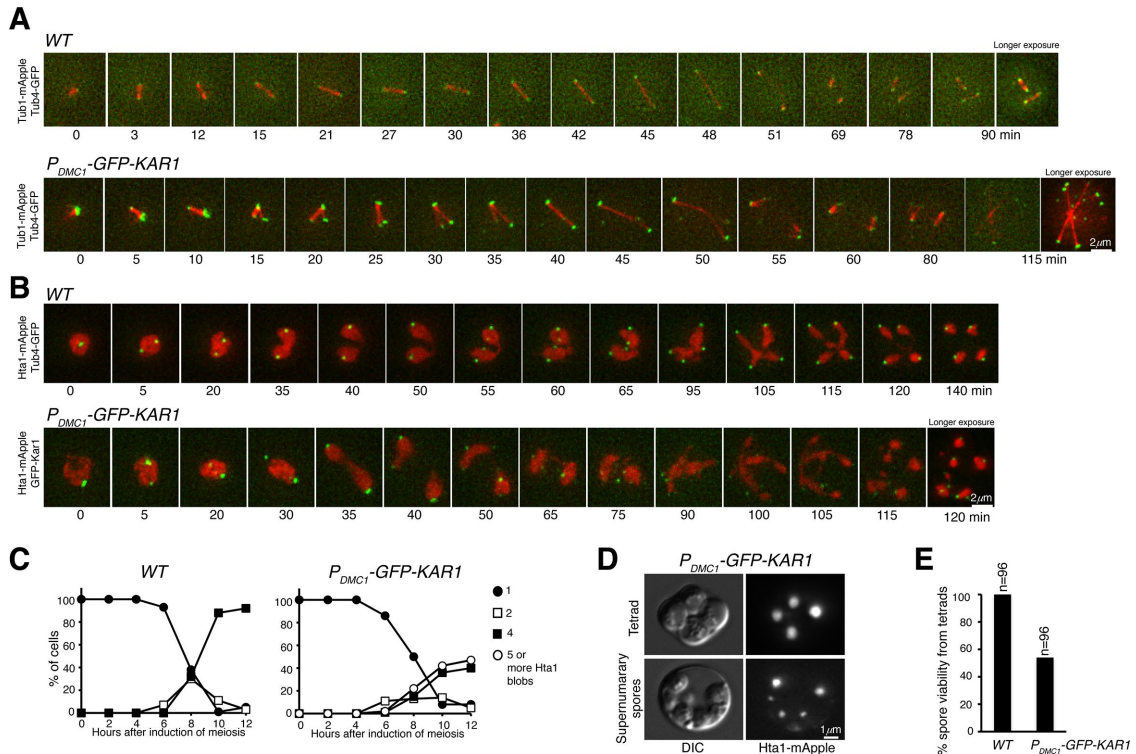


FIGURE 3: Supernumerary SPB and ascospore formation in Kar1-overproduced cells during yeast meiosis. (A) Spindle dynamics during yeast meiosis. Wild-type (WT, Tub1-mApple, Tub4-GFP) and P_{DMC1} -GFP-KAR1 cells were induced to undergo synchronous meiosis, and live-cell fluorescence microscopy was performed to monitor microtubule and SPB movement in meiosis. Time interval was set at either 3 or 5 min; time zero refers to the point of SPB separation in meiosis I. Merged images from 12 optical sections were shown. Extended data of A are shown in Supplemental Figure 2. Note that numerous spindles were formed in the P_{DMC1} -GFP-KAR1 cell. (B) Chromosome segregation in yeast meiosis. Wild-type (HTA1-mApple TUB4-GFP) and P_{DMC1} -GFP-KAR1 cells were induced to undergo synchronous meiosis, and live-cell microscopy was performed as in A. (C) Quantification of chromosome segregation in cells shown in B. Note that five or more Hta1 blobs were formed in the P_{DMC1} -GFP-KAR1 cell. (D) Ascospore formation during yeast meiosis. Selected images from P_{DMC1} -GFP-KAR1 cells show abnormal ascospore formation (lower panel). (E) Quantification of spore viability. One-day-old tetrads were dissected and grown on YPD for 3 d to determine viability.

Tub4 focus visible by fluorescence microscopy (Figure 5 and Shirk *et al.*, 2011). In contrast, more than 25% of P_{DMC1} -KAR1 *ndt80* Δ cells formed two Tub4 foci 8 h after the induction of meiosis (Figure 5, A and B). However, the double mutant rarely yielded more than two Tub4 foci (Figure 5B). Our findings demonstrate that SPBs separate prematurely at prophase I in the presence of an increased level of Kar1.

We and others have shown previously that in the absence of Ipl1, the Aurora kinase in budding yeast, SPBs separate prematurely at prophase I (Shirk *et al.*, 2011; Kim *et al.*, 2013). To determine whether Kar1 and Ipl1 regulate SPB separation in a similar manner, we generated a double mutant P_{DMC1} -KAR1 P_{CLB2} -IPL1, which overproduced Kar1 but depleted Ipl1 in meiotic cells (Figure 5C). As we found previously, ~40% of P_{CLB2} -IPL1 *ndt80* Δ cells prematurely separated their SPBs at prophase I 12 h after the induction of meiosis, whereas more than 50% of P_{DMC1} -KAR1 P_{CLB2} -IPL1 *ndt80* Δ cells separated prematurely (Figure 5C). More importantly, around 30% of P_{DMC1} -KAR1 P_{CLB2} -IPL1 *ndt80* Δ cells formed three Tub4 foci (Figure 5C). This result is in contrast to that of P_{DMC1} -KAR1 *ndt80* Δ , for which cells with three Tub4 foci were rarely observed (Figure 5B). Together, our findings support the idea that Kar1 promotes SPB separation, whereas Ipl1 inhibits SPB separation and to a lesser extent prevents SPB duplication.

Kar1 protein domains required for SPB duplication and separation in meiosis

Because Kar1 is an integral membrane protein of the outer nuclear envelope, we asked whether the transmembrane domain of Kar1, which is located at its very C-terminus, is required for mediating supernumerary SPB formation. We generated a P_{DMC1} -KAR1- Δ TMD allele, of which the C-terminal 22 amino acids were removed (Figure 6A). As shown in Figure 6B, GFP-Kar1- Δ TMD formed fluorescent foci inside the cell, but they failed to colocalize with Tub4, supporting the idea that the transmembrane domain is required for its localization to the SPB (Vallen *et al.*, 1992). Importantly, SPB duplication and separation, as visualized by Tub4-mApple, was on schedule in P_{DMC1} -KAR1- Δ TMD cells (Figure 6B). Of note, the endogenous wild-type copy of KAR1 was present in cells with P_{DMC1} -driven deletion alleles (Figure 6, B–H). Therefore, overproduction of Kar1- Δ TMD has no discernable negative effect on meiotic SPB duplication and cell progression, and we conclude that the transmembrane domain of Kar1 is critical for its function in regulating SPB duplication and separation in meiosis as shown previously in mitosis (Vallen *et al.*, 1992).

In addition to its role in SPB duplication, Kar1 is required for nucleating microtubules at the half bridge during karyogamy (Rose and Fink, 1987; Vallen *et al.*, 1992). To determine whether Kar1's role

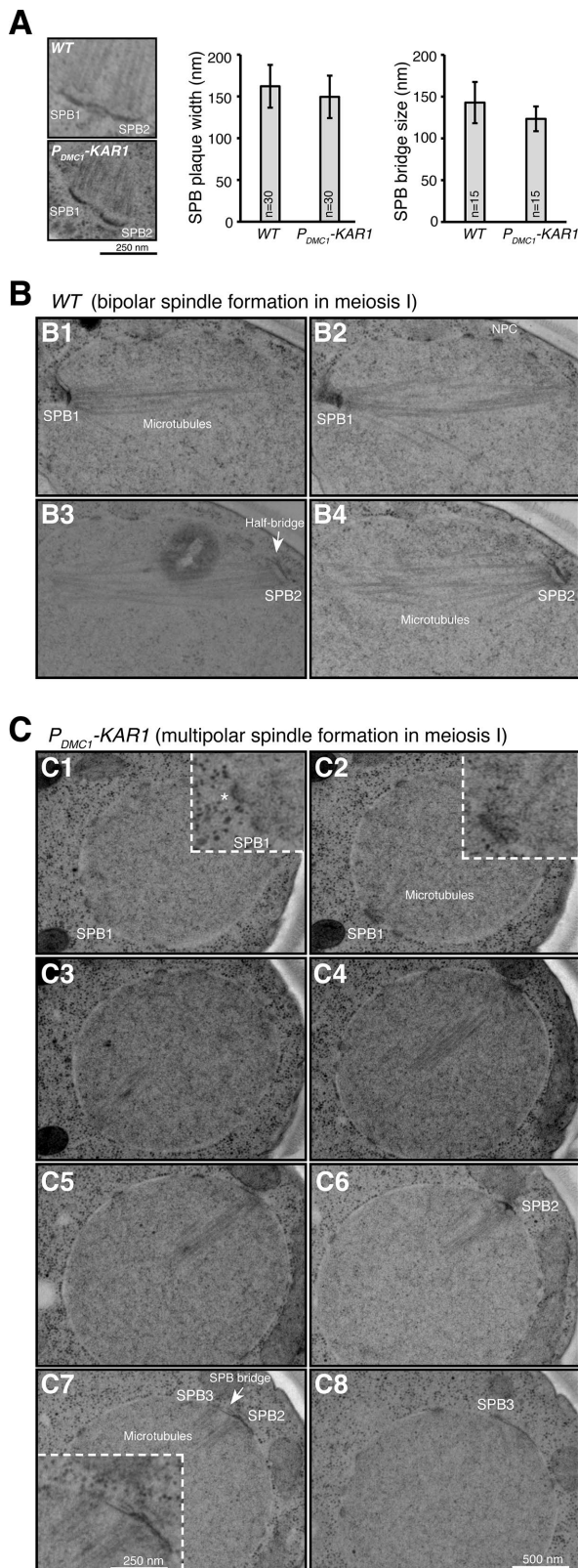


FIGURE 4: SPB ultrastructure visualized by electron microscopy (EM). (A) Side-by-side SPB configuration in meiosis I. Yeast cells were induced to undergo synchronous meiosis, and serial sectioning was performed to observe SPB morphology by EM. Representative images from the wild type (WT) and mutant are shown. Quantifications of SPB plaque width and bridge size are shown in the graphs to the right. For the t test for SPB plaque width, $p = 0.035$;

in microtubule nucleation is also required for regulating SPB duplication and separation in meiosis, we first used a separation-of-function mutant, *kar1-Δ15*, which abolishes microtubule nucleation and thus is defective in karyogamy but is fully competent for SPB duplication and cell growth (Figure 6A and Vallen *et al.*, 1992). When *Kar1-Δ15* was overproduced in meiosis by way of *P_{DMC1}-kar1-Δ15*, SPBs overduplicated to form more than four Tub4 foci just as observed in *P_{DMC1}-KAR1* cells (Figures 1B and 6E), indicating that microtubule-nucleating at the half bridge mediated by *Kar1* is not necessary for *Kar1*-mediated SPB duplication in meiosis. Of note, the karyogamy domain of *Kar1* appears to play a role in spore wall formation and maturation (Gordon *et al.*, 2006). Next, we determined whether deletion of region 1 (*Kar1-Δ18*), which abolishes SPB duplication in mitosis (Vallen *et al.*, 1992), also inhibits *Kar1*'s activity in promoting SPB overduplication (Figure 6, A and G). Indeed, ectopically expressing *P_{DMC1}-kar1-Δ18* had little effect on supernumerary SPB formation (Figure 6, A and G). Similarly, we found that *P_{DMC1}-KAR1-Δ15 ndt80Δ* but not *P_{DMC1}-KAR1-ΔTMD ndt80Δ* cells separated SPB prematurely at prophase I (Figure 6, F and H). Intriguingly, deletion of region 1 (*P_{DMC1}-kar1-Δ18*) increased the rate of premature SPB separation approximately fourfold (Figure 6H), indicating that region 1 plays an inhibitory role in SPB separation. Together, our findings indicate that the transmembrane domain and region 1 but not the karyogamy domain of *Kar1* are critical for generating the hypermorph of *P_{DMC1}-KAR1*.

Supernumerary SPBs form after prophase I in the excess of *Kar1*

To test the hypothesis that supernumerary SPBs form only after prophase I, we used an inducible *P_{GAL}-NDT80* allele in meiosis, first to arrest cells at prophase I and then to release yeast cells from prophase I arrest by inducing the expression of *P_{GAL}-NDT80* with estradiol (Carlile and Amon, 2008). In *P_{DMC1}-KAR1 P_{GAL}-NDT80* cells that were arrested at prophase I, we found that only two Tub4 foci were formed 12 h after induction of meiosis (Figure 5D). This observation is consistent with the idea that SPBs separate prematurely when *Kar1* is overproduced. On the addition of estradiol, yeast cells were released from prophase I arrest and continued meiosis (Figure 5D). As expected, supernumerary Tub4 foci were formed in *P_{DMC1}-KAR1 P_{GAL}-NDT80* cells (Figure 5D). In addition, we constructed a *P_{GAL}-KAR1 P_{GAL}-NDT80* strain, in which both *KAR1* and *NDT80* were under the control of the *GAL* promoter (Figure 5E). On the addition of estradiol, both *Kar1* and *Ndt80* were induced, leading to the formation of supernumerary Tub4 foci in ~40% of the cells (Figure 5E). The above findings demonstrate that in the presence of excessive *Kar1*, formation of supernumerary SPBs occurs only after prophase I.

To further determine the execution point of *Kar1*-mediated SPB duplication, we arrested yeast cells at metaphase I by way of *P_{CLB2}-CDC20* (Lee and Amon, 2003), overproduced *Kar1* by *P_{DMC1}-KAR1*, and determined the formation of supernumerary SPBs by observing Tub4 and/or *Kar1* foci formation (Figure 7A). After the induction of meiosis, *P_{CLB2}-CDC20* cells separated their SPBs and formed two

bridge size $p = 0.011$. (B, C) Spindle formation in meiosis I. (B) A bipolar spindle in a WT cell captured in four serial sections. NPC, nuclear pore complex. (C) A tripolar spindle in a *P_{DMC1}-KAR1* cell captured in eight serial sections. Insets show 2× enlarged images. Note that in the mutant cell, the number 2 SPB has already reduplicated before spindle elongation at anaphase I. The asterisk indicates that this nuclear structure may represent another SPB in the mutant cell.

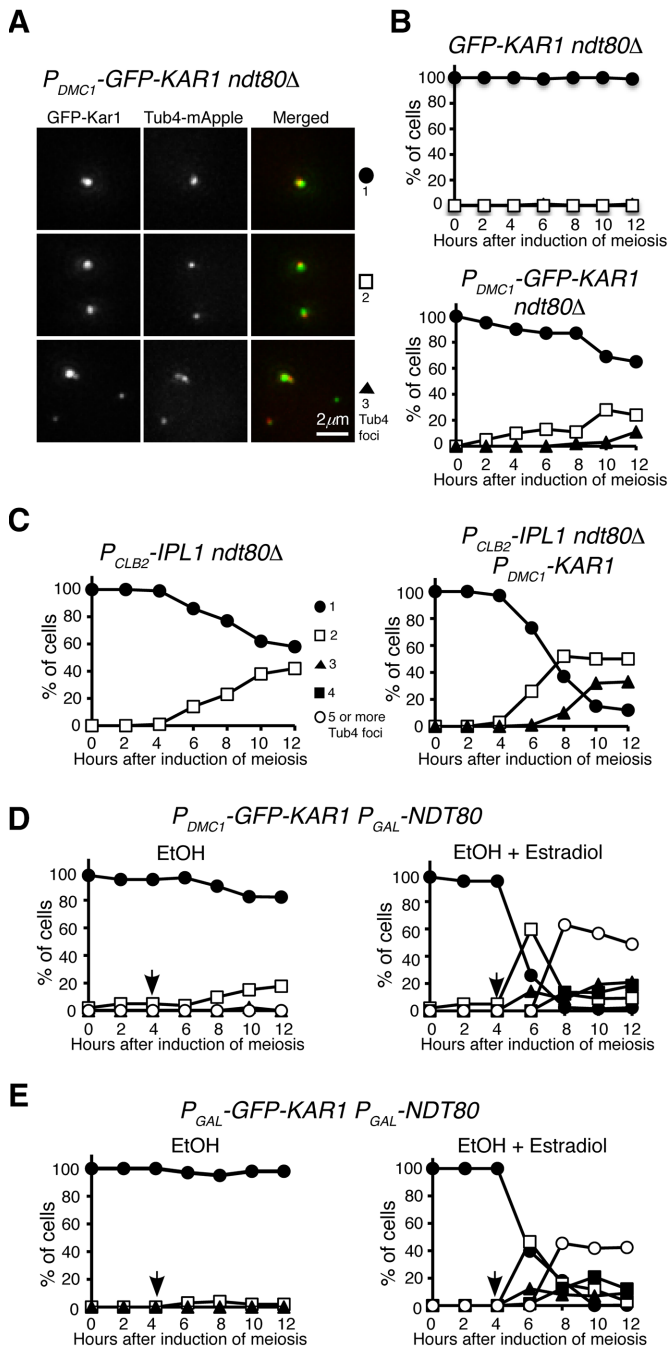


FIGURE 5: Kar1 promotes premature SPB separation at prophase I. (A) Representative images showing GFP-Kar1 localization at prophase I. Projected images are shown. (B) Quantification of SPB separation in *GFP-KAR1 ndt80Δ* and *P_{DMC1}-GFP-KAR1 ndt80Δ* cells. (C) Quantification of SPB separation in *P_{CLB2}-IPL1 ndt80Δ* and *P_{CLB2}-IPL1 ndt80Δ P_{DMC1}-KAR1* cells. (D) Quantification of SPB separation in *P_{DMC1}-GFP-KAR1 P_{GAL}-NDT80* cells. Arrows indicate the time of addition of EtOH and estradiol, which was dissolved in EtOH. (E) Quantification of SPB separation in *P_{GAL}-GFP-KAR1 P_{GAL}-NDT80* cells. As in D, arrows indicate the time of addition of EtOH and estradiol. The time-course experiments were repeated, and data from one representative experiment are shown.

Tub4 foci, but these cells never entered into anaphase I (Figure 7B). In contrast, in *P_{DMC1}-KAR1 P_{CLB2}-CDC20* cells, supernumerary Tub4 and Kar1 foci formed in ~60% of the cells, most of them with colo-

calizing Tub4 and Kar1 signals (Figure 7A). Additionally, in *P_{DMC1}-KAR1 P_{CLB2}-CDC20* cells, the supernumerary Tub4 foci were also associated with Sfi1, another half-bridge component, and the Kar1 foci were colocalized with Mps3 as well (Figures 6C and 7D). More importantly, the supernumerary Kar1 foci in *P_{DMC1}-KAR1 P_{CLB2}-CDC20* cells formed numerous spindles (Figure 7E), demonstrating their ability in microtubule nucleation. From these observations, we conclude that in the presence of an elevated level of Kar1, supernumerary SPBs can form only after prophase I but before the onset of anaphase I.

Cdc5 regulates Kar1-mediated SPB duplication

We have shown previously that depletion of the pololike kinase, Cdc5, in budding yeast meiosis inhibits SPB duplication during meiosis (Shirk *et al.*, 2011). To determine whether Kar1-mediated SPB overduplication requires Cdc5 activity, we used a meiosis-specific Cdc5 depletion mutant, *P_{CLB2}-CDC5* (Lee and Amon, 2003). After induction to undergo meiosis, *P_{CLB2}-CDC5* cells were arrested at a metaphase-I-like stage with two separated SPBs (Figure 7F and Shirk *et al.*, 2011). When Kar1 was overproduced in Cdc5-depleted (*P_{DMC1}-KAR1 P_{CLB2}-CDC5*) cells, SPBs separated, but their duplication was largely inhibited (Figure 7G), supporting the idea that Cdc5 is required for SPB duplication in meiosis (Shirk *et al.*, 2011).

Kar1 plays a role in licensing SPB duplication during yeast meiosis

Previous work with vegetative yeast cells has shown that phosphorylation at the C-terminus of the half-bridge component Sfi1, mediated by both Cdk1 and Cdc5, regulates SPB separation (Avena *et al.*, 2014; Elserafy *et al.*, 2014), whereas dephosphorylation of Sfi1, presumably by the phosphatase Cdc14 (Fox *et al.*, 2017), licenses SPB duplication. To determine the requirement of Sfi1 in meiotic cell division, we generated a meiosis-specific allele, *P_{CLB2}-SFI1*, to deplete Sfi1 in meiosis (Figure 8A). By fluorescence microscopy, we found that in *P_{CLB2}-SFI1* cells, SPBs, marked by Tub4-mApple, separated and formed an anaphase-I-like elongated spindle (Figure 8A), but mutant cells never underwent the second round of SPB duplication and separation (Figure 8A). Of note, 12 h after the induction of meiosis, more than 60% *P_{CLB2}-SFI1* cells formed dyads, therefore exiting meiosis prematurely (Figure 8A). We constructed *P_{DMC1}-SFI1* to express *SFI1* and its mutant forms specifically in meiosis (Figure 8B and see below). Importantly, we found that meiotic Sfi1 produced by *P_{DMC1}-SFI1* suppressed the phenotype of *P_{CLB2}-SFI1*, because cells of *P_{CLB2}-SFI1 P_{DMC1}-SFI1* completed meiosis with two rounds of SPB duplication and separation and formed tetrads at the end of meiosis (Figure 8B). We therefore conclude that Sfi1 is an essential SPB component required for SPB duplication in meiosis, as it is in mitosis (Kilmartin, 2003).

To determine the interplay between Kar1 and Sfi1 in meiotic SPB duplication, we overexpressed Kar1 in cells that were depleted of Sfi1 (*P_{DMC1}-KAR1 P_{CLB2}-SFI1*) and observed SPB dynamics using Tub4-mApple as a marker (Figure 8C). Surprisingly, more than 40% of *P_{DMC1}-KAR1 P_{CLB2}-SFI1* cells formed three or four Tub4 foci 8 h after the induction of meiosis (Figure 8C), demonstrating that overproduced Kar1 promotes SPB duplication even when Sfi1 is limited in quantity. However, five or more Tub4 foci were rarely formed in *P_{DMC1}-KAR1 P_{CLB2}-SFI1* cells, indicating that Sfi1, a structural component of the half bridge, is required for supernumerary SPB formation when Kar1 is overproduced.

To determine whether Kar1 can override the requirement of Sfi1 for licensing SPB duplication, we generated meiotic alleles *sfi1-6A* and *sfi1-6D*, in which the six phosphorylation sites located at the

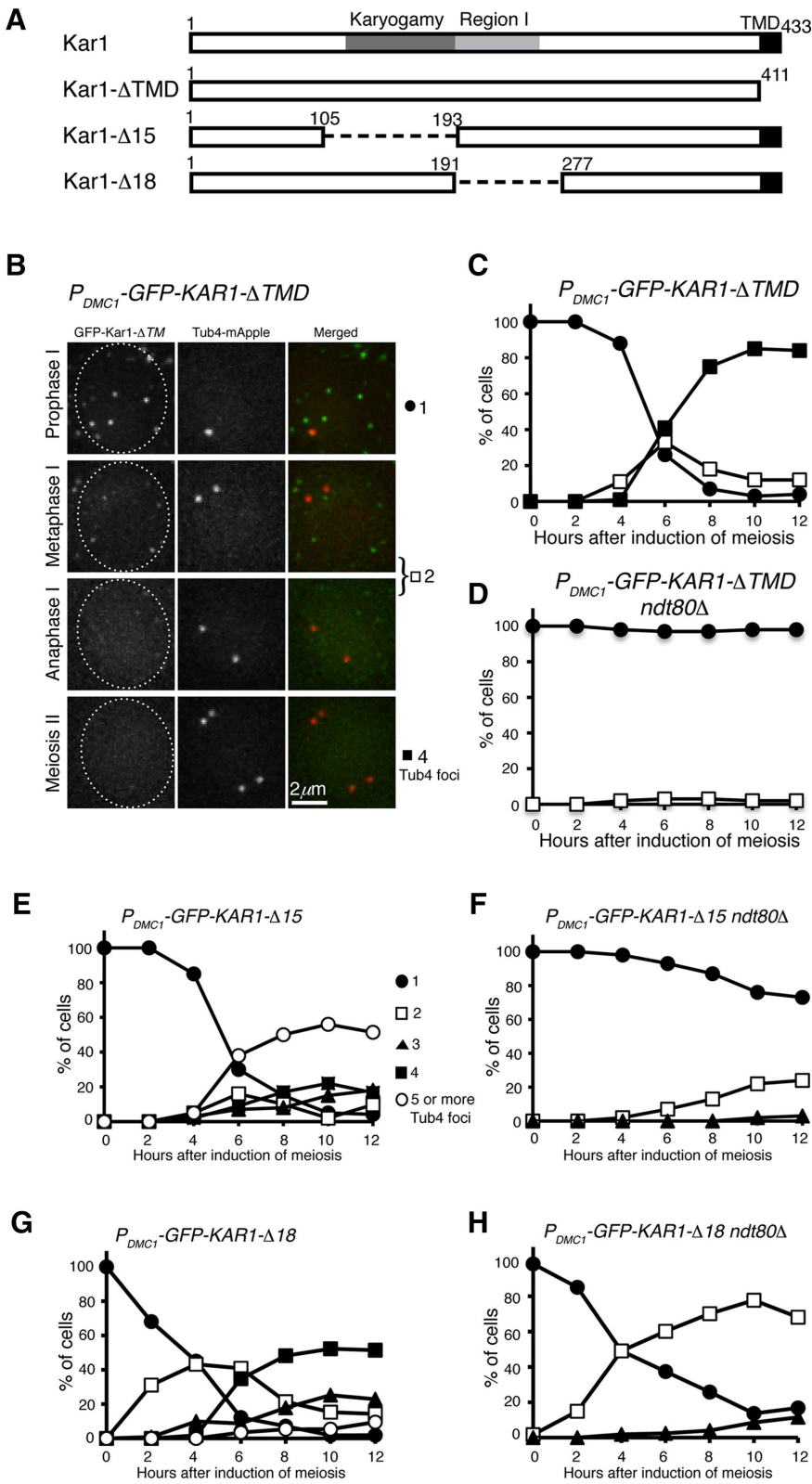


FIGURE 6: Protein domains of Kar1 required for yeast meiosis. (A) Diagram showing the Kar1 protein structure. Deletions of *kar1-Δ15* and *Δ18* have been described previously (Vallen *et al.*, 1992). (B) Representative images showing the localization of GFP-Kar1-ΔTMD in yeast meiosis. Note that without its transmembrane domain (TMD), Kar1 fails to localize to the SPB, which is marked by Tub4-mApple. (C, D) Quantification of SPB separation in *P_{DMC1}-GFP-KAR1-ΔTMD* cells. The *ndt80Δ* allele was used to arrest yeast cells at prophase I. (E, F) Quantification of SPB separation in *P_{DMC1}-GFP-KAR1-Δ15* cells. (G, H) Quantification

C-terminus of Sfi1 were mutated to alanine or aspartic acid to eliminate phosphorylation or mimic phosphorylation, respectively (Figure 8, D–G). Dephosphorylation of Sfi1 is required for licensing SPB duplication, because the phosphomimetic *sfi1-6D* mutant fails to duplicate SPB (Elserafy *et al.*, 2014). We found that *P_{CLB2}-SFI1 P_{DMC1}-sfi1-6D* cells mostly retained two Tub4 foci during meiosis (Figure 8D), indicating that dephosphorylation of Sfi1 is critical for SPB duplication in meiosis. Supporting this idea, only 50% *P_{CLB2}-SFI1 P_{DMC1}-sfi1-6A* cells formed three or four Tub4 foci (Figure 8E). If phosphorylation of Sfi1 is the rate-limiting factor for SPB duplication, then we would expect overproducing Kar1 to have no effect on SPB duplication in the *sfi1-6D* background. In contrast, when Kar1 was overproduced in both *P_{DMC1}-KAR1 P_{CLB2}-SFI1 P_{DMC1}-sfi1-6A* and *P_{DMC1}-KAR1 P_{CLB2}-SFI1 P_{DMC1}-sfi1-6D* cells, SPBs overduplicated, forming five or more Tub4-mApple foci (Figures 8, F and G). These findings demonstrate that Kar1 can override the requirement of Sfi1 dephosphorylation in licensing SPB duplication during meiosis.

To provide further evidence that Kar1 licenses SPB duplication in meiosis, we constructed the *P_{CLB2}-CDC14* mutant (Figure 8, H and I), which depleted Cdc14 in meiosis, thereby interfering with Sfi1 dephosphorylation (Fox *et al.*, 2017). After being induced to undergo meiosis, *P_{CLB2}-CDC14* cells were arrested at an anaphase-I-like stage with separated SPBs and elongated spindles (Figure 8H and unpublished data). Overproducing Kar1 in *P_{DMC1}-KAR1 P_{CLB2}-CDC14* cells led to the formation of five or more Tub4-mApple foci (Figure 8I). Therefore, we conclude that Kar1 plays an important role in licensing SPB duplication in yeast meiosis.

DISCUSSION

In this article, we have revealed an unexpected function of Kar1, which promotes SPB separation and licenses SPB duplication during budding yeast meiosis. We have shown that Kar1, when produced at an elevated level, induces SPB overduplication, resulting in supernumerary SPB formation during meiosis. In the absence of Kar1 or when Kar1 is overproduced, yeast cells fail to initiate SPB duplication during vegetative growth (Rose and Fink, 1987; Vallen *et al.*, 1994). Similarly, we have found that depletion of Kar1 in meiosis leads to defective SPB duplication and spore formation (our published data). However, overproduced Kar1 leads to premature

of SPB separation in *P_{DMC1}-GFP-KAR1-Δ18* cells. At least 100 cells were counted at each time point. The time-course experiments were repeated, and data from one representative experiment are shown.

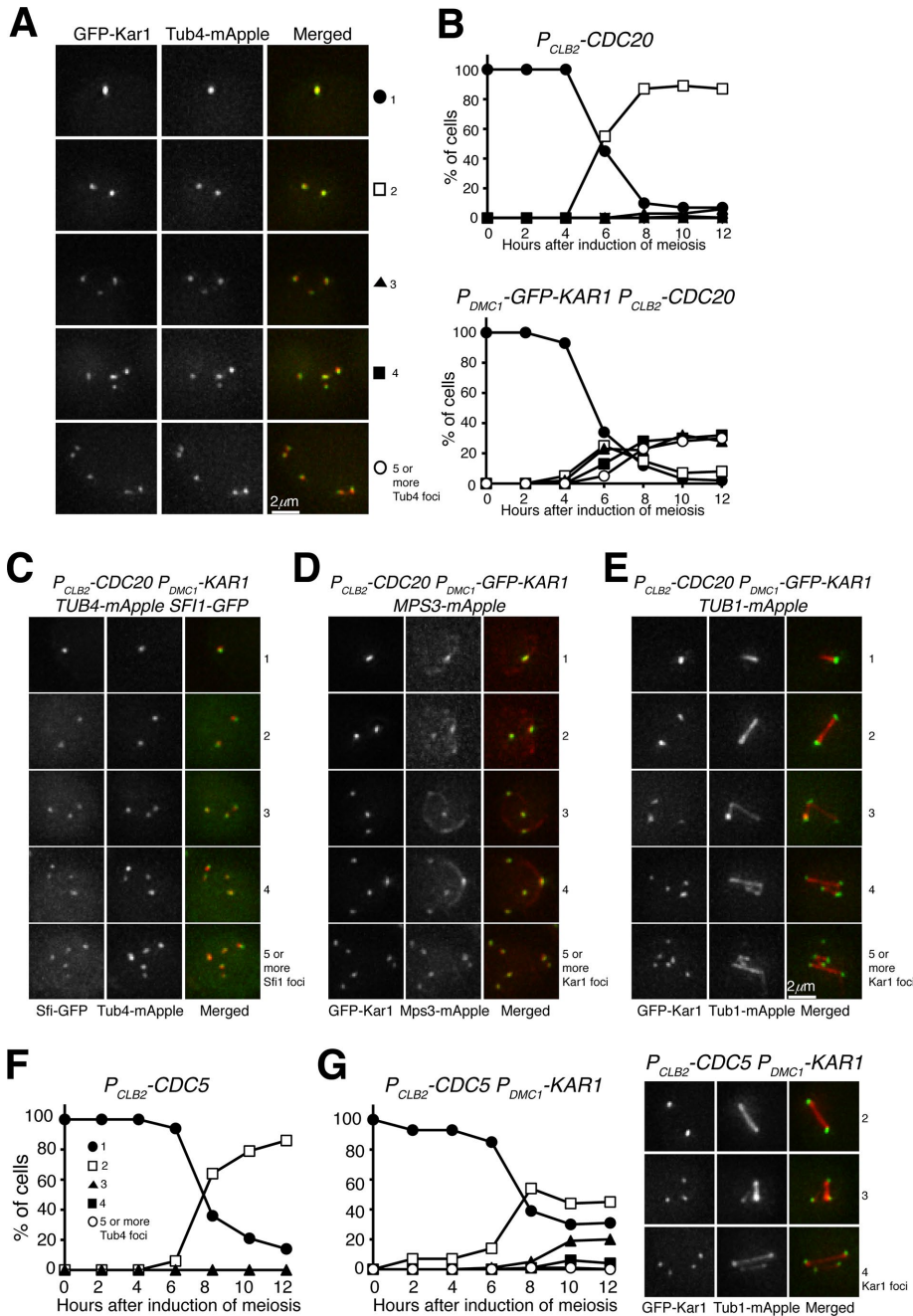


FIGURE 7: The execution point of Kar1-mediated SPB duplication in yeast meiosis. (A) Representative images showing GFP-Kar1 and Tub4 localization in $P_{DMC1}\text{-GFP-KAR1 } P_{CLB2}\text{-CDC20}$ cells. Depletion of Cdc20 arrests yeast cells at metaphase I. (B) Quantification of SPB separation in $P_{CLB2}\text{-CDC20}$ and $P_{DMC1}\text{-GFP-KAR1 } P_{CLB2}\text{-CDC20}$ cells. Note the formation of supernumerary SPBs in $P_{DMC1}\text{-GFP-KAR1 } P_{CLB2}\text{-CDC20}$ cells. (C) Representative images showing Sfi1 localization in $P_{DMC1}\text{-GFP-KAR1}$ cells at metaphase I. Note that Sfi1-GFP colocalizes with Tub4-mApple. (D) Representative images showing Mps3 localization in $P_{DMC1}\text{-GFP-KAR1}$ cells at metaphase I. (E) Representative images showing Tub1 localization in $P_{DMC1}\text{-GFP-KAR1}$ cells at metaphase I. Projected images are shown (C–E). (F) Quantification of SPB separation in Cdc5-depleted ($P_{CLB2}\text{-CDC5}$) cells in meiosis. (G) Quantification of SPB separation in $P_{DMC1}\text{-GFP-KAR1 } P_{CLB2}\text{-CDC5}$ cells. Representative images of GFP-Kar1 and Tub1-mApple are shown to the right. The time-course experiments were repeated, and data from one representative experiment are shown.

SPB separation at meiotic prophase I and supernumerary SPB formation on the onset of metaphase I. Kar1-induced supernumerary SPBs are functional because they can nucleate microtubules and

2015), abolishes Kar1's function in SPB duplication, consistent with the previous finding that region 1 is critical for SPB duplication (Vallen et al., 1992; Seybold et al., 2015).

form spindles that necessitate meiotic chromosome segregation. With excessive spindles formed in cells with overproduced Kar1, chromosome segregation becomes erroneous, forming inviable spores. During ascospore development, membranes are nucleated at the meiotic plaque, a modified outer plaque of the SPB, and then they extend to enclose the entire spore on the completion of meiosis (Neiman, 2011). Our observation that five or more ascospores frequently form in Kar1-overproduced cells further supports the idea that the supernumerary SPBs formed in these cells are functional in nucleating both microtubules for spindle formation and membranes for ascospore development. Finally, cells with excessive Kar1 overduplicate the SPB regardless of the phosphorylation status of Sfi1, demonstrating that in addition to Sfi1, Kar1 plays a role in licensing SPB duplication during yeast meiosis.

How does Kar1 promote SPB duplication?

We have shown that excessive Kar1 leads to SPB premature separation at prophase I, indicating that Kar1 destabilizes the SPB bridge, which links duplicated SPBs. Kar1 binds to the C-terminus of Sfi1, where dimerization of Sfi1 is achieved (Li et al., 2006). Dimerized Sfi1 molecules provide the structural support of the SPB bridge (Li et al., 2006; Anderson et al., 2007; Seybold et al., 2015). We favor a model in which excessive Kar1 interferes with the Sfi1 dimerization domain and therefore weakens the bridge structure and relieves the inhibitory signal for SPB duplication (Figure 9). Meiotic Kar1 appears to act differently from its mitotic version, because in vegetative yeast cells, overexpression of Kar1 forms a highly elongated SPB bridge (Seybold et al., 2015). Alternatively, excessive Kar1 in meiosis could activate motor proteins, such as Cin8 (Hoyt et al., 1992), to promote SPB separation. We note that the above two scenarios are not mutually exclusive. Finally, a possibility currently has not been ruled out that excessive Kar1 may cause SPB fragmentation and thereby leads to the formation of multinucleated sporelike structures. Nevertheless, removal of the karyogamy domain, Kar1- $\Delta 15$, retains Kar1's activity in promoting SPB separation, indicating that Kar1's role in promoting SPB disjunction is separable from its role in nucleating microtubules. On the other hand, deletion of the region 1 domain, which binds to Sfi1 (Seybold et al.,

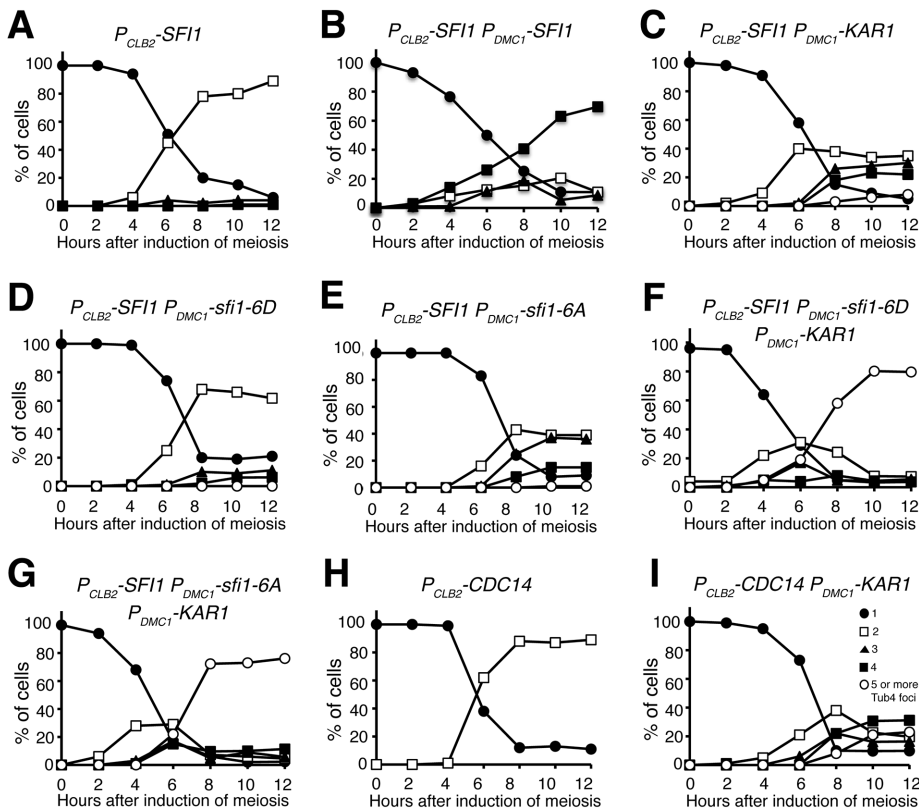


FIGURE 8: Kar1 bypasses the requirement of Sfi1 dephosphorylation for SPB duplication in meiosis. Yeast cells were induced to undergo synchronous meiosis, and cell aliquots were withdrawn at indicated times. Live-cell fluorescence microscopy was performed to determine Tub4-marked SPB dynamics. (A, B) Quantification of SPB separation in Sfi1-depleted ($P_{CLB2}\text{-SFI1}$) meiotic cells. Note that in the absence of Sfi1, SPBs were separated, but yeast cells were arrested in meiosis I. Ectopically produced Sfi1 permitted a second round of SPB duplication and cell progression in meiosis. (C) Quantification of SPB separation in $P_{CLB2}\text{-SFI1 } P_{DMC1}\text{-KAR1}$ cells. Note that more than 50% of cells underwent a second round of SPB duplication. (D, E) Quantification of SPB separation in $P_{CLB2}\text{-SFI1 } P_{DMC1}\text{-sfi1-6D}$ and $P_{CLB2}\text{-SFI1 } P_{DMC1}\text{-sfi1-6A}$ cells. Note that ectopically produced Sfi1-6A, but not Sfi1-6D, partially suppressed the phenotype of $P_{CLB2}\text{-SFI1}$ in meiosis. (F, G) Quantification of SPB separation in $P_{CLB2}\text{-SFI1 } P_{DMC1}\text{-sfi1-6D } P_{DMC1}\text{-KAR1}$ and $P_{CLB2}\text{-SFI1 } P_{DMC1}\text{-sfi1-6D } P_{DMC1}\text{-KAR1}$ cells. Note that five or more SPBs formed in the above strains in meiosis. (H, I) Quantification of SPB separation in $P_{CLB2}\text{-CDC14}$ and $P_{CLB2}\text{-CDC14 } P_{DMC1}\text{-KAR1}$ cells. Depletion of Cdc14 ($P_{CLB2}\text{-CDC14}$) in meiosis prevents cell exit from meiosis I. At least 100 cells were counted at each time point. The time-course experiments were repeated, and data from one representative experiment are shown.

We have found that overproduced Kar1 promotes SPB duplication to form supernumerary SPBs in meiosis, but SPB overduplicates only after metaphase I. These findings indicate that the execution point of Kar1-mediated SPB duplication occurs after the onset of metaphase I. Our EM analysis of the meiotic SPB reveals that morphologically, SPBs appear normal in Kar1-overproduced cells. This observation is consistent with the idea that the supernumerary SPBs found in Kar1-overproduced cells are competent in nucleating nuclear microtubules to form spindles and mediate chromosome segregation. But the rate of SPB duplication is accelerated in Kar1-overproduced cells, leading to supernumerary SPB formation. In Kar1-overproduced cells, the third SPB emerges within ~15 min after the first round of SPB separation (this article), which is about one-fourth of the duration of normal SPB duplication that is observed for meiosis II in wild-type cells (Shirk *et al.*, 2011). Duplication of the SPB starts with the deposition of the satellite material at the distal end of the SPB bridge (Cavanaugh and Jaspersen, 2017); we

speculate that increased protein level of Kar1 also promotes the nucleation of the satellite materials at the distal tip of the bridge.

We have shown that Sfi1 is essential for meiotic SPB duplication. However, in the presence of excessive Kar1, dephosphorylation of Sfi1 becomes less important to the regulation of SPB duplication. Our finding therefore is in contrast to that previously observed in mitotic cells where dephosphorylation of Sfi1 at anaphase licenses SPB duplication (Avena *et al.*, 2014; Elsefary *et al.*, 2014). Excessive Kar1 appears to override the requirement of changing the phosphorylation status of Sfi1 in meiosis. Supporting this idea, the SPB bridge appears intact in cells without the Sfi1 C-terminus, to which Kar1 binds and where the key Sfi1 phosphorylation sites are located (Avena *et al.*, 2014; Elsefary *et al.*, 2014; Seybold *et al.*, 2015). Importantly, removal of the C-terminus of Sfi1 maintains yeast cell viability at room temperature (Seybold *et al.*, 2015). We speculate that the C-terminus of Sfi1 becomes dispensable in the presence of excessive Kar1 in meiosis. Alternatively, independently of Sfi1, Kar1 may play a separate role in licensing SPB duplication in meiosis, which remains to be further determined.

Is meiosis unique for SPB duplication?

How, then, is SPB duplicated in meiosis? As in mitosis, SPB duplication requires Cdk1's kinase activity in meiosis (Carlile and Amon, 2008; Miller *et al.*, 2012). Activation of the B-type cyclin Cdk1 activity occurs early during the vegetative cell cycle; in contrast, the B-type cyclins, including Clb1, Clb3, Clb4, Clb5, and Clb6, are uniquely regulated in meiosis to ensure proper meiotic cell progression (Marston and Amon, 2004; Carlile and Amon, 2008; Miller *et al.*, 2012). Clb5- and Clb6-mediated Cdk1 activity is required for DNA replication and meiotic recombination and acts early in meiosis, whereas Clb1, Clb3, and Clb4 activate Cdk1 after the onset of metaphase I (Marston and Amon, 2004). In particular, *CLB3* is expressed in meiosis I, but its protein product only appears in meiosis II (Carlile and Amon, 2008). Our finding that supernumerary SPBs only form after the onset of metaphase I in Kar1-overproduced cells supports the idea that Clb1/3/4-Cdk1 activity is required for SPB duplication in meiosis (Miller *et al.*, 2012). In Kar1-overproduced cells, SPBs separate prematurely, and Kar1 licenses SPB duplication, therefore supernumerary SPB formation ensues. Finally, the SPB outer plaque is dramatically modified with meiosis-specific factors early on during meiosis (Neiman, 2011), which could exert a unique impact on SPB duplication in yeast meiosis.

The Kar1 protein appears to be highly modified during meiosis on the basis of its protein migration pattern (this work). Our preliminary study indicates that Kar1 is phosphorylated at 13 different sites in meiosis (unpublished data). Whether Kar1 phosphorylation plays

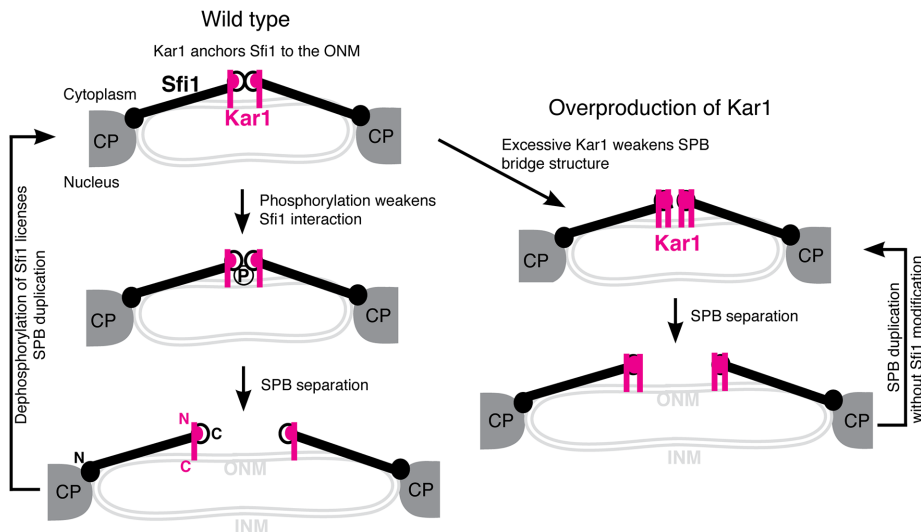


FIGURE 9: Model for excessive Kar1-mediated SPB separation and duplication in yeast meiosis. For simplicity, Cdc31 and Mps3 are omitted from the SPB half bridge. CP, central plaque; INM, inner nuclear membrane; ONM, out nuclear membrane; N, the N-terminus; C, the C-terminus.

a role in meiotic SPB duplication and separation will be determined in future studies.

Summary

This study reveals an unexpected role of Kar1 in licensing SPB duplication during yeast meiosis. We have shown that excessive Kar1 promotes SPB separation and induces supernumerary SPB formation, which causes chromosome missegregation. Kar1-mediated SPB duplication overrides the requirement of changing the phosphorylation status of Sfi1, demonstrating that Kar1 serves as an alternative licensing factor for SPB duplication during yeast meiosis.

MATERIALS AND METHODS

Yeast strains

All strains used in this study (Supplemental Table S1) are diploids isogenic to the SK1 genetic background. To generate meiosis-specific depletion alleles, including P_{CLB2} -*KAR1*, P_{CLB2} -*SFI1*, and P_{CLB2} -*CDC14*, we used a PCR-based strategy to replace the endogenous promoters with the mitosis-specific promoter from *CLB2* (Lee and Amon, 2003). A similar PCR-based strategy was used to tag Sfi1 with GFP to its C-terminus (Longtine *et al.*, 1998). The following alleles have been reported previously: P_{CLB2} -*IPL1*, P_{CLB2} -*CDC5*, P_{CLB2} -*CDC20*, $GAL4.ER$, P_{GAL} -*NDT80*, *TUB4-mApple*, *TUB4-GFP*, *MPS3-mApple*, and *TUB1-mApple* (Lee and Amon, 2003; Carlile and Amon, 2008; Shirk *et al.*, 2011; Li *et al.*, 2015). These gene mutations and fluorescent-protein-tagged alleles were first generated in Mata and Mat α haploid cells; homozygous diploids (zygotes) were obtained by mating the corresponding haploids.

Plasmids

Plasmids used in this study are included in Supplemental Table S2. To overproduce Kar1 in meiosis, we used the enhanced *DMC1* promoter (Fan *et al.*, 2017) to construct P_{DMC1} -*GFP-KAR1* (pHG433) and its variants (plasmids pHG465, pHG524, pHG549, and pHG614) by placing the *KAR1* open reading frame under the control of the *DMC1* promoter. The deletion alleles of *kar1-Δ15* and *kar1-Δ18* have been previously reported (Vallen *et al.*, 1992). To delete Kar1's transmembrane domain, we generated P_{DMC1} -*GFP-KAR1-ΔTMD* (pHG549) by removing the coding sequence of the C-terminal 22 amino acids of

Kar1. The P_{DMC1} -*GFP-KAR1* allele and its variants were linearized by *Afl*III and incorporated at the *LEU2* locus. Yeast strains with P_{DMC1} -*GFP-KAR1* and its variants retain the wild-type copy of the *KAR1* gene. To construct P_{KAR1} -*GFP-KAR1* (pHG465), we cloned a 418-base pair fragment upstream of the *KAR1* open reading frame to replace the *DMC1* promoter in pHG433. The plasmid pHG465, which harbors the *URA3* gene, was linearized by *Bgl*III and inserted at the *KAR1* locus. *URA3* positive colonies were counterselected in the 5'-FOA medium to remove the untagged copy of *KAR1* (Guthrie and Fink, 1991). Therefore, *GFP-KAR1*, which is under the control of its endogenous promoter, serves as the only copy of *KAR1* in the yeast genome (Figures 1B and 2 and Supplemental Figure S1B). Similarly, the P_{KAR1} -*V5-KAR1* allele (pHG559, Figure 1A and Supplemental Figure S1A) was under the control of its endogenous promoter and serves as the only copy of *KAR1* in the yeast

genome. The *SFI1* open reading frame was cloned and placed under the control of the *DMC1* promoter to generate P_{DMC1} -*SFI1* (pHG254). The alleles of *sfi1-6A* and *sfi1-6D* have been previously reported (Elserafy *et al.*, 2014), and they were under the control of P_{DMC1} for expression in meiosis (pHG434 and pHG435).

Yeast culture methods

A previously described method was used to induce yeast cells to undergo synchronous meiosis (Li *et al.*, 2015). Briefly, yeast colonies were inoculated in the yeast extract, peptone, dextrose (YPD) medium overnight and then diluted in the yeast extract, peptone, potassium acetate (YPA) medium to reach an OD₆₀₀ of 0.15–0.2. Yeast cells in YPA were vigorously shaken for ~16 h to reach an OD₆₀₀ of 1.4–1.6 and then transferred to the sporulation medium (2% potassium acetate); the time of transfer is referred to as $t = 0$ of meiosis in our time-course experiments. To induce the expression of the *GAL* promoter in meiosis, 2 μM β-estradiol (final concentration) was added to the culture medium 5 h after the transferring to the sporulation medium. An equal volume of ethanol was added to the control strains. All yeast cultures were grown at 30°C.

Live-cell fluorescence microscopy

A DeltaVision microscope system was used to acquire live-cell fluorescence images as before (Li *et al.*, 2015). Briefly, an agarose pad with 2% potassium acetate was prepared on a concave slide, and a small aliquot of yeast cells was placed on the agarose, sealed with a coverslip, and scoped for desired time duration (Shirk *et al.*, 2011). Images were acquired with a 63× (NA = 1.40) objective lens on an inverted microscope (IX-71, Olympus) equipped with a CoolSNAP HQ2 charge-coupled device camera (Photometrics). For time-lapse experiments, time interval was set at either 3 or 5 min, and 12 optical sections with 0.5-μm thickness were acquired at each time point. To reduce phototoxicity, we used a neutral density filter to reduce the intensity of the excitation light to ~30% or less of the equipment output.

Light microscopic data analysis

Microscopy images obtained from DeltaVision were deconvolved using SoftWorx. To determine the pole-to-pole distance in three

dimensions (Figure 1C and Supplemental Figure S2), we used the point-to-point measurement tool provided by SoftWorx. To determine the fluorescence intensity of GFP-Kar1 and Tub4-mApple at the SPB (Supplemental Figure 1), we defined a 6×6 pixel area and obtained the total fluorescence intensity from single optical sections. The net intensity was determined by subtracting the mean background from the total. We also used SoftWorx to generate the line-scan-intensity graphs shown in Figure 2. Projected images from multiple optical sections were used for display. For time-course experiments, we repeated at least twice, and data from one representative experiment are used for display.

Electron microscopy

Yeast cells were induced to undergo synchronous meiosis as described above. Eight hours after induction, cells were harvested by centrifugation and frozen on the Leica EM-PACT high-pressure freezer (Wetzlar, Germany) at ~ 2050 bar and then transferred to the Leica EM AFS for freeze substitution under liquid nitrogen into 2% osmium tetroxide/0.2% uranyl acetate/2% water/acetone. We used the following procedure for freeze substitution: -90°C to -80°C over 60 h, -80°C to -60°C over 5 h, -60°C for 4 h, -60°C to -20°C over 8 h, and -20°C to 0°C over 5 h, and, finally, samples were held at 0°C for 3 h. Samples were then removed from the AFS and brought to room temperature. Samples went through four changes of acetone over 1 h and were removed from the planchettes. They were embedded in acetone/Spurr's mixtures to a final concentration of 100% Spurr's over several days in a stepwise procedure as previously described (McDonald, 1999). Seventy-five-nanometer serial thin sections were cut on a Leica UC6 ultramicrotome, stained with uranyl acetate and Sato's lead, and imaged on a Zeiss Merlin SEM (Oberkochen, Germany) equipped with a STEM detector and Atlas software. The pixel size of acquired images was set at 4 nm. SPB plaque width and bridge size were determined on the basis of their pixel numbers. Original EM data can be downloaded from the Stowers Original Data Repository at <http://www.stowers.org/pubs/LIBPB-1305>.

Western blotting

We used a similar Western blotting procedure as described previously (Li *et al.*, 2015). Briefly, yeast cells were induced to undergo synchronous meiosis, cell aliquots were collected, and protein extracts were prepared for SDS-PAGE analysis. To determine the level of V5-Kar1, we used an anti-V5 antibody (1:10,000; Thermo Fisher Scientific, cat#CAB1001). The level of Pgk1 served as a loading control.

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