

Hop2 and Sae3 Are Required for Dmc1-Mediated Double-Strand Break Repair via Homolog Bias during Meiosis

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During meiosis, exchange of DNA segments occurs between paired homologous chromosomes in order to produce recombinant chromosomes, helping to increase genetic diversity within a species. This genetic exchange process is tightly controlled by the eukaryotic RecA homologs Rad51 and Dmc1, which are involved in strand exchange of meiotic recombination, with Rad51 participating specifically in mitotic recombination. Meiotic recombination requires an interaction between homologous chromosomes to repair programmed double-strand breaks (DSBs). In this study, we investigated the budding yeast meiosis-specific proteins Hop2 and Sae3, which function in the Dmc1-dependent pathway. This pathway mediates the homology searching and strand invasion processes. Mek1 kinase participates in switching meiotic recombination from sister bias to homolog bias after DSB formation. In the absence of Hop2 and Sae3, DSBs were produced normally, but showed defects in the DSB-to-single-end invasion transition mediated by Dmc1 and auxiliary factors, and mutant strains failed to complete proper chromosome segregation. However, in the absence of Mek1 kinase activity, Rad51-dependent recombination progressed via sister bias in the *hop2Δ* or *sae3Δ* mutants, even in the presence of Dmc1. Thus, Hop2 and Sae3 actively modulate Dmc1-dependent recombination, effectively progressing homolog bias, a process requiring Mek1 kinase activation.

INTRODUCTION

Meiosis employs two rounds of the cell division to produce haploid gametes (sperms or eggs), which are essential for sexual reproduction. During meiosis, programmed genetic recombination generates genetic diversity in organisms and ensures faithful chromosome segregation. Recombination events are

initiated by meiosis-specific programmed double-strand break (DSB) formation regulated by the topoisomerase-like protein Spo11 (Keeney, 2001). DSBs undergo an additional process to expose 3' single stranded DNA (ssDNA), which is mediated by the Exo1/Dna2 and Mre11/Rad50/Xrs2 complex (Cannavo and Cejka, 2014; Garcia et al., 2011). DSB ends with ~500 nucleotide (nt) 3' ssDNA tails preferentially interact with the homolog partner template rather than with the sister chromatid; these reactions are mediated by the RecA homolog proteins Dmc1 and Rad51 (Hong et al., 2013a; Lao et al., 2013). Dmc1 is a meiosis-specific recombinase found in most eukaryotes, including yeast, mice, and humans. Rad51 functions in homology searching and homolog pairing of DNAs during mitosis, but plays an auxiliary role in homology searching associated with Dmc1 during meiosis (Hong et al., 2013a). The *dmc1Δ* mutant in budding yeast shows an abnormal phenotype during recombination that reflects the essential role of this protein in meiotic recombination. A Dmc1-deficient strain exhibited defective DSB repair progression and deficient synaptonemal complex formation (Shinohara et al., 1997; Schwacha and Kleckner, 1997). It has been suggested that Dmc1 promotes DNA strand exchange to form synapsis exclusively between homologs, which is a process unique to meiotic recombination. In addition, DSBs accumulate in *dmc1Δ* mutants because of failed strand invasion, which triggers a checkpoint during meiotic prophase I. It has been reported that the Hop2-Mnd1 and Mei5-Sae3 complexes are dominant auxiliary factors for Dmc1 function and stimulate Dmc1-mediated ssDNA strand invasion during meiotic recombination (Chan et al., 2014; Ferrari et al., 2009). The Hop2-Mnd1 heterodimeric complex constructs a synaptic complex, which is a combination of 3' ssDNA and homologous double-strand DNA in the D-loop formation stage (Chi et al., 2007; Kang et al., 2015; Pezza et al., 2007). Previous studies showed that in *Saccharomyces cerevisiae*, *hop2Δ* or *sae3Δ* mutant exhibits prophase arrest and DSB accumulation (Henry et al., 2006; Leu et al., 1998; Tsubouchi and Roeder, 2002). The Mei5-Sae3 heterodimeric complex is specifically expressed in meiotic cells (Hayase et al., 2004; Tsubouchi and Roeder, 2004). *mei5Δ* or *sae3Δ* mutant exhibits inefficient spore viability, prophase arrest, and defective recombinant products (Hayase et al., 2004; Tsubouchi and Roeder, 2004). Biochemical studies have shown that the Mei5-Sae3 complex promotes nucleation of Dmc1 onto ssDNA (Tsubouchi and Roeder, 2004). Therefore, the Hop2-Mnd1 and Mei5-Sae3 complexes may be essential for homolog bias mediated by Dmc1-dependent recombination

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in early prophase I.

Mek1 kinase is expressed specifically during meiosis and is activated by the induction of DSBs (Niu et al., 2007). Mek1 kinase activity inhibits inter-sister DSB repair during meiosis by directly affecting the sister chromatid axes. The pattern of meiotic recombination by Dmc1 and Rad51 shows that Mek1 kinase involves in Rad51-mediated recombination (Hong et al., 2013; Lao et al., 2013). Related studies revealed that Rad51' strand exchange activity is inhibited by tightly controlled mechanisms that prevent the complex formation between Rad51 and Rad54 during meiotic recombination (Busygina et al., 2008). Thus, meiosis-specific Hed1 binds to Rad51, blocking the interaction with Rad54 and decreasing the binding affinity of Rad54 for Rad51 by phosphorylating Rad54 through Mek1 (Busygina et al., 2008; Niu et al., 2009). The choice of a partner for recombination during meiosis is highly modulated by inter-homolog repair pathways after DSB resection to expose the 3' end. Partner choice is modulated by Red1, Hop2, and Mek1 kinase activity (Hong et al., 2013a; Kim et al., 2010; Schwacha and Kleckner, 1997). Inactivation of Mek1 kinase promotes DSB repair to progress efficiently even in the absence of Dmc1; however, loss of homolog bias results in the repair of most DSBs via sister bias. In the absence of Mek1 kinase activity, cohesin channels the recombination to use the sister chromatid as a template, which is appropriate for mitotic DSB repair (mitotic mode) (Hong et al., 2013a). Thus, the sister chromatid is preferentially used as a partner template. This meiosis-specific pathway promotes homolog bias during the early stages post DSB, and additional pathways support that the bias is maintained during the interhomolog single-end invasion (IH-SEI) to interhomolog double-Holliday junctions (IH-dHJ) transition of interhomolog crossover (IH-CO) formation (Kim et al., 2010). The Hop2-Mnd1 complexes stimulate Dmc1-mediated strand exchange (Petukhova et al., 2005). Additionally, the Mei5-Sae3 complex co-localizes with Dmc1 and is required for Dmc1 function *in vivo* (Hayase et al., 2004; Tsubouchi and Roeder, 2004).

In this study, we investigated the roles of Hop2 and Sae3 during recombination in the presence or absence of Mek1 kinase activity, and found that both Hop2 and Sae3 promote homolog bias through a Dmc1-dependent pathway.

MATERIALS AND METHODS

Yeast strains

Saccharomyces cerevisiae strains used in this study included SK1 derivatives isogenic with homozygous *ho::hisG*, *leu2::hisG*, *ura3Δ* (*Pst1-Sma1*). All genotypes and strain details are described in Supplementary Table 1.

Culture media and time course

The culture media and meiotic time course were essentially performed as previously described (Kim et al., 2010). Cells were patched to YPG plates (1% yeast extract, 2% peptone, 3% glycerol, and 2% bactoagar) for 24 h at 30°C. To select single colonies, cells from the YPG plate were streaked onto YPD plates (1% yeast extract, 2% peptone, 2% glucose, and 2% bactoagar) and grown at 30°C for 2 days. A single diploid colony resulting from this streaking was inoculated into 2 ml liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose) and incubated at 30°C for 24 h. For synchronous meiosis, YPD cultures were inoculated in SPS medium (1% potassium acetate, 1% bacto-peptone, 0.5% yeast extract, 0.17% yeast nitrogen without amino acids, 0.5% ammonium sulfate, 0.05 M potassium biphthalate, and 2 drops/L antifoam [Sigma, USA],

pH 5.5) at a 1:500 dilution and cultured for 18 h. Meiosis was initiated in SPM medium (0.2% potassium acetate, 0.02% raffinose, and 2 drops/L antifoam). Meiotic cells were harvested and resuspended in 50 mM Tris-HCl and 50 mM EDTA. Cross-linking of cells was performed with psoralen under UV light for 10 min.

Physical analysis of meiotic recombination

Cultured cells at each time point were treated with Zymolyase (100T, US Biological, USA). Spheroplasted cells were subjected to guanidine-phenol extraction. DNA was precipitated with ethanol, and then resuspended in 50 mM Tris-HCl and 1 mM EDTA (Hong et al., 2013b; Kim et al., 2010). DNA concentration was measured using a Picogreen assay kit (Invitrogen, USA). For one-dimensional gel analysis, 2 μg genomic DNA was treated with *Xho*I (Enzymomics, Korea) for 3 h. Next, the DNA samples were loaded into a 0.6% Seakem LE agarose gel in TBE buffer at ~2 V/cm for 24 h. For two-dimensional gel analysis, DNA digested in the same manner was then loaded into a 0.4% Seakem Gold agarose gel without ethidium bromide in TBE buffer at ~1 V/cm for 21 h. After electrophoresis, the gel was stained for 30 min with 0.5 μg/ml ethidium bromide. Bands of interest were cut and arrayed on 2D gel trays. The gel was loaded in a two-dimensional manner. SeaKem LE agarose (0.8%) containing 0.5 μg/ml ethidium bromide was poured around the cut 1D gel array at 4°C. Gel electrophoresis was performed at ~6 V/cm for 6 h at 4°C. For crossover and non-crossover gel analysis, 2 μg of genomic DNA was treated with both *Xho*I and *Ngo*IV followed by 1D gel analysis. Enzyme digestion and 1D gel loading conditions were the same as those described above for 1D and 2D gel loading. Southern hybridization was conducted using ³²P-dCTP-labeled radioactive nucleotides reacted with a random primer labeling mixture (Agilent Technologies, USA). Radioactive signals were observed using a Bio-Rad phosphorimager and quantified using Quantity One (Bio-Rad, USA).

Meiotic division curves

Cells from SPM cultures were harvested and fixed in 40% ethanol containing 0.1 M sorbitol. To count the cells undergoing meiosis I and II, DAPI stock solution was added to each cell fraction. Nuclei stained with DAPI (approximately 200 cells per each time point) were counted under a fluorescence microscope as previously described (Kim et al., 2010).

RESULTS AND DISCUSSION

System for physical analysis of meiotic recombination

The progression of meiotic recombination was monitored by one-dimensional (1D) or two-dimensional (2D) agarose gel electrophoresis (Fig.1; Hunter and Kleckner, 2001; Kim et al., 2010; Oh et al., 2007). Specifically, programmed DSB is initiated at the *HIS4LEU2* locus only once in this position, and contains restriction polymorphisms to distinguish between the "Maternal" and "Parental" alleles as well as between meiotic recombination products (Fig. 1A). To obtain genomic DNA, the cells cultured in sporulation media were harvested at specific time points. The phenol-guanidine preparation method (Hong et al., 2013b; 2015; Kim et al., 2010; Koszul et al., 2008; Lee et al., 2015a) was used to prepare genomic DNA by Psoralen and UV cross-linking procedures, which involved treating each cell sample with psoralen and UV light exposure to fix the DNA interstrands. DNA was digested with the *Xho*I restriction enzyme and then subjected to 1D gel and 2D gel electrophoresis

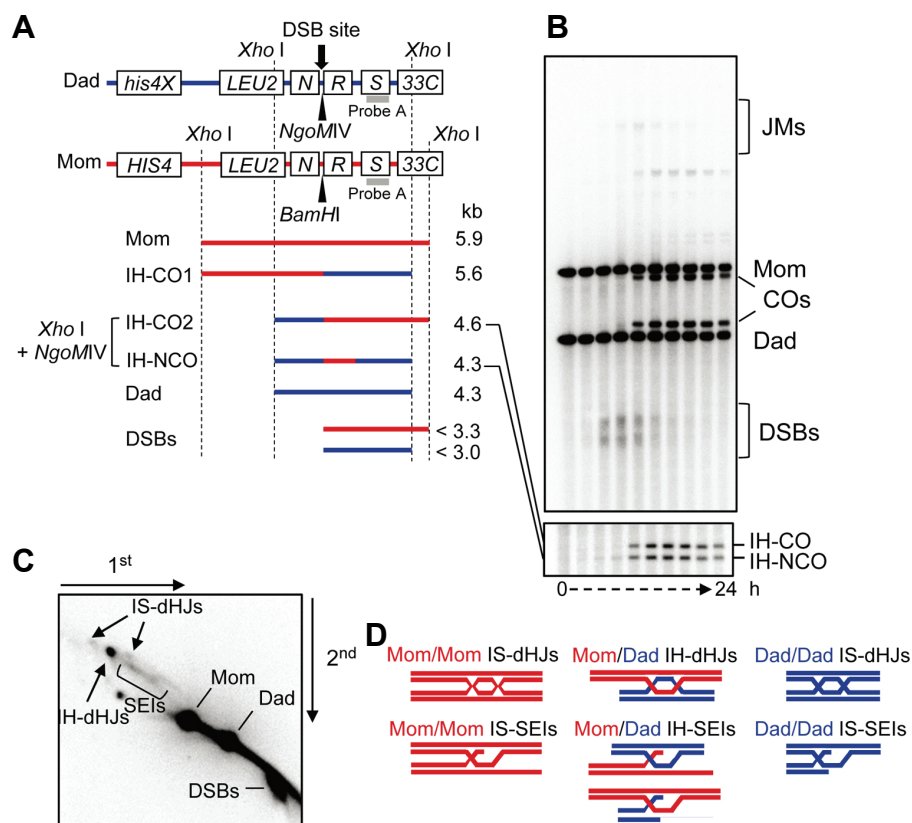


Fig. 1. Physical assay system for meiotic recombination. (A) Map of *HIS4LEU2* locus (Kim et al., 2010) with *XhoI* restriction enzyme sites and probe A position is shown below. “Dad” and “Mom” chromosomes were distinguished by *XhoI* restriction polymorphism. DSB, double-strand break; CO, crossover; IH-CO, interhomolog crossover; IH-NCO, interhomolog noncrossover. (B) 1D gel image of southern blot analysis in wild-type cells. Parental (Mom and Dad), COs, and DSBs shown in gel are described as in the figure. IH-COs and IH-NCOs were assayed using a *HIS4LEU2* locus containing *BamHI* (Mom) and *NgoMIV* (Dad) sites. (C) Images of native/native two-dimensional gel analysis showing joint molecules. (D) Structures of SEIs and dHJs in meiotic recombination. IS-SEIs, inter-sister single-end invasion; IH-SEIs, interhomolog single-end invasion. IS-dHJs, inter-sister double Holliday junctions; IH-dHJs, interhomolog double-Holliday junctions.

followed by Southern hybridization analysis to detect recombination intermediates and products (Figs. 1B and 1C). Meiotic recombination eventually produces IH-COs or interhomolog noncrossover (IH-NCO) without exchanging flanking genes (Fig. 1B; Börner et al., 2004; Hong et al., 2013; Kim et al., 2010). After digestion with both *XhoI* and *NgoMIV*, IH-COs and IH-NCOs from the *HIS4LEU2* locus were found to be 4.6 kb and 4.3 kb, respectively (Figs. 1A and 1B). IH and IS joint molecules (SEIs and dHJs) at the *HIS4LEU2* locus showed different molecular weights and shapes, enabling differentiation between the SEI and dHJ stages by 2D gel electrophoresis (Figs. 1C and 1D; Lee et al., 2015b; Hong et al., 2013a; Hunter and Kleckner, 2001; Kim et al., 2010).

Meiotic DSB repair is defective in the absence of Hop2 and Sae3

The absence of Hop2 and Sae3 eliminates homologous chromosome synapsis and causes defects in DSB repair during meiotic recombination (Petukhova et al., 2003; Neale and Keerey, 2006). In a previous *in vitro* study, Hop2 showed distinct activity; the Hop2-Mnd1 complex stimulated Dmc1 and Rad51 recombinase activity, and purified Hop2 promoted strand invasion (Pezza et al., 2014). The Mei5-Sae3 complex interacts with Dmc1 to promote assembly of 3' ssDNA on one side of a DSB (Gerton and Hawley, 2005). The physical analysis DNA in meiotic recombinant structures at the *HIS4LEU2* locus has been used to examine the functions of Hop2 and Sae3 during meiotic recombination and their relationships with Mek1 kinase activity to understand the partner choice pathway. DSB formation in *hop2Δ* and *sae3Δ* mutants was analyzed by

1D gel electrophoresis (Fig. 2A). We further introduced the *mek1as* allele that is inactivated Mek1 kinase activity in the presence of 1-NA-PP1. All *hop2Δ mek1as(-IN)* and *sae3Δ mek1as(-IN)* cells showed defective DSB turnover, and DSB levels accumulated up to ~36% compared to the control at 10 h, and hyper-resected DSBs were also observed (Fig. 2). These results suggest that Hop2 and Sae3 are essential for promoting the DSB-to-SEI transition during meiosis. However, in the presence of a chemical inhibitor (Mek1 kinase inactivation), DSBs were efficiently processed, but there was a large number of hyper-resected DSB. IH-CO levels were not effectively increased compared to wild-type levels. Additionally, this result implies that Mek1 kinase inactivation is associated with the progression of DSB repair, but IH-CO levels were not detectable (Fig. 2). Based on these results, we suggest that Hop2 and Sae3 are necessary for DSB repair during meiotic recombination.

Hop2 and Sae3 are involved in meiotic progression after DSB formation

DSBs were analyzed in the *dmc1Δ mek1as*, *hop2Δ mek1as*, and *sae3Δ mek1as* strains at the *HIS4LEU2* locus where programmed DSBs are produced at one site (Figs. 1 and 3). As essential mediators, Exo1 and Mre11 together with Dna2 execute meiotic DSB end resection, specifically in the absence of Dmc1 (Garcia et al., 2011; Hong et al., 2013a; Lukaszewicz et al., 2015; Zakharyevich et al., 2010). *dmc1Δ* displays DSB accumulation and hyper-resection (Fig. 3A), whereas in *dmc1Δ mek1as(+IN)* strains, significantly reduced DSBs were observed in 2D gel analysis. Since Dmc1 plays a role in meiotic

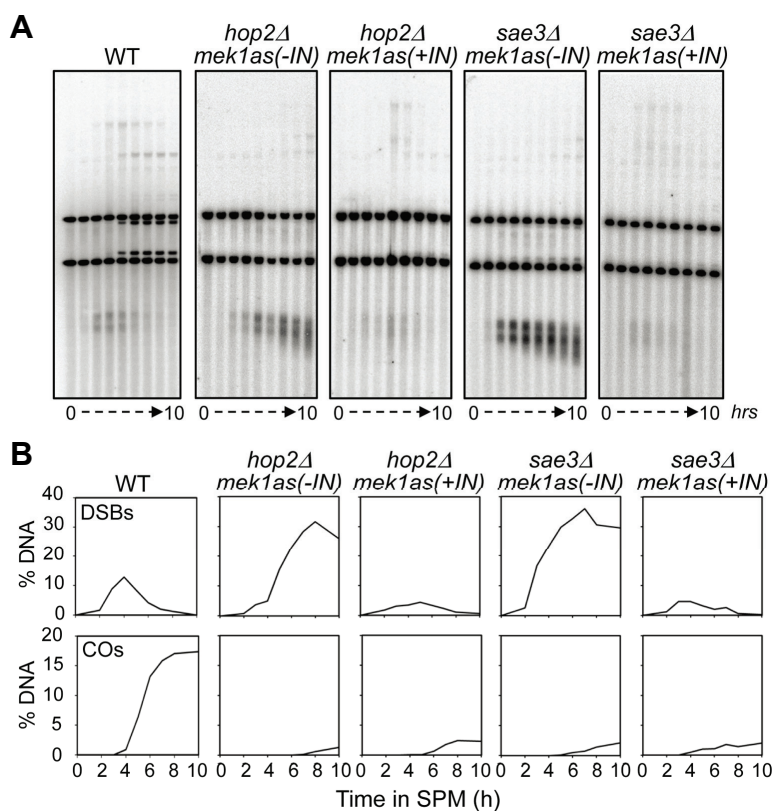


Fig. 2. Analysis of CO and DSB formation in *hop2Δ mek1as* and *sae3Δ mek1as* strains. (A) 1D gel analysis of DSB and CO in *hop2Δ mek1as* and *sae3Δ mek1as* strains. Synchronous meiosis was induced in SPM medium in the presence or absence of 1-NA-PP1. (-IN), absence of 1-NA-PP1; (+IN), presence of 1-NA-PP1, Mom, mom species; Dad, dad species; COs, crossover species; DSBs, double-strand breaks. (B) Quantitative analysis of DSB and CO in the meiotic cultures. Percentage of each DNA species versus total hybridizing DNA signals is plotted.

DSB repair, Rad51 critically functions in mitotic DSB repair in the absence of Mek1 kinase activity (Hong et al., 2013a). When Mek1 kinase was active in *hop2Δ mek1as(-IN)* and *sae3Δ mek1as(-IN)*, DSB resection patterns resulted in hyper-resection of the DSB ends, leading to the production of extensive single-strand DNA tails at the 3' end over time, as observed by 2D gel electrophoresis (Fig. 3). In the presence of Mek1 kinase activity, DSB levels remained elevated above the background signals detected in the wild-type strain because of a deficiency in DSB repair, causing DSB accumulation to higher steady-state levels. In contrast to *mek1as(+IN)*, where Mek1 kinase was inactive, *hop2Δ mek1as(-IN)* and *sae3Δ mek1as(-IN)* showed early DSB hyper-resection, reaching maximum levels approximately 8-10 h into meiosis; DSBs were processed after 8 h (Fig 3B). Thus, Mek1 kinase inhibits DSB repair in the absence of Hop2 and Sae3, but when Mek1 kinase is inactivated, the cells progress efficiently into a DSB repair state via a pathway other than IH-CO progression (Figs. 2 and 4).

Hop2 and Sae3 are not required for joint molecule formation in the absence of Mek1 kinase activity

Hop2 plays a role in the DSB-to-SEI transition through Dmc1-mediated strand invasion. Hop2 is a meiosis-specific protein that localizes to the chromosomes to prevent joining between nonhomologous partners and further promote joining with a homologous partner (Pezza et al., 2007). Moreover, Hop2 functions as a heterodimer complex with Mnd1 to recognize partner template DNA and to mediate its repair during meiosis, particularly by homologous pairing (Chan et al., 2014; Leu et al., 1998; Tsubouchi and Roeder, 2002). Sae3 is also an essential accessory factor that forms hetero-complex with Mei5 to assist

Dmc1 to promote the meiotic DSB repair process (Hayase et al., 2004; Tsubouchi and Roeder, 2004). We investigated SEI and dHJ formation in wild-type, *dmc1Δ mek1as*, *hop2Δ mek1as*, and *sae3Δ mek1as* strains that were inactivated in the presence of 1-NA-PPI inhibitor (Fig. 4). The wild-type cells showed 1.4% of IH-dHJ and 0.25% of IS-dHJ, and the ratio of IH:IS-dHJ was approximately 5:1. In the *dmc1Δ mek1as(-IN)* strain, both IH-dHJ and IS-dHJ were not detected in cells, whereas in *dmc1Δ mek1as(+IN)*, the IH-dHJ levels peaked at 0.3% but IS-dHJ remained undetected (Fig. 4A and 4B). In the *hop2Δ mek1as(-IN)* strain, IH-dHJ levels were approximately 0.1%, but IS-dHJs were undetectable in cells; whereas *hop2Δ mek1as(+IN)* showed ~0.1% IH-dHJ and ~1% IS-dHJ, with a ratio of IH-dHJ to IS-dHJ approximately 1:9 (Fig. 4C and 4D; Supplementary Table 2). In the *sae3Δ mek1as(-IN)* strain, cells exhibited 0.1% IH-dHJ and 0.25% IS-dHJ, whereas *sae3Δ mek1as(+IN)* cells displayed close to 0% IH-dHJ and 0.75% IS-dHJ levels (Figs. 4C and 4D). Thus, Mek1 kinase inactivation triggered DSB repair and JM formation by converting the recombinational mode from IH bias to IS bias. Further, in *mek1as(+IN)* cells, SEIs appeared to form inter-sister arcs of SEI going to IS-dHJ (Fig. 4; Kim et al., 2010). Taken together, these results indicate that when Mek1 kinase was inactivated, cells underwent DSB repair through the inter-sister crossover (IS-CO) pathway. Thus, Mek1 kinase regulates recombination in the functional stages of Hop2 and Sae3 to convert from the mitotic mode into the meiotic mode.

Both Hop2 and Sae3 are required for the production of interhomolog recombination products

Homologous recombination leads to CO or NCO, the outcomes

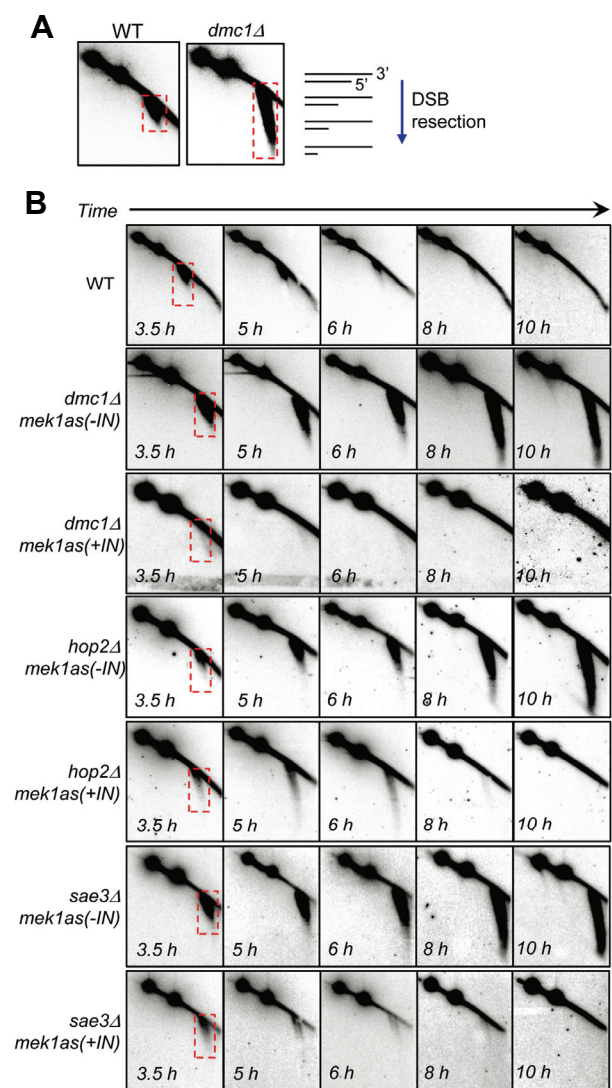


Fig. 3. DSB resection in WT, *dmc1Δ mek1as*, *hop2Δ mek1as*, and *sae3Δ mek1as* strains. (A) Representative images marking DSB resection location by two-dimensional gel analysis. (B) DSB resection patterns at each time point. Dot boxes indicate DSB area of the 2D gel.

of meiotic DSB repair, in the flanking region of paired chromosomes (Allers et al., 2001; Mancera et al., 2008; Martini et al., 2006). CO and NCO products can be distinguished at the *HIS4LEU2* locus after digestion with *XhoI* and *NgoMIV*. In wild-type cells, the maximum levels of CO and NCO were 3.8% and 3.2%, respectively. However, in the *hop2Δ mek1as* and *sae3Δ mek1as* strains, regardless of whether 1-NA-PP1 inhibitor was present, the CO and NCO levels were less than 0.3% (Figs. 5A and 5B). Because *hop2Δ mek1as(+IN)* and *sae3Δ mek1as(+IN)* exhibit DSB hyper-resection and accumulation (Fig. 3B) without ongoing repair, low levels of COs and NCOs are expected. In contrast, DSBs in the *hop2Δ mek1as(+IN)* and *sae3Δ mek1as(+IN)* strains were repaired as in the wild-type strain (Fig. 3B) and the IS-dHJ level was highly increased compared to levels in cells without a 1-NA-PP1 inhibitor. Thus, since 1D gel analysis can only detect IH-COs or IH-NCOs,

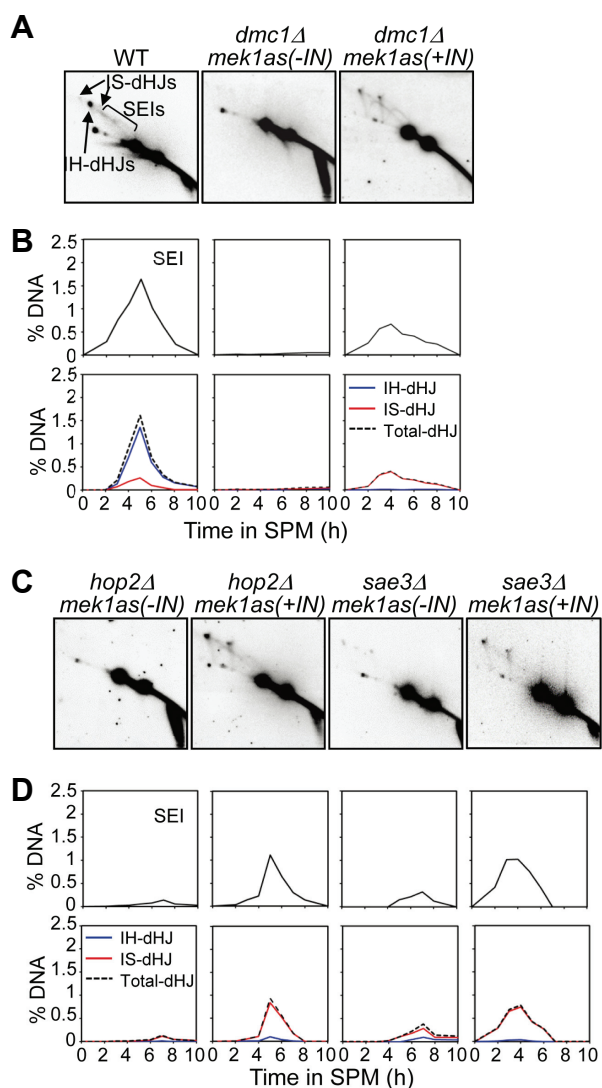


Fig. 4. Analysis of dHJ and SEI formation in WT, *dmc1Δ mek1as*, *hop2Δ mek1as*, and *sae3Δ mek1as* strains in the presence of 1-NA-PP1. (A) Representative images of 2D gel analysis in WT and *dmc1Δ mek1as* strains. (B) Quantitative analysis of SEIs and dHJs from two-dimensional gel images. Dot lines represent total dHJ levels. (C) Representative images of 2D gel analysis in *hop2Δ mek1as* and *sae3Δ mek1as* strains. (D) Quantitative analysis of SEIs and dHJs from two-dimensional gel images. Dot lines represent total dHJ levels.

DSBs processed into IS-dHJ were resolved to IS-COs or IS-NCOs (Fig. 5C). This explains why DSB repair enters the mitotic mode, which uses sister templates rather than homologous templates, as in meiotic mode. Hence, Hop2 and Sae3 play roles in the fate of meiotic DSBs and determine whether programmed DSBs are processed by homolog bias or sister bias by Mek1 kinase. When Mek1 kinase activity is inhibited, the meiotic DSB repair procedure is directed towards the sister bias mechanism that use sister chromatids as templates (Tracy et al., 2010). In this case, DSBs are processed in the mitotic mode and are finally resolved with IS-NCO or IS-CO. However, more precise methods are required to distinguish these products. If

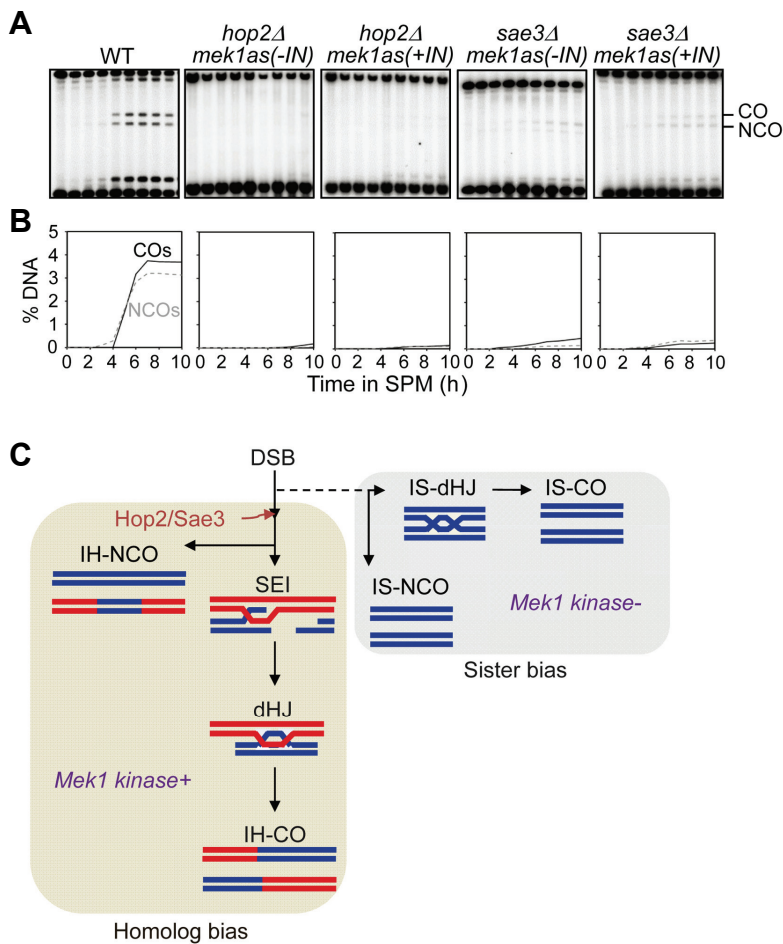


Fig. 5. CO and NCO formation in WT, *hop2Δ mek1as*, and *sae3Δ mek1as* strains. (A) Representative one-dimensional physical analysis gel images of WT, *hop2Δ mek1as*, and *sae3Δ mek1as* strains. (B) Quantitative analysis graphs showing COs and NCOs from one-dimensional gel images. (C) Proposed model for the roles of Hop2 and Sae3 in interhomolog recombination during meiosis.

Mek1 kinase plays the same role as in wild-type cells, the default option for programmed DSB repair is homologous bias, which uses homologous chromatids for recombination. When cells undergo defects in the SEI-to-dHJ transition, some of the SEIs may be converted into IH-NCO products, while SEI is stably converted into an IH-CO product when maintenance is sustained. Both Hop2 and Sae3 function during early prophase I before the SEI step as accessory factors of Dmc1, and therefore programmed DSBs are hyper-resected and accumulate because when subsequent pathways are blocked, such as in the absence of Hop2 or Sae3 (Fig. 5C).

In most organisms, recombinational interactions precisely occur between homologs to promote the efficient pairing of chromosomes and synapsis formation during meiosis. Dmc1 is a key factor in meiotic recombination and is required for homolog bias in the intrinsic pathway in meiosis. The budding yeast proteins Hop2 and Sae3 are assembled on DNA to promote heteroduplex DNA formation in a manner that affects Dmc1 strand exchange activity during meiotic recombination. Dmc1 and its accessory factors, Hop2 and Sae3, ensure that the first DSB ends are properly assembled onto the partner template to form a D-loop and that stably form SEI, which gives rise to the dHJ. These results suggest that during the DSB repair pathway, an early decision between the interhomolog and inter-sister pathway occurs, with most inter-sister CO arising from sister

bias, and interhomolog CO events progressing to form an IH-dHJ. Further, IH pathway is switched to IS pathway when Mek1 kinase becomes inactive (Hong et al., 2013b). Thus, these results indicate that Hop2 and Sae3 require Mek1 kinase activity, which also plays a role in the post-DSB stage to promote Dmc1-mediated meiotic recombination.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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