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The Logic and Mechanism of Homologous Recombination Partner Choice

Soogil Hong,[1] Youngjin Sung,[1] Mi Yu,[1] Minsu Lee,[1] Nancy Kleckner,[2,*] and Keun P. Kim[1,*]
[1]Department of Life Sciences, Chung-Ang University, Seoul 156-756, Korea
[2]Department of Molecular and Cellular Biology, Harvard University, 52 Oxford Street, Cambridge, MA 02138, USA
*Correspondence: kleckner@fas.harvard.edu (N.K.), kpkim@cau.ac.kr (K.P.K.)
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SUMMARY

Recombinational repair of spontaneous double-strand breaks (DSBs) exhibits sister bias. DSB-initiated meiotic recombination exhibits homolog bias. Physical analysis in yeast reveals that, in both cases, the recombination reaction intrinsically gives homolog bias. From this baseline default, cohesin intervenes to confer sister bias, likely independent of cohesion. In meiosis, cohesin's sister-biasing effect is counteracted by RecA homolog Rad51 and its mediators, plus meiotic RecA homolog Dmc1, which thereby restore intrinsic homolog bias. Meiotic axis complex Red1/Mek1/Hop1 participates by cleanly switching recombination from mitotic to meiotic mode, concomitantly activating Dmc1. We propose that a Rad51/DNA filament at one DSB end captures the intact sister, creating an anchor pad. This filament extends across the DSB site on the intact partner, precluding intersister strand exchange, thus forcing use of the homolog. Cohesin and Dmc1 interactively modulate this extension, with program-appropriate effects. In accord with this model, Rad51-mediated recombination in vivo requires the presence of a sister.

INTRODUCTION

Homologous recombination is essential for repair of double-strand breaks (DSBs) in all cells. During meiosis, recombination creates genetic diversity and promotes pairing and segregation of homologs (Kleckner et al., 2011). Due to their different biological imperatives, DSB repair and meiosis require qualitatively different partner choices. For DSB repair, the sister chromatid is the preferred partner (Kadyk and Hartwell, 1992; Johnson and Jasin, 2001; Bzymek et al., 2010; Rong and Golic, 2003). Sister bias minimizes the possibility that repair will alter the state of the genome by interactions between nonallelic pseudohomologous sequences. Moreover, crossovers between nonsister chromosomes create interchromosomal connections that can disrupt regular mitotic sister segregation (Beumer et al., 1998). For meiosis, all important roles of recombination require that interactions occur between homologs.

How these two alternative partner choices are differentially specified in the two programs remains mysterious. However, an implicit or explicit cornerstone of most considerations is the idea that the biochemical process of strand exchange is neutral with respect to partner selection and that the default option for partner choice is use of the sister simply because it is nearby (e.g., Kadyk and Hartwell, 1992; Johnson and Jasin, 2001). In contrast, homolog bias for meiosis requires special, program-specific features (e.g., Sheridan and Bishop, 2006). The results presented below suggest that this formulation is not correct.

Meiotic recombination (Figure 1A) initiates via programmed DSBs. One DSB end searches for a partner and engages a homolog chromatid duplex via a nascent D loop. The other DSB end remains associated with its sister, perhaps also in a nascent D loop. These DNA events are integrated with structural features of the developing chromosome structural axes, which are concomitantly drawn together in space. As the culmination of these events, recombinational interactions comprise ~400 nm bridges that link the homolog axes, with one DSB end and its associated recombinosome components associated with each axis (Storlazzi et al., 2010; Figure 1A). Formation of these bridges along the lengths of the chromosomes comprises homolog pairing, and the resulting configuration is presynaptic alignment. In some organisms, events at the two DSB ends may be controlled by two different RecA homologs: meiotic Dmc1 at the homolog-associated end and mitotic Rad51 at the sister-associated end (Shinohara et al., 2000; Hunter, 2006; Kurzbauer et al., 2012). An important implication of this bridge stage for recombination is that the two DSB ends are in direct physical, and thus presumptively functional, linkage.

As recombination progresses, the ends-apart bridge ensemble undergoes a differentiation step: a subset of these intermediates is designated to become interhomolog (IH) crossover (CO) recombination products, while the remainder matures in another way(s), primarily as IH noncrossovers (NCOs). Thereafter, along the CO branch of the pathway, DNA synthesis is initiated at one of the two ends. In the majority of cases, this extension occurs at the homolog-associated end (Figure 1A, top right). As extension progresses, the sister-associated end is released and anneals to the developing ensemble, thereby being drawn into the IH recombination complex. Further events then lead to IH double Holliday junctions (dHJs), which in turn mature specifically to IH COs. Importantly, it is extension at the homolog-associated DSB end that commits the reaction to making a dHJ (and then a CO) between homologs, rather than

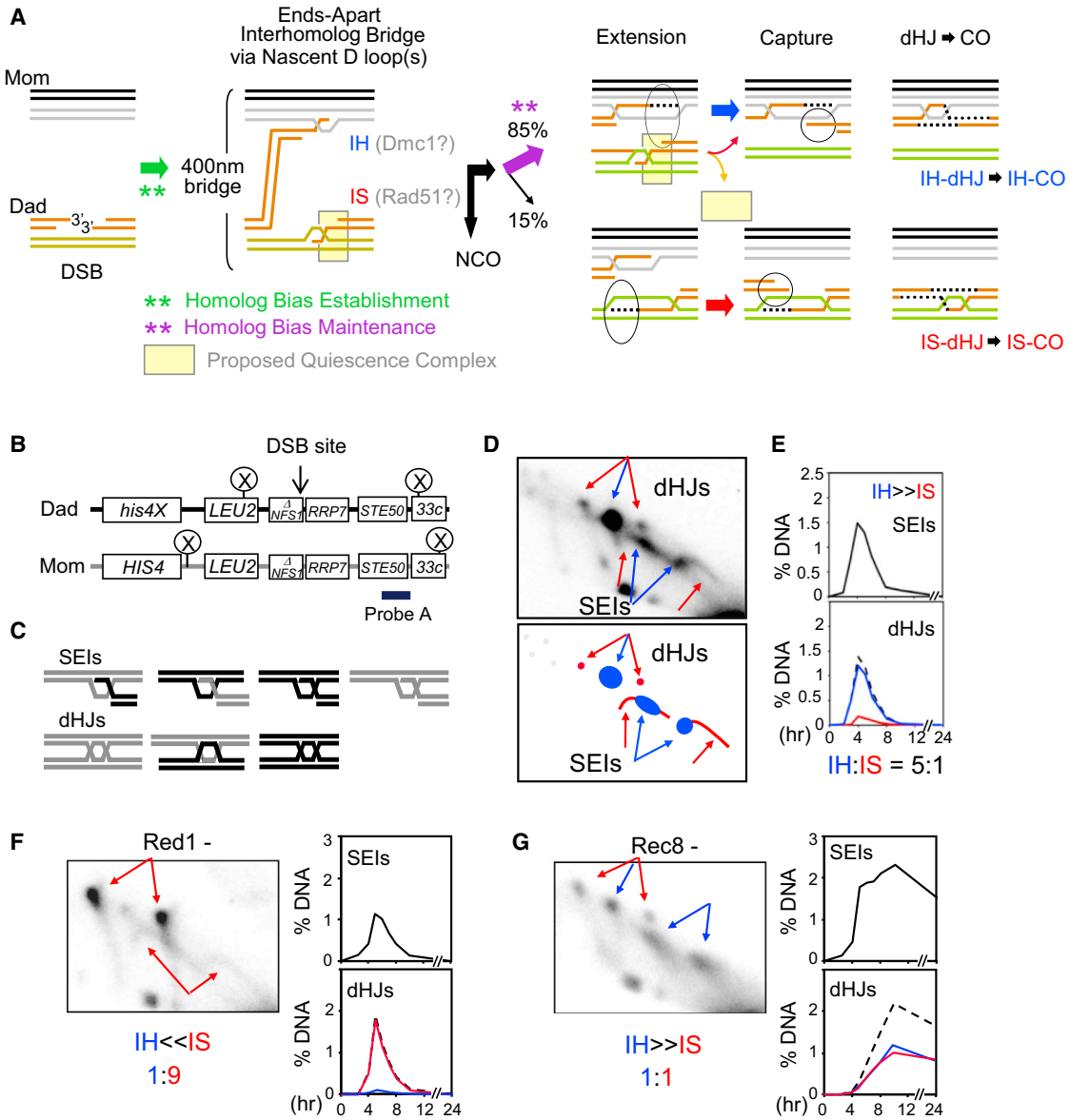


Figure 1. Meiotic Recombination: Pathway and Physical Analysis

(A) Key steps in meiotic recombination between one sister of each homolog (Hunter, 2006; Storlazzi et al., 2010; Kim et al., 2010).

(B) Physical map of *HIS4LEU2* double-strand break (DSB) hot spot. Parental homologs, Mom and Dad, are distinguished via *Xho*I restriction polymorphisms (X). For all data, DNA is digested with *Xho*I, species separated on 1D or 2D gels, and detected by Southern blot hybridization with probe A (Kim et al., 2010).

(C) Structures of SEI and dHJ intermediates. Mom- and Dad-derived DNA duplexes are in black and gray, respectively. SEI, single end invasion; dHJ, double Holliday junction.

(D) Representative 2D gel. IH/IS SEI and IH/IS dHJ species are indicated with arrows. Blue and red indicate IH and IS species, respectively.

(E) SEI and dHJ levels are shown as a percentage of total DNA over time during meiosis. Top inset: semiquantitative assessment of IH SEI versus IS SEI levels. Bottom: IH dHJs, IS dHJs, and their sum (blue, red, and dashed black lines, respectively). IH:IS dHJ ratio at time of maximal abundance is given below the graph.

(F and G) 2D gel analysis of SEIs and dHJs in *red1Δ* (F) and *rec8Δ* (G) strains (from Kim et al., 2010).

between sisters. In a minority of cases, extension occurs at the sister-associated end, leading to a dHJ between sisters (and then presumably to an intersister [IS] CO) (Figure 1A, bottom right). At the well-characterized yeast *HIS4LEU2* recombination hot spot, 85% of dHJs are IH dHJs, giving an IH:IS dHJ ratio of ~5:1.

Further insight into the control of recombination is provided by the finding that, in a number of different yeast mutants, the IH:IS dHJ ratio at *HIS4LEU2* is reduced from ~5:1 to exactly 1:1 (Kim et al., 2010). Occurrence of this precise 1:1 ratio, in many different situations (see also below), is explained as follows. The ends-apart bridge intermediate forms as usual. Then, with

the two DSB ends now in communication, one end or the other is selected to undergo synthesis-mediated extension, just as in wild-type (WT). However, in the mutant cases, the two ends are functionally symmetrized such that either end can initiate synthesis with equal probability (50% each, rather than 85% and 15% as in WT). As a result, dHJs form with equal probability between homologs or between sisters, irrespective of the fact that one end had formed a single end invasion (SEI) (Kim et al., 2010; Supplemental Experimental Procedures available online). Formation of the bridge intermediate implies that a DSB has already chosen a homolog partner, rather than its sister (i.e., homolog bias has been established). Thus, existence of a mutant phenotype involving a 1:1 IH:IS dHJ ratio implies the existence of some subsequent process that acts to maintain homolog bias after it is established by bridge formation (Figure 1, purple asterisks), with mutants exhibiting a 1:1 IH:IS dHJ ratio being defective in that process. A mutant exhibiting a 1:1 IH:IS dHJ phenotype is Establishment-Plus and Maintenance-Minus.

Maintenance of homolog bias is proposed to involve a quiescence complex that keeps the sister-associated end inactive until it is time for its incorporation into the evolving interhomolog reaction (Figure 1, yellow box; Kim et al., 2010). Without this complex, the sister-associated end and the homolog-associated end would have equivalent potential for undergoing extension, thus giving rise to IH and IS dHJs at an equal frequency.

Budding yeast studies also identify mutants in which recombination occurs efficiently, but only between sister chromatids (Kim et al., 2010). Such mutants are said to be defective in the establishment of homolog bias (Establishment-Minus). This defect is most simply attributed to a failure to form the ends-apart bridge complex, with ensuing events then occurring only between sisters (Figure 1, green asterisks).

Two types of molecules have been implicated in the establishment of homolog bias. First is the interacting complex of meiotic axis-associated proteins, Red1, Hop1, and Mek1 kinase (hereafter RMH) (Kim et al., 2010). Red1 and Hop1 are physically interacting abundant structural components; Mek1 is a Rad53-related kinase that associates with Red1/Hop1 along chromosome axes. Second are the RecA homologs. In WT meiosis, Dmc1 mediates strand exchange while mitotic RecA homolog Rad51 plays specialized roles, including a central role in homolog bias establishment (Cloud et al., 2012). Dmc1 is also implicated in homolog bias via an interhomolog interaction function of unknown nature (Schwacha and Kleckner, 1997; Sheridan and Bishop, 2006).

Rad51 activity is modulated by additional factors. For mitotic recombination, Rad51 activity is promoted by mediator proteins (Krejci et al., 2012), e.g., essential Rad51 loading factors (Rad55/57) and the PCSS complex (Psy1, Csm2, Shu1, and Shu2), which is important but not essential (Ball et al., 2009; Krejci et al., 2012; Shor et al., 2005; Tao et al., 2012; Qing et al., 2011; Sasanuma et al., 2013). In meiosis, Rad51 strand exchange is directly, specifically inhibited by meiosis-specific factor Hed1 (Busygina et al., 2012; Tsubouchi and Roeder, 2006). Modulator roles for partner choice are analyzed below.

Here, we further examine the logic and mechanism of recombination partner choice by physical analysis of recombination at the *HIS4LEU2* hot spot during meiosis in WT and various mutants. We identify diverse situations in which homolog bias establishment is defective, thus revealing the nature of the establishment process in RMH^+ conditions and the nature of recombination in the establishment-defective condition in which RMH functions are absent. In all of these situations, Rec8 (cohesin) is present. We show that in every Establishment-Minus mutant condition analyzed, if Rec8 is then also eliminated, a 1:1 IH:IS dHJ phenotype is observed. That is, elimination of Rec8 converts all Establishment-Minus conditions to the Establishment-Plus Maintenance-Minus condition. Thus, elimination of Rec8 renders all of the factors involved in establishment of bias in RMH^+ conditions irrelevant. Elimination of Rec8 also permits establishment of homolog bias in RMH^- conditions in which (as we show) meiotic components are not functionally involved. These patterns imply that (1) the default option for recombination is homolog bias, irrespective of whether strand exchange is promoted by Dmc1 or Rad51 and regardless of whether or not other meiotic recombination components are participating; (2) Rec8 (cohesin) actively enforces sister bias; and (iii) the role of meiotic components is to counteract this active effect of cohesin. Finally, we explore interplay among Rad51, Rec8, and the sister chromatid for dHJ formation. All findings are integrated into a coherent model.

RESULTS

Experimental System

Two-dimensional (2D) gel analysis of physical DNA recombination intermediates at the *HIS4LEU2* locus was carried out as described (Figures 1B–1E; Hunter and Kleckner, 2001; Kim et al., 2010; Oh et al., 2007). IH and IS SEIs and dHJs have diagnostic mobilities due to distinguishable molecular weights and shapes (Figures 1C and 1D). In WT meiosis, dominance of IH species over IS species characteristic of meiotic recombination is quantifiable at the dHJ stage (IH:IS dHJ ratio = 5:1; Figures 1D and 1E; Kim et al., 2010). IH SEIs also predominate over IS SEIs (Figures 1D and 1E). Partial overlap of different signals precludes precise quantification of relative levels (Supplemental Experimental Procedures).

Mutants defective in establishment of homolog bias (Introduction) exhibit a very low IH:IS ratio and strong prominence of IS SEIs over IH SEIs (e.g., IH:IS dHJ = 1:9 for *red1Δ*; Figure 1F) as well as reductions in all IH products, COs, and NCOs, as defined by one-dimensional (1D) gels (Figure S1; Kim et al., 2010).

Mutants defective in maintenance of homolog bias exhibit a diagnostic 1:1 IH:IS dHJ ratio (Introduction; Figure 1G for *rec8Δ*). In this particular mutant, IH SEIs predominate over IS SEIs as in WT meiosis. The opposite bias can also be observed, implying that SEI status is not relevant to ultimate symmetrization of the two DSB ends for dHJ formation (Supplemental Experimental Procedures). The observation of a precise 1:1 IH:IS dHJ ratio, reproducibly and in diverse situations (Kim et al., 2010; text below and Table S1), also suggests that IS and IH dHJs are equally long lived, contrary to other suggestions (Goldfarb and Lichten, 2010).

The primary phenotype used to define functional relationships is the IH:IS dHJ ratio. IH:IS dHJ phenotypes of all analyzed

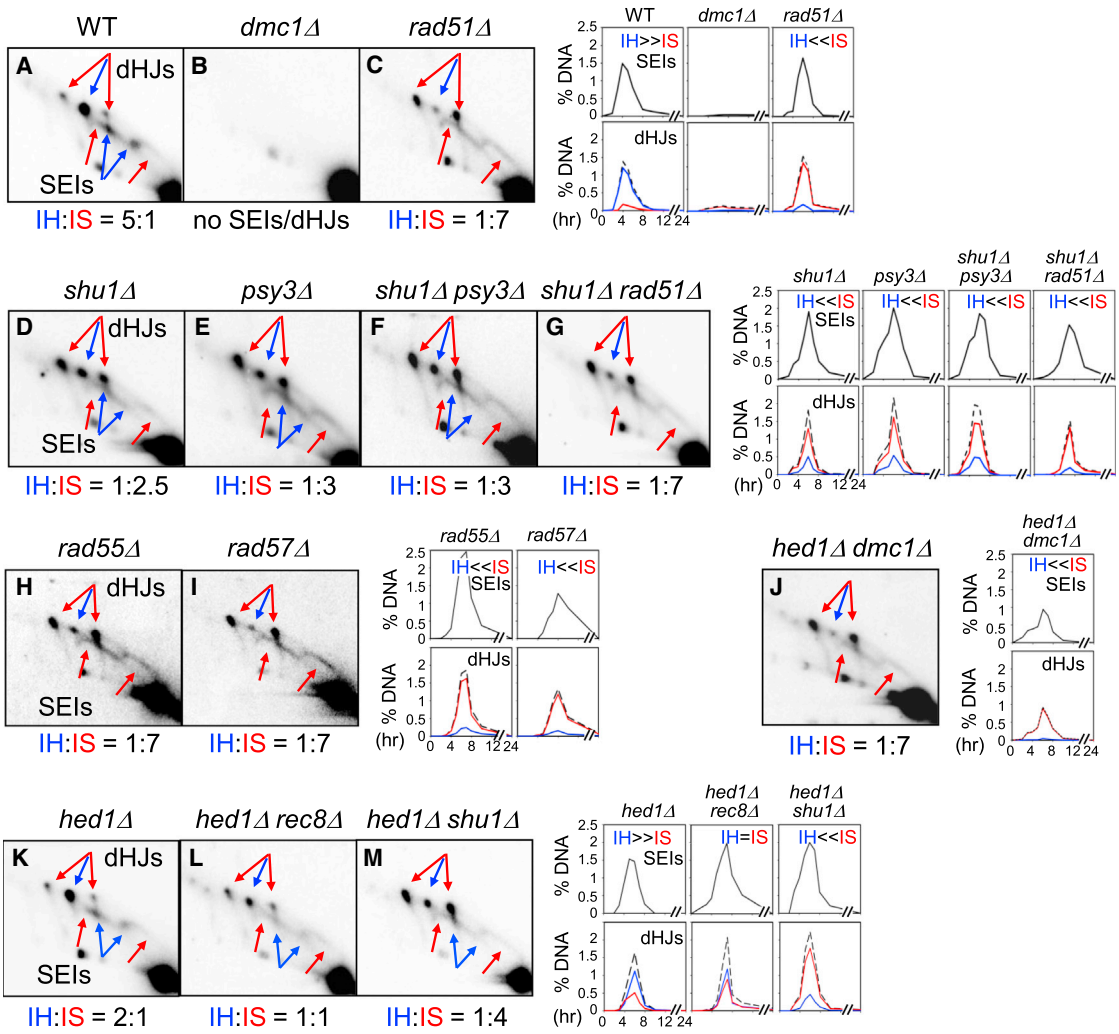


Figure 2. Roles of Dmc1, Rad51, and Rad51 Mediators in Meiotic Recombination

Analysis of SEIs and dHJs (as in Figures 1D and 1E) in RMH$^+$ meiosis (all strains were *mek1as [−IN]*; Experimental Procedures).

(A–C) Analysis of SEIs and dHJs in WT (A), *dmc1Δ* (B), and *rad51Δ* (C).

(D–G) Analysis of SEIs and dHJs in *shu1Δ* (D), *psy3Δ* (E), *shu1Δ psy3Δ* (F), and *shu1Δ rad51Δ* (G).

(H and I) Analysis of SEIs and dHJs in *rad55Δ* (H) and *rad57Δ* (I).

(J–M) Analysis of SEIs and dHJs in *hed1Δ* strains with *dmc1Δ* (J); without *dmc1Δ*, *rec8Δ*, or *shu1Δ* (K); with *rec8Δ* (L); and with *shu1Δ* (M).

strains are summarized in Table S1. Relative abundance of IH versus IS SEIs, though of secondary importance, has also been evaluated (Supplemental Experimental Procedures).

Partner Choice in RMH$^+$ Meiosis

The Entire Rad51 Mediator Ensemble Is Required for the Establishment of Homolog Bias

Distinct roles of Dmc1 and Rad51 in promoting strand exchange and mediating homolog bias (Cloud et al., 2012) are seen in 2D gel phenotypes. When Dmc1 is absent, no SEIs or dHJs are present, and IH COs and IH NCOs are reduced to low levels (Figure 2B versus Figure 2A and Figure S1E). When Rad51 is absent, high levels of SEIs and dHJs are seen but now occur almost entirely between sisters (IH:IS dHJ = 1:7 in *rad51Δ* versus 5:1 in WT meiosis; Figure 2C; Table S1). *rad51Δ* also exhibits sister bias at the SEI stage (Figure 2C) and reduced levels of both IH NCOs and IH COs (Figures S1E, S1G, and S1H; Schwacha and Kleckner, 1997).

All factors that assist Rad51 for mitotic DSB repair also assist Rad51 in promoting meiotic homolog bias. *shu1Δ* and *psy3Δ* single and the *shu1Δ psy3Δ* double mutant exhibit an IH:IS dHJ ratio intermediate between that of WT and *rad51Δ* (IH:IS = 1:2.5–1:3 in all three mutants, versus 5:1 in WT and 1:7 in *rad51Δ*; Figures 2D–2F; Table S1) and an intermediate reduction in IH COs and IH NCOs (Figure S1J–S1L). A *rad51Δ shu1Δ* double mutant exhibits the same, more severe defect of a *rad51Δ* mutant (IH:IS dHJ = 1:7); thus, Shu1/Psy3 acts in the Rad51 pathway (Figures 2G and S1I; Table S1). The same epistasis relationships are seen for meiotic progression timing and spore viability (Figure S2). Similar results have recently

been reported by Sasanuma et al., 2013. The Rad51 paralog mediators Rad55 and Rad57 are also required as strongly as Rad51 (IH:IS dHJs = 1:7 in *rad55Δ* and *rad57Δ*; Figures 2H and 2I; Table S1).

Relative roles and epistasis relationships among Rad51 and its mediators for meiotic homolog bias are the same as those for Rad51-mediated mitotic DSB repair: Rad55/Rad57 is essential; Shu1 and/or Psy3 are important, but less so, and act downstream of Rad51 (Figure S2; below; Mankouri et al., 2007). Thus, the entire mitotic RecA homolog ensemble has been coordinately utilized, as a single functional unit, for this meiosis-specific role.

Dmc1 Is Required for Establishment of Homolog Bias; Hed1 Is Required for Maintenance of Bias; and Dmc1 and Hed1 Play Sequential Roles for Suppression of Rad51-Mediated Strand Exchange

Dmc1 Is Required for the Establishment of Homolog Bias. Dmc1 is implicated as a player in homolog bias (Schwacha and Kleckner, 1994; Sheridan and Bishop, 2006), but documentation of a definitive role has been precluded by the fact that a *dmc1Δ* mutant does not carry out strand exchange. Evaluation of this possibility has been made possible by use of a *hed1Δ* mutation, which permits Rad51 to carry out strand exchange when Dmc1 is absent (Cloud et al, 2012; Tsubouchi and Roeder, 2006). We find that a *hed1*Δ *dmc1*Δ mutant exhibits a strong homolog bias defect. IS SEIs and IS dHJs strongly predominate over their IH counterparts (IH:IS dHJ = 1:7; Figure 2J). Thus, Dmc1 plays an important role for bias. The same observation has been made independently by N. Hunter and D.K. Bishop (personal communication). Restoration of strand exchange in this condition could result from the absence of Hed1; however, Hed1 is not essential for establishment of homolog bias (next section). Thus, activation of Rad51 in a *dmc1Δ hed1Δ* background results, in whole or in part, from the absence of Dmc1, which is thereby implicated as an inhibitor of Rad51. Another study provides further evidence for Dmc1-mediated inhibition of Rad51 activity (N. Hunter and D.K. Bishop, personal communication).

Role(s) of Rad51 Inhibitor Hed1. A *hed1Δ* mutant exhibits high levels of SEIs and dHJs, with an IH:IS dHJ ratio of 2:1 (Figure 2K; Table S1). This phenotype, also found independently by N. Hunter and D.K. Bishop (personal communication), could reflect a moderate defect in establishment and/or maintenance of homolog bias. Further, a *shu1Δ* mutation specifically affects establishment of bias (above), and a *hed1Δ shu1Δ* double mutant exhibits a greater defect in bias (IH:IS dHJ = 1:4; Figure 2M) than either single mutant (IH:IS = 2:1 and 1:2.5, respectively; Figures 2D and 2K; above). Thus, Shu1 and Hed1 have different (roughly additive) roles. If Shu1 acts during establishment, Hed1 might only act during maintenance, and since Hed1 acts specifically and directly to inhibit Rad51-mediated strand exchange, a role for Hed1 in maintenance of bias would implicate Rad51 in that process as well. Alternatively, or in addition, Hed1 and Shu1 might affect different aspects of bias establishment. If so, Hed1 might collaborate with Dmc1 for repression of Rad51 strand exchange.

Dmc1 Suppresses Rad51 Strand Exchange Activity to Promote Establishment of Homolog Bias. In meiosis, Rad51 mediates homolog bias rather than carrying out strand exchange (Introduction). Conversely, in a *hed1Δ dmc1Δ* mutant, establishment of homolog bias is defective and Rad51 is mediating strand exchange (above). These patterns imply the existence of two alternative Rad51 states. What molecule(s) is/are responsible for this switch? Hed1 might be the most obvious candidate. However, evidence above suggests that Dmc1 is involved in both suppression of Rad51 strand exchange and establishment of homolog bias. Thus, Dmc1 may be the critical component of the switch. Moreover, Dmc1 should be required for establishment of homolog bias, at least in part, because it is required to inhibit Rad51strand exchange activity, thereby enabling Rad51 to play its role in the establishment process.

These patterns suggest a two-phase process. Dmc1 suppresses Rad51 strand exchange activity during establishment of bias, perhaps with help from Hed1. Then, during maintenance of bias, Hed1 alone suppresses Rad51 strand exchange (e.g., as a component of the donor-associated quiescence complex; Figure 1A; Kim et al., 2010). Thus, in *hed1Δ*, Dmc1 inhibits Rad51 strand exchange transiently during bias establishment. Then, during maintenance of bias, Dmc1 no longer inhibits Rad51 strand exchange and, since Hed1 is absent, bias is defective. In *dmc1Δ*, no Rad51-mediated strand exchange occurs (Figure 2B). Since Dmc1 is absent, Hed1 carries out strong suppression prematurely, thus fully and permanently suppressing Rad51 strand exchange. In *hed1Δ dmc1Δ* (Figure 2J), Rad51-mediated strand exchange occurs because both meiotic inhibitors are absent, and strong sister bias is seen because Dmc1 is not there to promote bias establishment.

Homolog Bias Establishment Is Independent of Rad51/Dmc1 Interplay if Cohesin Is Absent

In WT meiosis, Rad51 and Dmc1 are both required for the establishment of homolog bias, with IH:IS dHJ ratios of 1:7 in *rad51Δ*, 1:2.5 in *shu1Δ*, and 1:7 in *hed1Δ dmc1Δ* (above; Figure 2). In contrast, in all three backgrounds, when Rec8 is also eliminated, a 1:1 IH:IS dHJ ratio is observed: *rad51Δ/shu1Δ rec8Δ* and *hed1Δ dmc1Δ rec8Δ* mutants both exhibit the same 1:1 IH:IS dHJ ratio seen in a *rec8Δ* single mutant (Figures 3A–3C and 3E). Two implications emerge: (1) when Rec8 is absent, homolog bias can efficiently be established (with the subsequent maintenance defect resulting in a 1:1 IH:IS dHJ ratio), regardless of which RecA homolog is promoting strand exchange (Dmc1 or Rad51); and (2) most importantly, even though Rad51 and Dmc1 are both required for the establishment of homolog bias in WT meiosis (above), neither is required when Rec8 is absent and the other molecule is promoting strand exchange (because both mutants get to the bridge stage required for a 1:1 IH:IS dHJ ratio). Thus, the role of Rad51/Dmc1 interplay for the establishment of homolog bias is to counteract a role of Rec8 that promotes sister bias. These findings further suggest the following general logic for meiotic partner choice: (1) homolog bias is the default option for meiotic recombination; (2) cohesin intervenes to confer sister bias; and (3) Rad51/Dmc1 further intervenes to oppose the intervening effect of cohesin, thus restoring the homolog bias default option.

The roles of Rec8 in meiotic partner choice can be substantially fulfilled by a cohesin complex containing mitotic kleisin subunit Mcd1/Scc1 (hereafter Mcd1). In yeast, Mcd1 is poorly expressed and much less abundant during meiosis than in mitotically dividing cells, although it does have discernible roles

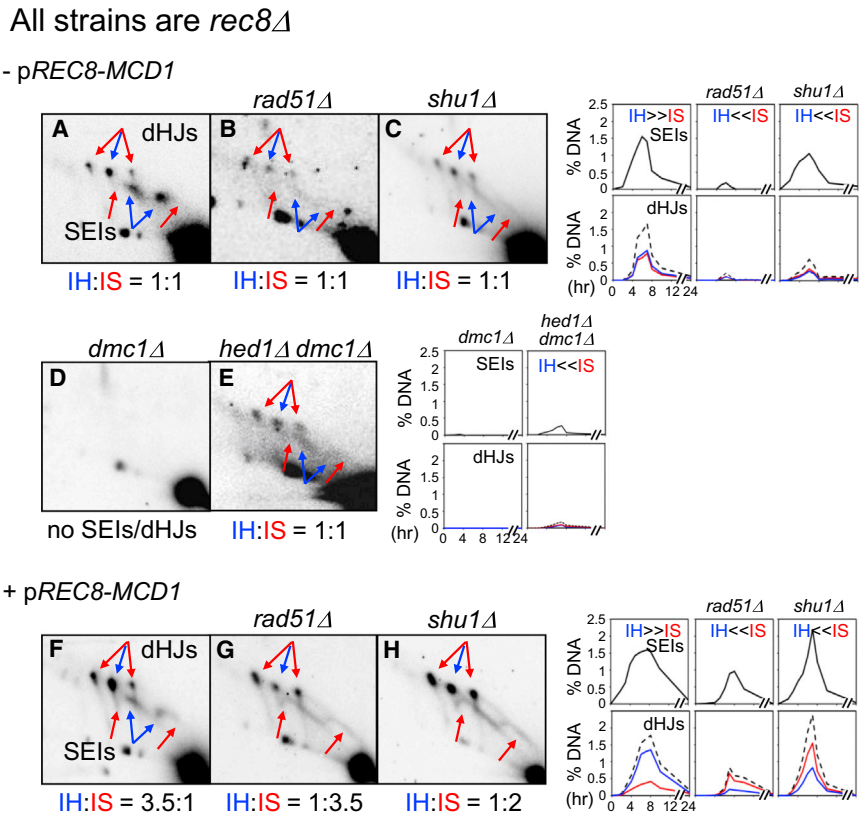


Figure 3. Rad51 and/or Shu1 Counteract an Inhibitory Effect of Cohesin

Analysis of SEIs and dHJs in RMH$^+$ Rec8$^-$ meiosis. (A–E) All strains were *mek1as (−IN) rec8Δ* strains without meiotically induced expression of mitotic kleisin Mcd1 and carrying either no additional mutation (A) or the indicated mutation: *rad51Δ* (B); *shu1Δ* (C); *dmc1Δ* (D); *hed1Δ dmc1Δ* (E).

(F–H) *mek1as(−IN) rec8Δ* strains with meiotically induced expression of mitotic kleisin Mcd1 and carrying either no additional mutation (F) or the indicated mutation: *rad51Δ* (G); *shu1Δ* (H).

(Kateneva et al., 2005). However, when Mcd1 is expressed at a high level (via a *pREC8-MCD1* fusion) in a *rad51Δ/shu1Δ rec8Δ* mutant, the IH:IS dHJ ratio decreases from 1:1 to 1:3.5/1:2 (Figures 3G and 3H), approaching the 1:7/1:2.5 observed in *rad51Δ/shu1Δ REC8* (Figures 2C and 2D). Thus, meiotic expression of Mcd1 substantially restores the requirement for Rad51/Shu1 in promoting the establishment of homolog bias. Furthermore, when Mcd1 is expressed at a high level in a *rec8Δ* mutant, the IH:IS ratio increases from 1:1 (in *rec8Δ*; Figure 3A) to 3.5:1 (Figure 3F), approaching the 5:1 ratio seen in WT (Figures 1D, 1E, and 2A). Thus, Mcd1 can also substantially substitute for Rec8 in promoting maintenance of homolog bias.

Partner Choice in RMH$^-$ Meiosis

Absence of the RMH Activity Toggles Recombination from Meiotic Mode to Mitotic-like DSB Repair Mode

The RMH complex is a central player in meiotic recombination partner choice. Diverse previous studies show that complete deletion mutations of axis components Hop1 or Red1 or chemically mediated elimination of Mek1 kinase activity all confer very strong sister bias (Kim et al., 2010). At *HIS4LEU2*, a *red1Δ* or a *mek1* strain with an inactive kinase (*mek1as +IN*; Experimental Procedures) exhibits an IH:IS dHJ ratio of ~1:9 (Kim et al., 2010; Figure 1F, 4A, and 4N), as also seen in a *hop1Δ* mutant (Schwacha and Kleckner, 1994).

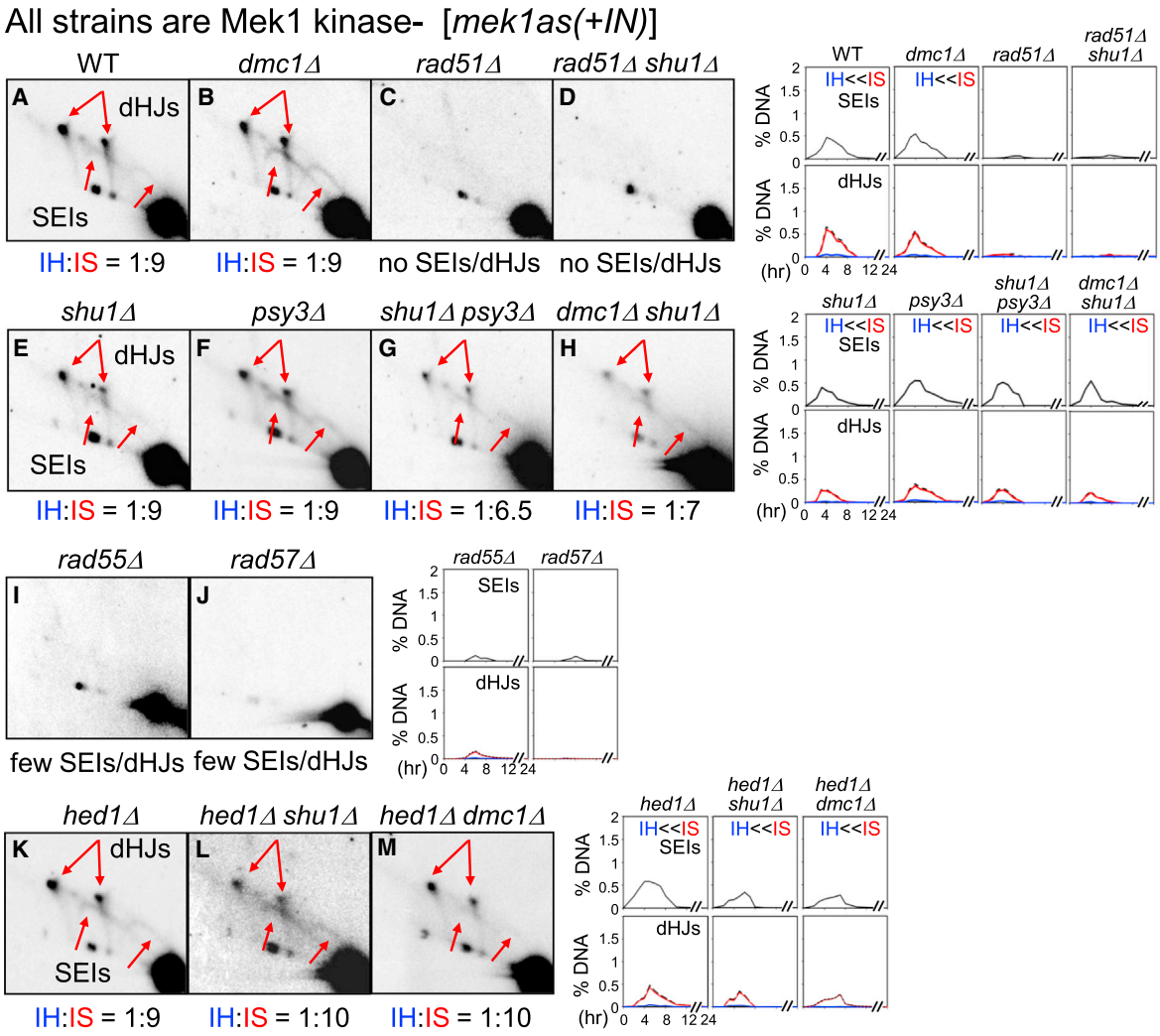
Further examination of these mutant conditions reveals a simple general explanation for their phenotype. Absence of RMH toggles the recombination process, as a single unit, from the normal meiotic mode to a mode that is very similar to mitotic DSB repair, according to diverse criteria:

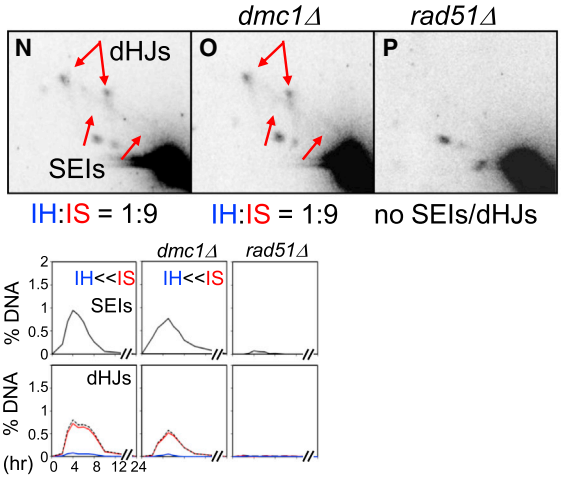
(1) The strong sister bias in RMH$^-$ meiosis (Figures 1F, 4A, and 4N) corresponds to the strong sister bias in mitotic DSB repair (Kadyk and Hartwell, 1992; Johnson and Jasin, 2001).

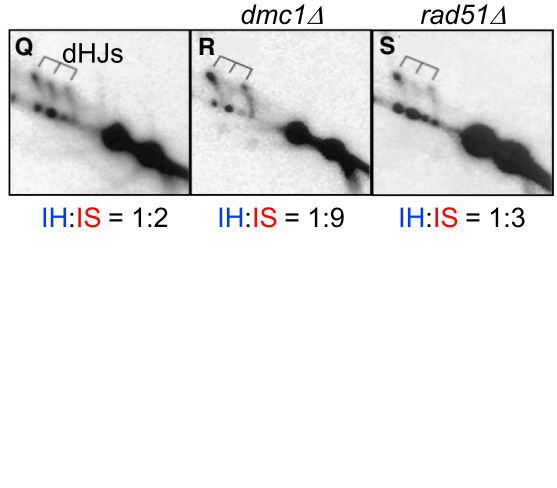
(2) RMH$^-$ meiosis is characterized by dramatic hyperresection of DSBs to give very long 3′ single-stranded DNA (ssDNA) tails (Kim et al., 2010), as compared to the carefully controlled resection of meiotic DSBs in WT meiosis (Hunter and Kleckner, 2001). Hyper-resection is also a prominent characteristic of mitotic DSB repair (Chung et al., 2010).

(3) In mitotic DSB repair, Rad51 promotes strand exchange. We now show that in RMH$^-$ conditions, elimination of Rad51 (in the presence or absence of Shu1), Rad55, or Rad57 abolishes SEIs and dHJs (Figures 4C, 4D, 4I, and 4J versus Figure 4A; Figure 4P versus Figure 4N). Further, elimination of Shu1, Psy3, or both reduces SEI and dHJ levels (in the presence or absence of Hed1) (Figures 4E–4G versus Figure 4A; Figure 4L versus Figure 4K). Furthermore, the *rad51II-3A* mutation, which diminishes but does not eliminate Rad51's strand exchange activity (Cloud et al., 2012), diminishes but does not eliminate SEIs and dHJs (Figure S3). In contrast, elimination of Dmc1 has little or no effect (in the presence or absence of Shu1 or Hed1) (Figure 4B versus Figure 4A; Figure 4H versus Figure 4E; Figure 4M versus Figure 4K; Figure 4O versus Figure 4N). Thus, in RMH$^-$ conditions, Rad51 is promoting strand exchange, as during mitotic DSB repair, while Dmc1 has no discernible influence.

(4) In mitotic DSB repair, meiosis-specific factors are not expressed and thus are not participating. In RMH$^-$ meiosis, meiosis-specific factors are presumably expressed but, nonetheless, are again not participating. Elimination of Dmc1, Hed1, or Dmc1 and Hed1 has no effect (above







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and Figure 4K versus Figure 4A). Since most other meiotic factors work in specific concert with Dmc1 (e.g., Hop2/Mnd1; Mei5/Sae3; Rdh54-Tid1; Cloud et al., 2012; Nimonkar et al., 2012; Pezza et al., 2007), those factors also should not be participating. Since all of these molecules are presumably expressed in RMH$^-$ meiosis, they appear to be present but unable to act in the absence of RMH function.

(5) During mitotic DSB repair, recombination tends to proceed to the NCO fate via synthesis-dependent strand annealing (SDSA) rather than to the CO fate via SEIs and dHJs (Bzymek et al., 2010). The same is true in RMH$^-$ meiosis, where the majority of IH recombination events appear to be resolved as NCOs rather than COs: CO:NCO = 1.3 in WT meiosis versus 0.5 in RMH$^-$ meiosis (Figure S1F).

RMH Function Is Required for Meiotic Homolog Bias Because It Activates Dmc1

Given that the RMH complex activates the entire meiotic recombination process, the next question is how this activation might occur, particularly regarding the ability of the RMH complex to promote homolog bias. Insight is provided by strains carrying a *red1::LEU2* insertion/disruption allele. In a previous analysis, we detected a significant level of IH dHJs in this mutant (Figure 4Q; Schwacha and Kleckner, 1997). Moreover, occurrence of these IH dHJs was specifically dependent on Dmc1: when Dmc1 is eliminated, Rad51 promotes dHJ formation, but only IS events occur (Figure 4R versus 4Q; Schwacha and Kleckner, 1997). Put another way, in the *red1::LEU2* background, Dmc1 is required specifically for IH events, not for strand exchange in general, which is promoted by Rad51. It was thus inferred that Dmc1 has a specific interhomolog interaction function (Schwacha and Kleckner, 1997). We now show above that Dmc1 is important for establishment of homolog bias. However, in contrast to the situation in *red1::LEU2*, no IH dHJs are observed in RMH$^-$ (*red1Δ*) meiosis, even when Dmc1 is present (Figure 4N). Importantly, our previous study used a more complex version of the *HIS4LEU2* locus. However, we have confirmed that this same intermediate defect occurs in a *red1::LEU2* strain carrying the *HIS4LEU2* allele used above for analysis of *red1Δ* and *mek1as+IN* null mutants (Figure S4).

Together, these results suggest that *red1::LEU2* is not a null allele and that it retains a subfunction that allows Dmc1 to now exert its influence on partner choice (i.e., the Dmc1's IH interaction function is conferred directly or indirectly by Red1). Thus, one role of the RMH complex for meiotic homolog bias is to activate Dmc1, directly or indirectly, as a factor for promotion of homolog bias establishment. Since all other meiosis-specific recombination components act via Dmc1, activation of Dmc1 would be a convenient key lynchpin for the switch from mitotic to meiotic mode.

The IH:IS dHJ ratio in *red1::LEU2 RAD51* is 1:2, while the ratio in *red1::LEU2 rad51Δ* is lower (1:3; Figure 4S versus 4Q), implying a role for Rad51 in homolog bias even in this compromised situation.

Mitotic-like RMH$^-$ Meiosis Also Exhibits Homolog Bias if Cohesin Is Absent

In RMH$^-$ conditions, strong sister bias for recombination is converted to normal homolog bias if cohesin is removed, as shown by a 1:1 IH:IS dHJ in RMH$^-$ *rec8Δ* strains (Kim et al., 2010; Figure 5A). Moreover, in these RMH$^-$ Rec8$^-$ conditions, if mitotic cohesin is then expressed at a high level, strong sister bias is restored (IH:IS dHJ = 1:5 in *rec8Δ pREC8-MCD1* versus 1:1 in RMH$^-$ *rec8Δ*; Kim et al., 2010; Figure 5G versus Figure 5A).

The results presented above now further reveal that in RMH$^-$ Rec8$^+$ conditions, recombination exhibits strong similarities with mitotic DSB repair, including the fact that Rad51 is responsible for strand exchange (Figures 4A–4Q).

Together, these two sets of results suggest that mitotic-like recombination, promoted by Rad51 and in the apparent absence of functional contributions from meiotic components, intrinsically tends to exhibit homolog bias when cohesin is absent; then, when cohesin is present, this intrinsic tendency for homolog bias is converted into sister bias.

This important conclusion critically depends on the assumption that the dHJ phenotypes seen in RMH$^-$ Rec8$^-$ conditions reflect recombination promoted by Rad51, just as in RMH$^-$ Rec8$^+$ conditions. We have confirmed this critical assumption. In RMH$^-$ Rec8$^-$ meiosis, absence of Dmc1 has no effect on partner choice, either in Hed1$^+$ (Figure 5B versus Figure 5A) or in Hed1$^-$ (Figure 5F versus Figure 5E); a 1:1 IH:IS dHJ ratio is again observed, as in the presence of Dmc1. Thus, in RMH$^-$ Rec8$^-$ conditions, Rad51 promotes strand exchange with normal homolog bias.

Elimination of Dmc1 does reduce steady-state dHJ levels somewhat. This likely reflects increased dHJ turnover rather than direct participation of Dmc1 in strand exchange. Accordingly, absence of Rad51 essentially completely eliminates SEIs and dHJs, and absence of Shu1 significantly reduces SEIs and dHJs (Figures 5C and 5D versus Figure 5A). Strand exchange is also strongly reduced by a *rad51* non-null allele that strongly reduces Rad51-mediated strand exchange while retaining Rad51-mediated functions for homolog bias (Figure S3).

Mcd1 can substantially substitute for Rec8 in RMH$^-$ conditions, just as in RMH$^+$ conditions (above). Mcd1 expression in RMH$^-$ Rec8$^-$ restores strong sister bias regardless of whether Dmc1 is present or absent (Figure 5G versus Figure 5A; Figure 5H versus Figure 5B) and even when strand exchange is reduced

Figure 4. Absence of Mek1 Kinase Activity or Red1 Protein Switches Recombination to Mitotic-like Mode

Analysis of SEIs/dHJs in RMH$^-$ Rec8$^+$ meiosis (A–P) and *red1::LEU2* Rec8$^+$ meiosis (Q–S).

(A–M) *mek1as(+IN)* strains carrying no additional mutation (A) or the indicated mutation: *dmc1Δ* (B); *rad51Δ* (C); *rad51Δ shu1Δ* (D); *shu1Δ* (E); *psy3Δ* (F); *shu1Δ psy3Δ* (G); *dmc1Δ shu1Δ* (H); *rad55Δ* (I); *rad57Δ* (J); *hed1Δ* (K); *hed1Δ shu1Δ* (L); *hed1Δ dmc1Δ* (M).

(N–P) *red1Δ* strains carrying no additional mutation (N) or the indicated mutation: *dmc1Δ* (O) or *rad51Δ* (P).

(Q–S) *red1::LEU2* strains carrying no additional mutation (Q) or the indicated mutation: *dmc1Δ* (R) or *rad51Δ* (S) (from Schwacha and Kleckner, 1997).

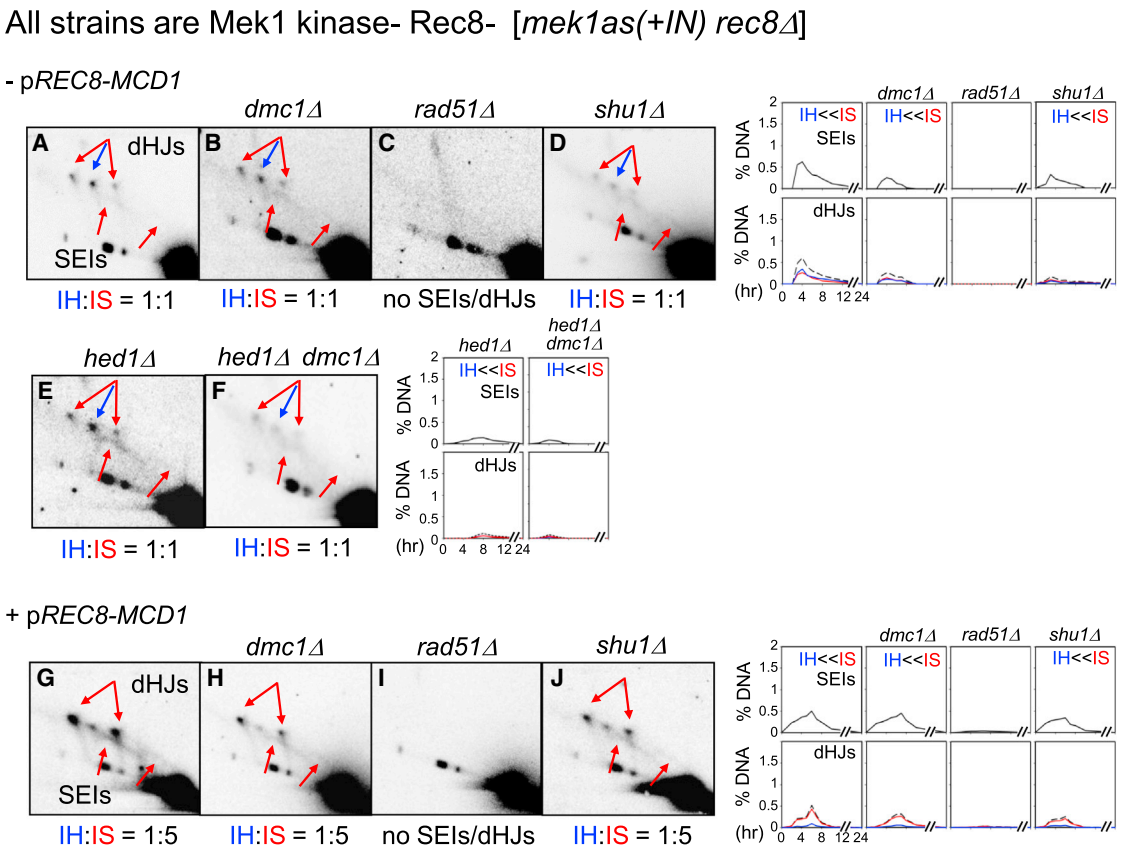

Figure 5. In the Absence of Cohesin, Rad51-Promoted Strand Exchange Exhibits Homolog Bias

Analysis of SEIs/dHJs in RMH$^-$ Rec8$^-$ meiosis.

(A–F) *mek1as(+IN) rec8Δ* strains not meiotically induced for expression of mitotic kleisin Mcd1 and carrying no additional mutation (A) or the indicated mutation: *dmc1Δ* (B); *rad51Δ* (C); *shu1Δ* (D); *hed1Δ* (E); *hed1Δ dmc1Δ* (F).

(G–J) *mek1as(+IN) rec8Δ* strains meiotically induced for expression of mitotic kleisin Mcd1 and carrying no additional mutation (G) or the indicated mutation: *dmc1Δ* (H); *rad51Δ* (I); *shu1Δ* (J).

by absence of Shu1 (Figure 5J versus Figure 5D), with strand exchange still fully dependent on Rad51 (Figure 5I).

These results show that in conditions in which meiotic factors are not participating and in which recombination has strong similarities to mitotic DSB repair (i.e., RMH$^-$ meiosis), Rad51-mediated strand exchange recombination exhibits homolog bias if cohesin is absent and sister bias if cohesin is present. The emergent suggestion is that the intrinsic tendency for Rad51-mediated mitotic-like recombination (i.e., the default option for this process) is homolog bias, not sister bias as is usually assumed. Moreover, cohesin actively intervenes in this default to promote sister bias.

Synthesis Thus Far

The observations above suggest a general scenario for RecA homolog-promoted partner choice. (1) The basic mechanistic default option for all RecA homolog-promoted recombination is selection of a homolog partner. (2) Cohesin interferes with this basic tendency to actively confer sister bias. (3) In meiosis, Dmc1/Rad51 collaboration, dependent on RMH activation of Dmc1, counteracts this cohesin-mediated sister bias activity, thereby restoring the intrinsic homolog bias default.

Requirements for Execution of dHJ Formation

Interplay among Rad51, Cohesin, and the Sister Chromatid Is Required for Stimulation of Dmc1-Mediated dHJ Formation in RMH$^+$ Meiosis

Rad51 and Cohesin Have Overlapping Roles. The levels of SEIs and dHJs are severely reduced when both Rec8 and Rad51 are absent (*rec8Δ rad51Δ/shu1Δ*; Figures 3B and 3C), but not when either component is absent alone (in *rec8Δ* or *rad51Δ/shu1Δ*; Figures 3A and 2C–2G). This pattern suggests that Rec8 and Rad51 have overlapping stimulatory roles in promoting dHJ formation for meiosis. Rad51 has been implicated previously as a positive activator of Dmc1 (Cloud et al., 2012). The role of Rec8 for this stimulatory effect can be carried out reasonably successfully by the mitotic kleisin Mcd1, as shown by increased levels of SEIs and dHJs in *rad51Δ/shu1Δ rec8Δ pREC8-MCD1* as compared to isogenic *rec8Δ* strains (Figures 3G and 3H versus Figures 3B and 3C).

The Sister and Cohesin Have Overlapping Roles. Meiotic recombination can occur reasonably efficiently in conditions in which prophase occurs without a preceding round of DNA replication (*pMCD1-CDC6*; Hochwagen et al., 2005). Since recombination

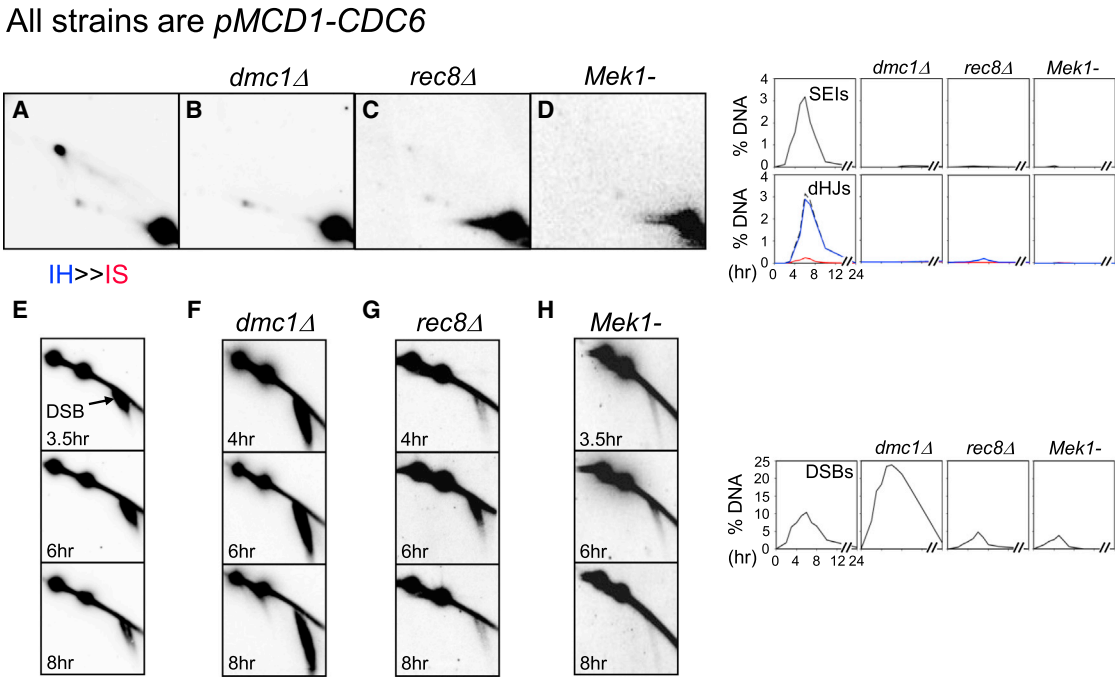

Figure 6. Analysis of Recombination in the Absence of a Sister

(A–D) 2D gel analysis of meiotic recombination in the absence of DNA replication (*pMCD1-CDC6*; Hochwagen et al., 2005) with no additional mutation (A) or the indicated additional genetic markers: *dmc1Δ* (B); *rec8Δ* (C); *Mek1*$^{-}$ (D). In (A), SEI and dHJ levels are multiplied by two so that the levels per chromatid are comparable to WT meiosis.

(E–H) Full 2D gels for strains in (A–D) showing DSB levels, with no additional mutation (E) or the indicated additional genetic markers: *dmc1Δ* (F); *rec8Δ* (G); *Mek1*$^{-}$ (H). Quantification is shown on the right.

in this condition occurs in the absence of a sister chromatid, examination of this condition permits evaluation of possible roles of the sister per se.

Recombination in the absence of a sister exhibits significant levels of SEIs, dHJs, and COs, confirming and extending earlier studies (Figure 6A; Hochwagen et al., 2005). Furthermore, this recombination is absolutely dependent on Dmc1, as in WT meiosis (Figure 6B), with DSBs accumulating in the absence of Dmc1 (Figure 6F versus 6E).

In addition, and in contrast to WT meiosis, recombination in this condition is absolutely dependent on Rec8 (Figure 6C). Since no sister is present, this result shows that Rec8 stimulates Dmc1-mediated recombination in the absence of a sister and thus via an effect that does not involve sister cohesion. Phenotypes of *rec8* separation-of-function alleles also show a cohesin-independent role of Rec8 (Brar et al., 2009). Since DSBs do not accumulate, initiated events proceed, but to some non-SEI/dHJ fate.

This result also permits a further conclusion. In the presence of a sister, absence of Rec8 does not dramatically reduce the levels of SEIs and dHJs (Figure 3A). In the presence of Rec8, absence of a sister does not dramatically reduce the levels of SEIs and dHJs (Figure 6A). However, in the absence of both Rec8 and a sister chromatid, only low levels of SEIs and dHJs occur (Figure 6C). Thus, the presence of a sister has an overlapping stimulatory role with Rec8 for Dmc1-mediated dHJ formation.

Rad51-Mediated Stimulation of Dmc1 in RMH^{+} *Meiosis Requires the Presence of the Sister*. Rad51 and Rec8 have overlapping roles for stimulation of Dmc1-mediated dHJ formation; the sister chromatid and Rec8 have overlapping roles for stimulation of Dmc1-mediated dHJ formation (above). The obvious suggestion that emerges from these two results is that Rad51 and the presence of a sister collaborate for the same role in stimulation of Dmc1-mediated dHJ formation; this role is overlapping with a sister-independent role of Rec8. Put another way, Rad51-mediated stimulation of Dmc1-promoted dHJ formation depends upon the presence of a sister chromatid (as seen in the absence of Rec8).

A prediction of this possibility is that in the absence of Rec8, elimination of Rad51, the sister chromatid, or both will all confer the same strong reduction of Dmc1-mediated dHJ formation. This prediction is fulfilled for the single-defect cases: SEIs and dHJs are virtually undetectable in both *rec8Δ rad51Δ* and in the absence of a cohesin and a sister (*rec8Δ pMCD1-CDC6*) (Figures 3B and 6C). The double-defect case is difficult to test because *pMCD1-CDC6 rad51Δ rec8Δ* exhibits mitotic growth defects.

Rad51-Mediated dHJ Formation in RMH^{-} *Meiosis Requires the Presence of the Sister*

The above results suggest that collaboration between Rad51 and the sister chromatid is important in promoting Dmc1-mediated dHJ formation in RMH^{+} meiosis. We were interested to know whether such collaboration might also be important

for Rad51-mediated dHJ formation under conditions in which meiotic recombination components have little or no effect. We thus examined recombination in RMH$^-$ conditions in which Rad51 is responsible for strand exchange and Dmc1 is not playing a prominent role (above). If the sister is required for Rad51 strand exchange activity, no SEIs or dHJs will occur in *cdc6* RMH$^-$ conditions. Remarkably, this prediction is fulfilled: no SEIs or dHJs occur in *pMCD1-CDC6 mek1as+IN* (Figure 6D). DSBs occur in all *pMCD1-CDC6* strains (Figures 6E–6H); thus, absence of SEIs and dHJs does not reflect a failure to enter meiosis or initiate recombination. Taken together, these findings strongly support the idea that the presence of a sister chromatid is essential for both Rad51-mediated dHJ formation in mitotic-like mode and for Rad51-mediated stimulation of Dmc1-mediated dHJ formation in meiotic mode.

DISCUSSION

The presented results permit a synthetic view of recombination partner choice for both general (mitotic) DSB repair and meiosis.

Homolog Bias in Wild-Type Meiosis

Analysis of recombination in RMH$^+$ conditions further defines the nature of meiotic homolog bias and elucidates the roles in this process of meiotic and mitotic RecA homologs and several associated modulators. The relationships defined above are summarized in Figure 7A. The default option for recombination is homolog bias; cohesin intervenes in the process to confer sister bias; and meiotic components counteract the effect of cohesin to restore homolog bias. For the meiotic process, the RMH ensemble activates Dmc1, which acts (at least in part) to inhibit Rad51 strand exchange activity, perhaps with help from Hed1. As a result, Rad51 and its entire ensemble of mediator proteins now function to antagonize cohesin. It is most straightforward to think that all of these effects occur on the DSB donor chromosome, prior to release of a DSB end to search for a homolog. However, alternative or additional effects are not excluded, including features required to ensure that DSB extension by synthesis occurs preferentially at the homolog-associated DSB end (Figure 1A).

Direct activation of Dmc1 by RMH could be the lynchpin for bringing other meiosis-specific molecules into play, since all of those molecules work via Dmc1 (e.g., Tid1/Rdh54, Mei5-Sae3, Hop2-Mnd1). However, it is likely that the RMH complex also plays other roles (e.g., to provide a locally cohesin-depleted zone; Kim et al., 2010) such that effects of cohesin on partner choice are more manageable.

Rad51 inhibitor Hed1 is likely required for the maintenance of bias. Hed1 would exert its role as part of the quiescence complex that keeps the sister-associated end from undergoing synthesis-mediated extension and ensuing IS dHJ formation, in collaboration with Rad51 (Kim et al., 2010; Figure 1A). A role for Hed1 at a later stage in recombination has also been suggested by Busygina et al. (2012). Hed1 may also act earlier, in concert with Dmc1 (above).

RMH Activity Toggles Recombination between Mitotic-like and Meiotic Modes

The presented results show that the RMH cleanly switches recombination from a mode that has strong similarities to general DSB repair to meiotic mode, with its additional features. It is well known that RMH$^-$ meiosis has mitotic-like qualities; however, the existence of such a clean switch was not previously appreciated. This feature is biologically interesting. It suggests that the RMH complex played a critical role in the evolution of meiotic recombination from mitotic DSB repair. Additionally, existence of such a switch underlies the fact that yeast cells can interrupt meiosis and return smoothly to the mitotic program in a process that involves (1) rapid loss of meiotic chromosome axis components and (2) diversion of recombination from IH to IS mode (Schwacha and Kleckner, 1997; Zenvirth et al., 1997; Goldfarb and Lichten, 2010). This return-to-growth regime thus appears to involve toggling of the RMH switch from meiotic mode back to a mitotic mode analogous to RMH$^-$ mutant conditions.

Homolog Bias Is the Mechanistically Specified Default Option for All RecA Homolog Recombination

In RMH$^-$ meiosis, strand exchange is promoted by Rad51. Known meiotic components can be explicitly deleted (*dmc1Δ/hed1Δ*) or can be present but apparently making no functional contribution to recombination. Nonetheless, when cohesin is absent, RMH$^-$ recombination exhibits establishment of homolog bias, as indicated by occurrence of a 1:1 IH:IS dHJ ratio; when cohesin is present, recombination exhibits establishment of sister bias, with virtual absence of all IH species. These observations imply that homolog bias is the default option for mitotic-like Rad51-promoted strand exchange and that cohesin overrides this default to actively promote sister bias (Figure 7A, bottom).

Correspondingly, in a study of bona fide mitotic DSB repair, lower and higher levels of cohesin resulted in higher and lower levels, respectively, of IH versus IS recombination (Covo et al., 2010).

The role of cohesin in actively promoting sister bias probably does not involve its role in mediating sister chromatid cohesion for three reasons. (1) Given that homolog bias is a built-in feature of Rad51-mediated recombination, the relative spatial proximities of the sister and the homolog should be irrelevant. There is no reason to suppose that holding the sister closer to the DSB would have any effect. (2) During meiotic recombination, cohesin can play roles for recombination independent of the presence of a sister and thus independent of its cohesion role (above). (3) A non-cohesion role of cohesin is required for limitation of DSB-initiated ectopic recombination yeast, an effect that could also involve channeling of the DSB into an IS interaction (D. Koshland and J. Heidinger-Pauli, personal communication).

The situation in meiosis is precisely analogous, except that in addition, Rad51- and Red1-activated Dmc1 collaborate to override the effect of cohesion, thereby restoring the homolog bias default, as described above. Moreover, homolog bias is observed in meiotic (RMH$^+$) conditions when cohesin is absent, regardless of whether strand exchange is being promoted by Rad51 (with Dmc1 absent) or Dmc1 (with Rad51 absent). We conclude that homolog bias is the basic mechanistic default option for RecA homolog-mediated recombination (Figure 7A).

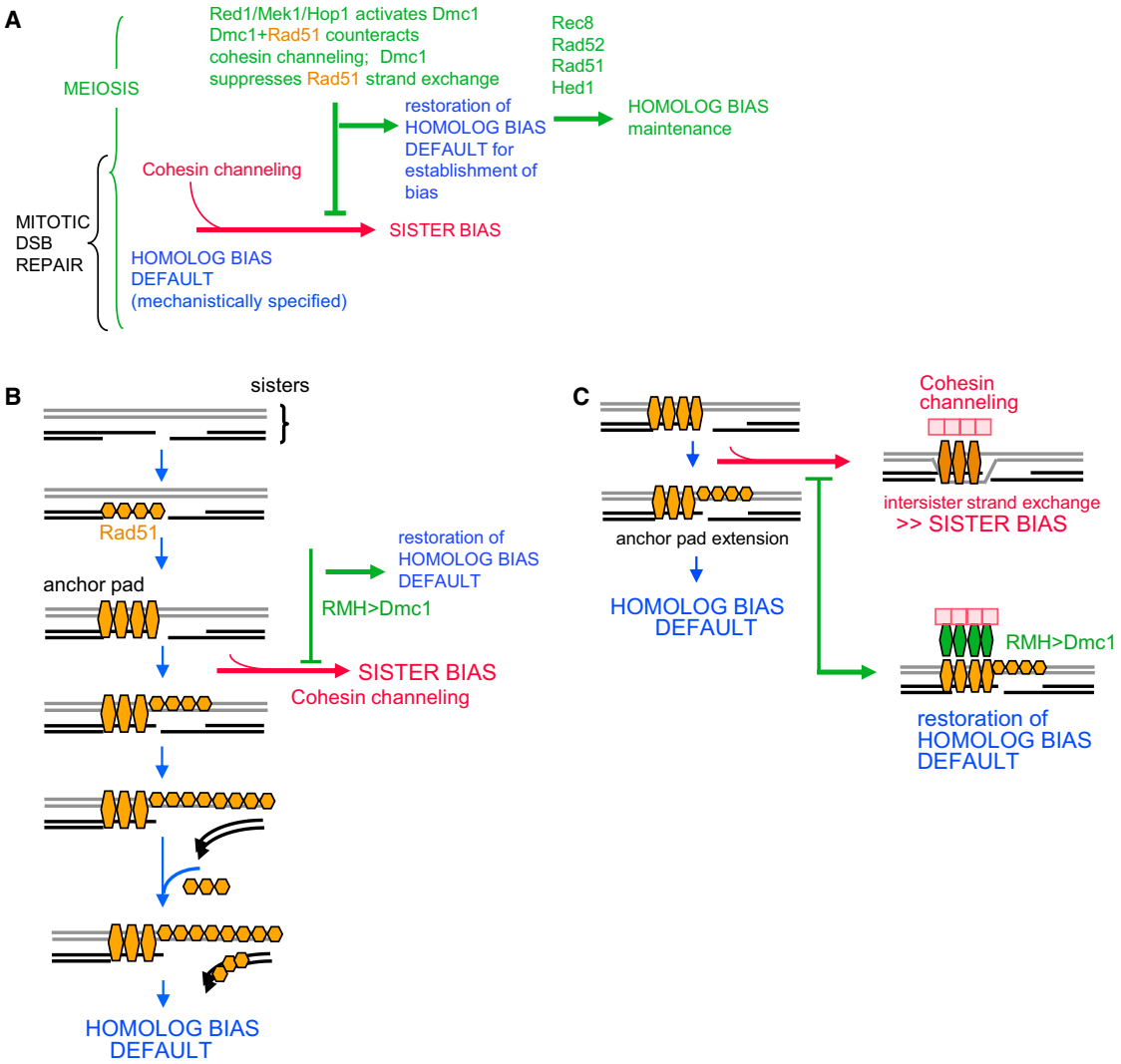

Figure 7. Logic and Proposed Anchor Pad Mechanism of RecA Homolog Recombination Partner Choice

(A) New partner choice paradigm. Recombination exhibits an intrinsic, mechanistically specified default toward selection of the homolog (homolog bias). Cohesin intervenes in this mechanism to channel recombination into use of the sister. This is the appropriate situation for mitotic DSB repair. However, in meiosis, homolog bias is required. This outcome is achieved by the collaborative action of Rad51 and Dmc1, with the RMH complex (and Red1 specifically) required to activate Dmc1. These factors also intervene to counteract the sister-channeling effect of cohesin.

(B) Anchor pad model for mechanistically specified homolog bias. Rad51 filament on the ssDNA of one DSB end captures the intact sister, creating the anchor pad. Extension of the filament along the intact sister, across the site corresponding to the DSB, precludes use of the sister, thus forcing use of the homolog. This effect can also promote release of the other DSB end from association with the sister, thus permitting loading of Rad51 on that end to promote IH strand exchange. Effects of cohesin and meiotic counteracting components come into play after anchor pad formation to modulate extension of the filament along the intact sister.

(C) Cohesin modulates the homolog bias default to give sister bias by altering the parameters of the Rad51 filament so that it promotes strand exchange rather than polymerizing along the intact sister. In meiosis, the same step is further modulated by RMH-activated Dmc1, which counteracts the sister-channeling effect of cohesin.

These findings further imply that program-appropriate outcomes in mitotic DSB repair and meiosis arise by modulation of the basic mechanistic IH default. For mitotic DSB repair, sister bias is the programmatically appropriate outcome and is ensured by cohesin. During meiosis, the appropriate outcome is homolog bias. However, cohesin cannot be globally eliminated because of its multiple additional roles. Thus, locally, at the sites of recombination, recombinosome components directly counteract the effects of cohesin, thereby providing the programmatically appropriate outcome by restoring the homolog bias default.

A Synthetic Model

How could a RecA homolog reaction exhibit intrinsic mechanistically specified homolog bias? We propose the following model

(Figures 7B and 7C). Assembly of Rad51 on the ssDNA tail of a DSB leads immediately to capture of a nearby DNA duplex, giving an anchor pad interaction. In G2 or during meiosis, this duplex will nearly always be the sister chromatid. Rad51 then polymerizes outward from this anchor pad, along the intact partner duplex, across the site opposite the DSB. This polymerization will preclude use of the sister as a partner, thus forcing use of the homolog. This polymerization could also have the effect of promoting release of the other DSB end from the sister, thus making it available for the loading of additional Rad51 or, in meiosis Dmc1, to create a leading end that can search for an available homologous partner DNA that will necessarily be on the homolog.

Program-appropriate partner bias could be imposed on this process by intervention at the step of polymerization outward from the anchor pad. Cohesin would intervene by altering the parameters of the filament so that polymerization across the DSB site is disfavored, whereas initiation of strand exchange would be favored. During meiosis, Dmc1 could also intervene at this step, eliminating the effect of cohesin while concomitantly blocking Rad51-mediated strand exchange (as observed) (e.g., by direct interaction with Rad51; Cloud et al., 2012).

This mechanism also has the attractive feature that it automatically provides for a functional and temporal sequence of events at the two DSB ends. This is a prominent feature of meiotic recombination (Figure 1A) and could also be an important feature in mitotic recombination, particularly during IH recombination. In this context, the Rad51 ensemble would be retained at the anchor pad end so that it can mediate second-end quiescence (in collaboration with Hed1; above) and also provide a possible backup for IS recombination if IH recombination goes awry, as previously suggested (Hunter, 2006).

Rad51 Activity for dHJ Formation Requires the Presence of a Sister Chromatid

The proposed anchor pad model predicts that the sister chromatid would play an important role in Rad51-mediated, homolog-mediated recombination. Mutant phenotypes suggest that Rad51 and the sister chromatid do collaborate to promote Rad51-mediated stimulation of Dmc1-mediated dHJ formation. Furthermore, there is no Rad51-mediated dHJ formation in mitotic mode (RMH^{-}) in a *cdc6* background where no sister is present, although this result could reflect some defect resulting from absence of DNA replication other than absence of the sister per se. Also, DSBs do progress to some other fate in the Cdc6-RMH^{-} condition, suggesting that some type of Rad51-mediated strand exchange remains possible. Nonetheless, these striking and unanticipated results, not predicted by previous considerations, encourage support for the central feature of our proposed model.

Conclusion

The presented results provide a synthetic view of how a DSB selects a partner in both the mitotic and meiotic programs, with inputs from the basic biochemical mechanism, chromosome structure components, and, during meiosis, direct interplay of the meiotic RecA homolog Dmc1 with its general counterpart Rad51.

EXPERIMENTAL PROCEDURES

Yeast Strains, Meiotic Time Courses, and DNA Physical Analysis

Detailed genotypes and strain constructions are listed in Table S2. Procedures for meiotic time course and recombination physical analyses have been described (Hunter and Kleckner, 2001; Kim et al., 2010; Supplemental Experimental Procedures). Most analyzed strains carry the inhibitor-sensitive *mek1as* allele (Wan et al., 2004). In all time courses with such strains, a single premeiotic culture was split into two sporulation medium (SPM) cultures, 1 μM fresh 1-NA-PP1 (USBiological) in DMSO was added directly to one of the two cultures (Kim et al., 2010), and the two cultures were then carried in parallel through meiosis to give directly comparable $Mek1^{+}$ and Mek1 $kinase^{-}$ results (*mek1as[−IN]* and *mek1as[+IN]*, respectively). For every genotype in which both $Mek1^{+}$ and Mek1 $kinase^{-}$ time courses are presented in the text, the $Mek1^{+}$ data were derived from a *mek1as(−IN)* culture analyzed in parallel with the corresponding Mek1 $kinase^{-}$ case in this way.

Calculation of IH:IS dHJ Ratios

For each time course, the IH:IS dHJ ratio is given for the time point at which dHJs were at their maximum level. Ratios denoted as 1:1 were, for all experiments with all analyzed strains, 1:1 ± 0.17 (n = 14; range = 1:0.8–1:1.2). All other values were rounded off to the nearest increment of 0.5.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2013.08.008.

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