Turning coldspots into hotspots: targeted recruitment of axis protein Hop1 stimulates meiotic recombination in Saccharomyces cerevisiae

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Abstract

The DNA double-strand breaks that initiate meiotic recombination are formed in the context of the meiotic chromosome axis, which in Saccharomyces cerevisiae contains a meiosis-specific cohesin isoform and the meiosis-specific proteins Hop1 and Red1. Hop1 and Red1 are important for double-strand break formation; double-strand break levels are reduced in their absence and their levels, which vary along the lengths of chromosomes, are positively correlated with double-strand break levels. How axis protein levels influence double-strand break formation and recombination remains unclear. To address this question, we developed a novel approach that uses a bacterial ParB-parS partition system to recruit axis proteins at high levels to inserts at recombination coldspots where Hop1 and Red1 levels are normally low. Recruiting Hop1 markedly increased double-strand breaks and homologous recombination at target loci, to levels equivalent to those observed at endogenous recombination hotspots. This local increase in double-strand breaks did not require Red1 or the meiosis-specific cohesin component Rec8, indicating that, of the axis proteins, Hop1 is sufficient to promote double-strand break formation. However, while most crossovers at endogenous recombination hotspots are formed by the meiosis-specific MutL resolvase, crossovers that formed at an insert locus were only modestly reduced in the absence of MutLγ, regardless of whether or not Hop1 was recruited to that locus. Thus, while local Hop1 levels determine local double-strand break levels, the recombination pathways that repair these breaks can be determined by other factors, raising the intriguing possibility that different recombination pathways operate in different parts of the genome.

Keywords: meiosis; meiotic recombination; double-strand breaks; chromosome axis; crossing over; Hop1; recombination pathways

Introduction

During meiosis, the diploid genome is reduced by half to form haploid gametes by the separation of homologous chromosomes of different parental origin (herein called homologs) during the first of 2 nuclear divisions (meiosis I). Faithful segregation of homologs requires that they must first identify and link with each other. This is achieved by homologous recombination, which first promotes homolog pairing and then forms crossovers (COs) that physically connect homologs and ensure their proper disjunction at meiosis I (Zickler and Kleckner 1999; Whitby 2005; Ur and Corbett 2021). Errors in homologous recombination cause aneuploidy in gametes, which in turn causes infertility, pregnancy loss, and genetic disorders (Hassold and Hunt 2001; Srivastava et al. 2012; Wang et al. 2017; Gao et al. 2018).

Meiotic recombination occurs in the context of a chromosome axis that contains 3 components: cohesin, an axis core protein; and HORMA domain-containing proteins (Hollingsworth and Ponte 1997; Zickler and Kleckner 1999; Blat et al. 2002; Glynn et al. 2004; Tsuouchi and Roeder 2006; Niu et al. 2007; Yang et al. 2008; Kugou et al. 2009; Niu et al. 2009; Callender and Hollingsworth 2010; Kim et al. 2010; Panizza et al. 2011; Chuang et al. 2012; Pyatnitskaya et al. 2019; Ur and Corbett 2021). The cohesin core holds the sister chromatids together and organizes them in a linear array of loops (Zickler and Kleckner 1999; Berezney et al. 2000; van Heemst and Heyting 2000; Hadjur et al. 2009; Nativio et al. 2009; Davidson et al. 2019; Kim et al. 2019; Gollier et al. 2020). Meiotic cohesin, which contains the meiosis-specific kleisin subunit Rec8, is important for most of the chromosomal localization of the other 2 axis proteins in wild-type cells (Smith and Roeder 1997; Klein et al. 1999; Blat et al. 2002; Riedel et al. 2006; Jin et al. 2009; Joshi et al. 2009; Katis et al. 2010; Panizza et al. 2011; Sun et al. 2015; Heldrich et al. 2020, 2022). Axis core proteins (Red1 in Saccharomyces cerevisiae, ASY3/4 in Arabidopsis, SYCP2/3 in mammals, Rec10 in S. pombe) have diverged considerably in sequence but have similar domain structures and are functionally conserved (Rockmill and Roeder 1990; Hollingsworth and Ponte 1997; Smith and Roeder 1997; de los Santos and Hollingsworth 1999; Lorentz et al. 2004; Yang et al. 2006; West et al. 2019; Ur and Corbett 2021). HORMA domain-containing proteins (Hop1 in S. cerevisiae and S. pombe, ASY1/PAIR2 in plants, HORMAD1/2 in mammals, HTP-1/2/HIM-3 in C. elegans) are also functionally conserved, and in most organisms contain a HORMA domain and a loop
containing a peptide sequence, called a closure motif, that binds either to its own HORMA domain to form a closed structure or to a HORMA domain on another protein to form oligomers (Hollingsworth and Byers 1989; Hollingsworth and Ponte 1997; Caryl et al. 2000; Woltering et al. 2000; Lorenz et al. 2004; Nonomura et al. 2004; Martinez-Perez and Villeneuve 2005; Yang et al. 2006; Baudat and de Massy 2007; Wojtasz et al. 2009; Kim et al. 2014; West et al. 2018). HORMA domain proteins are recruited to the axis by an interaction between their HORMA domain and a closure motif on the axis core protein (West et al. 2018, 2019). Although the main function of these proteins is similar in most organisms, there are also differences that have been discussed in detail elsewhere (Zickler and Kleckner 2015, 2016; Ur and Corbett 2021). For simplicity, the rest of this introduction will focus on the function of these proteins in meiotic recombination in S. cerevisiae.

Chromosome axis proteins are important for the first step of meiotic recombination, the formation of programmed DNA double-strand breaks (DSBs) by the meiosis-specific protein Spo11 and its co-factors: the RMM complex (Rec114, Mer2, Mei4); the MRX complex (Mre11, Rad50, Xrs2); Rec102-Rec104; and Sk8 (Malone et al. 1991; Bergerat et al. 1997; Uetz et al. 2000; Keone 2001; Kee and Keeney 2002; Tesse et al. 2003; Arora et al. 2004; Kee et al. 2004; Prieler et al. 2005; Henderson et al. 2006; Li et al. 2006; Maleki et al. 2007; Panizza et al. 2011; Stanzione et al. 2016). On a regional scale (on the order of 20–50 kb), enrichment levels for Spo11 and DSBs are closely related to those observed for Hop1 and Red1 (Hollingsworth and Ponte 1997; Blat et al. 2002; Pan et al. 2011; Panizza et al. 2011; Smagulova et al. 2011; Sun et al. 2015). In addition, mutant analyses have shown that the absence of any of the axis proteins results in a reduction in DSBs, although the extent of reduction can differ between genome regions (Zickler and Kleckner 1999; Blat et al. 2002; Glynn et al. 2004; Kugou et al. 2009; Kim et al. 2010; Panizza et al. 2011; Ur and Corbett 2021). hop1 mutants seem to show the most pronounced DSB reduction, at least when measured at loci where DSBs form frequently, called hotspots (Mao-Draayer et al. 1996; Schwacha and Kleckner 1997; Xu et al. 1997; Woltering et al. 2000; Pecifià et al. 2002; Niu et al. 2005). Hop1 is thought to promote DSB formation by interacting with Mer2, a member of the trimeric RMM complex, and this interaction is conserved in other species (Stanzione et al. 2016; Kariyazono et al. 2019; Claeyss Bouuaert et al. 2021; Rousová et al. 2021). Mer2, in turn, interacts with the other RMM components as well as other proteins that are important for Spo11-mediated DSB formation (Acquaviva et al. 2013; Sommermeyer et al. 2013; Rousová et al. 2021). In vitro studies indicate that although Red1 has no detectable affinity for Mer2, Red1 stimulates Hop1-Mer2 interaction by changing Hop1’s conformation and increasing its affinity for Mer2 (Rousová et al. 2021). Hop1 is also required for cohesin-independent enrichment of Red1 in certain parts of the genome (Panizza et al. 2011; Sun et al. 2015; Heldrich et al. 2020). Taken together, these observations suggest that Hop1 may be the primary axis protein promoting DSB formation, although this has not been directly demonstrated.

Once DSBs form, Hop1 and Red1 play subsequent roles in promoting interhomolog recombination and in promoting CO formation. DSBs promote Hop1 phosphorylation by the Mecl (ATR) and Tel1 (ATM) kinases (Carballo et al. 2008), and this promotes use of the homolog rather than the sister chromatid as a repair template (Hollingsworth and Ponte 1997; Tsubouchi and Roeder 2006; Niu et al. 2007, 2009; Callender and Hollingsworth 2010; Chuang et al. 2012). Once paired, homologs are held together by a tripartite proteinaceous structure called the synaptonemal complex and Hop1 is removed from the chromosome axis, curbing further DSB formation and removing the inter-sister recombination barrier to allow quick repair of any remaining breaks (Borner et al. 2008; Joshi et al. 2009; Wojtasz et al. 2009; Zanders and Alani 2009; Goldfarb and Lichten 2010; Daniel et al. 2011; Kauppi et al. 2013; Thacker et al. 2014; Lambing et al. 2015; Subramanian et al. 2016, 2019). Red1 interacts with Zip4 (Yang et al. 2008; De Muyt et al. 2018; Pyatnitskaya et al. 2019), a member of the ZMM protein ensemble (Zip1, Zip3, the Zip2-Zip4-Spo16 complex, the Msh4-Msh5 complex, and Mer3) that stabilizes double-Holliday junction intermediates and directs them toward resolution as COs by the meiosis-specific resolvase, MutLγ (Mih1-Mih3 and Exo1; Schwacha and Kleckner 1994; Wang et al. 1999; Khazanehdari and Borts 2000; Kirkpatrick et al. 2000; Tsubouchi and Ogawa 2000; Allers and Lichten 2001a, 2001b; Hoffmann et al. 2003; Bishop and Zickler 2004; Borner et al. 2004; Jessop et al. 2006; Lynn et al. 2007; Nishant et al. 2008; Zakharyevich et al. 2010; Cameron et al. 2012; Wang et al. 2012; Yang et al. 2012; Al-Sweel et al. 2017; De Muyt et al. 2018; Pyatnitskaya et al. 2019; Cannavo et al. 2020; Kulkarni et al. 2020; Sanchez et al. 2020). This is the major pathway for CO formation; a minority of COs are formed by the mitotic structure-selective nucleases (SSNs) Mus81-Mms4, S1x1-S1x4, Yen1 (de los Santos et al. 2003; Argueso et al. 2004; Hollingsworth and Brill 2004; Lynn et al. 2007; Jessop and Lichten 2008; De Muyt et al. 2012; Zakharyevich et al. 2012; Agostinho et al. 2013; Oke et al. 2014). Joint molecule resolution and CO formation in both pathways depend on the meiosis-specific transcription factor Nd80, which drives the mid-meiosis expression of many proteins required to complete meiosis and sporulation, including the polo-like kinase Cdc5 that stimulates resolvase activities (Xu et al. 1995; Chu and Herskowitz 1998; Allers and Lichten 2001a; Clyne et al. 2003; Sourirajan and Lichten 2008; Matos et al. 2011; De Muyt et al. 2012; Sanchez et al. 2020).

In summary, meiotic axis proteins play roles in various stages of meiotic recombination, with current data indicating that Hop1 has an early role in DSB formation and partner choice, while Red1 has a later role in recombination pathway choice. However, because Red1 and Hop1 are co-dependent for much of their localization, determining the specific role that each protein plays in meiotic recombination remains a challenge. Here, we used a novel approach based on a bacterial ParB-parS partition system (Khare et al. 2004; Dubarry et al. 2006, Murray et al. 2006, Sullivan et al. 2009; Graham et al. 2014; Attaiech et al. 2015), to recruit Hop1 to regions where meiotic axis proteins are normally depleted. We find that recruiting Hop1 at high levels is sufficient to dramatically increase both DSBs and homologous recombination, consistent with Hop1 being the most immediate determinant of where meiotic recombination occurs in the genome.

**Materials and methods**

**Yeast strains**

All S. cerevisiae strains (Supplementary File 1, sheet 1) used in this study are of SK1 background (Kane and Roth 1974) and were made by transformation or genetic crosses. To monitor the effect of axis protein recruitment via the ParB-parS system, 2 recombination reporter inserts were used (for schematics, see Figs. 2a and 9a). The first is a modification of the previously described URA3-ARG4-pBR322 insert (Wu and Lichtien 1995; Borde et al. 1999), and the second a modification of the previously described URA3-tel-ARG4 insert (Jessop et al. 2005; Ahuja et al. 2021). For both inserts, a 1-kb fragment containing the parSc2 element from chromosome c2 of Burkholderia cenocepacia J231 (Saad et al. 2014)
was synthesized and added downstream of the ARG4 gene. The URA3-ARG4-pBR322-parS construct was linearized by EcoRI and inserted 237 nt downstream of HXT1 and 150 nt downstream of YCR017c by ends-out 3-piece transformation (primers in Supplementary File 1, sheet 2). For insertion at URA3, the construct was linearized by ApaI, which cuts in the URA3 gene, and was inserted via ends-in 1-piece transformation. Hop1-ParB fusions are illustrated in Fig. 1a. The URA3-tel-ARG4-parS construct was inserted by ends-out transformation with a PCR-amplified copy with 60 nt termini homologous to URA3-flanking sequences (primers in Supplementary File 1, sheet 2). Sequences encoding ParBc2, which binds to parSc2 (Saad et al. 2014), were modified to include a V5 tag (Funakoshi and Hochstrasser 2009) and a stop codon at its C-terminus. This was combined with HOP1 flanking sequences in the following order to make pMJ1088 (sequence in Supplementary File 3): the HOP1 promoter (+652 to –1 nt); ParBc2-V5—stop codon; HOP1 3’UTR (131 bp starting at the 3’ end of HOP1 coding sequences); natMX4 (Lorenz 2015). PCR products (primers in Supplementary File 1, sheet 2) containing this element were integrated at HOP1 by single ends-in transformation to produce a HOP1 duplication where 1 copy was C-terminally tagged copy of HOP1 ([HOP1-parBsc2-V5]-natMX). Although both HOP1-V5-parBsc2 and HOP1 are expressed from the endogenous HOP1 promoter, levels of the Hop1-V5-ParB fusion protein were about 10–20% lower than of the corresponding wild-type Hop1 protein (Fig. 1b and Supplementary File 1, sheet 14).

To genetically monitor CO, markers flanking the URA3-arg4-pBR322-parS insert at URA3 were inserted by transformation (primers in Supplementary File 1, sheet 2): kanMX6 (Lorenz 2015) into the intergenic region between RIP1 and YEL023c (~14 kb to the right of the insert); and hygMX6 (Saad et al. 2014) into the intergenic region between NPF2 and EDC3 (~11 kb to the left).

**Sporulation, DNA extraction, and Southern blots**

Strains were grown in liquid presporulation medium and transferred to liquid sporulation medium as described (Goyon and Lichten 1993). Culture samples were collected and processed as described (Allers and Lichten 2000; Jessop et al. 2005, 2006). DNA was extracted as described (Goyon and Lichten 1993), digested with the appropriate restriction enzymes, displayed on agarose gels, transferred to membranes, hybridized to radioactive probes (Supplementary File 1, sheet 3), and analyzed as described (Wu and Lichten 1994, 1995; Allers and Lichten 2001b).

![Fig. 1. ParB fusion constructs. a) Illustration of protein fusions used. Dark arrows—coding sequences of tagged and untagged HOP1; vertical lines—fusion junction; light arrows—natMX6 drug resistance cassette; open boxes—5’ and 3’ HOP1 untranslated regions; thin black lines—flanking yeast chromosome sequences. b) Western blot of samples taken at indicated time in meiosis, probed with anti-Hop1. Bands corresponding to Hop1 and to Hop1-ParB are indicated; asterisk indicates nonspecific background band. Ratios of Hop1-ParB/Hop1 are indicated for strains where the 2 proteins are both present. See also Supplementary File 1, sheet 14.](https://academic.oup.com/genetics/article/222/1/iyac106/6649696)
Western blots

Protein was extracted from meiotic cultures, displayed on polyacrylamide gels, blotted to membranes and probed basically as described (Kaur et al. 2018), except that 5% nonfat dry milk was used in place of iBlock. Primary antisera and dilutions used were: rabbit anti-Hop1 (made for this work, 1:7,500) and goat anti-Arp7 (Santa Cruz Biotechnology Cat# sc-8961, RRID: AB_671730, 1:1,000). Secondary antisera were: goat polyclonal anti-rabbit conjugated with alkaline phosphatase (Abcam Cat# ab97048, RRID: AB_10680574, 1:10,000) and rabbit anti-goat IgG conjugated with alkaline phosphatase (Sigma-Aldrich Cat# A4187, RRID: AB_258141, 1:5,000). Chemiluminescence signals were captured using a BioRad Chemidoc MP imaging system and were quantified using the gel quantification tools in Fiji (Schindelin et al. 2012).

Cytology

Nuclear divisions were monitored by DAPI staining as described (Goyon and Lichten 1993). Meiotic chromosome spreads and staining with antisera were performed as described (Loidl et al. 1991). The primary antibodies were: rabbit polyclonal anti-Hop1 serum (prepared for this project), 1:7,500 and mouse anti-V5 (Bio-Rad Cat# MCA1360, RRID: AB_222378, 1:250). The secondary antibodies were: goat anti-rabbit conjugated to Alexa 488 (Molecular Probes, Cat# A-11034, RRID: AB_2576217, 1:350) and donkey anti-mouse conjugated to Cy3 (Jackson ImmunoResearch Labs Cat#, RRID: AB_2315777, 1:500). Images were taken on a Zeiss Axioplan 2 imaging microscope using a 100× plan apochromatic objective (440782-9902) and a Zeiss AxioCam HRm camera.

Genetic analysis

Frequencies of recombination between heteroalleles were determined by random spore analysis as described (Lichten et al. 1987). Map distances were determined by tetrad dissection, using the formula of Perkins (Perkins 1949) as implemented at https://eliza.bethhousworth.com/StahlLabOnlineTools/compare2.php.

Calibrated chromatin immunoprecipitation and sequencing

Chromatin immunoprecipitation and sequencing (ChIP-seq) experiments used a protocol that combined and modified previous methods (Murakami and Keeney 2014; Makrantoni et al. 2019; Murakami H, personal communication). Strains used contained URA3-tel-ARG4-parS (Makrantoni et al. 2019) by URA3-tel-ARG4-parS (Makrantoni et al. 2019) and vice versa to identify those reads that mapped to both genomes and those that mapped uniquely to a single genome. A calibration factor, called the occupancy ratio (OR), was then calculated from the counts of such reads as:

\[
\text{OR} = \frac{\text{ipSK1} / \text{inSK1}}{\text{ipSMIK} / \text{inSMIK}}
\]

where ipSK1 is the count of IP reads mapping uniquely to the SK1 genome, inSK1 is the count of input reads mapping uniquely to the SK1 genome, ipSMIK is the count of IP reads mapping uniquely to the S. mikatae genome, and inSMIK is the count of input reads mapping uniquely to the S. mikatae genome.

Calibrated depths for reads mapping uniquely to the SK1 genome were determined by multiplying read depths per million mapped reads by the OR computed in Equation (1). Data processing was performed on the NIH HPC Biowulf cluster (http://hpc.nih.gov). Scripts implementing the calibrated ChIP processing pipeline as a Snakemake (Mölder et al. 2021) workflow, suitable
for parallel execution on the Biowulf cluster, are included in Supplementary File 2; sequence reads are available at GEO, accession GSE201240.

**Data representation**

All values reported in figures are the mean of 2 or more independent experiments. Error bars denote the range in data values, except in Fig. 8b, where they denote calculated standard error.

**Results**

To recruit axis proteins to target loci, we used the bacterial ParB-parS chromosome segregation system, where the ParB protein binds to a <1-kb-long cluster of parS sites and then spreads to adjacent DNA (Lin and Grossman 1998; Dubarry et al. 2006; Breier and Grossman 2007; Attaiech et al. 2015; Soh et al. 2019). This system allows recruitment of multiple copies of ParB, fused to a protein of interest, with minimal disruption of chromosome integrity and function (Dubarry et al. 2006; Saad et al. 2014). We fused ParB and a V5 epitope tag to the C-terminus of Hop1 (hereafter called Hop1-ParB; Fig. 1a) to target this protein to 3 loci: URA3, HXT1; and YCR017c. All 3 are in regions of the yeast genome with low levels of occupancy by meiotic axis proteins and low levels of meiotic DSBs (Supplementary Fig. 1; Pan et al. 2011; Panizza et al. 2011).

**Recruiting Hop1 increases meiotic recombination**

To determine the effect of recruiting Hop1 on meiotic recombination, we initially used random spore analysis to examine recombination between arg4 heteroalleles in a URA3-ARG4-pBR322-parS recombination reporter inserted at URA3 (Fig. 2a). The same insert, but without parS, forms DSBs and undergoes recombination at levels that are location-dependent and that reflect underlying recombination levels in the region where it is inserted (Borde et al. 1999). As a no-insert control, we also measured recombination between heteroalleles at LEU2 (Fig. 2a). Initial experiments used a Hop1 gene duplication that contained both a tagged and a wild-type copy, to ensure normal function in the event that the tagged protein was only partially functional.

Recruiting Hop1-ParB caused a striking increase in recombination in the arg4 gene inserted at URA3 (Fig. 2b and Supplementary File 1, sheet 4). Inserts at HXT1 and YCR017c, 2 other axis protein/DSB coldspots (Supplementary Fig. 1), also displayed markedly increased Arg+ recombinant frequencies when Hop1-ParB was present (Fig. 2c). The presence or absence of a ParB-tagged axis protein did not markedly change recombination frequencies at the leu2 control locus (ranging from 3.1 \times 10^{-3} to 4.5 \times 10^{-3} across all crosses; Fig. 2, b and c and Supplementary File 1, sheet 4). These results suggest that levels of Hop1 in a region might be sufficient to determine levels of meiotic recombination in that region.

Recruiting Hop1-ParB also markedly increased crossing-over in a region containing the insert at URA3. Crossing-over was measured by analysis of tetrads from a diploid that contained a kanMX6 insert 14-kb centromere proximal to URA3-ARG4-pBR322-parS in 1 parent, and a hypMX6 insert 11-kb centromere distal in the other parent. The genetic distance for this ~35-kb interval was 12.3 \pm 1.78 cm in diploids lacking Hop1-ParB and 66.2 \pm 6.16 cm in diploids expressing Hop1-ParB, a ~5-fold stimulation of crossing-over (Supplementary File 1, sheet 10, also see Fig. 8). This remarkably high level of crossing-over indicates that most cells undergo recombination at this locus.

**Recruiting Hop1 increases DSB formation**

To confirm that recruiting Hop1 increases meiotic recombination by increasing levels of DSBs, we determined cumulative DSB levels in rec8A mutants, which accumulate un repaired DSBs with unresected ends (Keeney and Kleckner 1995; Prinz et al. 1997). Consistent with previous data (Borde et al. 1999; Pan et al. 2011), very few DSBs were present in reporter inserts at the 3 target loci (URA3, HXT1, and YCR017c) in the absence of Hop1-ParB or when ParB alone was expressed. The presence of Hop1-ParB increased DSBs in the reporter construct dramatically at all 3 loci (Fig. 3, a and b and Supplementary File 1, sheet 5), while the DSBs at the ARE1 control locus (Goldway et al. 1993) were relatively unchanged (Fig. 3b). Hop1-ParB recruitment caused the greatest increase in DSBs in the insert at URA3 locus, where DSB levels (~21% of chromatids) are consistent with most cells experiencing a break at this locus.

We also asked if Hop1 levels affect DSBs in cis or trans. In strains with parS on only 1 of the 2 homologs, the homolog with parS displayed insert DSBs at levels like those seen in strains lacking a parS-homoygous diploid, while the homolog lacking parS displayed DSBs at levels like those seen in strains without parS (Fig. 3, c and d and Supplementary File 1, sheet 6). Thus, the DSB increase observed is primarily due to recruited Hop1 acting in cis.

**Hop1-ParB-stimulated DSBs require Spo11 but not Rec8 or Red1**

According to current models, DSBs are formed by the Spo11 complex, which is recruited to the cohesin-based axis by interactions with Hop1, which in turn can be recruited to the axis via interactions with Red1 (Panizza et al. 2011; Sun et al. 2015; Zickler and Kleckner 2015; West et al. 2019; Roussová et al. 2021). This suggests that artificially recruiting Hop1 to chromosomes might bypass the need for Red1 or cohesin in DSB formation. To test this suggestion, DSBs in the insert at URA3 were examined in rec8A strains that were lacking Spo11, Red1, or the meiosis-specific cohesin component Rec8.

As expected, DSBs were abolished at all loci in spo11A strains, regardless of whether Hop1-ParB was present (Fig. 4, a and b and Supplementary File 1, sheet 5). Consistent with previous reports (Woltering et al. 2000; Peciná et al. 2002; Niu et al. 2005), red1A mutants displayed a substantial decrease (to ~40% of wild-type controls) in DSBs at the ARE1 control locus regardless of whether Hop1-ParB was present or absent. When only Hop1 was present, the parS insert locus showed a similar decrease in DSBs. However, when Hop1-ParB was present, DSB levels at the parS insert in red1A strains were similar to those in RED1 strains (Fig. 4, a and b and Supplementary File 1, sheet 5). Thus, direct recruitment of Hop1 appears to bypass the role of Red1 in DSB formation.

Previous studies have shown that rec8A strains display rearranged patterns of Red1, Hop1, and Spo11-complex components, with a tendency toward reducing occupancy at hotspots in the centers of large chromosomes while preserving occupancy, albeit at much-reduced levels, on short chromosomes and at certain loci on other chromosomes (Kugou et al. 2009; Panizza et al. 2011; Sun et al. 2015; Heldrich et al. 2022). Consistent with previous data showing that, at URA3 and ARE1, occupancy by these proteins is not substantially altered in rec8A mutants (Kugou et al. 2009; Panizza et al. 2011), DSBs at the parS insert locus and at the ARE1 control locus were similar in REC8 and in rec8A strains, regardless of the presence or absence of Hop1-ParB (Fig. 4, a and b and Supplementary File 1, sheet 5). Thus, unlike many DSB hotspots in the centers of long chromosomes, the hotspot created by
recruitment of Hop1-ParB to the insert at URA3 is not affected by loss of Rec8-cohesin.

The ParB-parS system specifically enriches Hop1 at the target locus

To confirm that the ParB-parS-dependent increase in meiotic recombination was associated with recruitment of Hop1, we used calibrated ChIP-seq to map Hop1 occupancy genome-wide, using a spike-in sample from meiotic cells of S. mikatae. S. mikatae is substantially diverged from S. cerevisiae (24% nucleotide divergence genome wide), but S. mikatae Hop1 shows 86.5% amino acid identity with S. cerevisiae Hop1, and cross-reacts with the antiserum against S. cerevisiae Hop1 used here for ChIP (Kellis et al. 2003; Dujon 2006; Liti et al. 2013; Lam and Keeney 2015; data not shown). Strains expressing either both Hop1 and Hop1-ParB (HOP1-ParB HOP1/HOP1) or Hop1 alone displayed similar Hop1 occupancy profiles genome-wide (Fig. 5, a and b and Supplementary Fig. 2). However, in strains expressing Hop1-ParB, Hop1 occupancy in a ~50-kb region surrounding the parS insert at URA3 was much greater than the Hop1 signal in the rest of the genome (Fig. 5, a and c). Quantitative interpretation of this pattern is complicated by the fact that strains expressing both Hop1-ParB and Hop1 have 3 modes of Hop1 chromosome binding: direct binding of the ParB domain in Hop1-ParB to chromosomal DNA; indirect binding of Hop1 through its interactions with itself and with Red1 and cohesin (Smith and Roeder 1997; Klein et al. 1999; Blat et al. 2002; Riedel et al. 2006; Panizza et al. 2011; Sun et al. 2015; West et al. 2018, 2019); and possible direct binding of Hop1 to DNA (Kironmai et al. 1998; Kshirsagar et al. 2017; Heldrich et al. 2020, 2022). Hop1 bound in these 3 modes is likely to be crosslinked to DNA with different efficiencies. Therefore, while the increased Hop1 ChIP signal in the vicinity of parS almost
certainly indicates that more Hop1 is bound in this region, the quantitative extent of that increase remains to be determined.

We also compared the distribution of Hop1 and Hop1-ParB on chromosome spreads of cells at the pachytene stage, using anti-Hop1 to detect both proteins and anti-V5 to specifically detect the Hop1-ParB fusion protein (Fig. 5d). Cells expressing Hop1 alone displayed a pattern of lines and punctate foci, as has been previously reported (Smith and Roeder 1997). Cells expressing both Hop1 and Hop1-ParB displayed a similar pattern, and similar staining patterns were obtained with anti-Hop1 (detecting Hop1 and Hop1-ParB) and anti-V5 (detecting Hop1-ParB only). Thus, Hop1-ParB appears to localize across the genome, at least when Hop1 is also present.

**Hop1-ParB provides partial Hop1 function**

The experiments described above used strains with a wild-type copy of the HOP1 gene in addition to the HOP1-ParB fusion (see Materials and methods). To determine whether Hop1-ParB was fully functional when expressed on its own, we examined meiotic spore viability, recombination, and DSB formation in strains containing a parS insert at URA3 on both homologs, where: (parS/parS)—both contain parS; (-/-)—both are without parS. DNA was digested with SbfI and probed with pBR322 sequences, which allows distinction between breaks at DSB1 on chromosomes with and without parS. Breaks at DSB2 cannot be resolved. Quantification of breaks at DSB1 in the parS hemizygous strain, as well as in control strains with homozygous inserts where both homologs either lacked or contained parS. DSB1 levels are normalized on a per chromatid basis. Because of overlapping signal, signals of DSB2 on chromosomes with parS could not be resolved from those on chromosomes without parS. Values in graphs are the average of 2 or more independent experiments, error bars denote range. See also Supplementary Fig. 3 and File 1, sheets 5 and 6.

**Fig. 3.** Hop1-ParB recruitment increases DSBs in reporter inserts. a) Southern blot of DNA from sae2Δ strains, which form DSBs but do not resect or repair them, with parS insert at indicated locus. Indicated restriction digests were probed with parS sequences to detect DSBs in the insert. These occur in pBR322 sequences on either side of ARG4 sequences (Wu and Lichten 1995), and will be called DSB1 and DSB2 as shown in the schematic. Strains were homozygous either for HOP1 (gray) or Hop1-parB HOP1 (salmon). b) Hop1-ParB increases DSBs (DSB1 + DSB2) at all 3 insert loci (top), but not at the ARE1 control locus (bottom). c) Hop1-parB acts primarily in cis: Southern blot with DNA from a sae2Δ strain with inserts at URA3 on both homologs, where: (parS/parS)—both contain parS; (-/-)—both are without parS. DNA was digested with SbfI and probed with pBR322 sequences, which allows distinction between breaks at DSB1 on chromosomes with and without parS. Breaks at DSB2 cannot be resolved. d) Quantification of breaks at DSB1 in the parS hemizygous strain, as well as in control strains with homozygous inserts where both homologs either lacked or contained parS. DSB1 levels are normalized on a per chromatid basis. Because of overlapping signal, signals of DSB2 on chromosomes with parS could not be resolved from those on chromosomes without parS. Values in graphs are the average of 2 or more independent experiments, error bars denote range. See also Supplementary Fig. 3 and File 1, sheets 5 and 6.
Altered DSB repair in the presence of Hop1-ParB and in inserts at URA3

The Hop1-ParB fusion protein also conferred an apparent defect in meiotic DSB repair. Cells expressing Hop1-ParB showed a 45–50-min delay in the disappearance of DSBs both at the parS insert and at the ARE1 control locus (Fig. 7, a, c, and d and Supplementary File 1, sheet 8). This delay was accompanied by a delay in meiotic divisions that increased with HOP1-parB gene dosage (Fig. 7b and Supplementary File 1, sheet 9), consistent with the presence of unrepaired DSBs activating the meiotic checkpoint (Lydall et al. 1996; Grushcow et al. 1999; Thompson and Stahl 1999; Roeder and Bailis 2000; Shimada et al. 2002).

In addition, Hop1-ParB-stimulated COs in inserts at URA3 do not appear to use the CO recombination pathway that is dominant at other DSB hotspots. Previous studies indicate that most meiotic COs form via a pathway that involves the ZMM proteins and the meiosis-specific MutLγ resolvase (Mlh1-Mlh3-Exo1), and a minor fraction are formed by SSNs (Mus81-Mms4, Yen1, Slx1-Slx4; Schwacha and Kleckner 1994; Wang et al. 1999; Khazanehdari and Borts 2000; Kirkpatrick et al. 2000; Tsubouchi and Ogawa 2000; Allers and Lichten 2001a, 2001b; de los Santos et al. 2003; Hoffmann et al. 2003; Argueso et al. 2004; Bishop and Zickler 2004; Borner et al. 2004; Hollingsworth and Brill 2004; Jessop et al. 2006; Lynn et al. 2007; Jessop and Lichten 2008; Nishant et al. 2008; Zakharyevich et al. 2010; Comeron et al. 2012; De Muyt et al. 2012; Yang et al. 2012; Zhang et al. 2012; Zakharyevich et al. 2012; Agostinho et al. 2013; Oke et al. 2014; Al-Sweel et al. 2017; De Muyt et al. 2018; Pyatnitskaya et al. 2019).

However, genetic crossing-over in a ∼35-kb interval containing the URA3-arg4-pBR322-parS was reduced only modestly in mhhΔ strains, both in strains where Hop1-ParB was expressed and where Hop1-parB was absent (Fig. 8 and Supplementary File 1, sheet 10).

We also measured CO and noncrossover (NCO) recombination at the molecular level, using a second parS-containing insert at URA3 (URA3-tel-ARG4-parB; Fig. 9a) that contains a single DSB site (J Jessop et al. 2005; Ahuja et al. 2021). Consistent with experiments described above that used URA3-arg4-pBR322-parS inserts, addition of a single copy of Hop1-ParB (HOP1-parB HOP1/HOP1) resulted in a marked increase in DSBs (∼7-fold; Fig. 9a and Supplementary File 1, sheet 12), in COs (∼5-fold; Fig. 9a, c, d and Supplementary File 1, sheet 11), and in NCOs (∼4.5-fold; Supplementary Fig. 5, a and c and File 1, sheet 11) within the insert at URA3.

Previous studies have shown that ndt80Δ mutants arrest at the pachytene stage of meiosis with markedly reduced CO levels, regardless of whether MutLγ or SSNs are the primary resolvase (Xu et al. 1995; Chu and Herskowitz 1998; Allers and Lichten 2001a; Jessop and Lichten 2008; Sourirajan and Lichten 2008;
De Muyt et al. (2012). Consistent with this, COs in the insert at URAT1 were reduced to about 1/3 to 1/4 of wild-type levels in ndt80Δ mutants, regardless of whether Hop1-ParB was present or absent (Fig. 9, c and d and Supplementary File 1, sheet 11). However, unlike at other hotspots, where mlh3Δ causes a ~50% reduction in COs (Hunter and Borts 1997; Wang et al. 1999; Argueso et al. 2004; Nishant et al. 2008; Al-Sweel et al. 2017), mlh3Δ caused a much more modest 10–15% decrease in COs (Fig. 9, c and d and Supplementary File 1, sheet 11), as was seen in genetic crosses (Fig. 8b). Inactivation of the major mitotic resolvase Mus81-Mms4, in mms4-md mutants, reduced COs in inserts at URAT1 by 30–40% (Fig. 9, c and d and Supplementary File 1, sheet 11), as compared to reductions of 10–20% (mus81Δ or mms4Δ) seen at recombination hotspots (Borner et al. 2004; Jessop et al. 2006; Oh et al. 2007; De Muyt et al. 2012; Zakharyevich et al. 2012; He et al. 2020). COs were reduced by about 50% in mlh3Δ mms4-md double mutants, as compared to the ~6-fold reduction reported in genetic studies (Argueso et al. 2004; Nishant et al. 2008; Brown et al. 2013). In these strains, COs were similarly affected whether Hop1-ParB was present or absent. Deletion of YEN1 from mlh3Δ mms4-md strains did not further reduce COs when Hop1-ParB was present but caused a further reduction (to about 1/3 of wild type) in COs when only Hop1 was present (mlh3Δ mms4-md yen1Δ; Fig. 9, c and d and Supplementary File 1, sheet 11). Thus, regardless of DSB levels, the canonical, MutLc-dependent pathway accounts for only a minor fraction of COs in inserts at URAT1.

Discussion

The meiotic chromosome axis lies at the center of meiotic chromosome transactions, including the initiation of recombination by DSB formation, recombination partner choice and homolog
pairing, CO designation and pathway choice, and CO assurance and spacing control (Hollingsworth and Ponte 1997; Zickler and Kleckner 1999; Blat et al. 2002; Glynn et al. 2004; Kleckner 2006; Tsubouchi and Roeder 2006; Niu et al. 2007; Yang et al. 2008; Joshi et al. 2009; Kugou et al. 2009; Niu et al. 2009; Callender and Hollingsworth 2010; Kim et al. 2010; Panizza et al. 2011; Youds and Boulton 2011; Kim et al. 2010; Panizza et al. 2011; Youds and Boulton 2011; Chuang et al. 2012; De Myt et al. 2018; Pyatnitskaya et al. 2019; Ur and Corbett 2021; Hyppa et al. 2022). While the ParB-parS system has previously been used as an alternative to operator-represser arrays to visually label specific loci (Saad et al. 2014; Germier et al. 2018), the use of this system to recruit meiotic axis proteins has presented a challenge to the identification of their individual roles in meiotic recombination. In this article, we used the bacterial ParB-parS system to independently enrich the axis protein Hop1 at target loci, and to identify a unique role for Hop1 in DSB formation.

Hop1 is efficiently recruited by the ParB-parS system

While the ParB-parS system has previously been used as an alternative to operator-repressor arrays to visually label specific loci (Saad et al. 2014; Germier et al. 2018), the use of this system to recruit meiotic axis proteins is, to our knowledge, the first time that it has been used to localize chromosomal proteins with the goal of understanding their function. Hop1-ParB expression caused a markedly greater increase in Hop1 ChIP signal at the parS site and for about 25 kb to either side of parS, consistent with the spread of ParB from parS observed in bacteria, which is facilitated by its ability to dimerize and form a clamp that slides along DNA (Walter et al. 2020) and by its ability to bridge DNA (Breier and Grossman 2007; Graham et al. 2014; Antar et al. 2021).

Cytological analysis showed that Hop1-ParB and the wild-type Hop1 protein show similar nucleus-wide localization patterns (Fig. 5d), suggesting that the C-terminal tag does not prevent Hop1-ParB loading via Hop1-Red1 or Hop1-Hop1 interactions (West et al. 2018, 2019).

Hop1 determines local DSB levels

Previous studies have reported a direct correlation between levels of Hop1 (and Red1) enrichment and levels of Spo11 DSBs in different regions of the genome (Pan et al. 2011; Panizza et al. 2011; Subramanian et al. 2019). Here, we have shown that ParB-parS-mediated recruitment of Hop1 to a locus causes a dramatic increase in Spo11-dependent DSBs at that locus. This increase in DSBs is independent of the other meiosis-specific axis proteins, Red1 and Rec8. Thus, while Red1 and Rec8 might be required for Hop1 loading under normal circumstances, it is the level of Hop1 enrichment that ultimately determines the local DSB levels. This suggests that Hop1 alone is sufficient to recruit the DSB-forming Spo11 complex, consistent with recent biochemical studies.
showing that the Spo11 complex protein Mer2 interacts directly with Hop1 and not with Red1 (Rousová et al. 2021).

We found that, while Hop1-ParB can stimulate DSB formation in the vicinity of par5, Hop1-ParB alone was insufficient for optimal DSB formation, recombination, and spore viability, and that full function required addition of 1 to 2 copies of wild-type HOP1, depending upon the assay (Fig. 6). Since Hop1-ParB is produced at about 80–90% of the levels of wild-type Hop1 protein (Fig. 1b), it is unlikely that these results can be explained by reduced levels of Hop1 protein alone, although it is possible that over-enrichment of Hop1 at par5 reduces Hop1 levels elsewhere in the genome. It also is possible that the presence of the C-terminal ParB tag creates a partially functional Hop1 protein. For example, recent in vitro studies have shown Mer2 preferentially binds to Hop1 with an unlocked closure motif (Rousová et al. 2021). Chromosome-bound Hop1 normally is in this unlocked configuration, due to closure motif-HORMA domain interactions that recruit it to the axis (West et al. 2018, 2019). However, Hop1 recruited to chromosomes by a ParB tag might frequently exist in the locked confirmation, and thus might recruit the Spo11 complex less efficiently. In addition, the ParB tag might interfere with interactions necessary for Hop1 posttranslational modification, and/or Hop1 loading/unloading (Carballo et al. 2008; Wojtasz et al. 2009; Thacker et al. 2014; Herruzo et al. 2016, 2021; Li and Shinohara 2021). For example, Hop1 is normally removed from the axis after homolog synapsis (Borner et al. 2008; Joshi et al. 2009; Wojtasz et al. 2009; Zanders and Alani 2009; Daniel et al. 2011; Kauppi et al. 2013; Thacker et al. 2014; Lambing et al. 2015; Subramanian et al. 2016, 2019); a failure to remove Hop1-ParB bound via the ParB tag might result in the persistent DSBs and delayed progression that we observed when Hop1-ParB is present (Fig. 7).

Noncanonical recombination pathway usage in inserts at the URA3 locus

Previous studies of CO formation have concluded that most meiotic COs are formed by MutLc-dependent double Holliday junction resolution, a minor fraction are formed by mitotic resolvases (Mus81-Mms4/Eme1, Yen1/Gen1, and Slx1-Slx4) and that both modes of resolution are CDC5- and NDT80-dependent (Xu et al.
1995; Chu and Herskowitz 1998; Allers and Lichten 2001a; Clyne et al. 2003; Jessop and Lichten 2008; Sourirajan and Lichten 2008; DeMuyt et al. 2012; Schwartz et al. 2012; Zakharyevich et al. 2012; Blanco and Matos 2015; Yoon et al. 2016). These studies either examined events at recombination hotspots or examined crossing-over in large genetic intervals, in which presumably most recombination is hotspot driven. We find that recombination in inserts at \textit{URA3} does not conform to these conclusions.

While ndt80\textsuperscript{D} substantially reduced COs (Fig. 9, c and d), consistent with crossing over in inserts at \textit{URA3} being resolvase driven, specific resolvase dependence of COs was substantially altered. Unlike in previous studies, where loss of MutL\textsuperscript{c} results in a CO reduction by a factor of 2 (Hunter and Borts 1997; Wang et al. 1999; Argueso et al. 2004; Nishant et al. 2008; Al-Sweel et al. 2017), mlh3\textsuperscript{D} mutants showed a substantially lower CO reduction (~20–25% when measured genetically, Fig. 8b; ~10–15% at the molecular level, Fig. 9, c and d). In addition, mms4-md mutants, which cause a meiosis-specific loss of Mus81-Mms4 activity, showed a substantial (30–40%) CO reduction in inserts at \textit{URA3}, which is greater than the minor CO reductions seen in the absence of Mus81-Mms4 in other studies (Argueso et al. 2004; Jessop and Lichten 2008; DeMuyt et al. 2012; Zakharyevich et al. 2012). Taken together, these data indicate a shift away from resolution by MutL\textsuperscript{c}, and toward resolution by mitotic resolvases during Spo11-induced recombination at \textit{URA3}. Of particular importance, similar MutL\textsuperscript{c}-independence was seen in \textit{HOP1/HOP1} and \textit{HOP1-parB HOP1/HOP1} strains, even though DSB levels and CO levels differ more than 5-fold between these strains (Fig. 9). Intriguingly, when all 3 major resolvase activities were absent

![Fig. 8. Effect of mlh3\textsuperscript{D} on CO at \textit{URA3}.](image)

(a) Schematic of the interval used to measure map distances by tetrad dissection. b) Map distances, calculated from marker segregation in tetrads, between kanMX and hygMX inserts flanking a \textit{URA3-ARG4-parS22} insert at the \textit{URA3} locus (shown in [a]; also see Materials and methods). Gray—\textit{HOP1/HOP1}, salmon—\textit{HOP1-parB HOP1/HOP1-parB HOP1}. Expression of Hop1-ParB results in a marked increase in map distances. Map distances are only modestly decreased in mlh3\textsuperscript{3A} strains. Error bars denote calculated standard error. See also Supplementary File 1, sheet 10.

![Fig. 9. Noncanonical CO pathway usage at \textit{URA3}.](image)

a) Schematic for the \textit{URA3-tel-arg4-parS} reporter insert at \textit{URA3}, showing product lengths in XmnI digests. Left—Southern blots showing DSBs in \textit{sae2} strains; right—CO (CO1 and CO2) products in \textit{SAE2} strains. Both blots were probed with \textit{URA3} sequences. b) Quantification of insert DSBs in \textit{sae2} \textit{HOP1/HOP1} (gray) or \textit{sae2} \textit{HOP1-parB HOP1/HOP1} (salmon) strains in samples taken 7h after meiotic induction. c) Quantification of COs (CO1 + CO2) in \textit{HOP1/HOP1} (gray) or \textit{HOP1-parB HOP1/HOP1} (salmon) strains in samples taken 8h after meiotic induction in the indicated mutants. d) Quantification of COs (CO1 + CO2) with a different scale for \textit{HOP1/HOP1} (gray, right Y axis) and \textit{HOP1-ParB HOP1/HOP1} (salmon, left Y axis) to compare relative levels in indicated mutants in the presence or absence of Hop1-ParB. Details as in panel (c). Values in graphs are the average of 2 or more independent experiments; error bars denote range. See also Supplementary Fig. 5 and File 1, sheets 11 and 12.
COs were markedly reduced when only Hop1 was present (to 15% of wild-type levels) but were still present at substantial levels (55% of wild type) when Hop1-ParB was present (Fig. 9, c and d and Supplementary File 1, sheet 11). This raises the possibility that either additional resolvases (such as Slx1-Slx4) or other repair pathways (such as break-induced replication) may be operating at loci with high DSB levels due to artificial Hop1 recruitment, as has been suggested for recombination in cells undergoing meiosis that is initiated by the VDE or HO site-specific nuclease (Medhi et al. 2016; Yisehak and MacQueen 2018; Shodhan et al. 2019).

One possible explanation for this is that Hop1-independent features of chromosome structure determine CO pathway choice. One such feature could be the axis proteins themselves. Red1 interacts with Zip4, a part of the ZZS complex and the larger ensemble of ZMM proteins that are important for the MutL-dependent CO pathway, and this interaction is conserved in other organisms (Yang et al. 2008; De Muyl et al. 2018; Pyatnitskaya et al. 2019). The meiotic cohesin component Rec8 has also been identified as playing a role in homolog bias and CO formation (Yoon et al. 2016; Hong et al. 2019), although this may simply reflect its role in recruiting Red1. If recruiting Hop1 to ‘cold’ regions increases DSB formation without increasing meiotic cohesin and/or Red1 levels, it is possible that insufficient ZMM proteins are recruited to promote MutL-dependent intermediate resolution, leading to an increased use of mitotic resolvases and of other pathways for CO formation.

In summary, we report here a novel use of the ParB-parS bacterial partition system, to study the role of chromosome structural proteins in meiotic recombination at a specific locus without substantially altering recombination elsewhere in the genome. The artificial recruitment of Hop1 to regions where meiotic axis proteins are normally low enabled the conversion of DSB/recombination coldspots into recombination hotspots by specifically increasing DSB formation independent of other axis proteins. Our data suggest an independent role for Hop1 in DSB formation, but also a need for the other axis proteins or other factors in normal repair pathway choice. It will be of interest to determine if recruiting both Red1 and Hop1 to these coldspots-turned hotspots can restore a more wild-type pattern of resolvasse usage during meiotic CO formation. We also anticipate that artificial Hop1 recruitment could facilitate analysis of the interactions between Hop1 and Spo11 complex proteins that promote DSB formation. In addition, artificial recruitment of Hop1 homologs in other organism may provide a targeted way to increase meiotic recombination in regions where recombination is normally low, both for mechanistic studies and for breeding purposes.

Supplementary File 3 contains the sequence of plasmid pMJ1088. ChIP sequence reads are available at GEO, accession GSE201240.

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Conflicts of interest

None declared.

Literature cited


Data availability

All strains and plasmids are available upon request. The authors affirm that all data necessary to confirm the conclusions of this article are represented within the article, tables, figures, supplementary materials, and publicly available database depositions. Supplemental files are available at figshare: https://doi.org/10.25386/genetics.20184515. Supplementary File 1 contains strain genotypes, primer and probe sequences, and numerical data underlying all graphs. Supplementary File 2 contains scripts implementing calibrated ChIP as a Snakemake workflow.

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