Current Biology, Vol. 14, 752–762, May 4, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.04.030

Mnd1 Is Required for Meiotic Interhomolog Repair

Christian Zierhut,[1,3,4] Marc Berlinger,[1,3] Christian Rupp,[1] Akira Shinohara,[2] and Franz Klein[1,*]

[1]Max F. Perutz Laboratories
Cell Biology and Genetics
Institute of Botany
University of Vienna
Rennweg 14, A-1030
Vienna

[2]Institute for Protein Research
Department of Biology
Osaka University
3-2 Yamadaoka
Suita, Osaka 565-0871
Japan

Summary

Background: While double-strand break (DSB) repair is vital to the survival of cells during both meiosis and mitosis, the preferred mechanism of repair differs drastically between the two types of cell cycle. Thus, during meiosis, it is the homologous chromosome rather than the sister chromatid that is used as a repair template.

Results: Cells attempting to undergo meiosis in the absence of Mnd1 arrest in prophase I due to the activation of the Mec1 DNA-damage checkpoint accumulating hyperresected DSBs and aberrant synapsis. Sporulation of *mnd1*Δ strains can be restored by deleting *RED1* or *HOP1,* which permits repair of DSBs by using the sister chromatid as a repair template. Mnd1 localizes to chromatin as foci independently of DSB formation, axial element (AE) formation, and synaptonemal complex (SC) formation and does not colocalize with Rad51. Mnd1 does not preferentially associate with hotspots of recombination.

Conclusions: Our results suggest that Mnd1 acts specifically to promote DSB repair by using the homologous chromosome as a repair template. The presence of Rec8, Red1, or Hop1 renders Mnd1 indispensable for DNA repair, presumably through the establishment of interhomolog (IH) bias. Localization studies suggest that Mnd1 carries out this function without being specifically recruited to the sites of DNA repair. We propose a model in which Mnd1 facilitates chromatin accessibility, which is required to allow strand invasion in meiotic chromatin.

Introduction

Meiosis is the specialized cell division undergone in sexually reproducing organisms during gamete formation in which chromosome number is halved by combining two rounds of chromosome segregation with a single round of DNA replication. Homologous recombination, which occurs during the prophase of meiosis I, physically links homologous chromosomes prior to their segregation at meiosis I, usually a pre-requisite for their correct segregation. Homologous recombination is initiated by DSBs [1, 2], the DNA ends of which invade a homologous chromatid for repair. This results in the formation of various intermediates including D loops and double Holliday junctions (dHJs), which are resolved to generate recombinant molecules (which can either be crossover or noncrossover). Only crossover recombinant molecules allow the physical association of homologous chromosomes through chiasmata, and it appears that pathways leading to crossover or noncrossover recombinant molecules diverge early, possibly at the time of DSB-formation [3].

Once homologous chromosomes are physically linked through chiasmata, differential loss of cohesion at chromosome arms, but not at centromeres, triggers the onset of anaphase I [4]. Meiotic sister chromatid cohesion is mediated by the meiotic cohesin complex in which Rec8 (which is able to support protection of centromeric cohesion and meiosis-specific DSB-repair [4, 5]) replaces the closely related Scc1 protein. While intersister (IS) repair is dominant during mitosis [6], it is suppressed during meiosis [7, 8], presumably because it fails to support pairing and reductional segregation of homologs. During meiotic IH recombination, the strand exchange protein Rad51 is assisted by a close meiotic homolog, Dmc1. Biochemical studies have implicated a second protein, Rad54, which exhibits sequence motives characteristic of Swi/Snf chromatin remodelling factors, in loading Rad51 onto ssDNA [9], D loop formation [10], and initiation of repair synthesis [11]. While Rad54 is required for IS repair in mitosis [12, 13] and for the IS-repair pathway of meiotic DSBs [14], Tid1/Rdh54, another Swi/Snf like protein that physically interacts with Dmc1 [15], is required for most meiotic IH recombination [16]. These examples demonstrate a remarkable distinction between IS- and IH-repair pathways in meiosis.

Progression through meiosis is under the tight control of DNA-damage checkpoints. Some DNA-damage checkpoint proteins, including the central checkpoint kinase Mec1, are common to both meiotic and mitotic checkpoint control, while others such as Pch2 are meiosis specific. In addition to their role in preventing nuclear division in the presence of DNA-repair intermediates, some checkpoint genes are also required for IH bias [17, 18].

The structure of meiotic chromosomes is critical for DSB formation, repair, and IH bias. After the establishment of cohesion, various proteins associate with each pair of sister chromatids to form AEs, important components of which in the budding yeast *Saccharomyces cerevisiae* are Red1 and Hop1. Red1 is required for formation of wild-type levels of DSBs and for establishing IH bias at dHJs [7]. By using chromatin immunoprecipi-

*Correspondence: franz.klein@univie.ac.at

[3]These authors contributed equally to this work.

[4]Present address: Cancer Research UK London Research Institute, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts, EN6 3LD, United Kingdom.

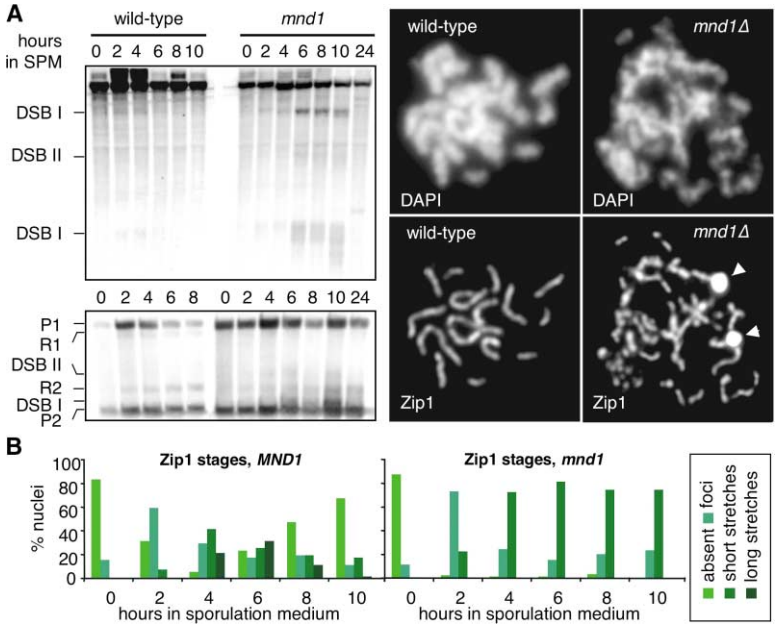

Figure 1. DSB Repair and Synapsis Are Impaired in *mnd1*Δ Cells

(A) Top: DSB assay from DNA extracted from wild-type (FKY1) and *mnd1*Δ (FKY1424) cells. DSBI and DSBII refer to the main sites of DSB formation at the *HIS4LEU2* hotspot. The bottom shows analysis of recombination products at *HIS4LEU2*. Abbreviations: P1, parental 1; R1, recombinant 1; P2, parental 2; R2, recombinant 2; DSBI and DSBII as above.

(B) Analysis of Zip1 staining. The panels on the upper right show maximal synapsis of a nucleus from wild-type (FKY1, left) and one of *mnd1*Δ (FKY1424, right) stained with α-Zip1 antiserum. Arrowheads point to brightly staining aggregates of Zip1 indicative of aberrant synapsis. The panels on the bottom show quantification of these data. 100 nuclei were scored per time point.

tation (ChIP; S. Prieler, A. Penker, V. Borde, and A.F., unpublished data), we recently discovered that the physical interaction of Spo11 with DNA is not as specific to recombination hotspots in the *red1* null mutant as in wild-type. As meiosis progresses, AEs of corresponding pairs of homologs synapse with the help of the transversal filament component Zip1 to form the tripartite SC structure (reviewed in [19]).

The mechanism governing the preference for IH repair during meiotic DSB-repair has yet to be fully understood. The *S. cerevisiae* protein Mnd1 may, however, be part of this system. We first identified *MND1* in an *S. cerevisiae* functional genomics screen in which genes specifically expressed early during meiosis were deleted [20]. In this study, we showed that *mnd1*Δ cells permanently arrest in prophase of meiosis I and, furthermore, that this arrest can be bypassed by deleting Spo11, hence implicating Mnd1 in meiotic DSB repair [20]. These findings have since been corroborated [21, 22] and, additionally, a physical interaction between Mnd1 and Hop2 [21] as well as a failure to repair DSBs in *mnd1*Δ cells [21, 22] have been reported. In this work, we present evidence that Mnd1 is involved in IH recombination in meiosis by allowing repair from the homolog in an environment coinhabited by IH-bias determinants such as Rec8, Red1, Hop1, and Dmc1.

Results

*mnd1*Δ Mutants Are Defective in Meiotic DSB Repair and Chromosome Synapsis

The prophase I arrest observed in *mnd1*Δ mutants, which could be bypassed by mutation of *SPO11* [20], suggested a role for Mnd1 in meiotic DSB repair. To confirm this, we performed a physical analysis of DSB formation in *mnd1*Δ cells at the well-characterized *his4-LEU2* recombination hotspot [23, 24]. In *mnd1*Δ cells, DSBs form with normal kinetics, but in contrast to the wild-type situation, do not disappear but accumulate, as previously observed (Figure 1A, [21, 22]). Moreover, in our preparations, the fragments corresponding to unrepaired DSBs show substantial degradation as determined by their diffuse, downward-trailing appearance. This phenomenon was also observed in strains mutated for either of the RecA homologs *DMC1* or *RAD51* [25, 26], suggesting that *mnd1*Δ mutants arrest with DSBs whose 5′ strands undergo extensive degradation. Extracted DNA was also tested for the formation of recombination-specific restriction fragments [24]. We confirm earlier reports [22] that the generation of recombination products is strongly reduced (Figure 1A) but also find a small degree of residual reciprocal recombination.

Synapsis and recombination are interdependent in *S. cerevisiae* and consequently SC formation is strongly compromised in *mnd1*Δ cells. Although early stages, such as nuclei containing Zip1 foci or very short synaptic stretches occur normally, many *mnd1*Δ cells arrest without or with immature SCs (Figure 1B). Nevertheless, isolated largely synapsed chromosomes can be detected and sometimes exhibit apparent axis cross-links (Figure 1B). Such interconnections have also been observed in several mutants defective in DSB repair such as *mre11S* [27], *com1/sae2*, [28] and *hop2* [29]. In these cases, an increase in nonhomologous synapsis was observed.

Mutation of *MEC1*, but Not of *PCH2*, Alleviates the Prophase Arrest of *mnd1*Δ Mutants

In order to examine whether the arrest observed in *mnd1*Δ mutants was the result of the activation of a DNA-damage checkpoint [30], we deleted the meiosis-specific checkpoint gene *PCH2* in *mnd1*Δ mutants. Deletion of *PCH2* was shown to partially restore nuclear divisions in *dmc1*Δ cells [31] whose DSB-phenotype is similar to that of *mnd1*Δ cells [26]. In the SK1 background, deletion of *PCH2* indeed restored nuclear division in ~24% of *dmc1*Δ cells (Table S2), a level lower than described in the BR strain background [31], but hardly in *mnd1*Δ cells (~4%). The *dmc1*Δ *mnd1*Δ *pch2*Δ

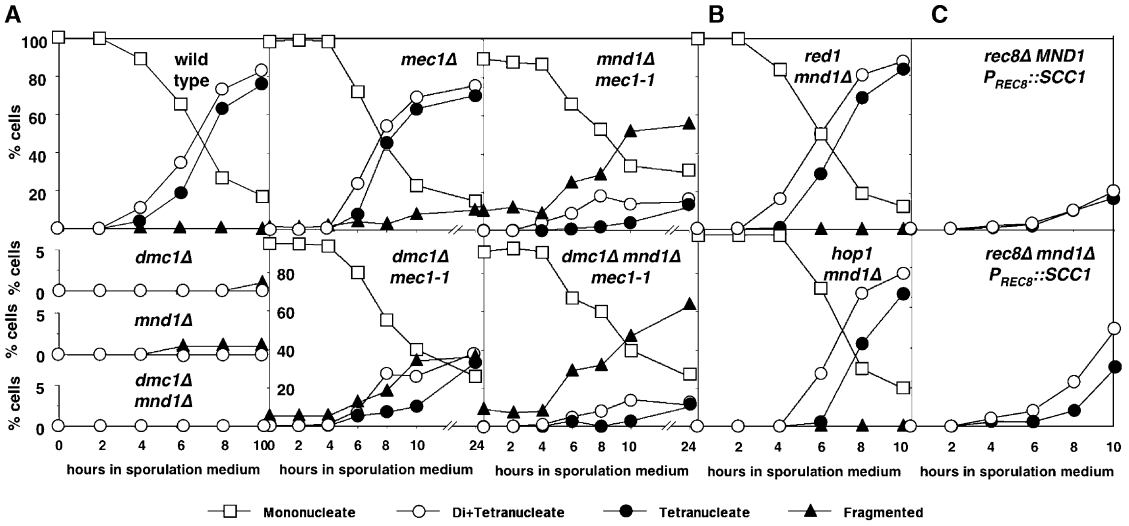

Figure 2. The Prophase Arrest of *mnd1*Δ Cells Is Bypassed by Mutation of *MEC1*, *RED1*, *HOP1*, and *REC8*

Nuclear divisions were scored after DAPI staining during meiotic time courses of the indicated strains (*dmc1*Δ, FKY1443; *mnd1*Δ, FKY1424; *dmc1*Δ *mnd1*Δ, FKY2054; *mec1-1*, FKY1484; *mnd1*Δ *mec1-1*, FKY2050; *dmc1*Δ *mec1-1*, FKY2049; *dmc1*Δ *mnd1*Δ *mec1-1*, FKY2051; *red1 mnd1*Δ, FKY2117; *hop1 mnd1*Δ FKY2118; *rec8*Δ P_{REC8}::*SCC1* FKY1889; *mnd1*Δ *rec8*Δ P_{REC8}::*SCC1* FKY2134). 100 cells were counted per time point.

triple mutant behaves identically to the *dmc1*Δ *pch2*Δ double mutant (Table S2), suggesting that the presence of Dmc1 on DSB ends accounts for some of the arrested *mnd1*Δ *pch2*Δ cells.

We also mutated *MEC1*, which encodes a highly conserved protein kinase whose function is required for most DNA-damage checkpoints (reviewed in [32]) in *mnd1*Δ mutants in yeast. Without functional Mec1 ~75% of the cells exited the mononucleate stage in *dmc1*Δ, *mnd1*Δ and *dmc1*Δ *mnd1*Δ strains by 24 hr in sporulation medium (SPM) (Figure 2A). However ~50% of progressive *dmc1*Δ *mec1-1* cells and over 80% of both *mnd1*Δ *mec1-1* double and *dmc1*Δ *mnd1*Δ *mec1-1* triple mutant cells underwent nuclear fragmentation (Figure 2A). Fragmentation probably derives from attempts to segregate fragmented, improperly attached chromosomes as 70% of *mnd1*Δ *mec1-1* cells proceeded to form anaphase spindles by 10 hr. In contrast, *mnd1*Δ single mutants arrest with a prophase I spindle. We conclude that the majority of *mnd1*Δ cells arrest in prophase I due to a DNA-damage checkpoint mechanism dependent on Mec1.

Interfering with Meiosis-Specific Chromosome Structure and DSB-Repair Mechanisms Allows Sporulation of *mnd1*Δ Mutants

It has been shown that the prophase arrest observed in several mutants defective for meiotic DSB-repair can be bypassed by deleting the AE protein-encoding genes *HOP1* or *RED1* (for example, [33–35]; reviewed in [30]). We found that meiotic nuclear division and spore formation is also restored to wild-type levels in the absence of Red1 or Hop1 in *mnd1*Δ cells (Figure 2B). The bypass of the prophase arrest observed in *mnd1*Δ *hop1* or *mnd1*Δ *red1* double mutants did not produce fragmented nuclei, suggesting that it involves elimination of breaks rather than loss of checkpoint control, as in *mnd1*Δ *mec1-1* mutants.

As a measure of break formation and persistence, we analyzed the distribution of Rad51 foci in *MND1*, *spo11*Δ, *mnd1*Δ, *mnd1*Δ *mec1-1*, *mnd1*Δ *red1*, and *mnd1*Δ *hop1* double mutants during meiotic time courses (Figure 3). In the absence of exogenous damage, the appearance of Rad51 foci is dependent on meiotic DSB formation, and several observations support the view that foci correspond to sites of functioning recombination complexes [2, 36–39]. Less than one Rad51 focus was detected on average in *spo11*Δ cells at 4 hr in meiosis, demonstrating that Rad51 foci depend on meiosis-specific DSBs. In *MND1* and *mnd1*Δ cells, Rad51 foci appear with similar kinetics, suggesting that early steps of repair, including Rad51 filament formation, are not affected in *mnd1*Δ mutants. However, while Rad51 foci appear only transiently and in intermediate numbers in wild-type, they accumulate in *mnd1*Δ mutants with the majority of nuclei containing more than 50 foci after 10 hr in SPM. No nucleus containing more than 50 foci was observed in wild-type.

Levels of Rad51 foci in the *mnd1*Δ *red1* double mutant reached 42% of those in the corresponding wild-type control. A similar reduction was observed by Shinohara et al. for the *red1* single mutant [38], consistent with our observation that Mnd1 has no role in break formation. More importantly, the transient occurrence of Rad51 foci in both the *mnd1*Δ *red1* and the *mnd1*Δ *hop1* double mutant (Figure 3B) supports the idea that breaks become repaired in these cells. *mnd1*Δ *mec1-1* double mutants illustrate that Rad51 foci persist in a checkpoint-defective situation. Figure 3A shows also examples of anaphase I nuclei from different mutants. If the *mnd1*Δ arrest was suppressed by *mec1-1*, anaphase I nuclei contained high levels of Rad51 foci. In contrast, *red1* or *hop1* anaphase I nuclei contained no foci. Resid-

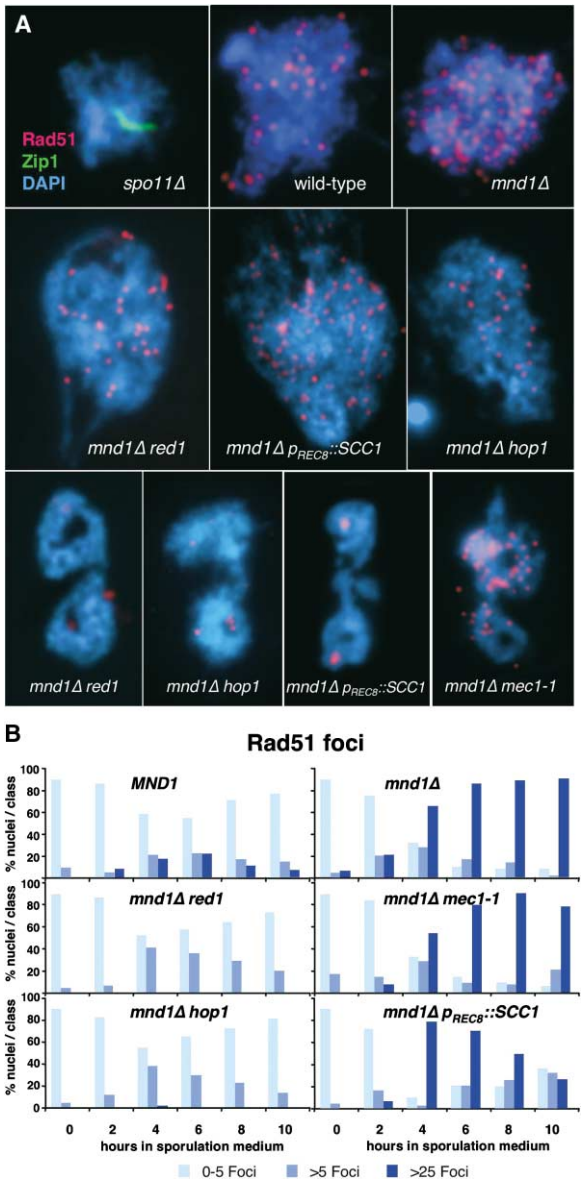

Figure 3. Rad51 Foci Accumulate in Nuclei from *mnd1*Δ or *mnd1*Δ *mec1-1* Cells, but Not in *mnd1*Δ *red1* or *mnd1*Δ *hop1* Double Mutants

(A) Typical examples of nuclei of wild-type or different mutants, stained with α-Rad51 antiserum 4 hr after induction of meiosis. *spo11*Δ (negative control), wild-type (FKY1), *mnd1*Δ (FKY1424), *red1 mnd1*Δ (FKY2117), *mnd1*Δ *rec8*Δ P_{REC8}::*SCC1* (FKY2134), and *hop1 mnd1*Δ (FKY2118). In the third row, typical examples of anaphase I nuclei are depicted (*mnd1*Δ *mec1-1* [FKY1890]). Zip1 was only labeled in the *spo11*Δ strain to demonstrate the absence of Rad51 foci in nuclei, which express a meiosis-specific protein normally.
(B) Statistical analysis of the distribution of foci from these experiments is presented in the panels below. 100 nuclei were scored for each time point.

ual Rad51 label often appeared as isolated blobs, mostly associated with DAPI-negative areas.

If breaks were indeed repaired in *mnd1*Δ *red1* (or *mnd1*Δ *hop1*) mutants, the production of some viable spores would be predicted if cells carry an additional *spo13* mutation, which restores viability in the absence of recombination. We determined the spore viability of *mnd1*Δ *red1 spo13* strains to be >16% (n = 40) and thus, similar to that of *red1 spo13*, ~25% (n = 40). This indicates that Mnd1 is not essential to repair the significant subset of DSBs that still occur in the *red1 spo13* strain, which is estimated to be about 40% that of the wild-type, as judged by Rad51 foci (Figure 3) and physical analysis [40]. *mnd1*Δ *spo13* cells, in contrast, do not sporulate at all. The *hop1* mutation had an analogous effect similar to *red1*. Neither *red1* nor *hop1* rescues spore viability in a *rad52 spo13* double mutant [35], indicating that the reduced initiation of breaks alone is not responsible for the suppression. These results indicate that deletion of *hop1* or *red1* allows DSB repair to proceed, probably via a mitosis-like IS-exchange pathway independent of the meiosis-specific repair machinery. Evidence for the existence of such a pathway comes from the physical analysis of dHJ repair intermediates [7, 8, 41] and genetic analyses of mutant strains [35, 41]. If Mnd1 were specific to the IH-repair pathway, deletion of *HOP1* or *RED1* would largely suppress repair defects in *mnd1*Δ.

Rad54 has been specifically implicated in the IS-repair pathway [14, 42], and its overexpression partially rescues sporulation in *dmc1*Δ mutant cells [41], presumably by channeling DSB-repair into the IS pathway. Overexpressing *RAD54* in the *dmc1*Δ and *mnd1*Δ single and *dmc1*Δ *mnd1*Δ double mutants revealed that restoration of sporulation in *mnd1*Δ cells required the absence of Dmc1 (Table S2). We interpret the selective effect of Rad54 overexpression as a result of its stimulation of IS repair and suggest that the presence of IH-specific Dmc1 at DSB sites, but not of Mnd1, may interfere with successful Rad54-mediated IS repair.

Why is Mnd1 required for repair specifically during meiosis? Although Scc1 is able to support cohesion during meiosis I [43], only the incorporation of Rec8 instead of Scc1 in the cohesin complex during premeiotic S phase guarantees full functionality in recombination and segregation. To test whether Mnd1 is specifically required for DNA repair in the presence of Rec8, we expressed Scc1 during meiosis from the *REC8* promoter [43] in an *mnd1*Δ *rec8*Δ mutant background. More than 50% of cells completed at least the first meiotic division by 10 hr in such a strain, although with slower kinetics than wild-type cells (Figure 2). Such cells accumulate Rad51 transiently to a greater extent than wild-type cells, suggesting that repair intermediates may be turned over more slowly. However, the number of foci eventually decreases and at least the fraction of cells that undergoes anaphase I contains no or very few Rad51 foci on the chromatin (Figure 3). Instead, a single accumulation of Rad51 signal can be located in each daughter nucleus or in the middle between them, suggesting that it may correspond to a deposit of superabundant material. We summarize that replacement of Rec8 by Scc1 improves meiotic progression in *mnd1*Δ strains without causing massive nuclear fragmentation or persistence of Rad51 foci past anaphase I.

Mnd1 Foci Do Not Colocalize with Zip1 or Rad51 and Form in the Absence of Premeiotic S Phase

In order to investigate whether Mnd1 is a chromatin-associated protein, we constructed a strain carrying the

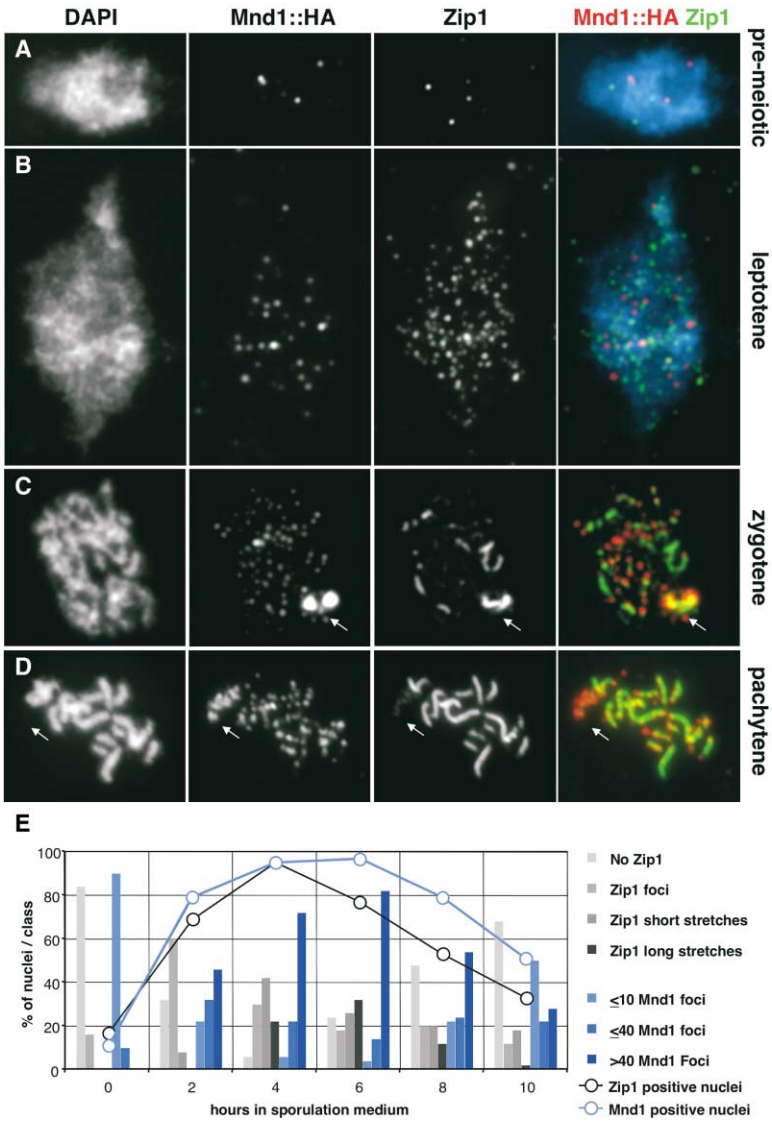

Figure 4. Mnd1 Localizes to Meiotic Chromosomes in the Form of Foci

(A–D) Spread meiotic nuclei were prepared from an *MND1::HA3* strain (FKY1800) and stained with antibodies against the HA epitope and Zip1. DAPI staining is only included in the merged panels of (A) and (B). Arrows in (C) indicate Zip1 polycomplex with Mnd1 aggregates attached. Arrows in (D) indicate nucleolar and rDNA region.

(E) Quantification of Zip1 and Mnd1 stages. 50 nuclei were scored per time point.

MND1 gene fused C-terminally to three copies of the hemagglutinin (HA) epitope at its original locus. This construct fully rescues the *mnd1*Δ-specific prophase arrest (data not shown) and yields viable spores. Nuclear spreads were prepared from this strain and stained with antibodies against both the HA epitope and Zip1. Consistent with the finding that the *MND1* transcript is absent from mitotic cells [20], Mnd1 staining was not observed above background levels at the 0 hr time point in SPM (Figure 4A). As cells enter meiosis, Mnd1 appears on chromatin as distinct foci (Figures 4B–4D), which are most abundant during pachytene and decrease during the subsequent meiotic divisions (Figure 4E). Early Mnd1 foci do not colocalize with Zip1 (Figures 4B and 4C). Wild-type cells appear quite heterogeneous as some of the nuclei show greater accumulations of Mnd1 staining, which appear as brighter blobs (Figure 4C). Analysis of pachytene nuclei showed that Mnd1 is present in the nucleolus and the rDNA region (arrows in Figure 4D).

In order to assess the localization dynamics of Mnd1, 50 randomly selected nuclei from each time point were analyzed for Mnd1 and Zip1 staining. While both proteins appear with similar kinetics, Mnd1 disappears slightly later (Figure 4E). After 2 hr in SPM, the majority of nuclei already contained more than ten Mnd1 foci. The number of Mnd1 foci increased as meiosis progressed and peaked at the 6 hr time point with ~80% of nuclei containing more than 40 Mnd1 foci. SC-containing cells also peaked at this time point, and 65% of cells were mononucleate. However, a significantly higher proportion of cells contained many Mnd1 foci than contained long stretches of SC at any time point, indicating that the period of high Mnd1 foci numbers starts well before pachytene.

If Mnd1 worked at the site of meiotic strand exchange, it might colocalize with Rad51 and Dmc1. To test this, meiotic spreads of the *MND1::HA3* strain were stained with antibodies against Rad51 (or Dmc1) and the HA-epitope and analyzed (Figure 5A). We only rarely observed colocalization of both labels, consistent with random overlaps. We do not, however, exclude that a fraction of Rad51 (or Dmc1) foci may align side by side with Mnd1 foci. Overall, the observation strongly suggests that Mnd1 does not associate specifically with the

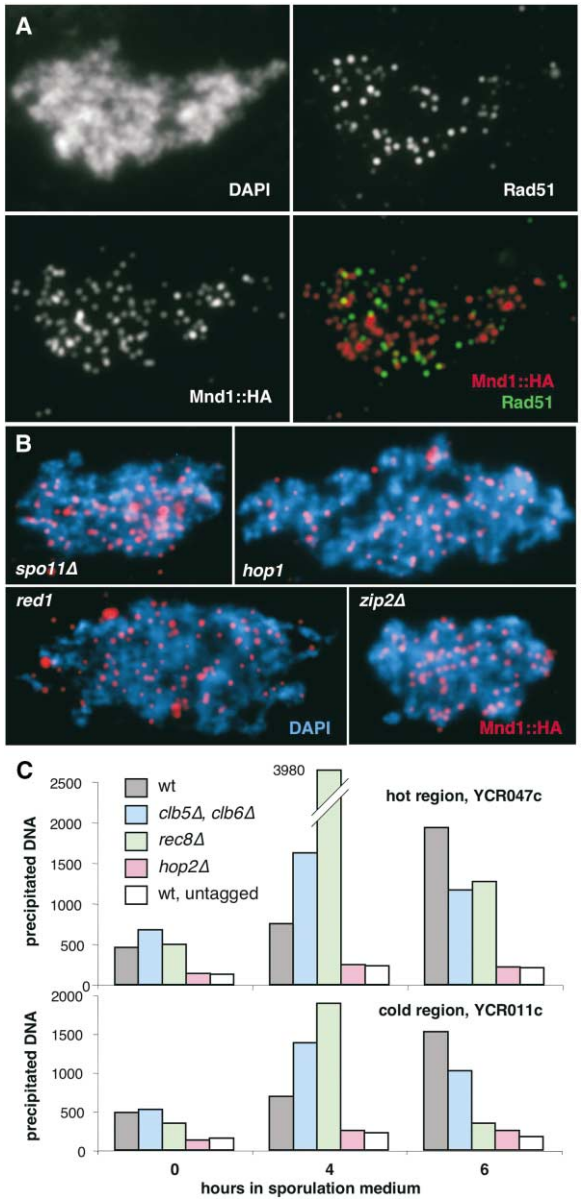

Figure 5. Mnd1 Does Not Colocalize with Rad51 nor Does It Differentiate Strongly between DSB Hot and Cold Regions

(A) Typical example of a spread nucleus of strain FKY1800 (*MND1::HA3*) stained with antibodies against the HA-epitope and Rad51.

(B) Association of Mnd1 with meiotic chromatin is independent of DSB, AE, and SC formation. An example of a spread meiotic nucleus stained for Mnd1-HA is shown (A) for a *spo11Δ MND1::HA3* (FKY2017), a *red1 MND1::HA3* (FKY2001), a *hop1 MND1::HA3* (FKY2002), and a *zip2Δ MND1::HA3* strain (FKY2004).

(C) Mnd1 associates with DSB hot and cold regions to a similar extent. Quantification of DNA from different strains by real-time PCR after coprecipitation with Mnd1-HA in two regions. The hot region is immediately adjacent to the YCR047c recombination hotspot, while the cold region is from YCR011c known for an absence of DSBs (for technical details see Experimental Procedures). Three different time points were analyzed.

sites of meiotic strand exchange at the same time as Rad51 and Dmc1.

When we analyzed the localization of Mnd1-HA in *spo11*, *hop1*, *hop2*, *red1*, *dmc1*, *zip1*, and *zip2* mutants, we confirmed earlier observations that association of Mnd1 with meiotic chromatin requires Hop2, but not Spo11 (Figure 5B) and [21], except that in our hands, Mnd1 forms foci on chromatin. This is in marked contrast to Rad51, Dmc1, and Rpa1, which all require DSBs for their localization as foci [37, 44]. We further find that neither the AE proteins Hop1 and Red1 nor Zip1, Zip2, or Dmc1 are required for Mnd1's localization on chromatin (Figure 5B and data not shown). We therefore conclude that interaction of Mnd1 with chromatin is independent of DSB formation and repair as well as axis formation and synapsis.

Premeiotic S phase differs from mitotic S phase by the loading of meiosis-specific proteins onto chromatin (reviewed in [45]). Rec8, the meiosis-specific cohesin subunit [4, 5], associates with chromatin and establishes meiotic sister chromatid cohesion. Lack of Rec8 causes accumulation of unrepaired meiotic DSBs very similar to that caused by lack of Mnd1. However, Mnd1 foci are correctly formed in the *rec8Δ* mutant. Finally, we utilized a *clb5Δ clb6Δ* double mutant strain to examine Mnd1-foci formation in a mutant defective in the initiation of premeiotic S phase [46]. Deletion of Clb5 and Clb6 abolishes meiotic DSB-formation and severely affects meiotic chromosome morphogenesis [47]. However, the distribution of Mnd1 foci appears to be essentially unchanged (Table S3). We did notice a subtle change in the pattern of Mnd1 localization in mutants with defects in synapsis. The nuclei had a more uniform appearance with regular-sized foci and showed one or several accumulations of foci, which almost always coincided with the presence of one or several Zip1 polycomplexes (PCs) (Figure 4C). However, the frequency of such accumulations of Mnd1 remains unaffected by deletion of *ZIP1* (data not shown), suggesting that the two aggregates have no causal relationship with each other.

Mnd1 Does Not Preferentially Associate with Hotspots of Meiotic Recombination

The observation that Mnd1 foci colocalize neither with Rad51 nor with early Zip1 foci suggests that the essential function of Mnd1 is physically or temporally removed from the strand transfer reaction. Quantitative chromatin immunoprecipitation (ChIP), using Mnd1-HA as the bait, was used to ascertain whether Mnd1 preferentially binds to hotspots of meiotic recombination. We used two pairs of primers, one immediately adjacent to the recombination hotspot close to YCR047c [48] (hot region) and one from a centromere proximal region of chromosome III (YCR011c) that is known for an absence of recombination and DSBs [49, 50] (cold region). The amount of precipitated template was determined by quantitative PCR and calibrated against a dilution series of template DNA before precipitation (whole-cell extract, WCE) at three different time points: 0, 4, and 6 hr after transfer to SPM. A strain lacking the HA-tag was used as negative control. It can be seen that Mnd1-HA associates with DNA in a time-dependent manner at both hot and cold regions but that there is no strong preference for binding at hot regions (Figure 5C). In contrast to Mnd1-HA, Spo11-MYC discriminates strongly between the two templates, yielding values of up to 7-fold over untagged

for the hotspot but only up to 1.2-fold for the cold region (S. Prieler, A. Penker, V. Borde, and A.F., unpublished data). A similar result was obtained for another pair of loci, YDR187c and YBR294w, a recombination active and an inactive region, respectively [50] (data not shown). Chromatin binding was absolutely dependent on the presence of Mnd1's partner Hop2, but not on cohesin (Rec8) or DNA replication (Clb5, Clb6). However, we noted a reproducible increase of coprecipitated DNA and an increased difference between hot and cold regions in the *rec8*Δ mutant (Figure 5C).

We summarize that Mnd1's association with chromatin is not limited to meiotic recombination hotspots is independent of most meiosis-specific processes and occurs even in the absence of premeiotic S phase. Moreover, the absolute requirement of Mnd1 for meiotic divisions is bypassed by replacing the meiotic cohesin apparatus with a more mitosis-like version and by promotion of IS-repair pathways. Hence, we propose that Mnd1's activity to allow DSB repair only becomes essential in the specific chromatin environment of meiosis, which promotes IH bias.

Human Mnd1 Is Ubiquitously Expressed

BLAST searches against Genbank using the protein sequence of *S. cerevisiae* Mnd1 yielded homologous proteins in *Encephalozoon cuniculi*, *Schizosaccharomyces pombe*, *Arabidopsis thaliana*, *Mus musculus*, and *Homo sapiens* (Figure S1). The presence of *MND1* in the gene-poor genome of *E. cuniculi* highlights the importance of its function.

To gain an overview of the expression patterns of *HsMND1*, cDNA libraries from various human tissues were analyzed by PCR for the presence of the *HsMND1*. The expression of ubiquitously expressed β-actin was used as a control. *HsMND1* was expressed in every tested tissue at varying levels (Figure S1), the weakest expression level being detected in peripheral leukocytes. The strongest PCR products were obtained from tonsil, thymus, fetal liver, and lymph node cDNA samples (Figure S1). As the level of β-actin expression was roughly equal in all tested tissues, the pattern of *HsMND1* expression levels are likely to reflect true differences in transcript abundance in the various tissues. The fact that Mnd1 is not exclusively expressed in the germline raises the possibility that Mnd1 has acquired additional functions in somatic tissue.

Discussion

*mnd1*Δ Is Defective in Meiotic DSB Repair and Activates the Mec1-Dependent DNA-Damage Checkpoint

Our physical analysis of recombination intermediates confirms earlier results that *mnd1*Δ cells are defective in the repair of meiotic DSBs [21, 22]. Furthermore, we observed the accumulation of intermediates with strongly resected DSB-ends (Figure 1A). Since the Mec1-dependent DNA-damage checkpoint is thought to respond to ssDNA [51], the tight arrest may well be a consequence of this massive production of ssDNA. The existence of residual crossovers (Figure 1A) also corroborates findings that a minor pathway for homolog interaction operates in the absence of Mnd1 [21]. We show that up to 90% of the cells eventually contain more than 25 Rad51 foci (Figure 3B), indicating that Mnd1 is required for Rad51 focus disassembly. Dmc1 foci accumulate in *mnd1*Δ meiosis in a similar manner (data not shown). Thus, Mnd1 may be required after loading of RecA homologs but before formation of a stably paired structure that prevents excessive 5′ end resection.

Foci Formation of Mnd1 Is Independent of Premeiotic S Phase

Immunocytological analysis of spread meiotic nuclei derived from a strain carrying a functional HA epitope-tagged version of Mnd1 revealed that Mnd1 localizes to meiotic chromosomes as foci (Figure 4). This contrasts with a previous report that Mnd1 tagged with green fluorescent protein (GFP) globally associates with chromatin in a manner reminiscent of DAPI staining [21]. This discrepancy could reflect a higher resolution of detection with Mnd1-HA as opposed to Mnd1-GFP or a difference in the applied preparation technique. However, even when we omitted any detergent from our spreading protocol to eliminate the major risk of losing antigen from the preparation, the staining pattern remained focal in most spread cells. Only pachytene chromosomes sometimes appeared to be covered by foci that could not be further resolved. We therefore believe that for the large majority of cells, the focal pattern very likely reflects the in vivo localization of our construct. That Mnd1-HA was resolved as well-separated foci allowed us to determine that Mnd1 foci do not colocalize extensively with Zip1, Dmc1, or Rad51 foci.

Formation of Mnd1 foci on chromatin was independent of DSB, AE, and SC formation and did not require cohesin or premeiotic DNA-synthesis. If Zip1 polycomplexes were formed, aggregates of Mnd1 were generally found associated with them (Table S3). However, aggregates of either gene product occur frequently in strains deleted for the other gene, suggesting that the two aggregates have no causal relationship with each other. Rather there might be a tendency to cohabitate the same nuclear compartment (possibly the nucleolus). The finding that Mnd1 coprecipitated both hotspot and coldspot DNA in a meiosis-specific manner suggests that also Mnd1 foci may not differentiate between regions hot or cold for recombination.

Mnd1 Operates in a Pathway Specific for IH Recombination

Establishment of meiotic sister chromatid cohesion by Rec8 is an important step in setting up meiosis-specific chromatin. Replacing Rec8 by its mitotic counterpart Scc1 by expressing Scc1 from the *REC8* promoter (P_{REC8}*::SCC1*) substantially improves the ability of *mnd1*Δ cells to progress through meiosis (Figure 2). Rad51 foci accumulate transiently but eventually disappear in those cells that undergo anaphase I (Figure 3A), suggesting that a repair pathway is operating. The fact that cell cycle progression is not fully restored in the double mutant is most likely due to the inefficiency of *rec8*ΔP_{REC8}*::SCC1 MND1* cells in meiotic DSB repair, since the sporulation

of this strain can be restored to ~80% by deleting *SPO11* [43]. The absence of Mnd1 may even improve repair, as deletion of *MND1* improves nuclear division in the *rec8*Δ*P*$_{REC8}$*::SCC1* strain background (Figure 2).

One explanation for the suppression of arrest in *mnd1*Δ cells by replacement of *REC8* by *SCC1*, is that the requirement for Mnd1 in meiotic DSB repair is largely bypassed. This raises the possibility that Mnd1 functions specifically within the environment of Rec8-dependent meiotic chromosome organization. The decrease of Rad51 foci in *P*$_{REC8}$*::SCC1* nuclei suggests that these cells are able to eliminate some of the breaks, albeit inefficiently. In contrast, Rad51 foci persist much longer in *mnd1*Δ *mec1-1* nuclei.

Rec8 is also required for formation of the AE and localization of the AE proteins, Red1 and Hop1 [5]. Deletion of either *RED1* or *HOP1* has been shown to alleviate the meiotic arrest observed in many recombination-deficient mutants such as *rad50S* and *dmc1*Δ [35, 41]. Similarly, disruption of either *HOP1* or *RED1* completely restored nuclear divisions and spore formation of the *mnd1*Δ mutant (Figure 2B). What is the mechanistic basis of this bypass? It has been shown [7] that Red1 plays a key role in establishing interhomolog bias for dHJs. Deletion of *RED1* reduces the number of initiating breaks and the number of IH-dHJs but hardly affects IS-dHJs. This suggests that the subset of breaks that depend on Red1 is predetermined for IH repair. The remaining DSBs show little preference for either repair template. The *dmc1*Δ and *dmc1*Δ *red1* double mutants were also analyzed [7]. In the *dmc1*Δ mutant, DSBs accumulate and become hyperresected while repair intermediates fail to appear, indicating that Dmc1 is essential for repair under wild-type conditions. Importantly, in the *red1 dmc1*Δ double mutant, repair is restored, albeit only IS repair. DSBs disappear in the *red1 dmc1*Δ double mutant as in wild-type, while IS-dHJs, but not IH-dHJs, appear [7]. Thus, Red1 represses IS repair in *dmc1*Δ mutants and Dmc1 promotes IH repair in *red1* mutants [7, 52].

Bypass of the meiotic prophase arrest of *mnd1*Δ cells by disruption of *red1* (or *hop1*) could be similar to that of *dmc1*Δ cells. The *red1* mutation may allow Mnd1-independent repair from the sister template, as viable spores are obtained in *mnd1*Δ *red1 spo13*Δ cells, where the requirement for reductional segregation is bypassed. These results are best explained by assuming that the axis components Red1 and Hop1 prevent repair from the sister chromatid in the *mnd1*Δ mutant, similar to Red1's effect on *dmc1*Δ mutants.

It has also been put forward that mutation of *HOP1* or *RED1* interferes with the DNA-damage checkpoint [30], which would provide an alternative explanation for the bypass of the prophase I arrest in *mnd1*Δ cells when *HOP1* or *RED1* are mutated. While the two genes may be required for full checkpoint response, solely eliminating a checkpoint arrest is not expected to improve the viability of *mnd1*Δ *spo13* cells. Eliminating the central checkpoint gene *MEC1* in *mnd1*Δ cells caused massive nuclear fragmentation, a feature not observed in *red1 mnd1*Δ or *hop1 mnd1*Δ cells. In addition, in contrast to the situation in the *mnd1*Δ *mec1-1* double mutant, Rad51 foci appear in *red1 mnd1*Δ or *hop1 mnd1*Δ double mutants but disappear before anaphase I, consistent with their being repaired (Figure 3). Hence, Mnd1 as Dmc1 is most likely involved in the IH-specific repair pathway.

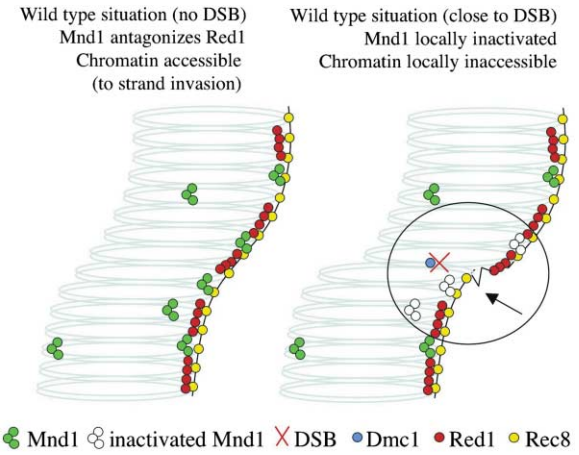

Figure 6. Local Inhibition Model for IH Bias

Two segments of chromatid pairs are shown, the right one displaying a DSB loaded with Dmc1. Mnd1 seems to antagonize structural constraints caused by Red1 and other axes components and thus keeps the chromosome available for Dmc1-dependent repair. Inhibition of Mnd1 (or a cooperating protein) on both chromatids close to a DSB would exclude the sister chromatid locally from serving as a repair template. Local signal transmission between the DSB and Mnd1 could, for instance, be explained by the segmented tension release model [55].

Differences between *mnd1*Δ and *dmc1*Δ phenotypes do, however, exist. In the BR strain background (but not in SK1) the *dmc1*Δ mutation is less efficient in triggering DNA damage arrest compared to *mnd1*Δ [53]. Suppression of spore inviability of a *hop2*Δ BR strain by overexpression of Rad51 is greatly improved by additional deletion of Dmc1 [53]. Similarly, overexpression of *RAD54* supports nuclear divisions in *mnd1*Δ mutants if *DMC1* is deleted (Table S2). Finally, the deletion of *PCH2*, a gene assigned to the pachytene checkpoint [31], also allows nuclear divisions in *mnd1*Δ mutants but only in the absence of *DMC1* (Table S2). Taken together, these observations suggest that specifically Dmc1-dependent processes require the assistance of Mnd1 and that the absence of Dmc1 allows alternative routes. Mnd1 may thus be essential for the repair of DSBs that are bound for IH repair through mechanisms dependent on Rec8, Red1, Hop1, and Dmc1.

A Model for the Function of Mnd1

To date, two models for the function of Mnd1 have been proposed. The first model suggests that Mnd1 might be directly involved in recombination as a cofactor of Dmc1 [22], whereas the second model envisions a role for Mnd1 in aborting interactions between nonhomologous chromosomes [21]. While we agree with certain aspects of both models, we present a slightly different view incorporating our additional data (Figure 6). Mnd1 clearly assists Dmc1-dependent processes, but it is unlikely that Mnd1 binds specifically at sites of strand exchange. The formation of Mnd1 foci on chromatin is independent of DSBs ([21], Figure 5), while the localization of factors

involved directly in the recombination process, such as Dmc1, Rad51 [44], Rad52, and RPA [37] usually depend on DSBs. Furthermore, Mnd1 foci do not colocalize with Rad51 or Dmc1 (Figure 5A), while all the biochemically active, strand exchange-mediating proteins tested so far show a significant degree of colocalization with Rad51 [37, 44, 54]. Finally, Mnd1 precipitates similar amounts of DNA from regions close to and remote from hotspots of DSB formation in ChIP-experiments (Figure 5C). We therefore favor a model in which Mnd1 acts on chromatin structure, but not necessarily at the hotspot to facilitate strand invasion. Although we acknowledge Mnd1's role in preventing nonhomologous alignments, this property could be a consequence of its strand invasion-promoting activity, as we have observed similar increases in nonhomologous interactions in *mre11S* [27] and in *com1*Δ/*sae2*Δ cells [28], which fail to undergo any strand invasion. Thus, we think it is possible that chromosome axes eventually synapse by default in yeast and that homolog alignment normally precedes this process. Many genes required for homolog alignment could, in such a scenario, contribute to preventing nonhomologous alignment.

Evidence in *S. cerevisiae* points to a role of Mnd1 specifically in IH-DSB repair. In meiosis, only interactions with the homologous chromosome are productive in pairing and in the formation of chiasmata, essential for the segregation of homologous chromosomes at anaphase I. The mechanisms, which prevent repair from the sister chromatid and promote the homolog as a repair template, thus contributing to IH bias (reviewed in [55]), have yet to be understood in detail. As DSBs occur at random and every DSB defines its sister chromatid locally, such a system is likely to ultimately be under the local control of the DSB. Nonetheless, it may largely consist of global components, which favor or disfavor genetic interactions in general. We propose that the Mnd1/Hop2 complex is such a global, positive regulator of recombination (Figure 6). Moreover, we believe that the Red1- and Hop1-dependent axial structures generally inhibit DSB-repair by exerting structural constraints on strand invasion. In addition to this negative role, Red1 and Hop1 also facilitate the introduction of breaks bound for IH repair. Mnd1/Hop2 may antagonize the negative Red1/Hop1-dependent constraint by a mechanism possibly involving the enhancement of chromatin accessibility. The presence of Mnd1/Hop2 could, for instance, favor underwound DNA, which would facilitate D loop formation at the repair template. As both Mnd1 and Hop2 [29] localize to chromatin independently of DSBs, they are likely to exert a genome-wide positive effect. However, local control could be introduced if Mnd1/Hop2 were inhibited or displaced in DNA regions close to a DSB on both chromatids (Figure 6). The DSB could propagate its signal along the chromatin by stress release, a mechanism suggested by Zickler and Kleckner [55] to explain other phenomena. If propagated via a physical bridge, the inhibitory signal would not reach the homolog and as a consequence, Mnd1/Hop2 would keep the homolog accessible for repair. The described model proposes a strand invasion-promoting role for Mnd1 specifically on the donor chromatid, which acts as the repair template, but further work will be required to decide between this and alternative models.

Experimental Procedures

Yeast Strains, Genetic Manipulation, and Meiotic Time-Course Experiments and Physical Assays

All strains used were isogenic to the SK1 strain background with the following exceptions: The *mec1-1* mutant strains were derived from David Lydall's original strains [56] and were backcrossed at least six times into SK1. The *mec1-1* strains are kept alive by an unlinked suppressor of lethality of *mec1* (*sml*X). The *zip2* mutant was derived from the BY strain background and was backcrossed three times with pure SK1 strains. Epitope-tagging and gene-deletion procedures were carried out by using a PCR-based homologous integration approach [20, 57] and confirmed by PCR and Southern blotting. A full list of strain genotype details is available in the Supplemental Data (Table S1). Standard yeast growth, crossing, and transformation procedures were used. Except for the strains carrying pFAT4-*RAD54*, sporulation time courses were carried out as described previously [27]. Strains carrying pFAT4-*RAD54* were taken from selective plates lacking leucine and resuspended directly in SPM. DSB and recombination product assays were performed as described previously [5].

Cytology

Spread meiotic nuclei were prepared and stained as described [27]. The following primary antibodies were used: mouse α-HA (16B12, 1:1500), rabbit polyclonal α-Zip1 (1:50), and rabbit polyclonal α-Rad51 (1:300). Secondary antibodies were goat α-mouse-CY3 conjugate (1:200, Dianova), goat α-rabbit-FITC conjugate (1:100, Sigma), goat α-rabbit-CY3 conjugate (1:250, Amersham), and goat α-rabbit-Alexa$_{488}$ conjugate (1:200, Molecular Probes). Images were taken with a Zeiss Axioskop fluorescence microscope equipped with a Photometrics CH250A b/w CCD camera. All pictures were captured with 250× magnification using IPLab 3.0 software (Scanalytics), which was also used for analysis. In order to minimize pixel shift between different channels, the Pinkel set arrangement was used for colocalizations in which only excitation filters, positioned very close to the light source, were switched, while a single multi-wavelength filter emission filter remained untouched.

ChIP

The protocol for this method is based on [58] with modifications according to S. Prieler, A. Penker, V. Borde, and A.F., unpublished data. Deviating from this protocol, crosslinking of meiotic samples (2×10^9 cells) was done in 1% formaldehyde for 30 min at 25°C. For the IP of Mnd1-HA, we used 12CA5 mouse α-HA antibody in combination with coated magnetic beads (Pan Mouse IgG Dynabeads, Dynal).

Quantitative PCR

Quantification of precipitated DNA was performed by real-time PCR by using primer pair 713/714, located 61bp upstream of the DSB site of the *YCR047* hotspot (713, 5′-CCGCAGAAGCCAACAAACGG-3′; 714, 5′-CTTTCGGTGGAACCTCGACC-3′) and by using primer pair 794/795, located within *YCR011*, from a region showing little or no localized DSBs (794, 5′-GGTGATGATTGCTCTCTGCC-3′; 795, 5′-CGTCACAATTGATCCCTCCC-3′). Real-time PCR was done with an ABI 7000 (ABI Prism) by measuring SYBR Green incorporation with the SYBR Green JumpStart Taq Ready Mix (SIGMA) and following the instructions of the manufacturer. Calculation of precipitated DNA: a series of 10-fold dilutions obtained from DNA of the WCE of $t = 0$ was used to calibrate amplification curves for the primer pairs for every strain, with DNA of 1 μl out of 470 set to 10,000. After the IP (of 470 μl) 10% of the precipitated DNA was quantified in duplicate and averaged. IP samples of all time points were quantified by using the calibration curve determined with WCE ($t = 0$) and finally normalized by multiplication with WCE(0)/WCE(t).

Supplemental Data

Supplemental Data including Tables S1–S3 and a figure depicting the alignment of Mnd1 homologs are available at http://www.current-biology.com/cgi/content/full/14/9/752/DC1/.

Acknowledgments

This work was funded by the Austrian Science Foundation grant S8203, by the Austrofan grant from the Austrian Ministry of Science to F.K. and a grant of the Japanese Ministry of Education and Science to A.S. We wish to thank Kim Nasmyth for constructs, Doug Bishop for the Rad54 plasmid, C. Erhart and S. Ferscha for skillful technical assistance, and A. Penkner for cytological expertise. Also thanks to a number of colleagues and lab members, in particular M. Siomos, for reading and discussing the manuscript.

Received: August 22, 2003
Revised: February 18, 2004
Accepted: March 17, 2004
Published: May 4, 2004

References

1. Sun, H., Treco, D., Schultes, N.P., and Szostak, J.W. (1989). Double-strand breaks at an initiation site for meiotic gene conversion. Nature *338*, 87–90.
2. Mahadevaiah, S.K., Turner, J.M., Baudat, F., Rogakou, E.P., De Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W.M., and Burgoyne, P.S. (2001). Recombinational DNA double-strand breaks in mice precede synapsis. Nat. Genet. *27*, 271–276.
3. Allers, T., and Lichten, M. (2001). Differential timing and control of noncrossover and crossover recombination during meiosis. Cell *106*, 47–57.
4. Buonomo, S.B., Clyne, R.K., Fuchs, J., Loidl, J., Uhlmann, F., and Nasmyth, K. (2000). Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell *103*, 387–398.
5. Klein, F., Mahr, P., Galova, M., Buonomo, S.B., Michaelis, C., Nairz, K., and Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell *98*, 91–103.
6. Kadyk, L.C., and Hartwell, L.H. (1992). Sister chromatids are preferred over homologs as substrates for recombinational repair in Saccharomyces cerevisiae. Genetics *132*, 387–402.
7. Schwacha, A., and Kleckner, N. (1997). Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell *90*, 1123–1135.
8. Schwacha, A., and Kleckner, N. (1994). Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell *76*, 51–63.
9. Wolner, B., Van Komen, S., Sung, P., and Peterson, C.L. (2003). Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. Mol. Cell *12*, 221–232.
10. Solinger, J.A., Kiianitsa, K., and Heyer, W.D. (2002). Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. Mol. Cell *10*, 1175–1188.
11. Sugawara, N., Wang, X., and Haber, J.E. (2003). In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol. Cell *12*, 209–219.
12. Game, J.C., and Mortimer, R.K. (1974). A genetic study of x-ray sensitive mutants in yeast. Mutat. Res. *24*, 281–292.
13. Sjogren, C., and Nasmyth, K. (2001). Sister chromatid cohesion is required for postreplicative double-strand break repair in Saccharomyces cerevisiae. Curr. Biol. *11*, 991–995.
14. Arbel, A., Zenvirth, D., and Simchen, G. (1999). Sister chromatid-based DNA repair is mediated by RAD54, not by DMC1 or TID1. EMBO J. *18*, 2648–2658.
15. Dresser, M.E., Ewing, D.J., Conrad, M.N., Dominguez, A.M., Barstead, R., Jiang, H., and Kodadek, T. (1997). DMC1 functions in a Saccharomyces cerevisiae meiotic pathway that is largely independent of the RAD51 pathway. Genetics *147*, 533–544.
16. Shinohara, M., Shita-Yamaguchi, E., Buerstedde, J.M., Shinagawa, H., Ogawa, H., and Shinohara, A. (1997). Characterization of the roles of the Saccharomyces cerevisiae RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. Genetics *147*, 1545–1556.
17. Grushcow, J.M., Holzen, T.M., Park, K.J., Weinert, T., Lichten, M., and Bishop, D.K. (1999). Saccharomyces cerevisiae checkpoint genes MEC1, RAD17 and RAD24 are required for normal meiotic recombination partner choice. Genetics *153*, 607–620.
18. Thompson, D.A., and Stahl, F.W. (1999). Genetic control of recombination partner preference in yeast meiosis. Isolation and characterization of mutants elevated for meiotic unequal sister-chromatid recombination. Genetics *153*, 621–641.
19. Heyting, C. (1996). Synaptonemal complexes: structure and function. Curr. Opin. Cell Biol. *8*, 389–396.
20. Rabitsch, K.P., Toth, A., Galova, M., Schleiffer, A., Schaffner, G., Aigner, E., Rupp, C., Penkner, A.M., Moreno-Borchart, A.C., Primig, M., et al. (2001). A screen for genes required for meiosis and spore formation based on whole-genome expression. Curr. Biol. *11*, 1001–1009.
21. Tsubouchi, H., and Roeder, G.S. (2002). The Mnd1 protein forms a complex with Hop2 to promote homologous chromosome pairing and meiotic double-strand break repair. Mol. Cell. Biol. *22*, 3078–3088.
22. Gerton, J.L., and Derisi, J.L. (2002). Mnd1p: an evolutionarily conserved protein required for meiotic recombination. Proc. Natl. Acad. Sci. USA *99*, 6895–6900.
23. Xu, L., and Kleckner, N. (1995). Sequence non-specific double-strand breaks and interhomolog interactions prior to double-strand break formation at a meiotic recombination hot spot in yeast. EMBO J. *14*, 5115–5128.
24. Cao, L., Alani, E., and Kleckner, N. (1990). A pathway for generation and processing of double-strand breaks during meiotic recombination in S. cerevisiae. Cell *61*, 1089–1101.
25. Shinohara, A., Ogawa, H., and Ogawa, T. (1992). Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. Cell *69*, 457–470.
26. Bishop, D.K., Park, D., Xu, L., and Kleckner, N. (1992). DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell *69*, 439–456.
27. Nairz, K., and Klein, F. (1997). *mre11S*—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. *11*, 2272–2290.
28. Prinz, S., Amon, A., and Klein, F. (1997). Isolation of *COM1*, a new gene required to complete meiotic double-strand break induced recombination in *S. cerevisiae*. Genetics *146*, 781–795.
29. Leu, J.Y., Chua, P.R., and Roeder, G.S. (1998). The meiosis-specific Hop2 protein of S. cerevisiae ensures synapsis between homologous chromosomes. Cell *94*, 375–386.
30. Roeder, G.S., and Bailis, J.M. (2000). The pachytene checkpoint. Trends Genet. *16*, 395–403.
31. San-Segundo, P.A., and Roeder, G.S. (1999). Pch2 links chromatin silencing to meiotic checkpoint control. Cell *97*, 313–324.
32. Rouse, J., and Jackson, S.P. (2002). Interfaces between the detection, signaling, and repair of DNA damage. Science *297*, 547–551.
33. Xu, L., Weiner, B.M., and Kleckner, N. (1997). Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. *11*, 106–118.
34. Woltering, D., Baumgartner, B., Bagchi, S., Larkin, B., Loidl, J., De, L.S.T., and Hollingsworth, N.M. (2000). Meiotic segregation, synapsis, and recombination checkpoint functions require physical interaction between the chromosomal proteins Red1p and Hop1p. Mol. Cell. Biol. *20*, 6646–6658.
35. Mao-Draayer, Y., Galbraith, A.M., Pittman, D.L., Cool, M., and Malone, R.E. (1996). Analysis of meiotic recombination pathways in the yeast Saccharomyces cerevisiae. Genetics *144*, 71–86.
36. Shinohara, A., Gasior, S., Ogawa, T., Kleckner, N., and Bishop, D.K. (1997). Saccharomyces cerevisiae recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. Genes Cells *2*, 615–629.
37. Gasior, S.L., Wong, A.K., Kora, Y., Shinohara, A., and Bishop, D.K. (1998). Rad52 associates with RPA and functions with

Rad55 and Rad57 to assemble meiotic recombination complexes. Genes Dev. *12*, 2208–2221.
38. Shinohara, M., Gasior, S.L., Bishop, D.K., and Shinohara, A. (2000). Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination. Proc. Natl. Acad. Sci. USA *97*, 10814–10819.
39. Raderschall, E., Golub, E.I., and Haaf, T. (1999). Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. Proc. Natl. Acad. Sci. USA *96*, 1921–1926.
40. Blat, Y., Protacio, R.U., Hunter, N., and Kleckner, N. (2002). Physical and functional interactions among basic chromosome organizational features govern early steps of meiotic chiasma formation. Cell *111*, 791–802.
41. Bishop, D.K., Nikolski, Y., Oshiro, J., Chon, J., Shinohara, M., and Chen, X. (1999). High copy number suppression of the meiotic arrest caused by a dmc1 mutation: REC114 imposes an early recombination block and RAD54 promotes a DMC1-independent DSB repair pathway. Genes Cells *4*, 425–444.
42. Schmuckli-Maurer, J., and Heyer, W.D. (2000). Meiotic recombination in RAD54 mutants of Saccharomyces cerevisiae. Chromosoma *109*, 86–93.
43. Toth, A., Rabitsch, K.P., Galova, M., Schleiffer, A., Buonomo, S.B., and Nasmyth, K. (2000). Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I. Cell *103*, 1155–1168.
44. Bishop, D.K. (1994). RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell *79*, 1081–1092.
45. Forsburg, S.L. (2002). Only connect: linking meiotic DNA replication to chromosome dynamics. Mol. Cell *9*, 703–711.
46. Stuart, D., and Wittenberg, C. (1998). CLB5 and CLB6 are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev. *12*, 2698–2710.
47. Smith, K.N., Penkner, A., Ohta, K., Klein, F., and Nicolas, A. (2001). B-type cyclins CLB5 and CLB6 control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr. Biol. *11*, 88–97.
48. Liu, J., Wu, T.C., and Lichten, M. (1995). The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. EMBO J. *14*, 4599–4608.
49. Baudat, F., and Nicolas, A. (1997). Clustering of meiotic double-strand breaks on yeast chromosome III. Proc. Natl. Acad. Sci. USA *94*, 5213–5218.
50. Gerton, J.L., Derisi, J., Shroff, R., Lichten, M., Brown, P.O., and Petes, T.D. (2000). Inaugural article: global mapping of meiotic recombination hotspots and coldspots in the yeast Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *97*, 11383–11390.
51. Nyberg, K.A., Michelson, R.J., Putnam, C.W., and Weinert, T.A. (2002). Toward maintaining the genome: DNA damage and replication checkpoints. Annu. Rev. Genet. *36*, 617–656.
52. Malone, R.E., Bullard, S., Hermiston, M., Rieger, R., Cool, M., and Galbraith, A. (1991). Isolation of mutants defective in early steps of meiotic recombination in the yeast Saccharomyces cerevisiae. Genetics *128*, 79–88.
53. Tsubouchi, H., and Roeder, G.S. (2003). The importance of genetic recombination for fidelity of chromosome pairing in meiosis. Dev. Cell *5*, 915–925.
54. Gasior, S.L., Olivares, H., Ear, U., Hari, D.M., Weichselbaum, R., and Bishop, D.K. (2001). Assembly of RecA-like recombinases: distinct roles for mediator proteins in mitosis and meiosis. Proc. Natl. Acad. Sci. USA *98*, 8411–8418.
55. Zickler, D., and Kleckner, N. (1999). Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. *33*, 603–754.
56. Lydall, D., Nikolsky, Y., Bishop, D.K., and Weinert, T. (1996). A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature *383*, 840–843.
57. Knop, M., Siegers, K., Pereira, G., Zachariae, W., Winsor, B., Nasmyth, K., and Schiebel, E. (1999). Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. Yeast *15*, 963–972.
58. Tanaka, T., Knapp, D., and Nasmyth, K. (1997). Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. Cell *90*, 649–660.