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A failure of meiotic chromosome segregation in a *fbh1Δ* mutant correlates with persistent Rad51-DNA associations

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ABSTRACT

The F-box DNA helicase Fbh1 constrains homologous recombination in vegetative cells, most likely through an ability to displace the Rad51 recombinase from DNA. Here, we provide the first evidence that Fbh1 also serves a vital meiotic role in fission yeast to promote normal chromosome segregation. In the absence of Fbh1, chromosomes remain entangled or segregate unevenly during meiosis, and genetic and cytological data suggest that this results in part from a failure to efficiently dismantle Rad51 nucleofilaments that form during meiotic double-strand break repair.

INTRODUCTION

Homologous recombination (HR) is important both in vegetative and meiotic cells for the repair of DNA double-strand breaks (DSBs). The majority of DSBs in most vegetative cells are accidental and result from the encounter of replication forks with single-stranded DNA (ssDNA) lesions or barriers, or following exposure to agents such as ionizing radiation (IR). Here HR provides a way of faithfully repairing the DSB by using the undamaged sister chromatid as a template to recover genetic information that might have been lost or corrupted as a consequence of DNA breakage. In diploid cells, recombination may also occur between the homologous chromosomes (homologues), however this can result in loss of heterozygosity (LOH), which is detrimental when it involves a disease-associated recessive allele (1). The risk of LOH is greatly increased if recombination intermediates are processed by endonucleolytic cleavage to give rise to reciprocal exchange of the DNAs that flank them (so-called crossover recombinants). Reassuringly there are mechanisms in vegetative cells that promote sister chromatid recombination and limit crossing over (2–6).

In contrast to vegetative cells, most DSBs in meiotic cells are the consequence of a deliberate attack by Spo11, which is related to the type II topoisomerase from archaea, Topo VI (7,8). Like in vegetative cells these DSBs are repaired by HR, however here both allelic recombination and crossing over are promoted for the establishment of chiasmata that help guide correct chromosome segregation during meiosis I (9).

The mechanism of DSB repair by HR first necessitates the resection of the broken DNA end to generate a 3′-OH-ended single-stranded tail. The exposed ssDNA is initially bound by RPA, but is later replaced by the Rad51 recombinase. Rad51 polymerises along the DNA forming a nucleoprotein filament that catalyzes the pairing and strand invasion/exchange between homologous DNA molecules (10). The nucleation of the Rad51 nucleofilament is negatively affected by RPA (11). Efficient filament formation therefore necessitates the involvement of so-called mediator proteins, such as Rad52 in the budding yeast *Saccharomyces cerevisiae* (12–14). Rad52 binds ssDNA and interacts both with Rad51 and RPA, and through these interactions is thought to promote the nucleation of Rad51 onto the RPA-coated ssDNA (14–18).

The formation and stability of the Rad51 nucleofilament can also be affected by DNA translocases that can displace Rad51 from DNA (19,20). In eukaryotes, the best-known example of this class of enzyme is the Superfamily 1 (SF1) DNA helicase Srs2 from *S. cerevisiae* (21,22). Srs2 promotes Rad51 removal through interaction via its C-terminal domain, which stimulates Rad51 to hydrolyze ATP and thereby dissociate from DNA (23). This activity is important for aborting HR at stalled replication forks and thereby enabling alternative repair pathways, governed by the ubiquitin conjugase Rad6

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and ubiquitin ligase Rad18, to operate (24–29). Rad51 nucleofilament disassembly is also important following strand invasion/exchange (i.e. post-synapsis) to promote the re-cycling of Rad51 and accessibility of the DNA for downstream processing. Rad51 removal from duplex DNA can be performed by the Swi/Snf-related protein Rad54, which *in vitro* has been shown to clear the invading 3′-strand end so that it can prime DNA synthesis (30–33). The importance of post-synaptic removal of Rad51 was also recently highlighted in *Caenorhabditis elegans* where the DNA helicase HELQ1 and Rad51 paralogue RFS1 were shown to provide independent mechanisms for displacing Rad51 from duplex DNA during meiotic DSB repair (34).

It is currently unclear whether Srs2 is needed to remove Rad51 from ssDNA post-synapsis, however it does appear to play a role in processing recombination intermediates into non-crossover recombinants during DSB repair in vegetative cells possibly by promoting synthesis-dependent strand annealing (SDSA) (2,3). SDSA involves the unwinding of the invading DNA strand following its extension by DNA synthesis so that it can anneal to the other end of the DSB. Potential roles for Srs2 here include catalysing the unwinding of the invading DNA strand and the removal of Rad51 from ssDNA to enable single-strand annealing (2,35). Whether it performs similar activities during meiotic DSB repair is currently unknown, although a reduction in spore viability in *srs2* mutants suggests that it does have a meiotic role (36).

Homologues of Srs2 have been detected in many eukaryotes, but are seemingly absent in mammals (37). There is, however, a close relative of Srs2 in mammals called F-box DNA helicase 1 (Fbh1), which is absent in *S. cerevisiae* but present in the fission yeast *Schizosaccharomyces pombe* (38,39). Like Srs2, Fbh1 appears to play a role in countering Rad51 activity. Evidence for this comes from observations both in *S. pombe* and humans showing that levels of spontaneous Rad51 nuclear foci increase in Fbh1 deficient cells, whilst DNA damage-induced Rad51 foci are reduced in cells over-expressing Fbh1 (40–42). Moreover human Fbh1 can rescue many of the phenotypes of a *srs2* null in *S. cerevisiae* (43), and in *S. pombe* the hypersensitivity to genotoxins, deficiency in HR and reduction in DNA damage-induced Rad51 foci that result from the loss of the Rad52 orthologue Rad22 are mostly suppressed by deletion of *fbh1* (42,44). The failure to constrain Rad51 in Fbh1 deficient cells results in elevated levels of both sister chromatid recombination and replication fork block-induced direct repeat recombination (41,42,45). Whether Fbh1 displaces Rad51 from DNA in the same way as Srs2 is not certain, however its role in controlling Rad51 does appear to depend on its DNA helicase/translocase activity because mutations that impair ATP hydrolysis result in a null-like phenotype in *S. pombe* (42,44,46). In contrast Fbh1's F-box, which mediates its interaction with Skp1 to form a Skp1-Cullin-F-box protein E3 ubiquitin ligase complex (47), appears to be largely dispensable for its role in HR (44), albeit mutations within Fbh1′s F-box can impede nuclear localization and recruitment to DNA damage sites in addition to attenuating the Skp1 interaction (46).

Similar to Srs2 it is currently unknown whether Fbh1′s role in constraining Rad51 activity is important during meiosis. Here we address this question by assessing the impact of *fbh1* deletion on meiosis in *S. pombe*.

MATERIALS AND METHODS

Yeast strains and plasmids

Schizosaccharomyces pombe strains used in this study are listed in Table 1. Plasmid pAN1 used in the plasmid gap repair assay has been described (3). pREP41-YFP-Fbh1 (= pMW651) has also been described previously (44).

Media and genetic methods

Schizosaccharomyces pombe strains were cultured in yeast extract plus supplements (YES) or Edinburgh minimal medium with 3.7 mg/ml sodium glutamate (EMMG) and amino acids (0.2475 mg/ml) as required. The sporulation of crosses was performed on malt extract (ME) agar at 25°C. For meiotic time courses *pombe* minimal media (PM) and *pombe* minimal media without a nitrogen source (PM-N), supplemented with leucine as required, were used (48). The plasmid gap repair assay (3), determination of spore viability by random spore analysis (49) and meiotic recombination assay (50,51) have been described previously. In each of these assays, the two sample *t*-test was used to determine whether the values obtained for each strain were statistically different.

Quantitative IR survival assay

Cell cultures were grown in YES broth to a density of 1×10^7 cells/ml, washed and resuspended in sterile MilliQ water, and then irradiated using a ^{137}Cs source (Gravitron RX30/55M). After irradiation dilutions of cells were plated in triplicate onto YES plates, which were incubated at 30°C for 6 days before colonies were counted. All data points represent the mean value from three independent cultures.

Meiotic time courses and cytology

The protocol for *pat1-114* haploid meiotic courses has been described (48). In brief, cells were grown in YES (or EMMG lacking leucine if selecting for pMW651) at 25°C before being transferred into PM and grown for a further 42 h. The cells were then incubated for 16 h in PM-N at 25°C before adding NH_4Cl (to 0.5 g/l) and adenine, uracil, lysine, histidine and leucine (leucine is omitted when selecting for pMW651) to a final concentration of 75 mg/l. Cultures were then shifted to 34°C to start the time course. Samples for monitoring YFP-Fbh1 foci and meiotic progression were fixed in 70% ethanol, stained with DAPI and analyzed using an Olympus BX50 epifluorescence microscope equipped with the appropriate filter sets to detect blue and green fluorescence. Samples for detecting YFP-Fbh1 and Rad51 foci by immunostaining of spread nuclei were processed and

Table 1. *Schizosaccharomyces pombe* strains used in this study

Strain	Relevant genotype	Source
MCW1203	*h⁻ ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M26*	Lab strain
MCW1784	*h⁻ fbh1Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M26*	This study
Sun63	*h⁻ˢᵐᵗ⁰ rad22Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M26*	This study
MCW1819	*h⁻ˢᵐᵗ⁰ fbh1Δ::kanMX6 rad22Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M26*	This study
MCW1193	*h⁺ ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M26*	(50)
MCW2496	*h⁺ rad51Δ::arg3⁺ ura4-D18 leu1-32 his3-D1 arg3-D4 ade6-M26*	(3)
MCW3811	*h⁺ srs2Δ::kanMX6 ura4-D18 his3-D1 leu1-32 arg3-D4 ade6-M26*	(3)
MCW2620	*h⁺ fbh1Δ::kanMX6 ura4-D18 his3-D1 leu1-32 arg3-D4 ade6-M26*	This study
ALP651	*h⁺ ura4-D18*	This study
MCW3139	*h⁻ ura4-D18*	This study
MCW3135	*h⁺ fbh1Δ::kanMX6 ura4-D18*	This study
MCW3136	*h⁻ fbh1Δ::kanMX6 ura4-D18*	This study
MCW1758	*h⁺ fbh1^{L14AP15A}::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	(44)
MCW5134	*h⁻ fbh1^{L14AP15A}::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	This study
MCW1759	*h⁺ fbh1^{D485N}::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	(44)
MCW5135	*h⁻ fbh1^{D485N}::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	This study
MCW3193	*h⁺ rec12-152::LEU2 ura4-D18 leu1-32*	This study
MCW3194	*h⁻ rec12-152::LEU2 ura4-D18 leu1-32*	This study
FO1706	*h⁻ fbh1Δ::kanMX6 rec12-152::LEU2 ura4-D18 leu1-32*	This study
FO1707	*h⁺ fbh1Δ::kanMX6 rec12-152::LEU2 ura4-D18 leu1-32*	This study
MCW352	*h⁺ pat1-114 ura4-D18 leu1-32 his3-D1*	Lab strain
MCW1989	*h⁺ rec12-171::ura4⁺ pat1-114 ura4-D18 leu1-32 his3-D1 arg3-D4*	This study
MCW2138	*h⁺ fbh1Δ::kanMX6 pat1-114 ura4-D18 leu1-32 his3-D1*	This study
MCW1195	*h⁻ ura4-D18 his3-D1 arg3-D4 ura4⁺-aim2 ade6-M26*	(50)
MCW1196	*h⁺ ura4-D18 his3-D1 leu1-32 his3⁺-aim ade6-L469*	(50)
MCW1785	*h⁻ fbh1Δ::kanMX6 ura4-D18 his3-D1 leu1-32 arg3-D4 his3⁺-aim ade6-L469*	This study
MCW1832	*h⁺ fbh1Δ::kanMX6 ura4-D18 his3-D1 leu1-32 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
MCW2738	*h⁻ mus81Δ::kanMX6 pat1-114 ade6-3074 his3⁺-aim ura4-D18 leu1-32 his3-D1 arg3-D4*	This study
MCW1519	*h⁺ rad22Δ::kanMX6 ura4-D18 his3-D1 leu1-32 his3⁺-aim ade6-L469*	This study
MCW1520	*h⁻ˢᵐᵗ⁰ rad22Δ::kanMX6 ura4-D18 his3-D1 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
FO1699	*h⁻ rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 his3⁺-aim ade6-L469*	This study
FO1700	*h⁺ rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
MCW1791	*h⁺ rad22Δ::kanMX6 rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 his3⁺-aim ade6-L469*	This study
MCW1792	*h⁻ˢᵐᵗ⁰ rad22Δ::kanMX6 rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
MCW1786	*h⁻ˢᵐᵗ⁰ rad22Δ::kanMX6 fbh1Δ::kanMX6 ura4-D18 his3-D1 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
MCW1787	*h⁺ rad22Δ::kanMX6 fbh1Δ::kanMX6 ura4-D18 his3-D1 leu1-32 his3⁺-aim ade6-L469*	This study
MCW1902	*h⁺ fbh1Δ::kanMX6 rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
MCW1924	*h⁻ fbh1Δ::kanMX6 rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 arg3-D4 his3⁺-aim ade6-L469*	This study
MCW1800	*h⁻ rad22Δ::kanMX6 fbh1Δ::arg3⁺ rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 arg3-D4 ura4⁺-aim2 ade6-M26*	This study
MCW1802	*h⁺ rad22Δ::kanMX6 fbh1Δ::arg3⁺ rti1Δ::LEU2 ura4-D18 his3-D1 leu1-32 arg3-D4 his3⁺-aim ade6-L469*	This study
MCW2128	*h⁺ rad22Δ::kanMX6 pat1-114 ura4-D18 his3-D1 leu1-32 arg3-D4*	This study
MCW2125	*h⁺ rti1Δ::LEU2 pat1-114 ura4-D18 his3-D1 leu1-32 arg3-D4*	This study
MCW2126	*h⁺ rad22Δ::kanMX6 rti1Δ::LEU2 pat1-114 ura4-D18 his3-D1 leu1-32 arg3-D4*	This study
MCW2148	*h⁺ fbh1Δ::arg3⁺ rad22Δ::kanMX6 pat1-114 ura4-D18 his3-D1 leu1-32 arg3-D4*	This study
MCW2140	*h⁺ fbh1Δ::kanMX6 rti1Δ::LEU2 pat1-114 ura4-D18 his3-D1 leu1-32*	This study
MCW2149	*h⁺ fbh1Δ::arg3⁺ rad22Δ::kanMX6 rti1Δ::LEU2 pat1-114 ura4-D18 his3-D1 leu1-32 arg3-D4*	This study
FO1033	*h⁺ rad51Δ::arg3⁺ ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
FO1541	*h⁻ˢᵐᵗ⁰ rad51Δ::arg3⁺ ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
FO1554	*h⁻ˢᵐᵗ⁰ rad51Δ::arg3⁺ fbh1Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
MCW1587	*h⁺ rad51Δ::arg3⁺ fbh1Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain

analyzed as described previously (42). For immunofluorescence in whole-cells yeast cultures were fixed for 40 min in 4% freshly prepared paraformaldehyde in the presence of 1.2 M sorbitol to preserve the cytoskeleton (52,53). Cells were stained with an antibody raised against fission yeast Rad51 (Cosmo Bio Co., Tokyo, Japan), TAT1 (54), which recognizes microtubules and appropriate secondary antibodies conjugated to FITC or Cy3. DNA was stained with Hoechst 33342 before analyzing cells using the above-mentioned Olympus BX50 microscope equipped with the required filter sets. Meiotic cells were classified using the microtubule staining defining prophase I, anaphase I and anaphase II as previously shown (55). In all time courses, meiotic progression was verified by assessing the relative numbers of uni-nucleate, horsetail and multi-nucleate cells at 1-h intervals.

Pulse-field gel electrophoresis

Genomic DNA from 2×10^7 cells was prepared in agarose plugs using a Bio-Rad plug kit (Bio-Rad, Hercules, CA) with modifications (56). PFGE was performed with a 0.8% chromosomal grade agarose gel in 1 × TAE buffer (40 mM Tris–acetate, 2 mM EDTA) and a CHEF III system (Bio-Rad, Hercules, CA). Gels were run for 48 h at 14°C with a switching time of 30 min and an angle of 106°. They were then stained with ethidium bromide and analyzed using a transilluminator and Kodak EDAS 290 gel documentation system.

RESULTS

Fbh1 prevents efficient DSB repair in vegetative cells in the absence of Rad22

Fbh1 and Rad22 play opposing roles in modulating Rad51 activity in vegetative cells of *S. pombe* (42,44). In the absence of Fbh1 Rad22 promotes unfettered Rad51 activity leading to poor growth and viability, as well as problems in chromosome segregation (40,44). In contrast, without Rad22, Rad51 is unable to support efficient DNA repair and recombination when Fbh1 is active (42,44). This genetic interplay between Rad22 and Fbh1 has previously been shown to hold true for DNA repair and recombination events that most likely stem from problems that arise during DNA replication. To see if it is also true for non-replication-associated DNA damage, we compared the sensitivity of wild-type, *fbh1Δ*, *rad22Δ* and *fbh1Δ rad22Δ* strains to the DSB inducing agent IR. At doses up to 100 Gy, the *fbh1Δ* mutant displays little or no sensitivity indicating that Fbh1 does not play a vital role in DSB repair (Figure 1A). In contrast, a *rad22Δ* mutant is very sensitive to IR (Figure 1A). Significantly, this sensitivity is suppressed by deleting *fbh1* (Figure 1A), which suggests that Fbh1 prevents Rad51-dependent DSB repair in the absence of Rad22. Overall these data suggest that whilst Fbh1 can act at DSBs to limit Rad51 activity, this activity is not essential for the repair itself.

Fbh1 limits crossing over during DSB repair in vegetative cells

Having established that Fbh1 limits Rad51 activity at DSBs in the absence of Rad22, we next sought to determine whether it has any measurable effect on repair when Rad22 is present. In both *S. cerevisiae* and *S. pombe* Srs2 suppresses the formation of crossover recombinants during DSB repair possibly by promoting SDSA (2,3). To see if the same is true for Fbh1, we compared the effect of *fbh1Δ* and *srs2Δ* on crossover formation in a plasmid gap repair assay, in which a plasmid containing a DSB within a copy of *ade6* is repaired through HR with a mutant copy (*ade6–M26*) on the chromosome (3) (Figure 1B). Both mutants exhibit similar levels of gap repair as wild type judged from the ratios of the numbers of cut plasmid transformants to uncut plasmid transformants (Figure 1C). In contrast, deletion of *rad51* results in a dramatic reduction in repair as observed previously (Figure 1C) (3). In both wild-type and *srs2Δ*- and *fbh1Δ*-mutant strains, the majority of repair events (~75%) occur by gene conversion of *ade6* to generate Ade^+ recombinants (Figure 1D). Such repair is dependent on Rad51 (Figure 1D). Repair events that are associated with crossing over result in the relatively stable integration of the plasmid into the chromosome (Figure 1B). Therefore to determine the proportion of Ade^+ recombinants that are crossovers, we assessed the stability of the plasmid-borne markers following a period of non-selective growth. Both mutants exhibit an increase in crossovers amongst Ade^+ recombinants compared to wild type (Figure 1E). In the case of *srs2Δ*, this is 1.6-fold ($P = 0.0002$), which is essentially the same as observed previously (3), and for *fbh1Δ* it is 1.8-fold ($P = 0.0006$) (Figure 1E). These data indicate that like Srs2, Fbh1 is required to constrain crossing over during DSB repair in vegetative cells. Unfortunately, the very poor viability of a *fbh1Δ srs2Δ* double mutant prevented us from establishing whether Fbh1 and Srs2 contribute to crossover suppression via separate or common pathways.

The *fbh1Δ* mutant exhibits poor spore viability that can be partially rescued by deleting *rec12*

Having established that Fbh1 can influence DSB repair in vegetative cells, we next investigated whether it might have a meiotic role. First, we assessed the viability of spores generated from a *fbh1Δ* homozygous cross (Figure 2A). In contrast to wild type, which shows relatively high levels of spore viability (~80.5%), only ~1.1% of the spores from the *fbh1Δ* cross were viable. To determine the importance of Fbh1′s F-box and DNA helicase/translocase activities we also measured the viability of spores from $fbh1^{L14AP15A}$ and $fbh1^{D485N}$ homozygous crosses (Figure 2A). The L14A + P15A mutations in Fbh1's F-box results in a loss of interaction with Skp1 (47), but this has a relatively mild effect on spore viability, which is reduced by ~50% compared to wild type. In contrast, the D485N mutation in helicase motif II, which should impair ATP hydrolysis and DNA unwinding based on the analysis of equivalent mutations in other DNA helicases, results in a reduction in spore viability similar to that of a *fbh1*-deletion mutant. These data indicate that Fbh1 plays an important role during meiosis and/or in proper spore formation, which is partially dependent on its F-box activity and totally dependent on its DNA helicase/translocase activity.

To see whether Fbh1′s meiotic role might be in DSB repair, we next determined whether preventing DSB formation, through deleting *rec12* (= *S. pombe* orthologue of Spo11), suppresses the poor spore viability of a *fbh1Δ* mutant. Even though HR is important for proper homologue segregation in meiosis I, *rec12Δ* mutants exhibit reasonably high levels of spore viability because of both random and achiasmate homologue segregation (57). Consequently a *rec12Δ* homozygous cross generates relatively high levels of viable spores (in this case ~24%) (Figure 2A). Although the spore viability of a *fbh1Δrec12Δ* double mutant is less than this (~5.5%), it is significantly greater ($P = 0.02$) than that of a *fbh1Δ* single mutant (Figure 2A). Overall these data establish that Fbh1 plays an important meiotic role, which is, at least in part, needed as a consequence of DSB formation.

Fbh1 forms Rec12-dependent nuclear foci during meiosis

Fbh1 forms nuclear foci in response to DNA damage in vegetative cells (42,44), and therefore if it truly functions during DSB repair in meiotic cells, it should similarly form foci, and moreover these foci should be Rec12-dependent. To test this, we transformed a plasmid that expresses yellow fluorescent protein (YFP)-tagged Fbh1 from the pREP41 *nmt* promoter into wild-type and *rec12Δ* strains containing the temperature sensitive *pat1–114* allele, which enables a synchronous meiosis to be thermally

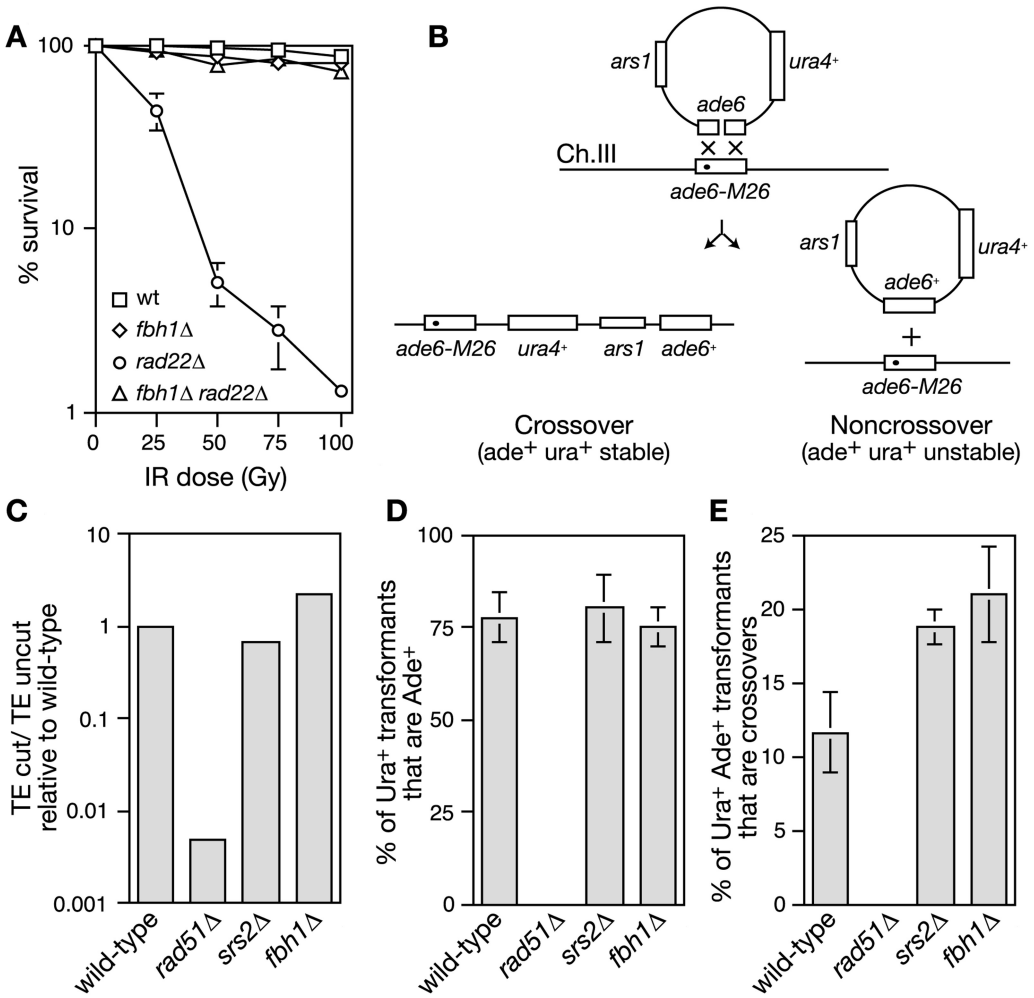


Figure 1. Fbh1 inhibits DSB repair in vegetative cells in the absence of Rad22 and limits the formation of crossovers in its presence. (**A**) IR survival curves for strains MCW1203, MCW1784, Sun63 and MCW1819. Error bars are the standard deviations about the mean. (**B**) Schematic of the plasmid gap repair assay. The black dot indicates the position of the *M26* mutation. (**C**) Histogram showing the mean relative transformation efficiency (TE) of cut versus uncut plasmid in strains MCW1193, MCW2496, MCW3811 and MCW2620. (**D**) Histogram showing the mean percentage of Ura^+ transformants that are Ade^+ in the same strains as in (C). (**E**) Histogram showing the mean percentage of Ade^+ recombinants that are crossovers in the same strains as in (C). Error bars in (D) and (E) are the standard deviations about the mean.

induced. Strains were grown at 25°C and then shifted to 34°C to induce meiosis following which samples were taken for epifluorescence microscopy at timed intervals (Figure 2B). In the wild-type strain, YFP-Fbh1 foci are detected in >10% of cells and peak at the 3-h time point, which is approximately when DSBs are formed (Figure 2B and data not shown). The number of cells with foci then gradually decreases over time, and is reduced to essentially zero by the 7-h time point (Figure 2B). In contrast, few if any foci are formed in the *rec12Δ* mutant. To confirm that this is not due to a failure in YFP-Fbh1 expression but rather the absence of DSB formation, we added the topoisomerase I poison camptothecin (CPT) at the start of the time course to substitute for DNA breakage by Rec12. CPT-treated *rec12Δ* cells display YFP-Fbh1 foci that first appear at 2 h, which is approximately coincident with the end of S-phase in a *pat1–114* meiosis, and peak at the 5-h time point (Figure 2B). Together these data show that Fbh1 forms foci in response to Rec12-dependent DSBs, which is consistent with it functioning during meiotic DSB repair.

Fbh1 foci co-localize with Rad51 foci during meiosis

The observation that Fbh1 forms Rec12-dependent foci suggests that it is recruited to DSB sites. If true then YFP-Fbh1 should co-localize with recombination proteins such as Rad51, as has been observed in vegetative cells (42,44). To test this, we prepared nuclear spreads from cells undergoing a *pat1–114* meiosis at the 3–5-h time points when DSBs are formed and repaired, and stained these using antibodies against Rad51 and the YFP tag on Fbh1 (Figure 2C). The majority of YFP-Fbh1 foci co-localize with Rad51 foci (Figure 2C and G). Likewise, the majority of Rad51 foci at the 4- and 5-h time points co-localize with a Fbh1 focus (Figure 2F). These data are consistent with Fbh1 being recruited to DSB sites that are undergoing repair by HR. Intriguingly Rad51 foci start to appear prior to

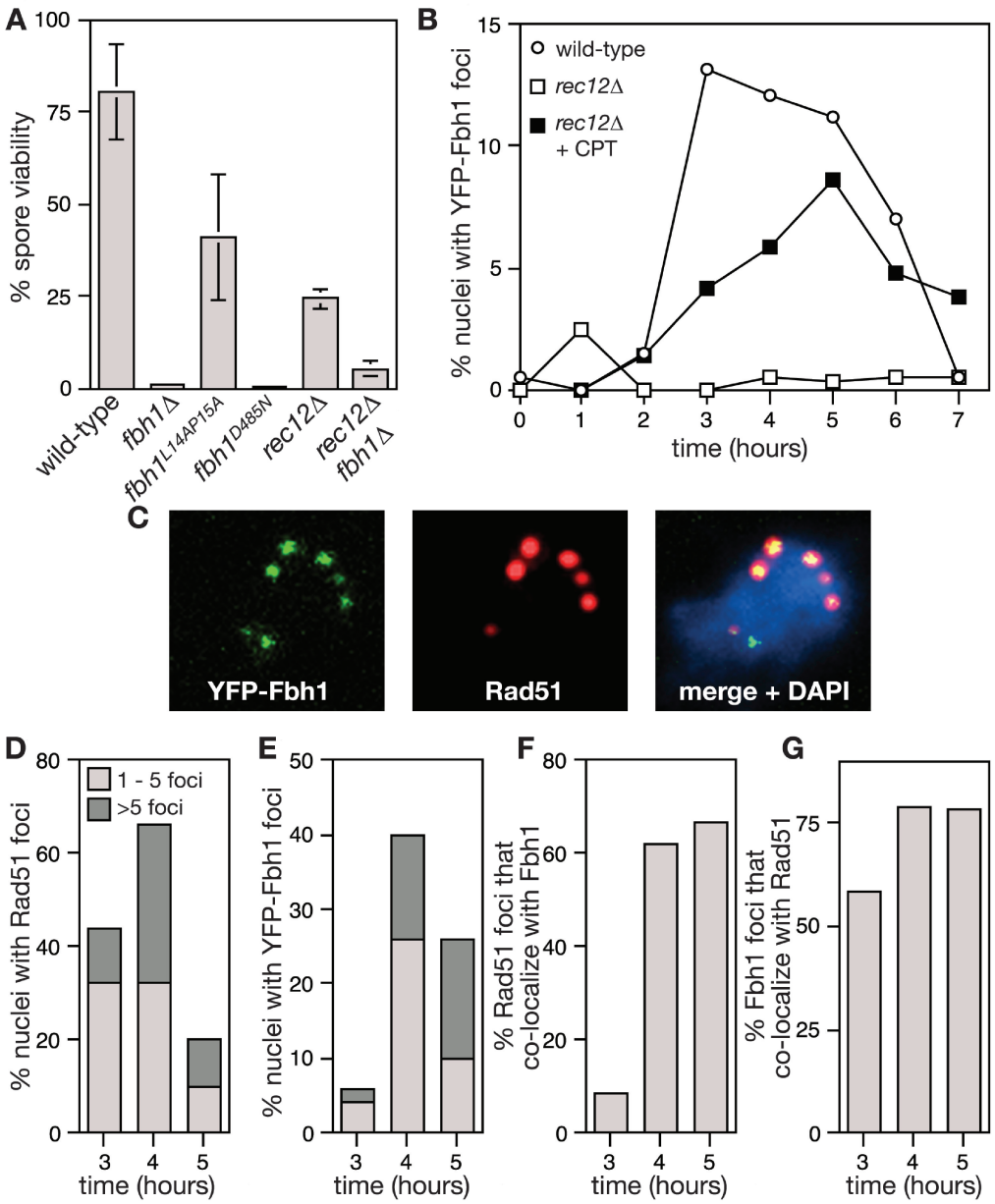


Figure 2. Fbh1 is required for meiotic success and co-localizes with Rad51 at discrete nuclear foci induced by Rec12. (**A**) Spore viability from crosses ALP651 × MCW3139, MCW3135 × MCW3136, MCW1758 × MCW5134, MCW1759 × MCW5135, MCW3193 × MCW3194 and FO1706 × FO1707. Values are the means from at least three independent experiments with error bars representing the standard deviations about the mean. (**B**) The percentage of nuclei with YFP-Fbh1 foci in wild-type (MCW352) and *rec12Δ* (MCW1989) haploid strains carrying plasmid pMW651 undergoing a *pat1–114* synchronous meiosis. To MCW1989, 10 μM CPT was added to confirm that YFP-Fbh1 can be induced in this strain. Values are the means from three independent experiments. (**C**) Representative spread nucleus from a wild-type (MCW352) cell carrying pMW651 undergoing a *pat1–114* meiosis. The spread was stained with antibodies against Rad51 and the YFP tag on Fbh1. The DNA was stained with DAPI (blue). (**D–G**) Quantification of data like in (C). Values are from a single experiment in which 100-spread nuclei were analyzed for each time point. The experiment was repeated twice to confirm that the data were reproducible.

YFP-Fbh1 foci (compare Figure 2D and E; note that in this experiment, the peak of Fbh1 foci is slightly later than in Figure 2B). This suggests that Fbh1 is recruited to meiotic DSBs after Rad51 nucleofilament formation.

Meiotic DSBs are repaired in a *fbh1Δ* mutant

To determine whether the poor spore viability of a *fbh1Δ* mutant was the result of failed DSB repair, we initially attempted to compare DSB formation and repair in diploid wild-type and *fbh1Δ* strains. However, *fbh1Δ* diploid cells were unstable and therefore we again made use of a haploid *pat1–114* meiosis. Samples of cells were taken every hour from which genomic DNA was prepared in agarose plugs and analyzed by pulse field gel electrophoresis on a CHEF gel (Figure 3). Typically in a *pat1–114* meiosis, DSBs are formed by the 3–4-h time points and are mostly repaired by the 5-h time point (58). This is

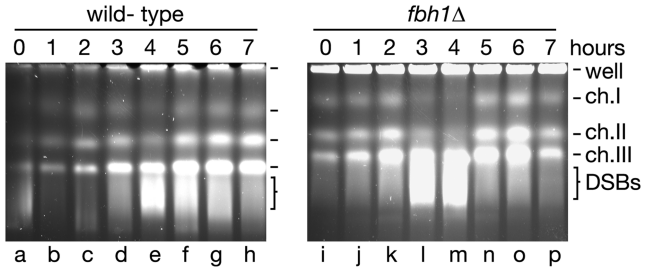


Figure 3. Pulse field-gel electrophoresis analysis of chromosomal DNA from a wild-type (MCW352) and *fbh1Δ* (MCW2138) *pat1–114* meiosis.

essentially what we observe for the wild-type in Figure 3 where a reduction in chromosome band intensity and appearance of a smear of DNA running ahead of chromosome III at the 4-h time point signifies DSB formation (lane e), and a reduction in the intensity of the smear concomitant with an increase in chromosome band intensity at the 5-h time point indicates that most of the DSBs have been repaired (lane f). Similarly in the *fbh1Δ* time course, DSBs are formed by the 3-h time point (lane l), and are repaired by the 5-h time point (lane n). The earlier appearance of DSBs here reflects variation between *pat1–114* meioses because in other time courses, fewer DSBs were noticeable at the 3-h time point (data not shown). Altogether these data show that meiotic DSBs are efficiently formed and repaired in a *fbh1Δ* mutant, and therefore Fbh1′s critical role in meiosis is not DSB repair itself but rather some other associated function.

The frequency of recombinants amongst viable progeny is normal in a *fbh1Δ* mutant

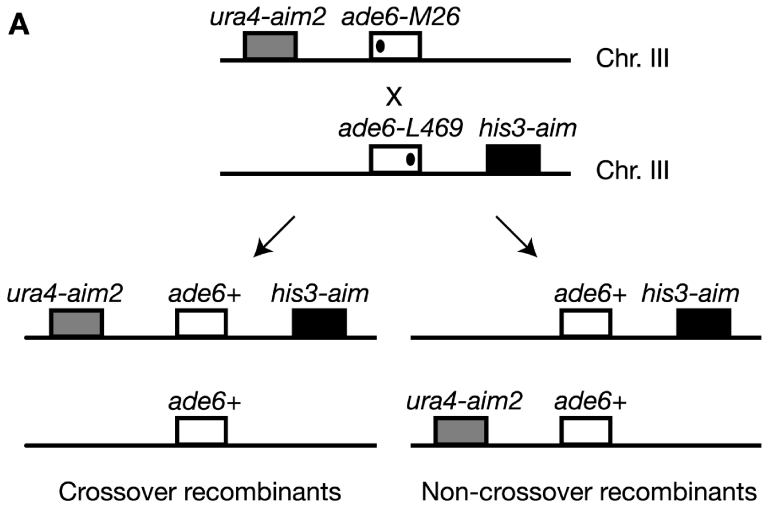
Similar to DSB repair in vegetative cells, the repair of meiotic DSBs gives rise to both crossover and non-crossover recombinants. In *S. pombe*, meiotic crossovers depend on the cleavage of DNA junctions by the Mus81-Eme1 structure-specific endonuclease (50,59). However, the identity of the enzymes that process meiotic recombination intermediates into non-crossover recombinants have generally not been defined, although recent data suggest that in *C. elegans* this function is performed by RTEL-1 (60). As Fbh1 is required for suppressing crossover formation during DSB repair in vegetative cells, we sought to determine whether it has a similar role in meiotic cells by assessing the effect of *fbh1* deletion on the percentage of crossover and non-crossover recombinants associated with gene conversion at *ade6* using the system outlined in Figure 4A. The frequency of Ade^+ recombinants (gene conversion) is slightly higher in a *fbh1Δ* mutant than in a wild type, however the proportion of crossover and non-crossover recombinants amongst these is essentially the same as wild type (Figure 4C). The same is also true for the overall frequency of crossing over in the *ura4-aim2* to *his3-aim* interval (Figure 4A and B). Together these data indicate that Fbh1 is not required for the formation of meiotic recombinants nor does it play a key role in determining crossover versus non-crossover pathway choice. However, it should be noted that our assay allows us to score only viable recombinants, and it is possible that these are not representative of recombination in the majority of *fbh1Δ* cells, which fail to give rise to viable progeny.

Meiotic chromosome segregation is impaired in a *fbh1Δ* mutant but not because of unresolved recombination junctions

The failure of Fbh1 to modulate Rad51 nucleofilament formation during vegetative growth correlates with problems in chromosome segregation, which may underlie much or all of the poor viability of a *fbh1Δ* mutant (44). To see whether the poor spore viability of a *fbh1Δ* mutant is similarly associated with problems in chromosome segregation, *fbh1Δ* mutant strains of opposite mating type were crossed and the resultant asci stained with DAPI and analyzed by fluorescence microscopy (Figure 5A). The majority of *fbh1Δ* asci are abnormal, with fewer than four spores (Figure 5A and B), and even amongst those asci that contain four spores the DNA is unequally distributed (Figure 5C). Indeed, >10% of these asci show a complete failure in chromosome segregation with the entire DNA contained within a single spore (Figure 5A and C). In contrast, a *fbh1Δ rad51Δ* double mutant exhibits a greater number of four-spore asci none of which contain a single DNA mass (Figure 5B and C). Instead ~25% of *fbh1Δ rad51Δ* four-spore asci contain more than four DNA masses, which is similar to what is seen in a *rad51Δ* single mutant and is indicative of failed DSB repair (Figure 5C). These data suggest that Rad51 is at least partly responsible for the failed chromosome segregation in a *fbh1Δ* mutant. Indeed Fbh1 might be needed to resolve recombination junctions that are established by Rad51, and in this regard the single DNA masses that are observed in some *fbh1Δ* four-spore asci is reminiscent of a *mus81Δ* mutant where chromosomes fail to segregate due to unresolved recombination junctions (50,61). However, when DNA from a *mus81Δ* mutant meiotic time course is run on a CHEF gel the unresolved recombination junctions cause it to remain trapped in the well following disappearance of the DSBs (Figure 5D, lanes f–h). This is not seen in a *fbh1Δ* mutant (Figure 3). Therefore the failure of chromosome segregation in a *fbh1Δ* mutant does not appear to be due to a failure in processing DNA junctions that are formed during HR.

Fbh1 constrains the amount of Rad51 that accumulates on DNA during meiosis

During vegetative cell growth, Fbh1 appears to be important for preventing the accumulation of Rad51 onto DNA, and the failure to keep this accumulation in check may be responsible for the pathologies associated with a *fbh1Δ* mutant (40,42). To see whether this might also be the case during meiosis, spread nuclei were prepared from wild-type and *fbh1Δ* mutant haploid cells undergoing a synchronized *pat1–114* meiosis and stained using an anti-Rad51 antibody (Figure 6). Similar to the data in Figure 2D, the number of cells with Rad51 foci peaks at 4 h concomitant with cells in the so-called horsetail stage when the nuclei appear elongated and move back and



B

Genotype	No. of crosses	Tested	P1 (ura+ his-)	P2 (ura- his+)	R1 (ura+ his+)	R2 (ura- his-)	% Rec.	cM
wild-type	44	9454	4044	3927	755	728	15.9 (+/- 3.9)	19.1
fbh1	18	1703	633	770	106	194	17.1 (+/- 6.2)	20.9

C

Genotype	Frequency of Ade$^+$ (x10^{-2})	Ade$^+$ tested	% Ade$^+$ non-crossovers		crossovers	
			Ura$^+$ His$^-$	Ura$^-$ His$^+$	Ura$^+$ His$^+$	Ura$^-$ His$^-$
wild-type	0.525 (+/- 0.110)	5944	34.3 (+/- 4.8)	9.1 (+/- 3.9)	5.6 (+/- 2.6)	50.1 (+/- 7.3)
fbh1	0.784 (+/- 0.263)	1499	32.0 (+/- 5.2)	11.6 (+/- 5.5)	7.3 (+/- 3.4)	49.1 (+/- 7.7)

Figure 4. Meiotic recombinant formation in a *fbh1Δ* mutant. (**A**) Schematic showing the cross used to assess gene conversion at *ade6*, crossing over in the *ura4-aim2–his3-aim* interval and percentage of ade$^+$ recombinants that are associated with a crossover of the flanking markers. The filled circles indicate the relative positions of the *M26* and *L469* mutations. (**B**) Frequency of meiotic recombination in wild-type (MCW1195 × MCW1196) and *fbh1Δ* mutant (MCW1785 × MCW1832) homozygous crosses. The number of crosses and total number of random spores analyzed is indicated. Recombinant frequencies are converted to centimorgans (cM) using the mapping function of Haldane (65). (**C**) Frequency of Ade$^+$ recombinants and their association with crossover in the *ura4-aim2–his3-aim* interval. Data are derived from the crosses in (B). The values in parentheses are the standard deviations.

forth in the cell, which is a marker for meiotic prophase (57) (Figure 6A and B). By 5 h, the horsetail stage is over and the majority of cells have undergone either one or two rounds of chromosome segregation as indicated by the appearance of cells with 2–4 nuclei (Figure 6A) and this correlates with a reduction in the number of nuclei with Rad51 foci. Like in the wild type, the number of Rad51 foci increases in the *fbh1Δ* mutant from the 3–4-h time points when DSBs are made and repaired, albeit the starting level of foci are higher most likely due to problems encountered during pre-meiotic S-phase (Figure 6D) (42). However, unlike in the wild type, the levels of Rad51 foci persist and even increase at the 5-h time point in the *fbh1Δ* mutant (Figure 6D). This accumulation and retention of Rad51 foci does not appear to be due to changes in overall meiotic progression as judged by the timing of the horsetail stage and formation of cells with two or more nuclei, which in multiple repeats show no significant difference to wild type (Figure 6C and data not shown). Indeed the similarity between wild type and *fbh1Δ* meiotic chromosome segregation in a haploid *pat1–114* background appears to be at odds with that observed in zygotic asci (Figure 5A and C). However, it should be noted that chromosome segregation is generally aberrant in haploid *pat1–114* meioses with 50% of wild-type cells displaying uneven segregation of DNA following meiosis I

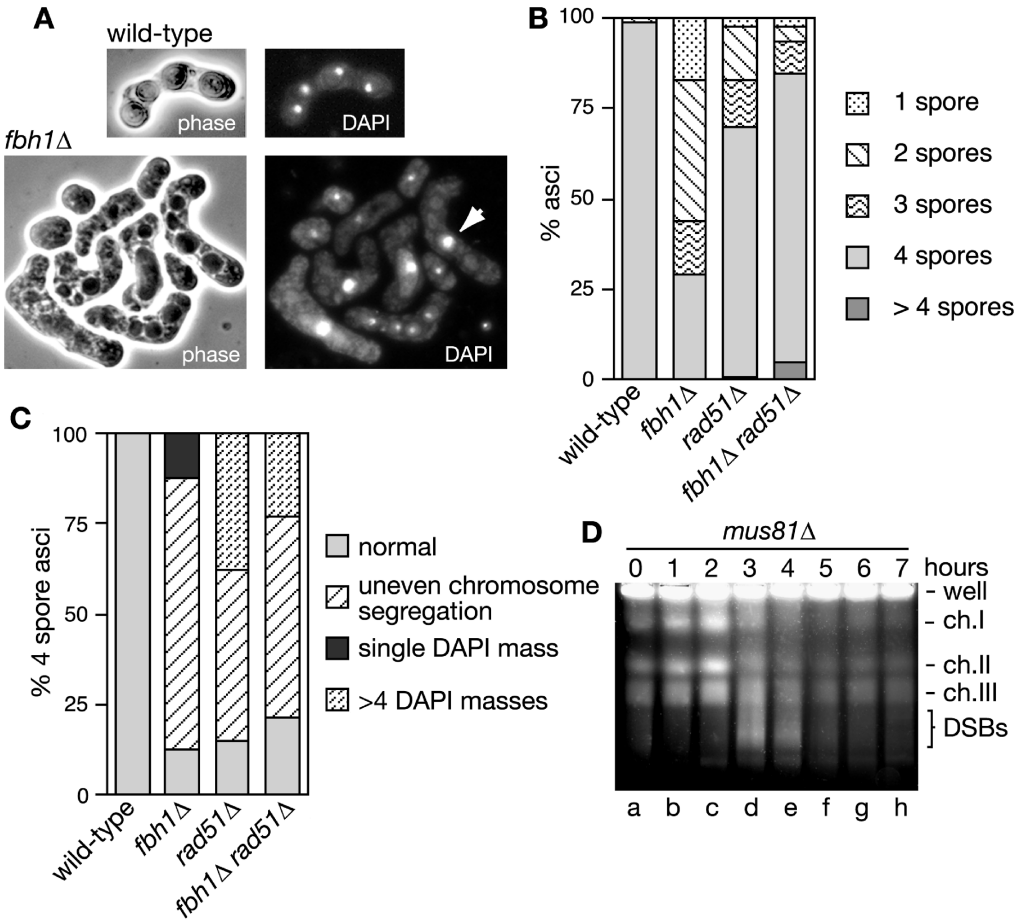


Figure 5. Meiotic chromosome segregation in a *fbh1Δ* mutant. (**A**) Phase contrast and epifluorescence microscopy of DAPI-stained asci from wild-type (MCW1195 × MCW1196) and *fbh1Δ* mutant (MCW1785 × MCW1832) homozygous crosses. The arrow indicates an example of where the DAPI-stained DNA in an ascus has failed to segregate and consequently is encapsulated in only one of the four spores. (**B**) Quantification of data like in (A) showing the percentage one-, two-, three- and four-spore asci obtained from wild type, *fbh1Δ*, *rad51Δ* (FO1033 × FO1541) and *fbh1Δ rad51Δ* (MCW1587 × FO1554) homozygous crosses. Values are based on the analysis of 150 asci from three independent crosses. (**C**) The percentage of four-spore asci obtained from wild-type and *fbh1Δ* homozygous crosses that have uneven chromosome segregation between the spores. Values are based on the analysis of 100-four-spore asci from three independent crosses. (**D**) Pulsed field gel-electrophoresis analysis of chromosomal DNA from a *mus81Δ* (MCW2738) *pat1–114* meiosis.

(Supplementary Figure S1). This high rate of chromosome mis-segregation in the wild type presumably masks much of the effect of *fbh1* deletion, which only increases mis-segregation by a further 10% in this background (Supplementary Figure S1).

The data in Figure 6B and D show that the levels of Rad51 foci are generally higher in a *fbh1Δ* mutant than in wild type during a *pat1–114* haploid meiosis. However, it is unclear from these data whether Rad51 foci are present at anaphase I and II because the nuclei are not staged and therefore there could be a bias towards those in prophase I if they happen to spread more efficiently. We therefore repeated the experiment, but instead of analyzing Rad51 foci in spread nuclei we used fixed whole cells, which were also stained for tubulin to determine the presence of the anaphase spindle(s) (Figure 6E). By this analysis, ~50% of *fbh1Δ* cells containing an anaphase I spindle also contained Rad51 foci, whereas this was true of <10% of wild-type cells (Figure 6F). In contrast, Rad51 foci were generally absent in both wild type and *fbh1Δ* cells in anaphase II (Figure 6F). These data indicate that a greater number of cells undergo anaphase I with Rad51 foci in the absence of *fbh1*, and we suspect that it is this retention of Rad51 on DNA, which in part stems from its loading at meiotic DSB sites, that is responsible for the aberrant chromosome segregation seen in zygotic asci.

Suppression of the poor spore viability and chromosome segregation defects of a *fbh1Δ* mutant by deleting *rad22* and *rti1*

During vegetative growth, Rad51 activity appears to be modulated by the pro-nucleofilament forming activity of Rad22 and the putative anti-nucleofilament forming activity of Fbh1 (42,44). An imbalance of these factors (e.g. by gene deletion), results in either a loss or excess of Rad51 activity depending on which way the balance is skewed. However, the loss of both proteins restores the balance, and Rad51 is therefore able to function relatively well. To see whether a similar situation pertains during meiosis, we sought to determine whether loss of

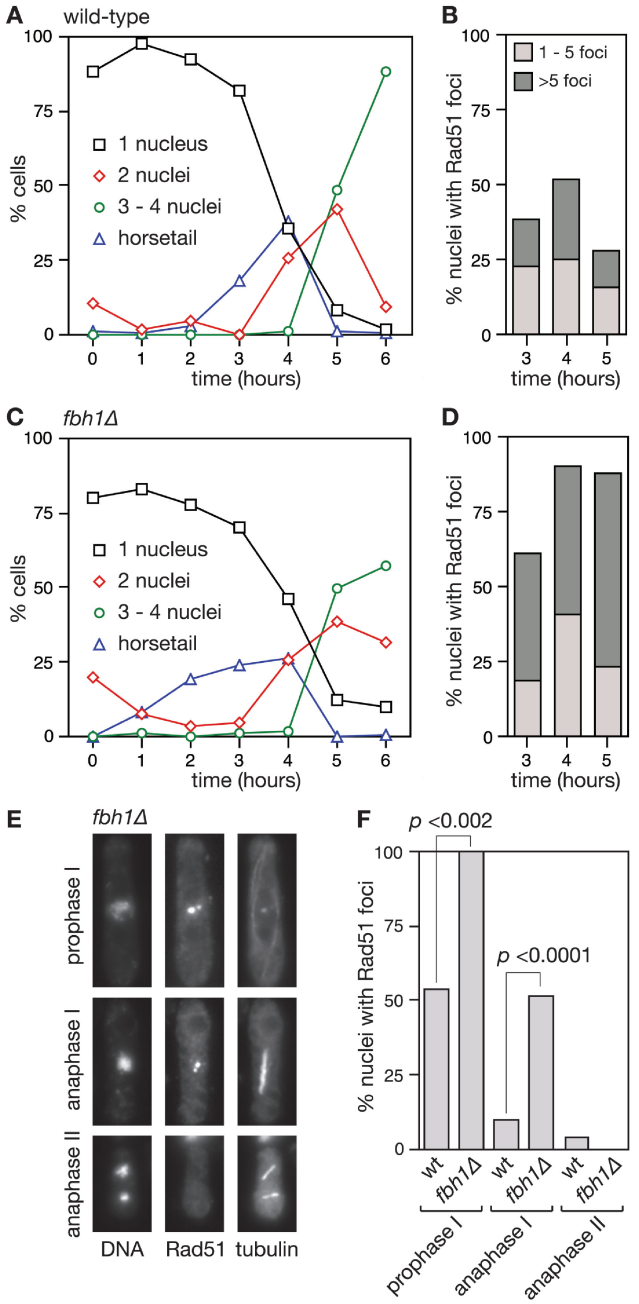


Figure 6. Rad51 foci accumulate to higher levels in a *fbh1Δ* mutant meiosis compared to wild type. (**A**) Cytological analysis of meiotic progression in a wild-type (MCW352) *pat1–114* haploid meiosis. (**B**) Quantification of Rad51 foci in nuclear spreads obtained from the time course in (A). The spreads were stained with an antibody against Rad51. (**C**) Cytological analysis of meiotic progression in a *fbh1Δ* (MCW2138) *pat1–114* haploid meiosis. (**D**) Quantification of Rad51 foci in nuclear spreads obtained from the time course in (C). The values in (B) and (D) are each from a single experiment in which 100-spread nuclei were analyzed for each time point. Experiments were repeated twice to confirm that the data were reproducible. (**E**) Representative images of fixed whole cells from a *fbh1Δ* mutant *pat1–114* haploid meiotic time course stained for Hoechst 33342, Rad51 and tubulin. (**F**) Quantification of the number of fixed whole cells that stain positive for Rad51 foci at different stages of a *pat1–114* haploid meiosis ($n = 30–40$). *P*-values were determined using Fisher's Exact Test (two-tailed).

Rad22 and/or its meiosis-specific paralogue Rti1 would suppress the meiotic defects of a *fbh1Δ* mutant.

First, we assessed the spore viability of *rad22Δ*, *rti1Δ* and *fbh1Δ* single-, double- and triple-mutant strains (Figure 7A). Consistent with previous findings spore viability was unaffected by loss of *rti1* and only reduced by ~40% in a *rad22Δ* single mutant (Figure 7A) (62). However, loss of both *rti1* and *rad22* caused a 27-fold reduction in spore viability consistent with the idea that there is functional redundancy between Rad22 and Rti1 for promoting DSB repair in meiosis (Figure 7A) (62). Importantly, deletion of either *rad22* or *rti1* improved the spore viability of a *fbh1Δ* mutant by as much as 15-fold, and this improvement was doubled if both were deleted (Figure 7A). Moreover, in the case of the triple-mutant suppression of poor spore viability is reciprocal because loss of *fbh1* improves the spore viability of a *rad22Δ rti1Δ* double mutant by ~14-fold. This improvement in spore viability in the triple mutant correlates with a reduction in abnormal asci and increased levels of normal chromosome segregation compared to both a *fbh1Δ* single mutant and *rad22Δ rti1Δ* double mutant (Supplementary Figure S2). Together these data provide further evidence that Rad51 activity, stimulated by Rad22 and Rti1, impedes chromosome segregation in a *fbh1Δ* mutant thereby giving rise to non-viable spores. Moreover the partial suppression of *rad22Δ rti1Δ* mutant poor spore viability by *fbh1* deletion is consistent with the notion that Fbh1 activity, in the absence of Rad22 and Rti1, prevents DSB repair by Rad51 in meiosis.

An *fbh1Δ rad22Δ rti1Δ* triple mutant displays wild-type levels of gene conversion

As noted above an *fbh1Δ* mutant exhibits a modest increase in the frequency of gene conversion between *ade6-M26* and *ade6-L469* compared to wild type ($P = 0.0007$) (Figure 4C). This is consistent with Fbh1 acting to limit Rad51 activity during meiotic recombination. To see whether this increased level of recombination depends on Rad22 and/or Rti1, we compared the frequency of Ade^+ recombinant formation in *fbh1Δ*, *rad22Δ* and *rti1Δ* single, double and triple mutants (Figure 7B). Loss of either *rad22* or *rti1* causes a reduction in gene conversion compared to wild type ($P = 0.0002$ and <0.0001, respectively), with a further reduction if both are deleted ($P < 0.0001$) (Figure 7B). Deletion of *fbh1* in combination with *rad22Δ* and/or *rti1Δ* restores recombination to at least wild-type levels consistent with the notion that Fbh1 is more able to inhibit Rad51-dependent gene conversion in the absence of Rad22 and/or Rti1. By the same token, the hyper-recombination in a *fbh1Δ* mutant is tempered by deleting either *rad22* ($P = 0.05$) or *rti1* ($P = 0.0004$), although intriguingly only *rti1* deletion (on its own or in combination with *rad22Δ*) reduces recombinant frequency to wild-type levels (Figure 7B). Altogether these data suggest that there is interplay between the pro-gene conversion activities of Rad22 and Rti1, and anti-gene conversion activity of Fbh1.

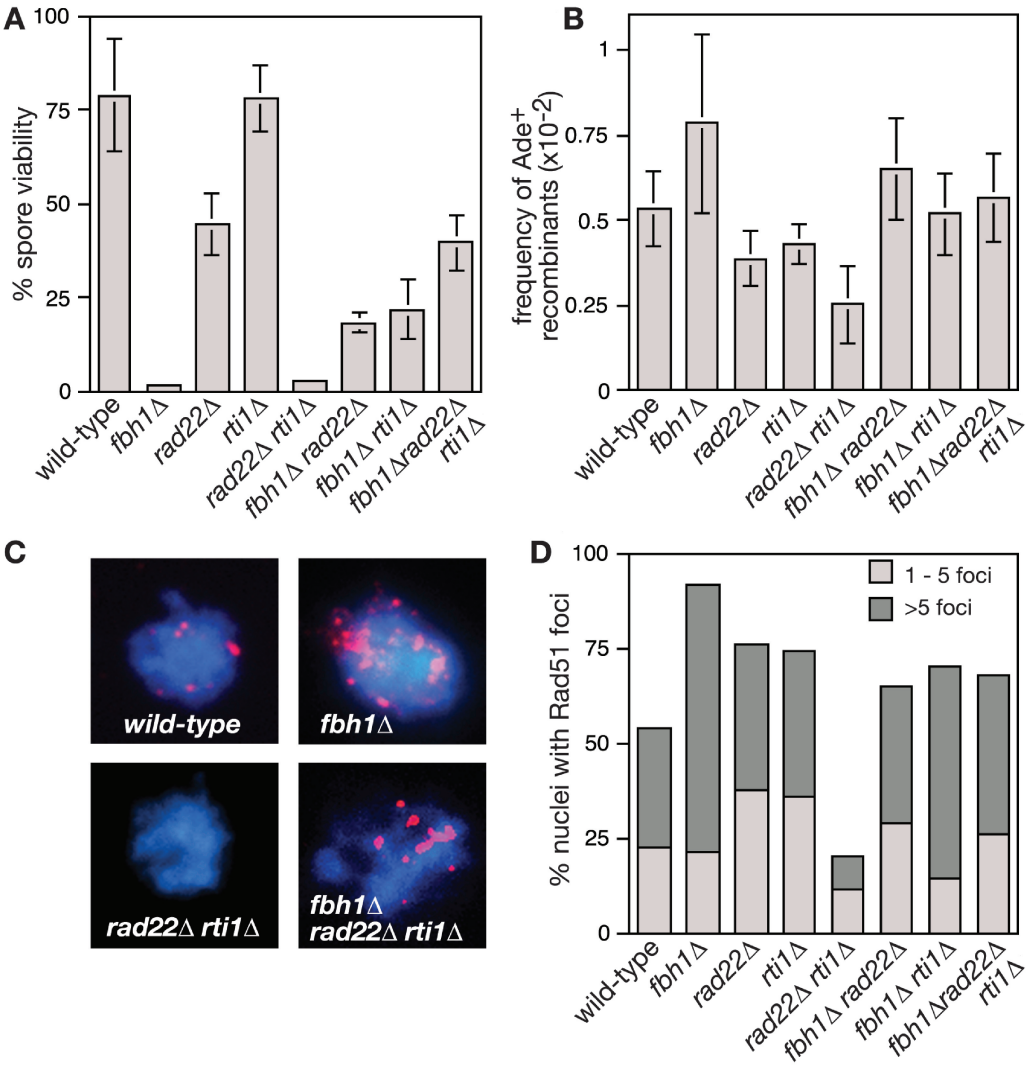


Figure 7. Genetic interaction between *fbh1Δ*, *rad22Δ* and *rti1Δ* with respect to spore viability and Rad51 focus formation. (**A**) Spore viability from crosses MCW1195 × MCW1196, MCW1785 × MCW1832, MCW1519 × MCW1520, FO1699 × FO1700, MCW1791 × MCW1792, MCW1786 × MCW1787, MCW1902 × MCW1924 and MCW1800 × MCW1802. Values are the means from at least three independent experiments with error bars representing the standard deviations about the mean. (**B**) Frequency of gene conversion between *ade6-M26* and *ade6-L469* alleles in single, double and triple *fbh1Δ*, *rad22Δ* and *rti1Δ* mutants. The data are derived from crosses MCW1195 × MCW1196, MCW1785 × MCW1832, MCW1519 × MCW1520, FO1699 × FO1700, MCW1791 × MCW1792, MCW1786 × MCW1787, MCW1902 × MCW1924 and MCW1800 × MCW1802. The error bars are the standard deviations about the mean. (**C**) Representative spread nuclei from wild type (MCW352), *fbh1Δ* (MCW2138), *rad22Δ rti1Δ* (MCW2126) and *fbh1Δ rad22Δ rti1Δ* (MCW2149) cells undergoing a *pat1–114* meiosis. The spreads were stained with antibodies against Rad51 and the DNA was stained with DAPI (blue). (**D**) Peak level of Rad51 foci in the indicated strains undergoing a *pat1–114* meiosis. The strains are: MCW352, MCW2138, MCW2128, MCW2125, MCW2126, MCW2148, MCW2140 and MCW2149. Values are from a single experiment in which spread nuclei were analyzed from each time point between 3–5 h. The time point with the highest level of Rad51 foci is shown for each strain. Values are the means from three independent experiments in which a total of 150 nuclei from each strain were assessed for Rad51 foci.

Restoration of wild-type levels of Rad51 foci in a *fbh1Δ rad22Δ rti1Δ* triple mutant

Without Fbh1 Rad51 foci accumulate during meiosis at higher levels and at later time points than in wild-type cells (Figure 6). As stated above, we suspect that it is this accumulation of Rad51 on DNA that is responsible for the failed chromosome segregation in a *fbh1Δ* mutant. The partial rescue of this defect by the deletion of Rad22 and Rti1, which promote Rad51 nucleofilament formation, is consistent with this idea. However, to gain more direct evidence for this we compared the levels of Rad51 foci at their peak time points in haploid *fbh1Δ*, *rad22Δ* and *rti1Δ* single, double and triple mutant strains induced for meiosis by means of the *pat1–114* allele (Figure 7C and D). As expected loss of both Rad22 and Rti1 results in a marked reduction in Rad51 foci relative to wild type ($P = 0.05$), whereas without Fbh1 they accumulate to higher levels than in the wild type ($P = 0.018$). Levels of Rad51 foci are restored to near wild-type levels in the *fbh1Δ rad22Δ rti1Δ* triple mutant ($P = 0.15$).

The heightened levels of Rad51 foci in the *fbh1Δ* mutant are also suppressed by deleting only *rad22* or *rti1*. These data tally with the spore viability and chromosome segregation data above, and are consistent with a model in which there is interplay between Fbh1, Rad22 and Rti1 for modulating Rad51 nucleofilament formation during meiotic DSB repair.

DISCUSSION

We have shown that Fbh1, a putative Rad51 disruptase, plays an important role during meiosis in promoting proper chromosome segregation, which is at least in part linked to DSB repair. The evidence for this is 3-fold. First, deletion of *fbh1* results in very low levels of spore viability, which correlate with a failure in proper chromosome segregation. Second, Fbh1 forms discrete nuclear foci in response to DSBs formed by Rec12, which co-localize with Rad51. Third, the poor-spore viability of a *fbh1Δ* mutant is partially suppressed by deleting the meiosis-specific factors *rec12* and *rti1*, which have no effect on suppressing its vegetative defects (including poor viability and aberrant mitotic chromosome segregation) (44).

In addition to showing that Fbh1 plays an important meiotic role, we have also shown that it functions during DSB repair in vegetative cells, where it acts to limit the formation of crossover recombinants. This ability is likely to be conserved in higher eukaryotes because in both chicken DT40 and human cells Fbh1 deficiency results in increased sister-chromatid exchange, which is indicative of elevated levels of crossing over (41,45). Crossover suppression is also a role performed by Fbh1′s relative Srs2, which is thought to achieve this by promoting the SDSA pathway of DSB repair in vegetative cells of both *S. cerevisiae* and *S. pombe* (2,3). Exactly how Srs2 might promote SDSA is still a matter of conjecture. One possibility is that it directly unwinds the D-loop formed by Rad51-mediated strand invasion (2,35). However, this function appears to be more ably performed by the Mph1/Fml1 DNA helicase, which strongly suppresses crossing over in *S. cerevisiae* and *S. pombe* (3,4,63). Instead of directly competing with Mph1/Fml1 for unwinding the D-loop, Srs2 might facilitate its action by using its Rad51 disruptase activity to limit the extent of the nucleofilament (21–23), and thereby generate shorter D-loops that are more easily unwound. Rad51 disruptase activity may also be used to remove Rad51 from the non-invading DNA end, which might serve to prevent the formation of a double Holliday junction by second-end capture (2). Like Srs2, Fbh1 might suppress crossover formation by promoting SDSA. Exactly how it would do this is uncertain as its biochemical properties have yet to be thoroughly investigated. However, *in vivo* data are consistent with Fbh1 acting as a Rad51 disruptase (41,42), and therefore it could perform similar roles as suggested for Srs2 above.

Even though Fbh1 functions to limit crossover formation during DSB repair in vegetative cells, it does not appear to do the same in meiotic cells (Figure 4). Moreover, CHEF-gel analysis of chromosomal DNA from a meiotic time course reveals no obvious deficiency in DSB repair in a *fbh1Δ* mutant (Figure 3). Nevertheless, Fbh1 clearly has a critical role to play in meiosis, which if not performed gives rise to aberrant chromosome segregation where most, or sometimes all, of the DNA remains clumped together. This is unlike Srs2 in *S. pombe*, which when deleted has no obvious effect on spore viability or meiotic recombination (64 and our unpublished data). During vegetative growth the activities of Fbh1 and Srs2 in processing Rad51-dependent recombination intermediates appear to partially overlap since a *fbh1Δ srs2Δ* double mutant is synthetically sick, a condition that is rescued by deleting *rad51* (40,44). It is possible therefore that Srs2 could partially compensate for loss of Fbh1 in meiotic cells. However, the very poor growth of a *fbh1Δ srs2Δ* double mutant together with its rapid acquisition of suppressor mutations makes this difficult to investigate.

Exactly how a failure of Fbh1 action gives rise to aberrant chromosome segregation is uncertain. It could relate to a hitherto uncharacterized role for Fbh1 in promoting the machinery of chromosome segregation itself. Alternatively, it could reflect a need for Fbh1 to process recombination intermediates, which if allowed to persist would physically impede chromosome segregation. From these two possibilities, the latter seems more likely based on our observation that abolition or attenuation of HR by deletion of *rad51* or *rti1* and/or *rad22* partially suppresses the aberrant chromosome segregation phenotype of a *fbh1Δ* mutant. However, the persistent recombination intermediates in question do not appear to be DNA junctions because unlike a *mus81Δ* mutant, which is known to process D-loops/Holliday junctions (50,51,59), chromosomes from a *fbh1Δ* mutant meiotic time-course are not retained in the well of a CHEF gel following DSB repair (compare Figures 3 and 5D). We suspect therefore that it is the retention of Rad51 on the DNA into anaphase I, which is observed in a *fbh1Δ* mutant, that interferes with chromosome segregation by holding sister chromatids and/or homologous chromosomes together. Since deletion of *rec12* only partially suppresses the poor spore viability of a *fbh1Δ* mutant the build-up of Rad51 on DNA likely stems from loading during pre-meiotic S-phase as well as during the repair of meiotic DSBs. Possibly Fbh1′s putative Rad51 disruptase activity is required for dismantling Rad51 nucleofilaments during both these stages of meiosis. In the case of DSB repair, a post-synaptic role for Fbh1 is consistent with our observation that Fbh1 foci peak slightly later than Rad51 foci during meiosis (Figure 2D and E). However, the fact that Fbh1 inhibits Rad51 foci in the absence of Rad22 and Rti1 (Figure 7) suggests that Fbh1 could also function during pre-synapsis to modulate Rad51 nucleofilament assembly. Possibly this modulation is important for limiting the growth of each nucleofilament, and thereby indirectly aids post-synaptic disassembly, which might be performed by other factors such as Rad54. Future studies will determine the validity of these ideas.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Lorenz,A. and Whitby,M.C. (2006) Crossover promotion and prevention. *Biochem. Soc. Trans.*, **34**, 537–541.
2. Ira,G., Malkova,A., Liberi,G., Foiani,M. and Haber,J.E. (2003) Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell*, **115**, 401–411.
3. Sun,W., Nandi,S., Osman,F., Ahn,J.S., Jakovleska,J., Lorenz,A. and Whitby,M.C. (2008) The fission yeast FANCM ortholog Fml1 promotes recombination at stalled replication forks and limits crossing over during double-strand break repair. *Mol. Cell*, **32**, 118–128.
4. Prakash,R., Satory,D., Dray,E., Papusha,A., Scheller,J., Kramer,W., Krejci,L., Klein,H., Haber,J.E., Sung,P. *et al.* (2009) Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. *Genes Dev.*, **23**, 67–79.
5. De Piccoli,G., Cortes-Ledesma,F., Ira,G., Torres-Rosell,J., Uhle,S., Farmer,S., Hwang,J.Y., Machin,F., Ceschia,A., McAleenan,A. *et al.* (2006) Smc5-Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. *Nat. Cell Biol.*, **8**, 1032–1034.
6. Cortes-Ledesma,F. and Aguilera,A. (2006) Double-strand breaks arising by replication through a nick are repaired by cohesin-dependent sister-chromatid exchange. *EMBO Rep.*, **7**, 919–926.
7. Bergerat,A., de Massy,B., Gadelle,D., Varoutas,P.C., Nicolas,A. and Forterre,P. (1997) An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature*, **386**, 414–417.
8. Keeney,S., Giroux,C.N. and Kleckner,N. (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell*, **88**, 375–384.
9. Whitby,M.C. (2005) Making crossovers during meiosis. *Biochem. Soc. Trans.*, **33**, 1451–1455.
10. San Filippo,J., Sung,P. and Klein,H. (2008) Mechanism of Eukaryotic Homologous Recombination. *Annu. Rev. Biochem*, **77**, 229–257.
11. Sung,P. (1997) Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev.*, **11**, 1111–1121.
12. Sung,P. (1997) Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *J. Biol. Chem.*, **272**, 28194–28197.
13. New,J.H., Sugiyama,T., Zaitseva,E. and Kowalczykowski,S.C. (1998) Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature*, **391**, 407–410.
14. Shinohara,A. and Ogawa,T. (1998) Stimulation by Rad52 of yeast Rad51-mediated recombination. *Nature*, **391**, 404–407.
15. Mortensen,U.H., Bendixen,C., Sunjevaric,I. and Rothstein,R. (1996) DNA strand annealing is promoted by the yeast Rad52 protein. *Proc. Natl Acad. Sci. USA*, **93**, 10729–10734.
16. Mortensen,U.H., Erdeniz,N., Feng,Q. and Rothstein,R. (2002) A molecular genetic dissection of the evolutionarily conserved N terminus of yeast Rad52. *Genetics*, **161**, 5491562.
17. Krejci,L., Song,B., Bussen,W., Rothstein,R., Mortensen,U.H. and Sung,P. (2002) Interaction with Rad51 is indispensable for recombination mediator function of Rad52. *J. Biol. Chem.*, **277**, 40132–40141.
18. Plate,I., Hallwyl,S.C., Shi,I., Krejci,L., Muller,C., Albertsen,L., Sung,P. and Mortensen,U.H. (2008) Interaction with RPA is necessary for Rad52 repair center formation and for its mediator activity. *J. Biol. Chem.*, **283**, 29077–29085.
19. Branzei,D. and Foiani,M. (2007) RecQ helicases queuing with Srs2 to disrupt Rad51 filaments and suppress recombination. *Genes Dev.*, **21**, 3019–3026.
20. Symington,L.S. and Heyer,W.D. (2006) Some disassembly required: role of DNA translocases in the disruption of recombination intermediates and dead-end complexes. *Genes Dev.*, **20**, 2479–2486.
21. Veaute,X., Jeusset,J., Soustelle,C., Kowalczykowski,S.C., Le Cam,E. and Fabre,F. (2003) The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature*, **423**, 309–312.
22. Krejci,L., Van Komen,S., Li,Y., Villemain,J., Reddy,M.S., Klein,H., Ellenberger,T. and Sung,P. (2003) DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature*, **423**, 305–309.
23. Antony,E., Tomko,E.J., Xiao,Q., Krejci,L., Lohman,T.M. and Ellenberger,T. (2009) Srs2 disassembles Rad51 filaments by a protein-protein interaction triggering ATP turnover and dissociation of Rad51 from DNA. *Mol. Cell*, **35**, 105–115.
24. Aboussekhra,A., Chanet,R., Zgaga,Z., Cassier-Chauvat,C., Heude,M. and Fabre,F. (1989) RADH, a gene of *Saccharomyces cerevisiae* encoding a putative DNA helicase involved in DNA repair. Characteristics of radH mutants and sequence of the gene. *Nucleic Acids Res.*, **17**, 7211–7219.
25. Aguilera,A. and Klein,H.L. (1988) Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. *Genetics.*, **119**, 779–790.
26. Lawrence,C.W. and Christensen,R.B. (1979) Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of *Saccharomyces cerevisiae rad6* mutants. *J. Bacteriol.*, **139**, 866–887.
27. Papouli,E., Chen,S., Davies,A.A., Huttner,D., Krejci,L., Sung,P. and Ulrich,H.D. (2005) Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell*, **19**, 123–133.
28. Pfander,B., Moldovan,G.L., Sacher,M., Hoege,C. and Jentsch,S. (2005) SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature*, **436**, 428–433.
29. Schiestl,R.H., Prakash,S. and Prakash,L. (1990) The SRS2 suppressor of *rad6* mutations of *Saccharomyces cerevisiae* acts by channeling DNA lesions into the RAD52 DNA repair pathway. *Genetics*, **124**, 817–831.
30. Kiianitsa,K., Solinger,J.A. and Heyer,W.D. (2006) Terminal association of Rad54 protein with the Rad51-dsDNA filament. *Proc. Natl Acad. Sci. USA*, **103**, 9767–9772.
31. Li,X., Zhang,X.P., Solinger,J.A., Kiianitsa,K., Yu,X., Egelman,E.H. and Heyer,W.D. (2007) Rad51 and Rad54 ATPase activities are both required to modulate Rad51-dsDNA filament dynamics. *Nucleic Acids Res.*, **35**, 4124–4140.
32. 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.
33. Li,X. and Heyer,W.D. (2009) RAD54 controls access to the invading 3′-OH end after RAD51-mediated DNA strand invasion

in homologous recombination in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **37**, 638–646.
34. Ward,J.D., Muzzini,D.M., Petalcorin,M.I., Martinez-Perez,E., Martin,J.S., Plevani,P., Cassata,G., Marini,F. and Boulton,S.J. (2010) Overlapping mechanisms promote postsynaptic RAD-51 filament disassembly during meiotic double-strand break repair. *Mol. Cell*, **37**, 259–272.
35. Dupaigne,P., Le Breton,C., Fabre,F., Gangloff,S., Le Cam,E. and Veaute,X. (2008) The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: implications for crossover incidence during mitotic recombination. *Mol. Cell*, **29**, 243–254.
36. Palladino,F. and Klein,H.L. (1992) Analysis of mitotic and meiotic defects in *Saccharomyces cerevisiae* SRS2 DNA helicase mutants. *Genetics*, **132**, 23–37.
37. Blanck,S., Kobbe,D., Hartung,F., Fengler,K., Focke,M. and Puchta,H. (2009) A SRS2 homolog from *Arabidopsis thaliana* disrupts recombinogenic DNA intermediates and facilitates single strand annealing. *Nucleic Acids Res.*, **37**, 7163–7176.
38. Kim,J., Kim,J.H., Lee,S.H., Kim,D.H., Kang,H.Y., Bae,S.H., Pan,Z.Q. and Seo,Y.S. (2002) The novel human DNA helicase hFBH1 is an F-box protein. *J. Biol. Chem.*, **277**, 24530–24537.
39. Park,J.S., Choi,E., Lee,S.H., Lee,C. and Seo,Y.S. (1997) A DNA helicase from *Schizosaccharomyces pombe* stimulated by single-stranded DNA-binding protein at low ATP concentration. *J. Biol. Chem.*, **272**, 18910–18919.
40. Morishita,T., Furukawa,F., Sakaguchi,C., Toda,T., Carr,A.M., Iwasaki,H. and Shinagawa,H. (2005) Role of the *Schizosaccharomyces pombe* F-Box DNA helicase in processing recombination intermediates. *Mol. Cell. Biol.*, **25**, 8074–8083.
41. Fugger,K., Mistrik,M., Danielsen,J.R., Dinant,C., Falck,J., Bartek,J., Lukas,J. and Mailand,N. (2009) Human Fbh1 helicase contributes to genome maintenance via pro- and anti-recombinase activities. *J. Cell Biol.*, **186**, 655–663.
42. Lorenz,A., Osman,F., Folkyte,V., Sofueva,S. and Whitby,M.C. (2009) Fbh1 Limits Rad51-dependent Recombination at Blocked Replication Forks. *Mol. Cell Biol.*, **29**, 4742–4756.
43. Chiolo,I., Saponaro,M., Baryshnikova,A., Kim,J.H., Seo,Y.S. and Liberi,G. (2007) The human F-Box DNA helicase FBH1 faces *Saccharomyces cerevisiae* Srs2 and postreplication repair pathway roles. *Mol. Cell Biol.*, **27**, 7439–7450.
44. Osman,F., Dixon,J., Barr,A.R. and Whitby,M.C. (2005) The F-Box DNA helicase Fbh1 prevents Rhp51-dependent recombination without mediator proteins. *Mol. Cell Biol.*, **25**, 8084–8096.
45. Kohzaki,M., Hatanaka,A., Sonoda,E., Yamazoe,M., Kikuchi,K., Vu Trung,N., Szuts,D., Sale,J.E., Shinagawa,H., Watanabe,M. *et al.* (2007) Cooperative roles of vertebrate Fbh1 and Blm DNA helicases in avoidance of crossovers during recombination initiated by replication fork collapse. *Mol. Cell Biol.*, **27**, 2812–2820.
46. Sakaguchi,C., Morishita,T., Shinagawa,H. and Hishida,T. (2008) Essential and distinct roles of the F-box and helicase domains of Fbh1 in DNA damage repair. *BMC Mol. Biol.*, **9**, 27.
47. Lawrence,C.L., Jones,N. and Wilkinson,C.R. (2009) Stress-induced phosphorylation of S. pombe Atf1 abrogates its interaction with F box protein Fbh1. *Curr. Biol.*, **19**, 1907–1911.
48. Loidl,J. and Lorenz,A. (2009) Analysis of *Schizosaccharomyces pombe* meiosis by nuclear spreading. *Methods Mol. Biol.*, **558**, 15–36.
49. Forsburg,S.L. and Rhind,N. (2006) Basic methods for fission yeast. *Yeast*, **23**, 173–183.
50. Osman,F., Dixon,J., Doe,C.L. and Whitby,M.C. (2003) Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. *Mol. Cell*, **12**, 761–774.
51. Gaskell,L.J., Osman,F., Gilbert,R.J. and Whitby,M.C. (2007) Mus81 cleavage of Holliday junctions: a failsafe for processing meiotic recombination intermediates? *EMBO J.*, **26**, 1891–1901.
52. Hagan,I.M. and Hyams,J.S. (1988) The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.*, **89**, 343–357.
53. Bridge,A.J., Morphew,M., Bartlett,R. and Hagan,I.M. (1998) The fission yeast SPB component Cut12 links bipolar spindle formation to mitotic control. *Genes Dev.*, **12**, 927–942.
54. Woods,A., Sherwin,T., Sasse,R., MacRae,T.H., Baines,A.J. and Gull,K. (1989) Definition of individual components within the cytoskeleton of *Trypanosoma brucei* by a library of monoclonal antibodies. *J. Cell Sci.*, **93**, 491–500.
55. Svoboda,A., Bahler,J. and Kohli,J. (1995) Microtubule-driven nuclear movements and linear elements as meiosis- specific characteristics of the fission yeasts *Schizosaccharomyces versatilis* and *Schizosaccharomyces pombe*. *Chromosoma*, **104**, 203–214.
56. Win,T.Z., Goodwin,A., Hickson,I.D., Norbury,C.J. and Wang,S.W. (2004) Requirement for *Schizosaccharomyces pombe* Top3 in the maintenance of chromosome integrity. *J. Cell Sci.*, **117**, 4769–4778.
57. Wells,J.L., Pryce,D.W. and McFarlane,R.J. (2006) Homologous chromosome pairing in *Schizosaccharomyces pombe*. *Yeast.*, **23**, 977–989.
58. Young,J.A., Hyppa,R.W., Smith,G.R., Schreckhise,R.W. and Steiner,W.W. (2004) Conserved and nonconserved proteins for meiotic DNA breakage and repair in yeasts. *Genetics*, **167**, 593–605.
59. Cromie,G.A., Hyppa,R.W., Taylor,A.F., Zakharyevich,K., Hunter,N. and Smith,G.R. (2006) Single Holliday junctions are intermediates of meiotic recombination. *Cell*, **127**, 1167–1178.
60. Youds,J.L., Mets,D.G., McIlwraith,M.J., Martin,J.S., Ward,J.D., ONeil,N.J., Rose,A.M., West,S.C., Meyer,B.J. and Boulton,S.J. (2010) RTEL-1 enforces meiotic crossover interference and homeostasis. *Science*, **327**, 1254–1258.
61. Boddy,M.N., Gaillard,P.-H.L., McDonald,W.H., Shanahan,P., Yates 3rd,J.R. and Russell,P. (2001) Mus81-Eme1 are essential components of a Holliday junction resolvase. *Cell*, **107**, 537–548.
62. Octobre,G., Lorenz,A., Loidl,J. and Kohli,J. (2008) The Rad52 homologs Rad22 and Rti1 of *Schizosaccharomyces pombe* are not essential for meiotic interhomolog recombination, but are required for meiotic intrachromosomal recombination and mating-type-related DNA repair. *Genetics*, **178**, 2399–2412.
63. Whitby,M.C. (2010) The FANCM family of DNA helicases/translocases. *DNA Repair*, **9**, 224–236.
64. Cromie,G.A., Hyppa,R.W. and Smith,G.R. (2008) The Fission Yeast BLM Homolog Rqh1 Promotes Meiotic Recombination. *Genetics.*, **179**, 1157–1167.
65. Young,J.A., Schreckhise,R.W., Steiner,W.W. and Smith,G.R. (2002) Meiotic recombination remote from prominent DNA break sites in *S. pombe*. *Mol. Cell*, **9**, 253–263.