The Fission Yeast BLM Homolog Rqh1

Promotes Meiotic Recombination

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ABSTRACT
RecQ helicases are found in organisms as diverse as bacteria, fungi and mammals. These proteins promote genome stability, and mutations affecting human RecQ proteins underlie premature aging and cancer predisposition syndromes, including Bloom Syndrome, caused by mutations affecting the BLM protein. In this study we show that mutants lacking the Rqh1 protein of the fission yeast Schizosaccharomyces pombe, a RecQ and BLM homolog, have substantially reduced meiotic recombination, both gene conversions and crossovers. The relative proportion of gene conversions having associated crossovers is unchanged from that in wild type. In rqh1 mutants, meiotic DNA double-strand breaks are formed and disappear with wild-type frequency and kinetics, and spore viability is only moderately reduced. Genetic analysis and the wild-type frequency of both intersister and interhomolog joint molecules argue against these phenotypes being explained by an increase in intersister recombination at the expense of interhomolog recombination. We suggest that Rqh1 extends hybrid DNA and biases the recombination outcome toward crossing over. Our results contrast dramatically with those from the budding yeast ortholog, Sgs1, which has a meiotic anti-recombination function that suppresses recombination events involving more than two DNA duplexes. These observations underscore the multiple recombination functions of RecQ homologs and emphasize that even conserved proteins can be adapted to play different roles in different organisms.
INTRODUCTION

Homologous recombination allows the faithful repair of DNA double-strand breaks (DSBs) by generating a joint molecule between the broken DNA and an intact homologous duplex. In this joint molecule, the intact duplex provides a template for repair of the broken DNA. This repair can lead to gene conversion events, which may or may not be accompanied by a crossover between the two interacting duplexes. Although many of the DNA intermediates and protein effectors of homologous recombination are now known, the regulation of this process is still poorly understood.

Although homologous recombination provides a method to faithfully repair DSBs, there are dangers associated with this process. Genome rearrangements or loss of heterozygosity can occur during mitotic growth if homologous recombination generates crossovers. This danger is lessened if recombination is directed to preferentially avoid crossovers. However, the danger can also be avoided by the use of alternative DNA repair mechanisms, instead of homologous recombination. In contrast to mitotic growth, during meiotic recombination DSBs are actively generated by the cell, using the Rec12 (Spo11) protein, precisely in order to produce crossovers. Therefore, depending on the situation, it may be advantageous for cells to promote or discourage the use of homologous recombination over other DNA repair mechanisms or to promote certain outcomes of homologous recombination over others (e.g. crossovers vs. non-crossovers).

Recent work has suggested that RecQ helicases are critical to understanding the interplay of pro- and anti-recombination factors in DSB repair. RecQ helicases are a conserved family of proteins that unwind DNA with a $3' \rightarrow 5'$ polarity and are found in organisms as diverse as mammals, fungi, and bacteria (reviewed by COBB and BJERGBAEK 2006). Mutations affecting RecQ proteins often lead to genome instability and associated diseases, such as cancer. In humans, there are five RecQ helicases, mutations affecting three of which, the BLM, WRN, and RECQL4 proteins, are associated with genetic instability and cancer predisposition (e.g. PURANAM and BLACKSHEAR 1994; ELLIS et al. 1995; YU et al. 1996; KITAO et al., 1999; reviewed by HANADA and HICKSON 2007).

There are several lines of evidence indicating that members of the RecQ family have an anti-recombination function, particularly in the suppression of “aberrant” recombination events. For instance, patients with Bloom Syndrome (caused by mutations in \textit{BLM}) have a greatly elevated frequency of chromosome rearrangements and breaks, which in turn can explain their predisposition to developing cancer (e.g. CHAGANTI et al. 1974; ELLIS et al. 1995; reviewed by HANADA and HICKSON 2007). Sgs1 and Rqh1 are BLM homologs found in budding and fission yeast, respectively.
All of these proteins have a domain structure consisting of highly acidic regions, a helicase domain, a helicase C-terminal domain, and an HRDC (helicase RNase D C-terminus) domain. Mutations affecting these proteins are also associated with elevated levels of homologous recombination during mitotic growth (WATT et al. 1996; STEWART et al. 1997). In addition, double mutants with sgs1 (or rqh1) and srs2, encoding another 3’ → 5’ helicase, have very low viability. This last phenotype is suppressed by mutations altering the Rad51 strand-exchange protein, suggesting that the inviability of the double sgs1 srs2 and rqh1 srs2 mutants is caused by the accumulation of toxic recombination intermediates (GANGLOFF et al. 2000; MAFTAHI et al. 2002; LIBERI et al. 2005).

The Srs2 helicase is structurally related to the UvrD helicase of bacteria, rather than to the RecQ family. This protein is found in both budding and fission yeast, but no mammalian homologs have been identified (LAWRENCE and CHRISTENSEN 1979; WANG et al. 2001). The function of Srs2 often seems to be closely linked to that of Sgs1 or Rqh1. Both Sgs1 (Rqh1) and Srs2 proteins have an anti-recombination function during mitotic growth of budding and fission yeast (RONG et al. 1991; WATT et al. 1996; STEWART et al. 1997; WANG et al. 2001; DOE and WHITBY 2004; J. VIRGIN, personal communication). In addition, both Sgs1 (Rqh1) and budding yeast Srs2 promote non-crossover outcomes over crossover outcomes during mitotic recombination, implicating them in this aspect of recombination regulation (IRA et al. 2003; HOPE et al. 2007; J. VIRGIN, personal communication). The elevated-crossover phenotype of either mutant can be suppressed by over-expression of the other protein, and the sensitivity to DNA damaging agents of srs2 mutants can be suppressed by over-expression of SGS1 (MANKOURI et al. 2002; IRA et al. 2003).

The anti-recombination function of RecQ proteins (and Srs2) is not fully understood. However, one possibility is that their helicase action reverses or destabilizes recombination intermediates, such as Holliday junctions (HJs). This reversal would prevent HJs being resolved to give crossovers and hence would reduce crossover recombination frequencies and the proportion of crossover to non-crossover outcomes of recombination. Consistent with this model, mutations affecting both Sgs1 (Rqh1) and the Mus81 HJ resolving protein are synthetically lethal (BODDY et al. 2000; MULLEN et al. 2001), and over-expression of a bacterial HJ resolvase can partially suppress some rqh1 mutant phenotypes, such as sensitivity to DNA damaging agents (DOE et al. 2000). In further support of this view, the helicase activity of the BLM protein, in conjunction with topoisomerase IIIα and the Bloom Syndrome complex protein BLAP75, can disassemble double HJs in vitro (WU et al. 2006). The Sgs1 protein, without accessory factors, can also unwind simple HJ structures in vitro (BENNETT et al. 1999). However, mutations affecting budding yeast Top3, the
to topoisomerase IIIα ortholog, have phenotypes similar to those of sgs1 mutants, supporting a DNA processing pathway involving both proteins rather than Sgs1 alone (IRA et al. 2003; LIBERI et al. 2005). In fission yeast, Rqh1 also interacts with the Top3 ortholog (AHMAD and STEWART 2005).

At least in budding yeast, RecQ proteins also have an anti-recombination function during meiosis. Budding yeast sgs1 mutants show mildly elevated frequencies of meiotic crossovers, apparently due to the formation of recombination intermediates involving more than two homologous duplexes, a unique phenotype (ROCKMILL et al. 2003; JESSOP et al. 2006; OH et al. 2007). This anti-recombination activity of Sgs1 appears to be antagonized by a pro-recombination activity of the ZMM group of proteins (JESSOP et al. 2006; OH et al. 2007), which appear to be specifically required for the formation of one subset of meiotic crossovers in budding yeast (reviewed by CROMIE and SMITH 2007; LYNN et al. 2007). The recombination-deficiency of several zmm mutants is at least partially suppressed by a further mutation in SGS1 (JESSOP et al. 2006; OH et al. 2007). Mutation of budding yeast SRS2 reduces meiotic spore viability, but this phenotype is not suppressed by additional mutations in SPO13 and MEI4, which bypass meiosis I and DSB formation and repair (PALLADINO and KLEIN 1992). This result suggests that budding yeast srs2 mutants have a meiotic defect unrelated to meiotic recombination.

We set out to investigate the roles of Rqh1 and Srs2 in fission yeast meiosis. Despite the similar mitotic roles of fission yeast Rqh1 and budding yeast Sgs1, it seemed plausible that the meiotic roles of these proteins would be distinct, because fission yeast lacks the ZMM proteins. In fact, we observe that rqh1 mutants are recombination-deficient, in complete contrast to the situation in budding yeast, where sgs1 mutants have elevated recombination frequencies. We see little effect of the srs2 mutation on recombination. The recombination-deficiency of the rqh1 mutant is not explained by a failure to generate or repair DSBs, and spore viability in this genetic background is only moderately reduced. Genetic and physical assays argue against the simplest explanation for these phenotypes: that DSBs are repaired more frequently against sister chromatids, rather than homologous chromosomes, in an rqh1 mutant. We suggest that, for meiotic DSB repair, Rqh1 is required to extend hybrid DNA and to bias the outcome toward crossing over.

**MATERIALS AND METHODS**

**Yeast strains and genetic techniques:** The *S. pombe* strains used in this study, and their genotypes, are listed in Table 1. Meiotic crosses were carried out by suspending single yeast colonies in 5 ml of supplemented yeast extract liquid medium, [YEL + adenine (100 µg/ml); SMITH 2008], and
growing at 30° until saturated. For each cross, aliquots of 100 µl from two saturated cultures were mixed, and the cells were washed twice with water and spotted on sporulation agar plates (SPA; SMITH 2008) supplemented with any required amino acids, purines, and pyrimidines. After two days of incubation at 25° the cell-ascus mixture from each spot was suspended in 1 ml of water and treated with glusulase and ethanol to kill vegetative cells, essentially as described by DEVEAUX et al. (1992).

For measurement of viable spore yield, crosses were carried out as above, and the titers of the two cultures used for the meiotic cross were measured at the time of crossing by assay on appropriately supplemented YEA plates. The titer of viable spores was measured in the final spore suspension. The viable spore yield was calculated as the number of viable spores per viable cell in the mated culture with the lower viable cell titer (ELLERMEIER et al. 2004).

For measurement of spore viability, spore suspensions were prepared essentially as described above, but with spore release by autolysis (i.e. without glusulase or ethanol treatment). The cell-ascus-spore suspension was spotted onto YEA-5S (YEA + adenine, uracil, lysine, histidine, and leucine) plates, and individual spores were micro-manipulated under a microscope onto a grid on another part of the plate. Plates were incubated at 32° for four days, and the proportion of spores that had formed visible colonies was calculated.

Auxotrophic markers were scored by transferring well-isolated yeast colonies to appropriately supplemented YEA plates, followed by replica-plating onto appropriately supplemented nitrogen-base minimal agar (NBA; SMITH 2008). Replica plating onto YEA + phloxin B plates (MORENO et al. 1991) at 37° was used to score tps16.

Measurement of gene conversion at ade6 and associated crossovers: Spores with gene conversion at the ade6 or ura1 loci were selected as intragenic recombinants (prototrophs) in crosses between different ade6 and ura1 point mutants (see SMITH 2008 and RESULTS section for rationale). Measurement of total viable spores and prototrophic spores, per unit volume of spore suspension, allowed calculation of gene conversion frequencies. Crossovers of flanking markers (ura4A+ and tps16) accompanying gene conversion at ade6 were measured among gene convertant spores, selected as above.

Determination of sister-chromatid recombination frequencies: Combined intrachromatid and unequal intersister-chromatid exchange frequencies were determined as follows. Appropriately diluted mitotic cultures of the ade6-Dup-containing strain were plated on YEA + adenine to determine the total number of viable cells and on YEA + guanine to determine the frequency of mitotic Ade+ recombinants (SCHUCHERT and KOHLI 1988; DAVIS and SMITH 2006). The ade6-Dup strain and the
appropriate ade6-D19 (complete ade6 deletion) strain were then mated on supplemented SPA. Spores were harvested, and spore suspensions were plated on YEA + adenine to determine the total frequency of viable cells and on YEA + guanine to determine the frequency of Ade+ recombinants. The mitotic frequency was subtracted from the meiotic frequency to give the final meiotic intra- and intersister recombinant frequency. Four crosses were performed for each genotype, and the statistical significance was calculated using Student’s t-test.

Analysis of meiotic DNA breaks: Meiotic inductions, flow cytometry, and preparation of DNA in agarose plugs were performed as described by Young et al. (2002). The agarose-embedded DNA was digested with restriction enzymes, separated by gel electrophoresis, Southern blotted, and hybridized with probe c139 (Young et al. 2002).

Detection and quantitation of crossover molecules and intersister and interhomolog joint molecules: Digestion of DNA in agarose plugs, separation by electrophoresis on one- or two-dimensional gels, Southern blotting, probing, and quantitation were carried out as described by Cromie et al. (2006) and Hyppa and Smith (2008).

RESULTS

rec9-109 is an allele of rqh1, with a mutation in the helicase C-terminal domain: The rec9-109 mutation reduces the frequencies of meiotic gene conversion (measured as ade6 intragenic recombination; see below and Gutz 1971; Smith 2008) and crossing over (measured as intergenic recombination; P. Munz, pers. comm., cited in Young et al. 2002; Smith 2008) approximately 5-fold (Ponticelli and Smith 1989; Deveaux et al. 1992). The rec9-109 allele is linked to ura1 and fails to complement the meiotic recombination defect of rqh1 (also known as hus2) mutants, identifying rec9-109 as an allele of rqh1 (Davis and Smith 2001; J. Young and G. Smith, unpublished data). The rec9-109 allele contains a missense mutation (G→A at position 2264 in the coding sequence) leading to the change Cys→Tyr at residue 755 of the protein, in the helicase C-terminal domain (C. Ellermeier and G. Smith, unpublished data).

Gene conversion and crossover frequencies are substantially reduced in an rqh1 mutant but not in an srs2 mutant: Meiotic recombination phenotypes of rqh1 mutants have been characterized only for the rec9-109 allele, which may affect only one of the domains of the multi-domain Rqh1 protein. Therefore, we began our investigation of the meiotic roles of Rqh1 by examining the effect of an rqh1 null mutant (a complete deletion of the rqh1 coding sequence) on meiotic recombination frequency. In addition, we examined the effect of an srs2 null mutation
because of the known interaction between Rqh1 (Sgs1) and Srs2 in mitotic growth and the role of both Rqh1 (Sgs1) and Srs2 proteins in promoting mitotic non-crossover vs. crossover events (Gangloff et al. 2000; Maftahi et al. 2002; IRA et al. 2003; DOE and Whitby 2004; Liberi et al. 2005; Hope et al. 2007; J. Virgin, personal communication).

Meiotic crossover frequencies were measured for three intervals, the $\text{ura}4A^+ – \text{tps}16$ and $\text{tps}16 – \text{arg}1$ intervals of chromosome III ($\text{ura}4A^+$ is also known as $\text{ura}4^+\text{-aim}$) and the $\text{lys}3 – \text{ural}$ interval of chromosome I. In each case, crossover frequencies (cM) were reduced to 10 – 20% of wt ($\text{rqh}1^+$) levels in the $\text{rqh}1$ mutant and to 60 – 80% of wt levels in the $\text{srs}2$ mutant (Figure 1A). The differences between wt and $\text{rqh}1\Delta$ recombination frequencies were significant ($p < 0.0001$ in all cases; Fisher’s exact test), but those between wt and $\text{srs}2\Delta$ were significant only for the $\text{ura}4A^+ – \text{tps}16$ interval ($p < 0.01$; Fisher’s exact test). Gene conversion frequencies were measured as intragenic recombination frequencies at two loci: $\text{ural}$ on chromosome I and $\text{ade}6$ on chromosome III. For intragenic markers spanning most of $\text{ade}6$, all of 123 recombinants tested arose from gene conversion; none arose from crossing over (Gutz 1971). In the $\text{rqh}1$ mutant, gene conversion frequencies were significantly reduced to 10 – 20% of wt levels ($p < 0.01$ in both cases; Student’s t-test, 2 tailed), similar to the reduction in crossover frequencies. In the $\text{srs}2$ mutant, effects on $\text{ural}$ and $\text{ade}6$ gene conversion frequencies were modest and of marginal significance ($p = 0.028$ and $p = 0.085$ respectively; Student’s t-test, 2 tailed), and no consistent direction of effect was observed (Figure 1B). The $\text{rqh}1$ mutant values are similar to those observed in both inter- and intragenic crosses with $\text{rec}9-109$ (Ponticelli and Smith 1989; Deveaux et al. 1992), suggesting that $\text{rec}9-109$ is a null mutation with respect to its effect on meiotic crossing over and gene conversion.

Possible explanations for the recombination deficiency of $\text{rqh}1$ mutants: Crossovers occur when meiotic DSBs are repaired against homologous chromosomes, with appropriate resolution of the Holliday junction(s) in the DNA intermediates. The reduction in crossover frequencies seen in the $\text{rqh}1$ mutant could therefore have several explanations. DSB frequency could be reduced, or a proportion of DSBs could remain unrepaired. Repair could occur between identical sister chromatids, rather than homologous chromosomes, a process that cannot produce genetic crossovers. Alternatively, repair could still occur against the homologous chromosome but by mechanisms such as synthesis-dependent strand annealing (SDSA) that avoid Holliday junctions and produce only non-crossovers. We set out to test these possibilities.

The relative frequencies of crossover and non-crossover recombination are not altered in $\text{rqh}1$ or $\text{srs}2$ mutants: One of the mitotic phenotypes of both $\text{rqh}1$ and $\text{srs}2$ mutants is an increase in
crossover vs. non-crossover outcomes to recombination (IRA et al. 2003; HOPE et al. 2007; J. VIRGIN, personal communication). As discussed above, if the rqh1 mutant had the opposite effect in meiosis, this would explain the reduced levels of crossovers seen in this background. Therefore, we examined the effect of the rqh1 and srs2 null mutants on the relative frequencies of these two outcomes during meiotic recombination.

When a DSB is repaired by recombination, gene conversion can occur with or without an associated crossover. The proportion of gene conversions at a particular site that have an associated crossover therefore gives an estimate of the proportion of all recombination events at that site that give rise to crossovers (but see DISCUSSION). In wt meiotic crosses, when a gene conversion occurs at ade6, approximately 65% of the time it is accompanied by a crossover between the flanking markers ura4A+ and tps16 (GRIMM et al. 1994; CROMIE et al. 2005). There is no significant change in this frequency in either the srs2 or rqh1 mutant backgrounds (p = 0.34 and p = 0.62 respectively; Fisher’s exact test; Figure 2). Therefore, neither Rqh1 nor Srs2 appears to regulate the frequency of crossover vs. non-crossover recombination during meiosis. This conclusion is further supported by the observation that, in both the rqh1 and srs2 mutant backgrounds, gene conversion frequencies are affected to a similar extent as crossover frequencies.

**Spore viability is only moderately reduced in an rqh1 mutant:** Defects in meiotic DSB formation or repair are expected to result in reduced spore viability, with DSB-repair mutants showing the greater impairment (reviewed in CROMIE and SMITH 2008). To help identify the nature of their respective recombination defects, we tested the effect of the rqh1 and srs2 null mutations on spore viability. The srs2 mutation had essentially no effect (p = 0.84; Fisher’s exact test), consistent with its mild effect on meiotic recombination. The rqh1 mutation had a more pronounced, and significant, defect, with spore viability reduced to approximately 50% of wt levels (p < 0.0001; Fisher’s exact test). However, this effect is considerably weaker than that in mutants, such as rec12 (p < 0.0001; Fisher’s exact test), that fail to form breaks (Figure 3; DAVIS and SMITH 2003) and may be exaggerated by the mild mitotic growth defect of rqh1Δ cells (STEWART et al. 1997; unpublished data). The moderate spore viability defect of the rqh1 mutant is consistent with a chromosome segregation problem, expected due to the reduced levels of crossing over seen in the mutant (Figure 1A).

Viable spore yield, measured as the number of viable spores produced per cell in the mating mixture, was also reduced to ~50% of wt levels in the rqh1 mutant, similar to the effect on spore
viability (Figure 3). This similarity suggests that the rqh1 mutant does not have a substantial defect in mating or sporulation.

**Meiotic DSBs are formed at normal frequencies and repaired with normal kinetics in the rqh1 null mutant:** Mutants that fail to repair meiotic DSBs, such as those affecting the MRN complex, have a lower spore viability than those, such as rec12 mutants, that do not form breaks at all (TAVASSOLI et al. 1995; DAVIS and SMITH 2003; ELLERMEIER and SMITH 2005). As the rqh1 mutant had a higher spore viability than that of the rec12 mutant, this suggests that the reduced spore viability seen in the rqh1 mutant is not caused by a failure to make or repair meiotic DSBs. Rather, it is consistent with the slight chromosome segregation defect expected due to reduced crossover frequencies in the rqh1 mutant background.

This idea was tested directly by examining the formation and repair of meiotic DSBs in wt and rqh1 mutant cultures induced to undergo meiosis. Meiotic DSBs appeared and disappeared at the same times and at approximately the same frequencies in both genetic backgrounds (Figure 4). The cumulative frequency of meiotic DSBs in the rqh1 mutant could not be measured because the rad50S allele, often used to accumulate meiotic DSBs (e.g. YOUNG et al. 2002), leads to poor viability and slow growth of cultures when combined with the rqh1 null mutation (unpublished data). Nevertheless, the rad50+ results indicate that DSBs are formed and repaired in the rqh1 null mutant at a similar frequency as in wt. As expected from the mild phenotypes reported above, breaks are also formed and repaired normally in the srs2 mutant (unpublished data).

**Intersister recombination is not elevated in an rqh1 mutant:** The simplest explanation for the phenotypes of the rqh1 null mutant described above is that, in the mutant, there is a specific defect in repairing DSBs using the homologous chromosome, and instead breaks are repaired using other homologous targets, such as the sister chromatid. Intersister recombination would explain the high spore viability and repair of meiotic DSBs without leading to detectable crossovers and gene conversions. This is consistent with mitotic studies, where rqh1 mutants show elevated levels of intersister recombination (HOPE et al. 2006).

Two assays were used to test this hypothesis. First, we used a genetic assay based on recombination between duplicated DNA sequences to measure the frequency of intersister (or intra-chromosomal) recombination, i.e. non-interhomolog recombination, in wt and rqh1 mutant backgrounds (Figure 5). In wt this assay produces about equal numbers of recombinants through unequal exchange (intersister) and “popout” (intrasister) events (SCHUCHERT and KOHLI 1988). No
elevation of Ade\textsuperscript{+} recombinant frequency was seen by this assay in the \textit{rqh1} mutant compared to \textit{wt}. The observed reduction was not statistically significant (p = 0.36; Student’s t-test; Figure 5).

A second, physical assay was used to examine directly the effect of the \textit{rqh1} null mutation on the relative frequency of recombination intermediates involving sister chromatids vs. homologous chromosomes. Diploids with heterozygous restriction sites flanking the \textit{mbs1} DSB and recombination hotspot (CROMIE \textit{et al.} 2005; CROMIE \textit{et al.} 2006) allow intersister and interhomolog recombination intermediates at this site to be detected and distinguished from one another. This is done by isolation of DNA from meiotically induced diploids after DSB formation, followed by restriction enzyme digestion, resolution on 2-D gels, and probing for \textit{mbs1} (CROMIE \textit{et al.} 2006; HYPPA and SMITH 2008). The 2-D gels separate DNA based mostly on molecular mass in the first dimension and on both molecular mass and shape in the second dimension. Parental DNA and intersister and interhomolog joint molecules run at distinctive positions on these gels (Figures 6A and B). In \textit{wt}, recombination-derived joint molecules are maximal at \textasciitilde4.5 hr after meiotic induction, after which they are resolved (CROMIE \textit{et al.} 2006). The frequencies of intersister and interhomolog joint molecules, measured 4.5 hr after meiotic induction, were similar in \textit{wt} and the \textit{rqh1} mutant (Figure 6C). In particular, there was no substantial increase in intersister species at the expense of interhomolog joint molecules.

The crossover products of recombination can be measured physically at \textit{mbs1} using the same restriction site markers as above (Figures 6A and D; CROMIE \textit{et al.} 2006). In contrast to joint molecules, the final level of crossovers at \textit{mbs1} was reduced substantially in the \textit{rqh1} mutant, compared to \textit{wt} (Figure 6D), consistent with the genetic analyses of crossover frequencies in other regions (Figure 1). Therefore, it appears that the reduction in meiotic crossover frequencies seen in an \textit{rqh1} mutant is not explained by a change in partner choice, with more intersister events in the \textit{rqh1} mutant.

**DISCUSSION**

In this study we examined the effect on meiotic recombination of null mutations affecting the helicases Rqh1 and Srs2. We observed that the \textit{srs2} mutation has at most a mild effect on recombination. In contrast, the \textit{rqh1} mutation substantially impaired both gene conversion and crossing over (Figure 1), whereas mutations in the corresponding gene in budding yeast, \textit{SGS1}, slightly increase meiotic recombination frequencies (ROCKMILL \textit{et al.} 2003; JESSOP \textit{et al.} 2006; OH \textit{et al.} 2007). Despite the substantial recombination defect in the \textit{rqh1} mutant, the maximal level of meiotic DSBs is not reduced, and the breaks are successfully repaired (Figure 4). Spore viability is
only moderately reduced in the \textit{rqh1} mutant (Figure 3), consistent with a chromosome segregation defect caused simply by the reduced level of meiotic crossovers. Both a physical assay (Figure 6) and a genetic assay for non-interhomolog recombination (Figure 5) argue against one possible explanation for these phenotypes, that meiotic DSB repair is redirected from interhomolog to intersister recombination in the \textit{rqh1} mutant. Although the molecular basis of the meiotic recombination-defect in \textit{rqh1} mutants remains undetermined, below we suggest one possibility, that Rqh1 extends hybrid DNA in joint DNA molecules and favors their resolution to crossovers rather than non-crossovers. What is clear, however, is that Rqh1 in fission yeast plays a different role in meiotic recombination than does Sgs1 in budding yeast. Below, we discuss the implications of these findings.

**Role of Rqh1 in meiotic recombination in fission yeast:** Starting with the observation that meiotic crossover frequencies were reduced in an \textit{rqh1} mutant, we set out to identify the meiotic role of the Rqh1 protein using a range of recombination and repair assays. The results of these assays argued against any of the possible roles for Rqh1 that we tested directly. This leaves two possibilities, either Rqh1 has a role different from any we have tested or our assays do not properly test one of these roles for Rqh1.

We think our gene conversion, crossover, and spore viability assays are unlikely to be misleading. Therefore, we are confident that gene conversion and crossover levels are reduced in the \textit{rqh1} mutant, and that this phenotype cannot be explained by a failure to repair meiotic DSBs.

Based on physical analysis of meiotic DNA, we also conclude that the frequency of meiotic DSB formation and the frequency of intersister and interhomolog joint molecule formation are not changed in the \textit{rqh1} mutant. However, these conclusions are based on measuring the levels of transient intermediates, so that comparisons of frequencies in \textit{rqh1}\textsuperscript{+} and \textit{rqh1}\textsuperscript{Δ} could be misleading, but only if recombination events occurring in the two genetic backgrounds have fundamentally different properties. Specifically, if the frequency of DSBs is reduced in the \textit{rqh1} mutant, their life-span must be increased to obtain approximately the same transient level as that seen in \textit{rqh1}\textsuperscript{+}. Similarly, if the \textit{rqh1} mutant increases the formation of intersister joint molecules at the expense of interhomolog joint molecules, these effects must both be counteracted by changes in life-spans. We prefer the simpler interpretation that DSBs and interhomolog joint molecules form at normal frequency in the \textit{rqh1} mutant.

The line of argument presented above suggests that the \textit{rqh1} mutation causes a defect in a step in the normal recombination pathway that occurs after interhomolog joint molecule formation. We can envisage two possibilities that are consistent with, although not directly tested by, our data.
First, Rqh1 could be required to stabilize interhomolog joint molecules, perhaps through extension of regions of hybrid DNA using its helicase activity. In the absence of Rqh1 most of these interhomolog joint molecules would fall apart. Final repair might then occur, in a second step, against the sister chromatid. This step would be unique to the \textit{rqh1} mutant. These putative secondary events might not be detected by our 2-D gel assay if, for example, they arise via a synthesis-dependent strand annealing (SDSA) pathway, whose characteristic physical intermediates are not detectable by current methods. In this scheme, a minority of interhomolog joint molecules in the \textit{rqh1} mutant would progress to give crossovers with a normal frequency of association with gene conversion (Figure 2).

Second, Rqh1 could be required to promote interhomolog crossovers vs. non-crossovers. This would predict that gene conversion frequencies would be unaffected in an \textit{rqh1} mutant while the association of crossovers with gene conversion would be reduced, in both cases contrary to our observations. However, gene conversions occur only in regions of heteroduplex DNA. Therefore, Rqh1 promotion of interhomolog crossovers rather than non-crossovers could be reconciled with our data if the additional non-crossovers in the \textit{rqh1} mutant are associated with very short regions of hybrid DNA. Again, this would suggest the role of Rqh1 is to extend regions of hybrid DNA, using its helicase activity. In this scheme, a minority of interhomolog joint molecules in the \textit{rqh1} mutant would still progress to give extensive heteroduplex DNA, with normal levels of gene conversion and associated crossovers.

**Differences between the phenotypes of \textit{rqh1} and \textit{sgs1} mutants:** Why are the meiotic phenotypes of fission and budding yeast mutants lacking RecQ homologs so different? Budding yeast Sgs1 and fission yeast Rqh1, in common with human BLM, have the same domain structure, and \textit{sgs1} and \textit{rqh1} mutants share many mitotic phenotypes, including sensitivity to DNA damaging agents, synthetic lethality with \textit{srs2} or \textit{mus81} mutations, and increased frequencies of mitotic recombination (Stewart et al. 1997; Boddy et al. 2000; Gangloff et al. 2000; Mullen et al. 2000; Mullen et al. 2001; Maftah et al. 2002). The amino acid sequences of the two proteins are 29% identical. However, during meiosis, Rqh1 appears to have a pro-recombination role while Sgs1 has an anti-recombination role.

Recent evidence indicates that an important meiotic role for Sgs1 is an interaction with the ZMM group of proteins whose pro-recombination activity antagonizes an anti-recombination activity of Sgs1 (Jessop et al. 2006; Oh et al. 2007). The ZMM proteins are specifically required for the formation of the class of budding yeast crossovers that are subject to interference, the process by which one crossover discourages the occurrence of further crossovers nearby (reviewed by Cromie...
and SMITH 2007; LYNN et al. 2007). zmm mutants are substantially recombination-defective, but this effect is largely suppressed by a further mutation affecting SGS1 (JESSOP et al. 2006; OH et al. 2007).

The ZMM proteins do not seem to be present in fission yeast, which also lacks the class of crossovers that are subject to interference (reviewed by CROMIE and SMITH 2007). Therefore, it is perhaps not surprising that rqh1 mutants lack those phenotypes of sgs1 mutants that are associated with the interplay between Sgs1 and the ZMM proteins. However, it is more surprising that Rqh1 appears to have an additional and completely different role in fission yeast meiosis, the promotion of homologous recombination.

These observations imply that Sgs1 has a meiotic role that Rqh1 lacks and vice versa. The budding yeast-specific role of Sgs1 appears to be related to features of that organism (the ZMM proteins and crossover interference) that are absent from fission yeast meiosis. It is tempting to speculate that the meiotic role of Rqh1 is similarly related to a fission yeast-specific process. One possibility is that Rqh1 operates on fission yeast-specific DNA intermediates. This is consistent with the recent observation that the majority of joint molecules in fission yeast meiosis contain single Holliday junctions, rather than the predominant double Holliday junctions found in budding yeast (CROMIE et al. 2006). Perhaps Rqh1 and Sgs1 carry out the same mitotic function, but, because the meiotic function of Sgs1 is unnecessary in fission yeast, Rqh1 has been recruited to process fission yeast meiotic recombination intermediates that do not have equivalents in budding yeast.

We thank Greg Freyer for S. pombe strains; Jeff Virgin, Jennifer Young, and Chad Ellermeier for unpublished results on rqh1 mutants; and Susan Amundsen, Luther Davis, and Jennifer Geelhood for critical reading of the manuscript. This work was supported by research grants GM031693 and GM032194 from the National Institutes of Health to G. R. S.

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**TABLE 1**

*S. pombe* strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>GP2</td>
<td>h&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>GP19</td>
<td>h&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>GP64</td>
<td>h&lt;sup&gt;+&lt;/sup&gt; pat1-114</td>
</tr>
<tr>
<td>GP65</td>
<td>h&lt;sup&gt;-&lt;/sup&gt; pat1-114</td>
</tr>
<tr>
<td>GP747</td>
<td>h&lt;sup&gt;-&lt;/sup&gt; ura1-171</td>
</tr>
<tr>
<td>GP850</td>
<td>h&lt;sup&gt;+&lt;/sup&gt; lys3-37 ura1-61</td>
</tr>
<tr>
<td>GP1040</td>
<td>h&lt;sup&gt;+&lt;/sup&gt; ura4-D18 ade6-52 ura4A&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>GP5086</td>
<td>h&lt;sup&gt;+&lt;/sup&gt; h&lt;sup&gt;-&lt;/sup&gt; ura1-61/ h&lt;sup&gt;-&lt;/sup&gt; pat1-114/ ade6-210/ ade6-216</td>
</tr>
<tr>
<td>GP5263</td>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>h&lt;sup&gt;-&lt;/sup&gt; srs2::kan&lt;sup&gt;R&lt;/sup&gt;</td>
</tr>
<tr>
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<td>h&lt;sup&gt;-&lt;/sup&gt; srs2::kan&lt;sup&gt;R&lt;/sup&gt;</td>
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<tr>
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<tr>
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<tr>
<td>GP5869</td>
<td>h&lt;sup&gt;-&lt;/sup&gt; ade6-D19 leu1-32 rec12-152::LEU2</td>
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</table>
Mutations other than commonly used auxotrophies and mating-type alleles are described in the following references: *ade6-Dup*(M26-ura4\(^+\)-469) (SCHUCHERT and KOHLI 1988), *mbsl* alleles (CROMIE et al. 2006), *pat1-114* (INO and YAMAMOTO 1985), *rqh1::kan\(^R\)* and *srs2::kan\(^R\)* (MAFTAHI et al. 2002), *tps16-23* (GYGAX and THURIAUX 1984), *ura4\(^+\)-aim* (ZAHN-ZABAL et al. 1995).

*tps16* mutations map to the *agsl* gene (F. Hochstenbach, personal communication).
FIGURE 1.—srs2 and rqh1 null mutants have reduced crossover and gene conversion frequencies. (A) Crossover frequencies were measured in three intergenic intervals among the products of meiotic crosses and converted to cM by the method of Haldane (1919). Data represent combined numbers from at least two independent experiments (537 - 600 spore colonies, total, for each cross), normalized to the wt genetic distance. Error bars indicate 95% binomial proportion confidence intervals calculated by the Wilson score interval method (Wilson 1927). The strains used for the lys3 – ura1 measurements were: GP2 and GP850 (wt), GP5348 and GP5352 (srs2Δ), GP5350 and GP5356 (rqh1Δ). The strains used for both the ura4A+ – tps16 and the tps16 – arg1 measurements were: GP1040 and GP5495 (wt), GP5494 and GP5559 (srs2Δ), GP5493 and GP5557 (rqh1Δ). The wt genetic distances for lys3 – ura1, ura4A+ – tps16, and tps16 – arg1 were 25, 17, and 55 cM, respectively. (B) Gene conversion frequencies at ade6 and ura1 were measured as the frequency of prototrophs in meiotic crosses involving point mutations of each gene (ade6-52 x ade6-M375 and ura1-61 x ura1-171). Data are the average of three independent experiments, normalized to the prototroph frequency observed in the wt crosses. Error bars indicate SEM (n = 3). The strains used in crosses for the ade6 measurements were: GP1040 and GP5495 (wt), GP5494 and GP5559 (srs2Δ), GP5493 and GP5557 (rqh1Δ). The strains used in crosses for the ura1 measurements were: GP850 and GP747 (wt), GP5348 and GP5354 (srs2Δ), GP5350 and GP5357 (rqh1Δ). The wt prototroph frequencies, per viable spore, for ura1 and ade6 were 0.00020 and 0.0033, respectively.

FIGURE 2.—The srs2 and rqh1 null mutants have no effect on the frequency with which gene conversions at ade6 are accompanied by crossovers between flanking markers. Gene convertants were selected as prototrophs arising in meiotic crosses involving the ade6-52 and ade6-M375 point mutations. Among these gene convertants (589 - 605 spore colonies, total, for each cross), the percentage of spores displaying crossovers between the markers ura4A+ and tps16, flanking ade6, was measured. Percentages represent combined data from two independent experiments. Error bars indicate 95% binomial proportion confidence intervals calculated by the Wilson score interval method. The strains used for the crosses were: GP1040 and GP5495 (wt), GP5494 and GP5559 (srs2Δ), GP5493 and GP5557 (rqh1Δ).

FIGURE 3.—The rqh1 null mutation has moderately reduced spore viability and viable spore yield, while there is no significant effect of the srs2 null mutation on spore viability. Left: spore viability was measured as the percentage of spores, relative to wt, successfully germinating into a visible colony after gridding by micromanipulation on YEA-5S plates. The wt spore viability was 78%. From each of two independent crosses 152 spores were micro-manipulated for each genotype.
Error bars indicate 95% binomial proportion confidence intervals calculated by the Wilson score interval method using the combined data. Crosses were GP2 x GP19 (wt), GP5352 x GP5353 (srs2Δ), GP5355 x GP5356 (rqh1Δ) and GP5868 x GP5869 (rec12Δ). Right: viable spore yield was measured as the number of viable spores produced per number of cells of the less numerous parent in the cross. The strains used were GP2 x GP19 (wt) and GP5355 x GP5356 (rqh1Δ). Data, expressed as a percentage of rqh1+, which produced 7.2 spores per less numerous cell, are the mean of at least four independent experiments ± SEM.

**FIGURE 4.**—DSB formation and repair are normal in the rqh1 null mutant. (A) Southern blots of NotI fragment J on chromosome I, probed from the right side, showing formation and repair of DSBs at the mbs1 and mbs2 meiotic DSB hotspots in the wt and rqh1Δ backgrounds. Synchronous meiosis was induced by temperature-shifting pat1-114 cultures, and DNA, isolated at the times indicated, was analyzed as described by YOUNG et al. (2002). The strains used were GP65 (wt) and GP5263 (rqh1Δ). The asterisk indicates an additional break site introduced by the kanR substitution at the rqh1 locus ~70 kb from mbs1. The markers are MidRange I PFG (right) and Lambda Ladder PFG Marker (left) (New England Biolabs). (B) DSB frequencies at the mbs1 and mbs2 hotspot sites, from DNA samples harvested four hr after meiotic induction (when breaks are close to maximal) and probed as in Figure 4A. Quantitation was done using a phosphorimager. For each genetic background, values are from one h+ and one h− induction, each assayed on multiple Southern blots. The mean ± SEM is shown; n = 5 for wt and 7 for rqh1Δ. The strains used were: GP65 and GP64 (wt), GP5263 and GP5489 (rqh1Δ).

**FIGURE 5.**—Frequency of meiotic recombination between tandemly duplicated copies of the ade6 gene, one marked with M26 and the other with 469, to give Ade+ spores. In this assay, recombination cannot use the homologous chromosome as a template, since ade6 is deleted from one parent. The tandem 1.9 kb duplications of ade6 are separated by ~4.4 kb of vector DNA and the ura4 gene, cloned into the pUC18 vector (SCHUCHERT and KOHLI 1988). The strains used were GP5837 and GP5860 (wt) and GP5835 and GP5859 (rqh1Δ). Data are the mean of four independent experiments ± SEM.

**FIGURE 6.**—The rqh1Δ mutation does not affect the frequency of intersister vs. interhomolog joint molecules at mbs1 but does substantially reduce the frequency of crossovers at mbs1. (A) The mbs1 region of chromosome I from diploids heterozygous for PmlI and XbaI restriction sites, flanking the mbs1 hotspot. PvuII, PmlI (“L”’) and XbaI (“R”)’ digestion and probing as shown reveals two parental (9.2 and 6.8 kb) and two recombinant (11.2 and 4.8 kb) fragments. Black arrows indicate
DSB sites. (B) Predicted migration during 2D gel electrophoresis of mbs1-probed DNA digested with PvuII, PmlI, and XbaI (Figure 6A). X-shaped molecules arise during recombination and can be distinguished based on their sizes. Intersister joint molecules (IS) arising from either parent (P1 X or P2 X) migrate as spikes, whereas interhomolog JMs (IH X) migrate as two spots connected by an arc and run at a position between the two intersister spikes (CROMIE et al. 2006). (C) DNA isolated 5 hr after meiotic induction of strains GP5086 (rqh1+) and GP6489 (rqh1Δ) was analyzed as in Figure 6B. Black arrowheads indicate X-form JM species. The thin arrow indicates a partial digestion product. The mean intersister (IS) and interhomolog (IH) JM frequencies are indicated. Values are means of at least two independent experiments ± SEM (n = 3, GP5086) or with the range shown (n = 2, GP6489). The GP5086 data were previously published in CROMIE et al. (2006). (D) DNA was isolated at the indicated times after meiotic induction of strains GP5086 (rqh1+) and GP6489 (rqh1Δ) and analyzed by gel electrophoresis and Southern blotting with digestion and probing at mbs1 as in Figure 6A. Asterisks indicate cross-hybridizing DNA not specific to meiosis. The mean crossover frequency among DNA species at 6 hr was calculated as 2xR2/total. Values are means of at least two independent experiments ± SEM (n = 3, GP5086) or with the range shown (n = 2, GP6489). The GP5086 data were previously published in CROMIE et al. (2006).
<table>
<thead>
<tr>
<th>Genetic Interval</th>
<th>Crossover Frequency (% of wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ura4A+ – tps16</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>tps16 – arg1</strong></td>
<td>80.0</td>
</tr>
<tr>
<td><strong>lys3 – ura1</strong></td>
<td>70.0</td>
</tr>
</tbody>
</table>

The bar chart shows the crossover frequency for different genetic intervals: 
- **ura4A+ – tps16**
- **tps16 – arg1**
- **lys3 – ura1**
Converting Locus Prototroph Frequency (% of wt)

<table>
<thead>
<tr>
<th>Locus</th>
<th>wt</th>
<th>srs2Δ</th>
<th>rgh1Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ura1</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ade6</td>
<td>100</td>
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</tr>
</tbody>
</table>

The diagram shows the prototroph frequency for different loci, comparing wt, srs2Δ, and rgh1Δ genotypes.
Crossovers Among ade6 Gene Conversions

% Crossovers Among ade6 Gene Conversions

Graph showing % crossovers among ade6 gene conversions with different strains.

Diagram with genetic loci:
- O
- ade6-M375
- tps16-23
- ura4^+
- ade6-52
- tps16^+
- ade6^+

Legend:
- wt
- srs2\(\Delta\)
- rqh1\(\Delta\)
Relative Spore Viability

Relative Viable Spore Yield

wt | srs2Δ | rqh1Δ | rec12Δ | wt | rqh1Δ

Relative Spore Viability or Yield (% wt)
Meiotic SCE Recombination Frequency (% Ade+ Spores)

Genotype

- **wt**
- **rqh1Δ**

Diagram:
- **ade6-469**
- **ade6-M26**
- **parent 1**
- **cross**
- **parent 2**
- **ade6Δ**
- **ade6+**
A Pvu II Pvu II Pvu II Pvu II

Parent 1 (P1)
Parent 2 (P2)
Recombinant 1 (R1)
Recombinant 2 (R2)

9.2 kb
6.8 kb
11.2 kb
4.8 kb

B Genotype % JM Relative to Linear

rqh1+ 0.53 ± 0.16 0.14 ± 0.01
rqh1Δ 0.67 ± 0.05 0.12 ± 0.03

C

D Genotype % Crossovers, 6 hr

rqh1+ 3.3 ± 0.5
rqh1Δ 0.70 ± 0.03