Overlapping Mechanisms Promote Postsynaptic RAD-51 Filament Disassembly during Meiotic Double-Strand Break Repair

Jordan D. Ward 1,6 Diego M. Muzzini,2,5 Mark I.R. Petalcorin,1,5 Enrique Martinez-Perez,3 Julie S. Martin,1 Paolo Plevani,2 Giuseppe Cassata,4 Federica Marini,2,* and Simon J. Boulton1,*

1DNA Damage Response Laboratory, Cancer Research UK, Clare Hall Laboratories, South Mimms EN6 3LD, UK
2Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy
3Clinical Sciences Division, Imperial College, Du Cane Road, London W12 0NN, UK
4FIRC Institute of Molecular Oncology Foundation, Via Adamello 16, 20139 Milano, Italy
5These authors contributed equally to this work

DOI 10.1016/j.molcel.2009.12.026

SUMMARY

Homologous recombination (HR) is essential for repair of meiotic DNA double-strand breaks (DSBs). Although the mechanisms of RAD-51-DNA filament assembly and strand exchange are well characterized, the subsequent steps of HR are less well defined. Here, we describe a synthetic lethal interaction between the C. elegans helicase helq-1 and RAD-51 paralog rfs-1, which results in a block to meiotic DSB repair after strand invasion. Whereas RAD-51-ssDNA filaments assemble at meiotic DSBs with normal kinetics in helq-1, rfs-1 double mutants, persistence of RAD-51 foci and genetic interactions with rtel-1 suggest a failure to disassemble RAD-51 from strand invasion intermediates. Indeed, purified HELO-1 and RFS-1 independently bind to and promote the disassembly of RAD-51 from double-stranded, but not single-stranded, DNA filaments via distinct mechanisms in vitro. These results indicate that two compensating activities are required to promote postsynaptic RAD-51 filament disassembly, which are collectively essential for completion of meiotic DSB repair.

INTRODUCTION

Homologous recombination (HR) plays a critical role in maintaining genome stability and preventing carcinogenesis through error-free repair of DNA damage. HR also plays an essential role in meiotic double-strand break (DSB) repair, which is required for establishing biorientation between homologous chromosomes and maintaining a physical connection (chiasma) between homologs at metaphase (Zickler and Kleckner, 1999). It is well established that DSB repair by HR requires nucleolytic processing of the DSB to generate a 3’ single-stranded DNA (ssDNA) tail. Following break resection, a recombinase protein of the RecA/Rad51 family is loaded onto the ssDNA overhang to form a nucleoprotein filament, which is the catalyst for strand invasion into homologous duplex DNA to form a displacement (D) loop. The 3’ end of the invading strand can then be extended by DNA synthesis to correct the DNA damage. Completion of HR by either dissolution or nucleolytic processing followed by ligation yields noncrossover or crossover repair products (Krogh and Symington, 2004; West, 2003). The mechanisms that control DSB resection, facilitate recruitment and nucleation of the RecA/Rad51 filaments, and regulate strand invasion and D loop formation are well characterized. However, the poststrand invasion steps of HR remain poorly defined at the molecular level, although various enzymatic factors have been identified recently that process late HR intermediates (Symington and Holloman, 2008).

C. elegans is a powerful system for studying the control of meiotic prophase progression, as these events are temporally and spatially polarized in the germline. Meiosis begins with the alignment and pairing of homologous chromosomes, which allows for the formation of the synaptonemal complex (SC), a proteinaceous structure that maintains the homologs in close proximity to facilitate meiotic recombination (reviewed in Garcia-Muse and Boulton, 2007). In early prophase, SPO-11 protein introduces DSBs that are repaired via HR (Dernburg et al., 1998). If crossing over is prevented either through loss of the SC or a block to meiotic DSB formation, then 12 unattached chromosomes (univalents) are observed at the end of meiotic prophase I (diakinesis) instead of the six bivalents (homologs linked by chiasma) seen in wild-type animals. Complete failure to repair meiotic DSBs, as seen in Cebrc-2 and rad-51 mutants, results in aggregated chromosomes at diakinesis (Martin et al., 2005; Rinaldo et al., 2002).

The Rad51 paralogs exhibit 20%–30% sequence identity to RAD51, and although existing data support both early and late roles in HR, their precise function remains unclear (Thacker, 2005). An early role is suggested by their stimulatory effect on Rad51-mediated strand exchange reactions in vitro and their requirement for efficient RAD51 focus formation in vivo, and a late role is suggested by Holliday junction resolution activity associated with the Rad51C/XRCC3 complex and their
localization during diplonete to late HR intermediates on mouse meiotic chromosomes (Lio et al., 2003; Liu et al., 2004, 2007; Sigurdsson et al., 2001). C. elegans possess a single Rad51 paralog, RFS-1, which is dispensable for RAD-51 recruitment to meiotic and ionizing radiation (IR)-induced DSBs but is necessary for RAD-51 loading onto ssDNA gaps and/or one-ended DSBs formed at replication fork barriers (Ward et al., 2007). Although RFS-1 is not required for RAD-51 loading onto meiotic DSBs, rfs-1 mutants have a weak high incidence of male (Him; increased X chromosome nondisjunction) phenotype and low levels of embryonic lethality (Ward et al., 2007; Yanowitz, 2008), which suggest that RFS-1 may play a nonessential or overlapping role in C. elegans meiosis.

HELQ-1 is a helicase belonging to the mus308 gene family. Previous studies have demonstrated that C. elegans HELQ-1 plays a role in strand crosslink (ICL) repair (Muzzini et al., 2008). Mutants in the Drosophila melanogaster homolog, mus301/spn-C, are also sensitive to ICL-inducing agents and weakly to ionizing radiation. A defect in DSB repair during meiosis is suggested by the accumulation of γ-HIS2AV (Drosophila H2A histone variant) and by DNA damage checkpoint activation in mus301 mutant oocytes (McCaffrey et al., 2006). mus301 also genetically interacts with genes involved in HR, such as Spn-A, Spn-B, and Spn-D, all members of the Drosophila Rad51 family (Ghabrial et al., 1998; Gonzalez-Reyes et al., 1997). In vitro, human HELQ1 is a 3′-to-5′ helicase stimulated by the ssDNA-binding protein RPA (Marini et al., 2003; Marini and Wood, 2002). Currently, the function of Mus308/HELQ1 in HR remains unclear.

Here, we show that C. elegans HELQ-1 and RFS-1 perform overlapping roles during meiotic DSB repair. Whereas meiotic DSBs are effectively repaired in rfs-1 and helq-1 single mutants, helq-1, rfs-1 double mutants accumulate stalled recombination intermediates at a poststrand invasion step of HR and thus fail to complete meiotic DSB repair. In vitro analysis reveals that HELQ-1 and RFS-1 bind to and promote disassembly of double-strand RAD-51-DNA filaments via distinct mechanisms. We therefore propose that HELQ-1 and RFS-1 represent compensating activities that function to clear RAD-51 from post-synaptic HR intermediates, which is essential for the completion of meiotic DSB repair.

RESULTS

helq-1, rfs-1 Double Mutants Are Synthetic Lethal

Recently, we demonstrated that helq-1 and polq-1, another mus308 family member, function in genetically distinct pathways of ICL repair in C. elegans (Muzzini et al., 2008). To further explore the roles of HELQ-1 and POLQ-1 in ICL repair, we assessed the consequences of combining helq-1 or polq-1 mutants with other characterized ICL repair mutants, including dog-1 (FANCJ), fci-1 (FANCI), brc-1 (BRCA1), brd-1 (BARD1), rfs-1, mus-81, and xpf-1 (Youds et al., 2009). Surprisingly, helq-1, but not polq-1, mutants exhibited synthetic lethality when combined with deletions in the single C. elegans Rad51 paralog rfs-1, but not with other ICL repair defective mutants (Table S1 available online and data not shown). Whereas helq-1 and rfs-1 single mutants exhibited low levels of embryonic lethality (3.3% and 6.7%, respectively), the helq-1, rfs-1 double mutant displayed embryonic lethality in 92% of F1 progeny (Table S1). The 8% of F1 progeny that survive likely do so through maternal rescue, as the F2 progeny are completely unviable (data not shown). Cytological analyses of the helq-1, rfs-1 double mutant failed to reveal evidence of a mitotic phenotype (Figure S1). This suggested that the synthetic lethality of the helq-1, rfs-1 strain may be caused by a meiotic defect.

helq-1, rfs-1 Double Mutants Exhibit a Defect in Meiotic DSB Repair

To investigate a possible meiotic defect, we first examined N2(WT), helq-1, and rfs-1 single mutants and the helq-1, rfs-1 double mutant for chromosomal abnormalities at diakinesis of meiotic prophase I. N2(WT), rfs-1, and helq-1 single mutants displayed the normal complement of six DAPI-stained bivalents at diakinesis, indicating that meiotic DSBs had been successfully repaired with the formation of chiasmata (Figures 1A and 1B). However, the helq-1, rfs-1 double mutant exhibited chromosome aberrations in nearly all diakinesis nuclei scored, which were highly reminiscent of the defects observed in rad-51, rad-54, and Cebrc-2 mutants (Figures 1A, 1B, and S2) (Martin et al., 2005; Rinaldo et al., 2002).

To address whether the chromosomal abnormalities at diakinesis in helq-1, rfs-1 double mutants are caused by a meiotic DSB repair defect, we blocked meiotic DSB formation in the double mutant in two ways: (1) by making a triple mutant spo-11, helq-1, rfs-1 and (2) through htp-3 deletion by dsRNA injection. SPO-11 is a topoisomerase II-like protein that generates meiotic DSBs, and HTP-3 is an axial element protein required to couple homolog pairing and synopsis with DSB induction (Dernburg et al., 1998; Goodyer et al., 2008). Elimination of SPO-11 or HTP-3 prevents meiotic DSB formation and results in 12 univalents at diakinesis (Figures 1C and 1D). Strikingly, the chromosomal abnormalities at diakinesis in the helq-1, rfs-1 double mutants were largely suppressed by spo-11 deletion or htp-3 deletion and resulted in 12 univalent chromosomes present in the majority of diakinesis nuclei (Figures 1C and 1D). These results underscored a problem in the repair of meiotic DSBs in the absence of both HELQ-1 and RFS-1.

Homologous Pairing and Synapsis Are Unaffected in helq-1, rfs-1 Double Mutants

To further examine the meiotic phenotype of helq-1, rfs-1 double mutants, we analyzed the establishment and maintenance of homologous chromosome pairing in early prophase. Homolog alignment and pairing in the helq-1, rfs-1 double mutant was comparable to wild-type as evidenced by fluorescence in situ hybridization to the SS rDNA locus on chromosome V. Efficient pairing was also confirmed by immunostaining for HIM-8, which marks pairing centers on the X chromosome (Figures 2A and 2B) (Phillips et al., 2005). Finally, we analyzed the loading of synaptonemal components HIM-3 and SYP-1 in germlines of helq-1, rfs-1 double mutants. Both proteins displayed a normal staining pattern during early- and midpachytene, suggesting that SC morphogenesis is not affected in helq-1, rfs-1 double mutants.

Following pachytene exit, it is known that chromosome compaction is accompanied by the asymmetric disassembly of
the SC (Nabeshima et al., 2005). In WT animals, this manifests as six short stretches of SC per nuclei, one stretch per bivalent (Figure 2C). In contrast, helq-1, rfs-1 double mutants displayed abnormal SC disassembly, similar to that observed in rad-51 and other crossover defective mutants (Figure 2C; Nabeshima et al., 2005). Thus, the chromosomal aberrations at diakinesis in the helq-1, rfs-1 double mutant could not be attributed to a defect in chromosome pairing or synapsis but were consistent with a defect in crossing over.

**HR Intermediates Persist in the Absence of Both HELQ-1 and RFS-1**

To test for a possible overlapping role for RFS-1 and HELQ-1 in promoting RAD-51 loading onto meiotic DSBs, we performed immunostaining against RAD-51. rfs-1 mutants had no detectable difference in meiotic RAD-51 focus formation compared to N2 (WT), whereas helq-1 animals had a slight reduction in the number of RAD-51 foci in early pachytene (Figures 3B). Surprisingly, instead of the complete loss of RAD-51 foci that occurs in the absence of CebRC-2 (Martin et al., 2005), the initial recruitment of RAD-51 to meiotic DSBs in early pachytene was unaffected in the helq-1, rfs-1 double mutants (Figure 3). However, whereas RAD-51 foci disappear during late pachytene in WT germlines, RAD-51 foci persisted until diplotene in helq-1, rfs-1 double mutants (Figures 3A and 3B).

Importantly, the accumulation and persistence of RAD-51 foci in the helq-1, rfs-1 double mutant were found to be largely suppressed by eliminating meiotic DSB formation with either the spo-11(ok79) mutation or by htp-3 RNAi depletion (Figures 4A and 4B). Defects in meiotic DSB repair that lead to persistent lesions are known to trigger the pachytene checkpoint, which results in increased germ cell apoptosis (Colaiáécovo et al., 2003; Gartner et al., 2000; Martin et al., 2005). Whereas helq-1 and rfs-1 single mutants exhibited a small increase in the numbers of apoptotic corpses per gonad arm relative to wild-type animals (helq-1, 2.1; rfs-1, 2.6; N2 [WT], 0.5), the helq-1, rfs-1 double mutant exhibited a considerable increase in germ cell apoptosis (7.4 corpses/gonad arm) (Figure 4C).
suppressed by eliminating spo-11 (Figure 4C). Hence, in the absence of both HELQ-1 and RFS-1, HR repair of SPO-11 induced DSBs stalls at a stage following the loading of RAD-51, which leads to persistent HR intermediates, increased apoptosis, and chromosomal aberrations at diakinesis.

**helq-1 and rfs-1 Mutants Exhibit Synthetic Growth Defects when Combined with rtel-1**

The persistence of HR intermediates in helq-1, rfs-1 double mutants may reflect a defect in either a pre- or poststrand invasion step of HR. In an attempt to discriminate between these two possibilities, we employed a genetic approach based on our recent analysis of the antirecombinase rtel-1, which counteracts recombination reactions by disrupting the D loop poststrand invasion intermediate (Barber et al., 2008). We reasoned that, if HELQ-1 and RFS-1 perform overlapping roles to promote a poststrand invasion step of HR, the viability of helq-1 and rfs-1 single mutants might depend on RTEL-1 activity to reverse stalled D loop intermediates. This possibility predicts that both helq-1 and rfs-1 single mutants should exhibit synthetic growth defects.

![Figure 2. Homologous Chromosome Pairing and Synapsis Is Unaffected in helq-1, rfs-1 Mutants, but Synaptonemal Complex Disassembly Is Abnormal](image-url)

(A) Representative images of pachytene nuclei in animals of the indicated genotype immunostained with antibodies against HIM-8 (green, X chromosome pairing center binding protein), HIM-3 (red, axial element component), and SYP-1 (green, SC central region component) and FISH analysis using a probe to the 5S rDNA locus on chromosome V. DNA is counterstained with DAPI (blue). Scale bar = 5 μm.

(B) Percentage of X chromosome (HIM-8 staining) and chromosome V (5S rDNA FISH) pairing in pachytene nuclei. Single foci or overlapping foci for each nucleus were scored as paired. n = number of scored nuclei.

(C) Representative images of diplotene and early diakinesis nuclei in animals of the indicated genotype immunostained with SYP-1 (white on the left; green in the merge with DAPI on the right). DNA is counterstained with DAPI (blue).
and persistent meiotic RAD-51 foci when combined with the rtel-1 mutation. Indeed, rtel-1; helq-1 and rtel-1; rfs-1 double mutants displayed a dramatic reduction in brood size (increased sterility), an increase in embryonic lethality (Table S2), and persistent meiotic RAD-51 foci, indicative of stalled HR intermediates, when compared to the respective single mutants (Figure S3). Given that RTEL-1 acts to disassemble D loops but has no detectable activity toward single-stranded RAD51-DNA...
Figure 4. Blocking Meiotic DSB Formation Prevents RAD-51 Accumulation and Germ Cell Apoptosis in helq-1, rfs-1 Mutants

(A) Representative images of RAD-51 staining (red) in fixed germlines of the indicated genotype. DNA was counterstained with DAPI (blue). The mitotic tip of the germline is oriented to the left of each panel. For each genotype, RAD-51 staining is shown in the top of the panel, and a DAPI merge with RAD-51 is provided in the bottom of the panel.

(B) Graphical representation of RAD-51 foci quantification in animals of the indicated genotype. The legend indicates which class (number of RAD-51 foci/nucleus) each colored column represents, the x axis indicates the position within the germline (zone), and the y axis indicates the percentage of nuclei that falls in each class per zone. A minimum of ten germ lines were scored for each genotype.

(C) The number of germ cell apoptotic corpses in animals of the indicated genotype. A minimum of 25 animals were scored per genotype.
filaments (Barber et al., 2008), the genetic interactions between *helq-1*/*rfs-1* and *rte1-1* suggested a role for HELQ-1 and RFS-1 in promoting a poststrand invasion step of HR.

**RFS-1 and HELQ-1 Interact Directly with RAD-51**
The phenotype of the *helq-1, rfs-1* double mutant may reflect overlapping roles for HELQ-1 and RFS-1 in directly binding to and regulating RAD-51 activities, which we next sought to investigate. Previous studies have shown that RFS-1 and RAD-51 can interact in the yeast two-hybrid system (Boulton et al., 2002; Ward et al., 2007). To confirm this interaction and to define a minimal RAD-51 interaction site in RFS-1, we utilized a combination of yeast two-hybrid mapping and peptide array binding experiments, as previously described (Thorlund et al., 2007). Yeast two-hybrid approaches mapped the RAD-51 interaction site in RFS-1 to the last 125 amino acids of the protein (data not shown). Incubation of recombinant RAD-51 with a 30 mer peptide-scanning array encompassing the C-terminal 125 amino acids of RFS-1 identified a single RAD-51 binding peptide within this region (Figure 5A). To identify residues critical for this interaction, we constructed a peptide substitution array in which each residue in the 30 mer peptide was sequentially mutated to each of the 20 amino acids in a 20 x 30 matrix. A significant reduction in binding by RAD-51 was observed when residues L134–E138 were mutated to proline, cysteine, or, to a lesser extent, lysine (Figure 5B). Introduction of a single L134P mutation into the full-length RAD-51 coding region compromised binding to RAD-51 in the yeast two-hybrid system (Figure 5C). Furthermore, a biotinylated wild-type peptide (PepWT) efficiently pulled down recombinant RAD-51, whereas a peptide containing a single L134P substitution (PepL134P) did not (Figure 5D). These data revealed that RFS-1 interacts directly with RAD-51 and that this association can be abolished by a single L134P amino acid substitution.

HELQ-1 has two confirmed isoforms, including a 923 amino acid form (HELQ-1A) containing all recognized helicase motifs and a second 486 amino acid truncated form (HELQ-1B), which results from an internal start site that removes the first five recognized helicase motifs, including the ATP-binding site (Figure S4A). To investigate a possible interaction with RAD-51, GST, GST-HELQ-1A, or GST-HELQ-1B and RAD-51-V5 were individually expressed in insect cells using the baculovirus system, purified, and used for in vitro binding experiments. Western blotting revealed that RAD-51, whereas a peptide containing a single L134P substitution (PepL134P) did not (Figure 5D). These data revealed that RFS-1 interacts directly with RAD-51 and that this association can be abolished by a single L134P amino acid substitution.

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**HELQ-1 Promotes Disassembly of RAD-51 from Double-Stranded, but Not Single-Stranded, DNA Filaments In Vitro**

Our genetic data suggested that HELQ-1 and RFS-1 play overlapping roles in regulating RAD-51 at a poststrand invasion step of HR, which we next sought to test biochemically. In addition to the purified HELQ-1A and HELQ-1B isoforms described above, we also expressed and purified a mutant in the ATP-binding pocket (K197R) (Figure S4B) using the baculovirus system. As expected, the full-length HELQ-1A isoform possessed DNA-dependent ATPase activity with both single- and double-stranded DNA, whereas the truncated HELQ-1B isoform and the K197R mutant were ATPase-dead (Figure S4C).

To investigate a potential role for HELQ-1 in regulating RAD-51 function poststrand invasion, we performed gel shifts to examine whether HELQ-1 is able to disassemble dsDNA-RAD-51 nuclei protein filaments. dsDNA-RAD-51 filaments were preformed by incubating RAD-51 for 10 min with a 5' 32P end-labeled linear 5.1 Kb φX174 dsDNA before incubation with HELQ-1A and then fixation with glutaraldehyde to terminate the reaction. In the absence of HELQ308A, fixation resulted in dsDNA-RAD-51 complexes (PCs), which failed to enter the gel (Figure 6A, lane 2). Strikingly, addition of HELQ-1A resulted in the rapid disassociation of RAD-51 and the release of naked dsDNA. Complete disassociation of the 5.1 Kb dsDNA-RAD-51 filament occurred within 10 s of addition of stoichiometric levels of HELQ-1 to the reaction (Figure 6A, lanes 3–8). In contrast, addition of CcBrcC-2, which binds directly to RAD-51 (Martin et al., 2005), or RECoQ-5, which disrupts ssDNA-RAD51 filaments (Hu et al., 2007), failed to disrupt preformed dsDNA-RAD-51 filaments (Figure S6B). To determine whether the activity of HELQ-1 is specific for dsDNA-RAD-51 filaments, we also performed gel shifts to examine whether HELQ-1 is able to dissociate ssDNA-RAD51 filaments. Surprisingly, we were unable to detect any measurable effect of HELQ-1A on the stability of the ssDNA-RAD51 filaments formed with a 100 mer ssDNA, even following addition of a 200-fold excess of unlabeled φX174 ssDNA competitor (Figure 6B and data not shown). Addition of increasing concentrations of HELQ-1A to ssDNA-RAD51 filaments formed with 5.1 Kb φX174 ssDNA resulted in more-defined filaments with slight reduced mobility, which suggested that HELQ-1A may act to stabilize ssDNA-RAD51 filaments (Figure S5A). These results suggested that HELQ-1 specifically disrupts RAD-51 from dsDNA, but not ssDNA, filaments.

**Disassembly of dsDNA-RAD51 Filaments Does Not Require the ATPase Activity of HELQ-1 or a DNA End**

We next investigated the role of the DNA-dependent ATPase activity of HELQ-1 in promoting dsDNA-RAD-51 filament disassembly. Surprisingly, incubation of preformed dsDNA-RAD-51 filaments.
Figure 5. HELQ-1 and RFS-1 Bind Directly to RAD-51

(A) Western blotting and Ponceau staining of a scanning peptide array for the C-terminal 125 amino acids of RFS-1 probed with recombinant RAD-51 and analyzed by immunoblotting. Ponceau stain was used to visualize position of the peptides within the array. A control in which no RAD-51 was added was also performed (mock). The RAD-51-binding peptide is indicated by a blue box.
filaments with the HELOQ-1A K197R mutant, which is defective for ATP hydrolysis, resulted in rapid filament disassembly comparable to the wild-type HELOQ-1A (Figure 6C). Furthermore, incubation of preformed dsDNA-RAD-51 filaments with the truncated HELOQ-1B isoform, which lacks the entire N terminus, including the ATP-binding pocket, also resulted in rapid filament disassembly (Figure 6B).

Because HELOQ-1 can disassemble dsDNA-RAD-51 filaments independently of its ATPase activity, it was possible that this activity may not require a DNA end. To investigate a requirement for DNA ends for dsDNA-RAD-51 filament disassembly and to determine whether HELOQ-1 exhibits species specificity for RAD-51, we employed a restriction enzyme protection assay similar to that previously described (Solinger et al., 2002). Both budding yeast (Sc) Rad51 and worm (Ce) RAD-51 are weak ATPases that form stable nucleoprotein filaments, whereas RecA is a potent ATPase that forms dynamic filaments (Bauermann et al., 1996; Howard-Flanders et al., 1984; Petalcorin et al., 2006). ScRad51 and CeRad51, but not RecA, protected supercoiled (sc) ϕX174 plasmid DNA from restriction enzyme cleavage (Figure 6D, lanes 4, 9, and 14). Incubation of preformed scDNA nucleoprotein filaments with HELQ-1A resulted in protection and restriction enzyme cleavage for the CeRad51 filaments (Figure 6E, lane 8), but not significantly for the ScRad51 filaments (Figure 6E, lane 3). These results suggested that HELQ-1A does not require a DNA end and also preferentially disassembles C. elegans RAD-51 from dsDNA filaments, thus demonstrating species specificity.

**DISCUSSION**

Here, we report that HELQ-1 and RFS-1 represent distinct activities that are individually dispensable for meiosis yet are collectively essential for completion of meiotic DSB repair by HR. Our findings that helq-1 and rfs-1 exhibit synthetic lethality with mutations in retel-1 suggest that the defect in HR occurs at a step following formation of a stable strand invasion intermediate. Indeed, we demonstrate that both HELQ-1 and RFS-1 interact directly with RAD-51 and can each promote its disassembly from dsDNA-RAD-51 filaments via distinct mechanisms. We hypothesize that HELQ-1 and RFS-1 perform overlapping roles in promoting the disassembly of dsDNA-RAD-51 nucleoprotein filaments following completion of strand exchange reactions, which is an essential step in the HR reaction.

RAD-51 filament assembly and disassembly are subject to strict control to ensure the correct timing and placement of recombination reactions, yet the mechanisms involved in promoting filament disassembly remain poorly defined (Symington and Heyer, 2006). BRCA2 is known to promote the nucleation
and stabilization of ssDNA-RAD-51 filaments, and this is achieved by targeting Rad51 to resected DSBs and by directly inhibiting ATP turnover of Rad51, respectively (Petalcorin et al., 2007; Yang et al., 2005). A number of eukaryotic helicases and translocases are also known to regulate Rad51 within the context of the nucleoprotein filament. Yeast Srs2 and vertebrate BLM and RECQL5 counteract the formation of aberrant recombination structures by promoting disassembly of ssDNA-Rad51 filaments, thus reversing the HR reaction (Bugreev et al., 2007b; Hu et al., 2007; Krejci et al., 2003; Veaute et al., 2003). Yeast MPH1 and Srs2 and vertebrate BLM and RTEL1 can, under certain reaction conditions, promote nonrecombinogenic outcomes by disrupting the D loop intermediate (Barber et al., 2008; Bugreev et al., 2007b; Dupaigne et al., 2008; Prakash et al., 2009). In all reported cases, the action of Srs2, Mph1, BLM, RECQ5, and RTEL1 toward HR intermediates is strictly dependent on their intrinsic ATPase activity. However, a recent study has shown that Rad51 displacement from ssDNA filaments by Srs2 is not only dependent on the ATP-dependent motor activity of Srs2, but also on its ability to bind to and trigger ATP turnover of Rad51, which results in dissociation of RAD51 from ssDNA (Antony et al., 2009). Our biochemical studies reveal that the helicase HELQ-1 specifically disrupts RAD-51 from double-stranded, but not single-stranded, DNA filaments in vitro. In contrast to other helicases and translocases that regulate Rad51, C. elegans HELQ-1 can disrupt dsDNA-RAD51 filaments through an ATP-independent mechanism (Figures 6A–6C). We show that the ATPase dead HELQ-1 K197R mutant and the truncated HELQ-1B isoform, which lacks the ATP-binding pocket, display dsDNA-RAD-51 disruption activity.

Figure 6. HELQ-1 Promotes Disassembly of dsDNA-Rad-51 Filaments, but Not ssDNA-RAD-51 Filaments

(A) Schematic of the dsDNA-RAD51 filament disassembly assay. Preformed linear φX174 dsDNA-RAD51 filaments incubated with HELQ-1A for the indicated time. PCs, dsDNA-RAD51 complexes.

(B) Schematic of the ssDNA-RAD51 filament disassembly assay. Preformed linear 100 mer ssDNA-RAD51 filaments were incubated with HELQ-1A for the indicated time.

(C) Schematic of the dsDNA-RAD51 filament disassembly assay. Preformed linear φX174 dsDNA-RAD51 filaments were incubated with WT or K197R mutant of HELQ-1A for the indicated time. PCs, dsDNA-RAD51 complexes.

(D) Schematic of the φX174 supercoiled DNA-RAD51 filament deprotection assay. Supercoiled φX174 dsDNA filaments were preformed with the indicated Rad51/RecA protein (1 μM) and then incubated with or without HELQ-1A for 5 min prior to addition of restriction enzyme. nc, nick circles; sc, supercoiled. See also Figure S5.
Although both mutant proteins are deficient for ATP-dependent motor activity, these proteins retain the C-terminal region that interacts directly with RAD-51 (Figure 5E). Attempts to generate a HELQ-1 mutant deficient for RAD-51 binding have been hampered by the presence of multiple RAD-51 binding sites within this region (data not shown). Nevertheless, our results indicate that HELQ-1 does not simply use its motor activity to displace RAD-51 from dsDNA. Instead, we propose that the C-terminal region of HELQ-1 binds to and invokes an ATPase-independent allosteric change in RAD-51 that drives filament disassembly. At least in vitro, disassembly of dsDNA-RAD-51 filaments requires the addition of stoichiometric levels of HELQ-1 relative to RAD-51 (Figure S6A). In its in vivo context, we envisage that the motor activity of HELQ-1, possibly on the displaced ssDNA strand of the D loop, may allow HELQ1 to act substoichiometrically to facilitate processive ATP-dependent 3’-to-5’ clearing of the filament. The motor activity on ssDNA may simply position HELQ-1 for...
binding to successive RAD-51 molecules within the dsDNA filament, as has been proposed for the action of Srs2 on ssDNA-Rad51 filaments (Antony et al., 2009). Our genetic data demonstrate that RFS-1 is able to compensate for the loss of HELQ-1 in vivo, implying that RFS-1 may also promote dsDNA-RAD-51 filament disassembly. Despite the lack of soluble RFS-1, we were able to show that a peptide corresponding to the minimal RAD-51-binding site in RFS-1 (Figure 5) is capable of promoting dsDNA-RAD-51 filament, but not ssDNA-RAD-51 filament, disassembly in vitro. Critically, this disruption activity is abolished by a single L134P substitution mutation in the peptide (Figure 7), which eliminates direct binding to RAD-51 (Figure 5D). Furthermore and in contrast to HELQ-1, the disruption activity of the RFS-1 wild-type peptide requires ATP hydrolysis, as dsDNA-RAD-51 filaments formed in the presence of AMP-PNP are resistant to disruption (Figure 7B). Collectively, our data demonstrate that HELQ-1 and RFS-1 display mechanistically distinct disrupting activities that can promote clearance of RAD-51 from dsDNA filaments, but not from ssDNA filaments.

Although HELQ-1 displayed no measurable disruption activity toward ssDNA-RAD-51 filaments, we noted that addition of HELQ-1 to preformed ssDNA-RAD-51 filaments resulted in more-defined species with slower mobility (Figure S3A). Thus, HELQ-1 may also stabilize ssDNA-RAD-51 filaments prior to strand invasion, similar to CeBRC-2 (Petalcorin et al., 2007). This possibility is supported by the observation that helq-1 mutants exhibit reduced numbers of RAD-51 foci at both mitotic and meiotic DSBs when compared to wild-type (Figure 3B) (Muzzini et al., 2008). Furthermore, helq-1 mutant animals exhibit a modest but significant reduction in meiotic recombination frequencies (Table S3). Thus, HELQ-1 plays a prorecombinogenic role and may act both prior to strand invasion in ssDNA-RAD-51 filament stabilization and poststrand invasion to promote RAD-51 clearance from stable synaptic products.

It is intriguing that the phenotypes of the helq-1, rfs-1 double mutant (persistence of meiotic Rad51 foci and chromosome aggregates at diakinesis) are very similar to those observed in C. elegans rad-54 mutants (Figure S2). The genetic interactions of helq-1 and rfs-1 with retl-1 are suggestive of a poststrand invasion defect during HR, which is further supported by the activities of HELQ-1 and RFS-1 toward dsDNA-RAD-51 filaments (Figures 6 and 7). The fact that rad-54 is an essential gene in C. elegans precludes a similar genetic analysis with retl-1. Of interest, like RFS-1, S. cerevisiae Rad54 can promote ATP-dependent displacement of Rad51 from dsDNA filaments, a function consistent with the persistence of RAD-51 foci at meiotic DSBs in yeast and in C. elegans rad-54 mutants (Slinger and Heyer, 2001). However, Rad54 has also been implicated in many other steps of the HR pathway, including cross-bridging dsDNA molecules, stimulating Rad51-mediated strand exchange, extension of heteroduplexes, and remodeling of chromatin to facilitate HR (Heyer et al., 2006). Perturbation of any one of these activities is also predicted to lead to persistent RAD-51 foci and a complete failure to repair meiotic DSBs. It is currently unclear which of these biochemical activities correspond to the essential function of Rad54 in vivo.

We envisage two possible models consistent with our data and those previously reported for Rad54: (1) RAD-54 and HELQ-1/RFS-1 catalyze distinct, nonoverlapping steps in the removal of RAD-51 from dsDNA filaments, and (2) RAD-54 plays an essential role in a synaptic and/or postsynaptic stage of HR, which is distinct from dsDNA-RAD-51 filament disassembly. Because rad-54 single mutants and helq-1, rfs-1 double mutants are both deficient for meiotic DSB repair and all three proteins can promote dsDNA-RAD-51 filament disruption, it is possible that their sequential action is required for complete filament disassembly. It is conceivable that, following completion of strand invasion, the primary function of Rad54 is to remove Rad51 from the 3' end of the invading DNA strand to permit D loop extension by DNA polymerases, which is suggested by ChiP experiments and is known to require Rad54 motor activity (Li and Heyer, 2009; Sugasawa et al., 2003; Wolner et al., 2003). Once disassembly is initiated and a free 3' OH is accessible, Rad54 may promote branch migration while HELQ-1/RFS-1 complete the removal of Rad51 from the synaptic product. Alternatively, dsDNA-RAD-51 filament disassembly may not be the essential function of Rad54 in HR. It is possible that the compensating activities of HELQ-1 and RFS-1 are sufficient for complete dsDNA-RAD-51 filament disassembly and that this process is Rad54 independent. Instead, the phenotype of rad-54 mutants may reflect a role at another step in the HR process, such as cross-bridging ssDNA molecules, stimulating strand exchange, branch migration and extension of heteroduplexes, or restoration of chromatin postsynapsis, which are all possibilities supported by biochemical data in vitro (Bugreev et al., 2006, 2007a; Sigurdsson et al., 2002; Wesoły et al., 2006; Zhang et al., 2007).

In summary, our study has identified an unexpected and striking genetic interaction between helq-1 and rfs-1. Mutation of both of these genes in the same animal results in a defect in the HR-mediated repair of meiotic DSBs and the subsequent formation of chiasmata, which we attribute to a failure to disassemble dsDNA-RAD-51 filaments. Our results reveal that post-synaptic filament disassembly can occur by two distinct yet compensating activities, and thus this step in the HR reaction is considerably more complex than previously realized.

**EXPERIMENTAL PROCEDURES**

**Cytological Preparation and Immunostaining**

Adult hermaphrodites were dissected using standard procedures and then processed for DAPI and immunostaining as previously described (Martin et al., 2009). Anti-RAD-51 and anti-HIM-3 primary antibodies were diluted 1:200 in TBSB-T (TBS + 0.5% BSA), and anti-HIM-8 and anti-SYP-1 primary antibodies were diluted 1:500 in TBSB-T (TBS + 0.5% BSA), and anti-rabbit antibodies conjugated to Cy3 (Sigma, UK), 1:10,000 dilution; anti-guinea pig antibodies conjugated to FITC (Sigma, UK), 1:5,000 dilution. The germlines were washed three times for 30 min each time with TBSS before incubation with secondary antibodies for 2 hr at room temperature (anti-rabbit antibodies conjugated to Cy3 [Sigma, UK], 1:10,000 dilution; anti-guinea pig antibodies conjugated to FITC [Sigma, UK], 1:5,000 dilution). The germlines were washed three times in TBSS for 30 min before being mounted on coverslips with Vectashield containing 1 µg/ml of DAPI.

**Protein Interaction Studies and Peptide Array Analysis**

Using the baculovirus system, 1 l of S99 insect cells (10⁶ cells/ml) was coinfected with either of the two types of baculoviruses expressing GST_CeHELQ-1a or V5_CeRAD-51. After 72 hr at 27°C, cells were harvested, resuspended in...
buffer A, and lysed by sonication. After 30,000 × g centrifugation at 4°C for 30 min, the supernatant was bound to glutathione Sepharose 4B (GE Healthcare). The affinity matrix was washed extensively (6 × 10 ml buffer A), and GST-HELQ-1a/V5 RAD51 complex was eluted in buffer containing 20 mM glutathione and loaded onto a Superdex 200 gel filtration column (GE Healthcare) for fractionation. Fractions were analyzed by SDS-PAGE, and western blotting was done with antibody against GST or V5 tags to analyze protein interaction. Interactions were also examined following transient transfection of HEK293T cells of the indicated expression constructs as previously described (Boulton et al., 2002). Purified proteins consisting of GST_CeHELQ-1a or CeRAD-51 were mixed together at equimolar ratio and loaded onto 50 ml GST Sepharose 4B beads (GE Healthcare). After 2 h at 4°C, the beads were washed extensively (6 × 10 ml buffer A), and the bead-bound proteins were analyzed by SDS-PAGE and western blot using antibody against GST or CeRAD-51. For peptide array studies, 410 peptides (each is 30 mer in length, representing a single-shift amino acid sequence corresponding to CeHELQ-1b1–410) were synthesized and spotted onto cellulose membranes. The membrane was activated in 50% methanol and blocked overnight in TBS + 0.1% BSA on ice and then further incubated to reach the designated time point after which the reaction was terminated by adding 0.1% glutaraldehyde prior to gel loading. Products were analyzed in 1% agarose gel electrophoresis at 4°C. After 2 hr at 4°C, the beads were washed extensively (6 × 10 ml buffer A), and the bead-bound proteins were analyzed by SDS-PAGE and western blot using antibody against GST or CeRAD-51. For peptide array studies, 410 peptides (each is 30 mer in length, representing a single-shift amino acid sequence corresponding to CeHELQ-1b1–410) were synthesized and spotted onto cellulose membranes. The membrane was activated in 50% methanol and blocked overnight in TBS + 0.1% BSA on ice and then further incubated to reach the designated time point after which the reaction was terminated by adding 0.1% glutaraldehyde prior to gel loading. Products were analyzed in 1% agarose gel electrophoresis at 4°C with Tris-borate-EDTA buffer and visualized by autoradiography.

**REFERENCES**


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