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Eviction Notice: New Insights into Rad51 Removal from DNA during Homologous Recombination

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In this issue of *Molecular Cell*, Ward et al. (2010) identify two genes whose products act redundantly to clear Rad51 from DNA after successful strand invasion, thereby enabling the downstream events of homologous recombination to go smoothly.

Homologous recombination (HR) represents an error-free form of DNA double-strand break (DSB) repair in mitotically dividing cells and the means by which homologous chromosomes exchange information during meiosis. The early steps of HR are well characterized (San Filippo et al., 2008); the reaction is initiated by resection of the 5′ terminated strands to form long 3′ single-stranded DNA (ssDNA) overhangs, and the resulting ssDNA is then coated with replication protein A (RPA). Several other proteins then facilitate replacement of RPA with Rad51 recombinase to produce Rad51 nucleoprotein filaments, which go on to bind duplex DNA and search for homology. Once a homologous sequence is located, Rad51 facilitates invasion of the 3′ ssDNA end into the intact duplex DNA, forming a D loop structure. A critical next step is removal of Rad51 from the duplex DNA so that downstream events such as DNA synthesis and Holliday junction processing may occur. What is the mechanism of Rad51 removal prior to strand extension? In this issue of *Molecular Cell*, Ward et al. (2010) report that, in *Caenorhabditis elegans*, RAD-51 is removed from recombination structures during DSB repair by the redundant activities of the products of two genes, *helq-1* and *rfs-1*.

The *helq-1* gene encodes a DNA helicase homologous to human HEL308 and *Drosophila mus301* (Marini and Wood, 2002; McCaffrey et al., 2006; Muzzini et al., 2008). This protein family functions in DNA crosslink repair, and previous work on *mus301* implicated the gene in repair of both damage-induced and meiotic double-strand breaks (McCaffrey et al., 2006). The *rfs-1* gene encodes the sole *C. elegans* member of the Rad51 paralog family of DNA repair factors (Ward et al., 2007; Yanowitz, 2008). Rad51 paralogs earn their name by virtue of limited homology to Rad51, and though their precise biochemical function(s) remain enigmatic, various studies have implicated the paralogs in multiple aspects of HR, including Rad51 filament assembly, and Holliday junction resolution. In *C. elegans*, RFS-1 is required for RAD-51 filament assembly during DNA replication stress, but not during meiotic DSB repair (Ward et al., 2007). Previous work, however, had revealed a weak high incidence of male (Him) phenotype for *rfs-1* mutants (Yanowitz, 2008), a phenotype that often correlates with problems during meiosis. These previous studies thus hinted at a role for RFS-1 in meiotic DSB repair that is independent of RAD-51 filament assembly.

To gain further insight into the roles of *helq-1* in DNA repair, Ward et al. (2010) initiated their study by searching for synthetic lethal genetic interactions between *helq-1* and other DNA repair genes. They hit pay dirt with the observation that a *helq-1*, *rfs-1* double mutant displayed a more severe phenotype than either single mutant. The relatively low levels of embryonic lethality observed in the single mutants (less than 7%) were dramatically elevated in the double mutant, to 92%. Follow-up analysis revealed that the embryonic lethality in the *helq-1*, *rfs-1* double mutant was due to problems during meiosis. During meiosis, homologous chromosomes pair and form synaptonemal complexes (SCs), a kind of proteinaceous glue that holds the homologs together as they prepare for meiotic recombination. The SPO-11 protein then induces DSBs, which are repaired through HR. The resulting repair products can produce chiasmata, physical linkages between homologs that hold them

together after the SC disassembles following the pachytene stage of meiosis. In the *helq-1*, *rfs-1* double mutants, Ward et al. (2010) observed that early processes, such as homolog pairing and SC assembly, occurred normally; however, as meiosis proceeded, the chromosomes in the double mutant formed aberrant structures, and SC disassembly was delayed. Importantly, these phenotypes were suppressed by coinactivation of *spo-11*, which strongly suggested that, whereas the *helq-1*, *rfs-1* double mutant could initiate DSB repair, it could not complete repair.

To further buttress the assignment of *helq-1* and *rfs-1* to meiotic DSB repair, Ward et al. (2010) next turned to an old standby in the DNA repair field, cytological analysis of RAD-51 repair foci. These experiments allowed the conclusion that RAD-51 foci persisted longer in *helq-1*, *rfs-1* double mutants than in wild-type, suggesting a problem in completing repair after RAD-51 was loaded onto the broken DNA. Furthermore, the data suggested that HELQ-1 and RFS-1 act redundantly to process HR intermediates that form after RAD-51-mediated strand invasion. If so, the authors proposed, then both *helq-1* and *rfs-1* could display genetic interactions with *rtel-1*, which encodes an antirecombinase protein. Previous work showed that RTEL-1 disrupts D loops formed after RAD-51-mediated strand invasion (Barber et al., 2008) and, thus, that its activity may be essential for the survival of *helq-1* or *rfs-1* single mutants through its ability to reverse "dead-end" D loops predicted to form in the single mutants. This prediction was borne out beautifully with the demonstration that both *helq-1*, *rtel-1* and *rfs-1*, *rtel-1* double mutants showed high lethality and persistence of RAD-51 foci, phenotypes that were not observed in *helq-1, rfs-1*, or *rtel-1* single mutants.

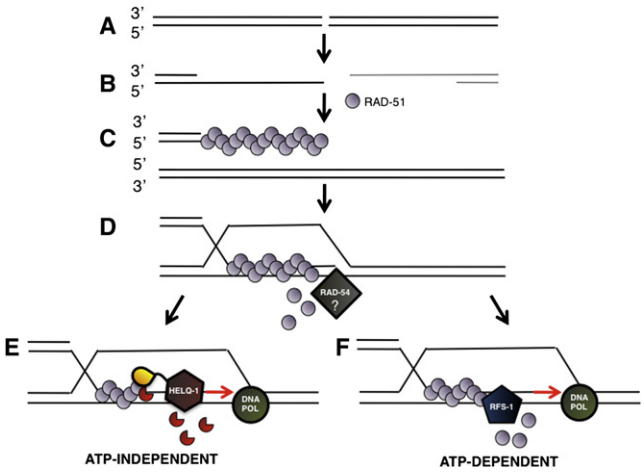

Figure 1. Model for Postsynaptic Removal of RAD-51 during HR-Dependent DSB Repair

(A) After a DNA DSB, the DNA ends are processed to produce 3′ ssDNA overhangs.

(B) These 3′ ssDNA overhangs are substrates for HR.

(C) An ssDNA-RAD-51 filament forms and mediates strand invasion and homology searching.

(D) After strand invasion, RAD-54 may displace RAD-51 from the 3′ terminus of the invading strand, providing a free 3′ OH for extension by DNA polymerases and free dsDNA, a substrate for HELQ-1.

(E) Via an ATP-independent mechanism, the C-terminal domain of HELQ-1 (yellow) interacts specifically with RAD-51 bound to dsDNA and induces a conformational change in RAD-51, causing it to disengage the DNA (red). The 3′-to-5′ helicase activity of HELQ-1 then translocates it along the RAD-51-coated dsDNA, where it continues to remove RAD-51 substoichiometrically.

(F) Alternatively, RFS-1 removes RAD-51 from the dsDNA via an ATP-dependent mechanism.

The genetic and cytological data suggested that HELQ-1 and RFS-1 function at sites of RAD-51-mediated HR; however, how they are recruited to their substrates and what they do once they arrive remained unknown. Using yeast two-hybrid analysis and biochemical techniques, the authors showed that HELQ-1 and RFS-1 directly interact with nonoverlapping regions of RAD-51. Ward et al. (2010) also devised in vitro experiments to show that both HELQ-1 and RFS-1 displace RAD-51 specifically from dsDNA-RAD-51 filaments and not ssDNA-RAD-51 structures. Of interest, however, their mechanisms differed. Filament disassembly by HELQ-1 did not require ATP hydrolysis by HELQ-1 or RAD-51; in contrast, filament disassembly mediated by RFS-1 required ATP hydrolysis by RAD-51.

Taken together, the data reported by Ward et al. (2010) represent an elegant example of combining the utility of *C. elegans* for the genetic dissection of meiotic DSB repair with biochemical approaches and suggest that HELQ-1 and RFS-1 function to remove RAD-51 after successful strand invasion (Figure 1). Previous data suggest that RAD-54 also stimulates RAD-51 turnover during HR (Heyer et al., 2006), leading to the question of why three proteins are required to manage RAD-51 during HR. The authors suggest that RAD-54 may remove RAD-51 at 3′ termini to allow access by DNA polymerases and that HELQ-1 and RFS-1 may remove the remaining RAD-51. An interesting question then is whether the functions of HELQ-1 and RFS-1 are truly redundant or whether they are required specifically in other HR-dependent pathways. Nevertheless, the results presented here provide an important contribution to our understanding of the late events during HR.

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