Characterization of the Interaction between the \textit{Saccharomyces cerevisiae} Rad51 Recombinase and the DNA Translocase Rdh54*

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### Background

Rdh54 is a motor protein that is able to dissociate Rad51 recombinase from chromatin.

### Results

The Rad51-binding domain of Rdh54 resides within its N terminus and is required for Rad51 removal from DNA.

### Conclusion

Rdh54-Rad51 interaction is important for Rad51 dissociation from chromatin but not for DNA repair.

### Significance

Rdh54 inhibits accumulation of toxic Rad51-DNA complexes.

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The \textit{Saccharomyces cerevisiae} Rdh54 protein is a member of the Swi2/Snf2 family of DNA translocases required for meiotic and mitotic recombination and DNA repair. Rdh54 interacts with the general recombinases Rad51 and Dmc1 and promotes D-loop formation with either recombinase. Rdh54 also mediates the removal of Rad51 from undamaged chromatin in mitotic cells, which prevents formation of nonrecombinogenic complexes that can otherwise become toxic for cell growth. To determine which of the mitotic roles of Rdh54 are dependent on Rad51 complex formation, we finely mapped the Rad51 interaction domain in Rdh54, generated N-terminal truncation variants, and characterized their attributes biochemically and in cells. Here, we provide evidence suggesting that the N-terminal region of Rdh54 is not necessary for the response to the DNA-damaging agent methyl methanesulfonate. However, truncation variants missing 75–200 residues at the N terminus are sensitive to Rad51 overexpression. Interestingly, a hybrid protein containing the N-terminal region of Rad54, responsible for Rad51 interaction, fused to the Swi2/Snf2 core of Rdh54 is able to effectively complement the sensitivity to both methyl methanesulfonate and excess Rad51 in \textit{rdh54} null cells. Altogether, these results reveal a distinction between damage sensitivity and Rad51 removal with regard to Rdh54 interaction with Rad51.

Homologous recombination (HR)$^4$ mediated by the recombinase Rad51 and its meiotic homolog Dmc1 (1) is essential for genome stability and meiotic chromosome segregation. DNA double-strand breaks (DSBs) (2, 3) can occur spontaneously from stalled and reversed replication forks, from trapped topoisomerase complexes, and from replication transcription collisions. Exogenous DSBs arise from exposure to high energy radiation and chemicals, and programmed breaks also occur, especially during meiosis. Accordingly, HR defects have been correlated with human cancer-prone diseases and various forms of cancer (4).

During DSB repair by HR, Rad51 first binds to single-stranded DNA (ssDNA) derived from the processing of a primary lesion to form a nucleoprotein filament. Once assembled, the filament is capable of conducting a search for a homologous target in double-stranded DNA (dsDNA) and catalyzing invasion of the target to form a DNA joint called the displacement loop (D-loop). Subsequent steps entail extension of the 3′-end of the invading strand by DNA synthesis, followed by resolution of DNA intermediates. However, Rad51 by itself is inefficient in D-loop formation and requires the activity of other proteins to facilitate this process. Rad54 and Rdh54, members of the Swi2/Snf2 family of DNA translocases, have been shown to enhance the efficiency of Rad51-mediated D-loop formation. Importantly, these translocases are also capable of displacing Rad51 from dsDNA, an attribute that is likely important for clearing Rad51 from the nascent D-loop to facilitate repair DNA synthesis and also for preventing the nonspecific accumulation of Rad51 on bulk chromatin (5, 6). Moreover, \textit{in vitro}, both Rad54 and Rdh54 are able to remodel chromatin via nucleosome mobilization in an ATP hydrolysis-dependent manner (7, 8).

Despite their biochemical similarities, there are important differences in the biological functions of Rad54 versus Rdh54. Whereas Rad54 has been proposed to participate in mitotic DSB repair and intrachromosomal recombination, Rdh54 seems to work primarily in interhomolog recombination in both mitotic and meiotic cells. Cytological studies have shown that Rdh54 is normally found at kinetochores and that exposure to DNA damage promotes its recruitment to repair foci in a Rad51- and Rad52-dependent manner (9). Because Rdh54 has a role in resuming cell growth after DSB-induced checkpoint arrest (i.e. adaptation), it has been suggested that Rdh54 facilitates communication between DNA repair and checkpoint control (10).
Yeast two-hybrid and biochemical analyses have shown that both Rad54 and Rdh54 physically and functionally interact with the Rad51 recombinase. Rdh54 has also been found to interact with the meiosis-specific recombinase Dmc1 (1). Although its role in meiosis is not fully understood, Rdh54 is thought to promote colocalization of Rad51 and Dmc1 and to have a role in the dissolution of sister chromatid cohesion to facilitate appropriate interhomolog recombination and chromosome segregation (11–13). Importantly, the translocase function of Rdh54 acts to promote dissociation of Dmc1 from nonrecombinogenic sites on chromatin to allow Dmc1 to work in meiotic recombination (14). Recent studies suggest that Rdh54 has a similar role in preventing sequestration of Rad51 at non-DSB sites in mitotic cells. Indeed, biochemical and cytological analyses have shown that Rdh54 removes Rad51 from dsDNA and chromatin to promote the intracellular recycling of Rad51 and frees the primer end in the nascent D-loop structure to facilitate DNA synthesis initiation (15, 16).

In vitro studies have shown that the Rad51-binding domain of Rdh54 resides within its N terminus and suggested that complex formation is required for promoting efficient D-loop reaction, for its chromatin-remodeling activity, and for Rad51 removal from dsDNA (8, 15).

To better understand the biological significance of the interaction between Rdh54 and Rad51, we generated yeast mutants expressing N-terminal truncations of Rdh54. By genetic analysis, we provide evidence that the N-terminal region of Rdh54 is required to overcome the toxic effect of Rad51 overexpression but is dispensable for the repair of DNA damaged by methyl methanesulfonate (MMS). Furthermore, we demonstrate that the N-terminal domain of Rdh54 is necessary for Rad51 binding in vitro and is required for the removal of Rad51 from dsDNA. Interestingly, a chimeric protein harboring the Rad54 N-terminal region that interacts with Rad51 and the Swi2/Snf2 core domain of Rdh54 is fully active in vitro and in cells. The functional significance of these results is discussed.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains**—All of the yeast strains used in the genetic experiments are isogenic to the W303 RADS5 background (ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 ura3-1).

**Plasmid Constructs**—DNA plasmids for bacterial expression and protein purification were generated by PCR amplification and cloning of wild-type RDH54, rdh54 truncation alleles, and RAD54-RDH54 hybrid sequence into the NcoI and XhoI sites of the pET32a vector (Novagen) to add thioredoxin and His6 tags to the N terminus of each protein. The DNA templates for these PCRs were plasmid pHK489 (full-length RDH54 with its endogenous promoter in vector pRS314) and plasmid pHK471 (the N-terminal 600 bp of RDH54 fused to C-terminal RDH54 with an Ndel linker in between and the endogenous RDH54 promoter into vector pRS414). It should be noted that the RDH54 coding sequence has two apparent ATG start sites, 34 amino acid residues apart. Although the first ATG site was initially thought to be the in vivo start site, it has become apparent that the downstream ATG is used in vivo. Thus, old nomenclature should be corrected by -34 amino acid residues. Here, we used the downstream ATG as residue 1. This becomes important for comparison and discussion of the N-terminal region and its conservation with the human Rad54B sequence.

The forward PCR oligonucleotides used to generate the constructs were as follow: 5'-CTATAGGGAGAGCCACCCATGCACAGATACCGAAATATGAG-3' for full-length wild-type Rdh54, 5'-CATGCCATGCAATAATTACC-3' for the rdh54Δ2 truncation mutant, 5'-CATGCCATGGCAAAAGTACTGTCAACTTTGAAAGA-3' for the rdh54Δ4 truncation mutant, and 5'-CATGCCATGGAATCATGTACAG-3' for the rdh54Δ5 truncation mutant, 5'-CATGCCATGGCATTCAATAATGAAAGCAAAA-3' for the rdh54Δ2 truncation mutant, 5'-CATGCCATGGCATTCAATAATGAAAGCAAAA-3' for the rdh54Δ5 truncation mutant, and 5'-CATGCATGGCAACGCAAGATTACC-3' for the Rad54-Rdh54 hybrid. The reverse PCR primer used for all expression constructs was 5'-GATCCGCTCGAGATAATGGTCTCCGAGAC-3'.

To generate constructs for genetic analyses in yeast, DNA fragments containing the endogenous RDH54 promoter and RDH54 or rAD54 sequences were amplified by PCR and cloned into the CEN TRP1 vector pRS314. The endogenous promoter of RDH54 (1240-bp fragment upstream of the ORF) was amplified using forward primer 5'-TATCCGCTCGAGATCTC-TATTAAACAGGTATCC-3' and reverse primer 5'-CATGCCATGGCATTCAATAATGAAAGCAAAA-3'. Additionally, a SacI restriction site was introduced for the radh54 truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively. The forward PCR primers used to amplify the truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively. The forward PCR primers used to amplify the truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively. The forward PCR primers used to amplify the truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively. The forward PCR primers used to amplify the truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively.

**Functional Interaction between Rdh54 and Rad51**

To generate constructs for genetic analyses in yeast, DNA fragments containing the endogenous RDH54 promoter and RDH54 or rAD54 sequences were amplified by PCR and cloned into the CEN TRP1 vector pRS314. The endogenous promoter of RDH54 (1240-bp fragment upstream of the ORF) was amplified using forward primer 5'-TATCCGCTCGAGATCTC-TATTAAACAGGTATCC-3' and reverse primer 5'-CATGCCATGGCATTCAATAATGAAAGCAAAA-3'. Additionally, a SacI restriction site was introduced for the radh54 truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively. The forward PCR primers used to amplify the truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively. The forward PCR primers used to amplify the truncation alleles, introducing an NcoI site for cloning, were the same ones used to generate the bacterial expression plasmids, and the reverse oligonucleotide used was 5'-TCCCGCGGTTATCATTGTTCTCTTGACATATTACC-3', which add XhoI and NcoI restriction sites, respectively.
SC—Trp solid medium containing different doses of the alkylating agent MMS or the DNA-cross-linking agent cisplatin. Additionally, SC—Trp plates were irradiated with UV light or \( \gamma \)-ionizing radiation using a Gammacell-220 60Co irradiator. Pictures were taken after 3 days of incubation at 30 °C.

**Rad51 Overexpression Assay**—To determine the sensitivities of yeast cells to Rad51 or rad51 K191A overexpression, strains were freshly transformed with pYES-GAL-RAD51, pYES-GAL-rad51 K191A, or the empty vector pYES2 (2 \( \mu \)g) together with the pRS314-based RDH54 constructs. Cells were then grown overnight in liquid SC—Ura—Trp—100 \( \mu \)g/ml creatine kinase) for 10 min at 30 °C. Next, the indicated amounts of Rdh54 and its variants were added to the mixture with a 3-min incubation at 30 °C, followed by the addition of \( \Phi X174 \) ssDNA (150 \( \mu \)m nucleotides) and a 10-min incubation to trap dissociated Rad51. The beads and supernatant were separated using a magnetic particle separator (Roche Applied Science). Bound proteins were eluted from the beads with 20 \( \mu \)l of 2% SDS. The supernatant and SDS eluate (8 \( \mu \)l each) were analyzed by SDS-PAGE and Coomassie Blue staining.

**RESULTS**

**Generation of rdh54 N-terminal Truncation Mutant Constructs**—Biochemical studies have shown that Rdh54 interacts with the Rad51 recombinase and that the binding domain resides within the N terminus (Fig. 1) (15). To obtain genetic evidence that complex formation is required for the roles of Rdh54 in vivo, we cloned N-terminal truncation mutants of RDH54 into a CEN TRP1 vector such that protein expression was driven by the native RDH54 promoter. These mutant alleles encode truncation variants missing 25–200 amino acid residues (Fig. 1).

**The N-terminal Truncations Do Not Affect DNA Damage Repair**—Plasmids encoding the various rdh54 alleles were introduced into an rdh54 null strain. We first examined whether rdh54 mutants encoding truncations of up to 200 amino acid residues show sensitivity to the DNA-alkylating agent MMS, which induces DNA damage that is repaired by...
HR. For comparison, the Rdh54 ATPase-deficient strain rdh54 K318R, which is damage-sensitive, was included. Expression of the truncated proteins was verified by Western analysis (data not shown). As shown in Fig. 2A, rdh54 truncation mutants were just as resistant as cells harboring the RDH54 allele, whereas rdh54 null cells and cells expressing the rdh54 K318R mutant showed sensitivity to high doses of MMS. We also examined the various strains for sensitivity to UV light, ionizing radiation, and cisplatin, but we saw no sensitivity to any of these agents (Fig. 2A). In addition, we replaced the chromosomal RDH54 locus with mutant alleles containing these truncations and obtained the same results with regard to MMS sensitivity (data not shown). Interestingly, none of these truncation mutants was able to interact with Rad51 by yeast two-hybrid assay (data not shown), and consistent with this observation, in vitro pulldown experiments revealed that some of the truncated rdh54 proteins were defective for interaction with Rad51 (see Fig. 5C). Moreover, these truncated rdh54 proteins did not enhance the damage sensitivity of a rad54Δ strain (Fig. 2B).

The N-terminal Domain of Rdh54 Is Required for Resistance to Rad51 Overexpression—Overexpression of Rad51 is detrimental to cell growth in the absence of Rdh54, as Rad51 can associate with undamaged chromatin and form cytotoxic complexes (Fig. 3A) (16). In addition, biochemical studies have shown that an rdh54 protein variant missing the first 99 amino acid residues has a reduced efficiency for Rad51 removal from dsDNA (15). To determine whether Rdh54-Rad51 complex formation is necessary for removal of excess Rad51 bound to chromatin in vivo, we introduced the plasmids that encode the rdh54 truncation mutants into rdh54 null cells together with a GAL1-RAD51 plasmid for Rad51 overexpression. We also overexpressed the ATPase-defective rad51 mutant, rad51 K191A, which possesses reduced DNA-binding affinity and thus is unable to bind chromatin effectively and is less able to induce growth defects. In our experimental setting, overexpression of Rad51 and rad51 K191A occurred only in medium containing galactose. As shown in Fig. 3B, functional interaction between Rdh54 and Rad51 was necessary for the response to Rad51 overexpression. The first 50 amino acid residues of Rdh54 do not seem to be required for this activity, as cells carrying mutant alleles encoding truncation variants lacking 50 or fewer residues did not show cell growth defects upon Rad51 overexpression. On the other hand, rdh54 mutant alleles encoding truncations ranging from 75 to 200 residues of Rdh54 showed the same growth defect as the null mutant (Fig. 3B). Sensitivity to Rad51 overexpression correlated with a defect in interaction with Rad51 (see Fig. 5C).

The N-terminal Domain of Rad54 Confers Resistance in rdh54 Mutant Cells upon Rad51 Overexpression—To better understand the role of the Rad51-binding domain of Rdh54 in the response to Rad51 overexpression, we generated a hybrid construct by replacing the first 200 amino acid residues of Rdh54 with the N-terminal domain of Rad54 (Fig. 1B). Previous studies have shown that similar to Rdh54, the N-terminal domain of Rad54 is responsible for the interaction with Rad51 (17, 18). The Rad54-Rdh54 hybrid protein is functional, as a CEN TRP1 construct that expresses it was able to complement the MMS sensitivity of rdh54 null cells (Fig. 4A). Increased copy of RAD54 (CEN RAD54) did not alter rdh54Δ resistance to MMS. Importantly, the hybrid construct was also able to complement the growth defect observed in the rdh54 truncation mutants after Rad51 overexpression in a manner similar to wild-type RDH54 (Fig. 4B). We obtained similar results by replacing the chromosomal RDH54 gene with the hybrid gene (data not shown).

In Vitro Properties of Truncated and Chimeric Rdh54 Proteins—We decided to further study the truncation variants missing 50 or 75 amino acid residues from the N terminus of Rdh54, as these two mutants showed a difference in the ability to counteract high Rad51 levels. We also examined the chimeric
Rad54-Rdh54 protein, as it behaved as functional Rdh54 in Rad51 overexpression studies. The three variant proteins were expressed in an N-terminally thioredoxin-His<sub>6</sub>-tagged form in <i>E. coli</i> and purified to near homogeneity (Fig. 5A) using the multistep purification procedure that has been described previously (15).

As all three variants possess the seven intact Swi2/Snf2 motifs and ATPase core domain of Rdh54 (Fig. 1), we expected them to have a wild-type level of ATPase activity. In congruence with this premise, the three rdh54 variants exhibited no significant difference in ATP hydrolysis activity compared with the wild-type protein (Fig. 5B). Next, the capacity of the variant proteins to interact with Rad51 was examined by <i>in vitro</i> affinity pulldown. The His<sub>6</sub>-tagged rdh54 variants were first incubated with Rad51 and then with Ni-NTA-agarose beads. Examination of the Rad51 content eluted from the Ni-NTA resin (Fig. 5C) showed that deletion of either 50 or 75 residues reduced Rad51 interaction, with the latter mutant being more strongly impaired in this regard, whereas Rad54-Rdh54 interacted with Rad51 as avidly as full-length Rdh54.

Previously, we showed that Rdh54 is able to disrupt Rad51-dsDNA nucleoprotein filaments (15). ATP hydrolysis by Rdh54 and a strong interaction with Rad51 are required for maximal reaction efficiency (15). Here, we asked whether variations in the N-terminal domain of Rdh54 lead to changes in the robustness of Rad51 removal. We conducted the Rad51 removal assay as described previously (15). Briefly, Rad51-dsDNA filaments were assembled on biotin-dsDNA-streptavidin magnetic beads, followed by incubation with Rdh54 or one of its variants and addition of excess of ssDNA that could sequester dissociated Rad51 molecules in the supernatant (Fig. 5D). Thus, the amount of Rad51 transferred to the supernatant provided an indication of Rad51 removal from dsDNA. The results show that wild-type Rdh54, rdh54<sup>Δ200</sup>, and Rad54-Rdh54 removed Rad51 from the dsDNA magnetic beads with a similar efficiency (Fig. 5D). Interestingly, the rdh54<sup>Δ75</sup> variant had a significantly reduced activity in this regard. Taken together, the results reveal that deletion of 75 residues from the N terminus of Rdh54 severely reduces the interaction between Rdh54 and Rad51 and impairs the ability of Rdh54 to dissociate the Rad51-dsDNA nucleoprotein filament.

**DISCUSSION**

Previous studies have shown a role for Rdh54 in interhomolog recombination and in the regulation of the Dmc1 recombinase during meiosis (1, 11, 19, 20). Similar to other members of the Swi2/Snf2 family of chromatin-remodeling proteins, Rdh54 possesses a DNA translocase activity that is fueled by ATP hydrolysis (21). The meiotic roles of Rdh54 are consistent with its ability to interact with Dmc1 and to translocate on DNA to prevent the accumulation of Dmc1 on chromatin, thus allowing its intracellular recycling for meiotic recombination processes (14). Moreover, Rdh54 promotes Dmc1-mediated DNA strand invasion (22). The translocase activity of Rdh54 is also required to counteract Rad51 that accumulates on chromatins, either from overexpression of Rad51 or from loss of two other translocases, namely, Rad54 and Uls1 (16). Additionally, Rdh54 is required for the adaptation to DSB-induced checkpoint arrest (10). Rdh54 is induced by persistent DNA
The above activities are dependent on the interaction of Rdh54 with Rad51. Biochemical studies have shown that the Rad51 interaction domain of Rdh54 resides within its N terminus and that complex formation is indispensable for its D-loop-promoting activity (8, 15). Maximal efficiency of Rad51 removal from dsDNA is reliant on this N-terminal domain, as a truncation variant of Rdh54 missing the first 99 amino acid residues is significantly impaired in this functional attribute (15). Here, we have defined the Rad51 interaction domain further and asked which activities are dependent on this domain. For this purpose, we generated yeast mutant strains expressing N-terminal truncation variants of Rdh54 of up to 200 residues and tested them in DNA damage repair and the response to Rad51 overexpression. We have provided evidence that the N-terminal region of Rdh54 is required for an appropriate response to excess Rad51 but is actually dispensable for the response to MMS damage. As the deletion mutant and the ATPase-defective mutant are sensitive to MMS damage, whereas the N-terminal truncation mutants are not sensitive, this suggests that ATPase activity is necessary for a full response to MMS damage. This conclusion correlates with our measures of ATPase activity, which are unchanged in the N-terminal truncation mutants (Fig. 5).

Mutant rdh54 proteins with up to 50 of the N-terminal residues deleted retained significant Rad51 interaction in vitro and were able to counteract the deleterious effects of Rad51 overexpression and the formation of Rad51-dsDNA complexes. Deletions removing 75 or more residues of the N-terminal region rendered cells sensitive to Rad51 overexpression and impaired the ability of Rdh54 to dissociate Rad51-dsDNA complexes. The interaction with Rad51 mediated via the N-terminal region of Rdh54 is thus crucial for the ability of Rdh54 to remove Rad51 from undamaged chromatin. It remains to be established whether these truncation mutants are also unable to bind Dmc1.

FIGURE 5. Rad51 binding and Rad51 removal activities of purified rdh54 variants. A, purified wild-type Rdh54, rdh54Δ50, rdh54Δ75, and Rad54-Rdh54 (R-rdh54), 1 µg each, were analyzed by SDS-PAGE and Coomassie Blue staining. M, molecular mass markers. B, ATP hydrolysis by Rdh54 and the rdh54 variants was examined. C, pulldown assays to examine the interaction between rdh54 variants and Rad51. After mixing Rad51 with His6-tagged Rdh54 or its variants, protein complexes were captured with Ni-NTA-agarose. The supernatant (S), wash (W), and SDS eluate (E) were analyzed by SDS-PAGE and Coomassie Blue staining. % Rad51 indicates the percentage of bound Rad51. D, panel i, a schematic of the protocol to examine Rad51 removal from dsDNA is shown. Panel ii, Rad51-dsDNA magnetic beads were incubated with 30, 60, and 120 nm wild-type Rdh54 (lanes 2–4), rdh54Δ50 (lanes 5–7), rdh54Δ75 (lanes 8–10), and Rad54-Rdh54 (lanes 11–13), and the supernatant and bead fractions were analyzed by SDS-PAGE and Coomassie Blue staining. The results were quantified and plotted. The amount of Rad51 that was spontaneously released from the beads (lane 1) has been subtracted from lanes 2–13.

CK, creatine kinase used in the reaction buffer as part of the ATP-regenerating system.
Previously, we purified and characterized two truncation mutants, rdh54Δ68 and rdh54Δ99 (15). The results showed that the rdh54Δ68 variant is slightly impaired inRad51 interaction and the promotion of D-loop formation but is proficient inRad51 removal from dsDNA. In contrast, the rdh54Δ99 variantis quite defective inRad51 interaction and D-loop reaction andis partially impaired inRad51 removal (15). These observationsaresufficient with the results we report here. Regarding therole ofRdh54 in DNA damage repair, it is possible that theinteraction region for Rad51 bound to dsDNA in a recombina-
tion intermediate is distinct from the interaction domainofRad51 bound to chromatin or that the role ofRdh54 in damage
can be constructed by fusing motor and interaction factors(23). The fact that the Rdh54 N terminus isrequired for D-loop formation with chromatinized DNA (8)yet is dispensable for repair of MMS damage further reinforcethe idea that the Rdh54 function in MMS damage repair isnot directly through HR.

Rdh54 has also been shown to physically interact with Rad51and to promote D-loop formation via its N-terminal domain(18). As Rad54 and Rdh54 share no homology at their N ter-
mini, the substitution of the Rad54 N-terminal region in placeof the Rdh54 N terminus can give clues as to the function of thisdomain in vivo. This is particularly interesting, as this domainappears to be conserved among Rdh54 orthologs (24). Our data
show that aRAD54-RDH54 hybrid construct is able to comple-
ment the sensitivity to MMS and Rad51 overexpression of an
rdh54Δ mutant strain. The in vitro data presented here also
demonstrates the targeting of the Rdh54 translocase domainby the Rad54 N-terminal domain in the dissociation of Rad51-
dsDNA complexes.

In conclusion, the results from our study have unexpectedlyrevealed a differential requirement for Rad51 interaction inthe abilities of Rdh54 to function as a Rad51-dsDNA dissociative
motor and in chromatid damage repair. Moreover, these results
show that a functional Rdh54 protein can be constructed by fusingthe Rad54 N terminus to the Swi2/Snf2 core of Rdh54.

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