DOI: 10.1534/genetics.104.030551

Note

A Novel Yeast Mutation, *rad52-L89F*, Causes a Specific Defect in Rad51-Independent Recombination That Correlates With a Reduced Ability of Rad52-L89F to Interact With Rad59

Felipe Cortés-Ledesma, Francisco Malagón[1] and Andrés Aguilera[2]

Departamento de Genética, Facultad de Biología, Universidad de Sevilla, 41012 Sevilla, Spain

Manuscript received April 26, 2004
Accepted for publication May 17, 2004

ABSTRACT

We isolated a novel *rad52* mutation, *rad52-L89F*, which specifically impairs recombination in *rad51*Δ cells. *rad52-L89F* displays phenotypes similar to *rad59*Δ and encodes a mutant protein impaired in its ability to interact with Rad59. These results support the idea that Rad59 acts in homologous recombination via physical interaction with Rad52.

RAD52 is the only gene required for virtually all homologous recombination events in *Saccharomyces cerevisiae*. Null mutations in this gene display the most severe phenotype in many different recombination assays (Paques and Haber 1999). The Rad52 protein shows DNA-binding and strand-annealing activities *in vitro* (Mortensen *et al.* 1996). In addition, Rad52 physically interacts with both the RecA ortholog Rad51 and Rad59 (Symington 2002).

In contrast to *RAD52*, *RAD51* is required for allelic recombination but not for recombination between DNA repeats (Prado *et al.* 2003). One-ended recombination events are *RAD51* dependent (Davis and Symington 2004), but also occur in the absence of Rad51 (Malkova *et al.* 1996). Thus, in the absence of Rad51, recombination may occur by single-strand annealing (SSA), which leads to deletions between direct repeats (Lin *et al.* 1984; Paques and Haber 1999) or break-induced replication, which could give rise to inversions between inverted repeats if followed by SSA (Bartsch *et al.* 2000; Malagon and Aguilera 2001).

Rad59 is homologous to the amino-terminal half of Rad52 and shares several *in vitro* activities with Rad52, such as DNA binding and strand annealing (Petukhova *et al.* 1999; Davis and Symington 2001). It plays an important role in recombination occurring in the absence of Rad51. Thus, *rad59*Δ mutants present only a slight decrease in inverted-repeat recombination, whereas *rad51*Δ *rad59*Δ double mutants show a strong decrease similar to *rad52*Δ (Bai and Symington 1996).

Isolation of the new *rad52-L89F* mutation: To understand recombination occurring in the absence of Rad51, we searched for mutants with reduced recombination levels in *rad51*Δ background. To facilitate the search we used a *rad51*Δ *spt6-140* double mutant, which shows high levels of *RAD51*-independent recombination (Malagon and Aguilera 2001). UV-irradiated cells carrying the chromosomic *his3$^{\beta}$::INV* inverted repeat system (Aguilera and Klein 1988) were screened for low levels of His^{+} recombinants. This led to the identification of a new *rad52* allele. Sequence analysis showed that the mutant allele carried a single T-to-C substitution at position 165, which results in a Leu-to-Phe change in residue 89 (see Figure 1). This residue is located in the amino terminus of Rad52, which is the most conserved part of the protein, in a domain described as being necessary for DNA binding, self-association, and Rad59 interaction (Symington 2002). Interestingly, the *rad52-1* mutation, which confers a *rad52* null phenotype, is at position 90 (Adzuma *et al.* 1984). The new mutant allele was named *rad52-L89F*.

Homologous recombination in *rad52-L89F*: To understand the types of homologous recombination impaired by *rad52-L89F*, we determined the effect of *rad52-L89F* on the frequency of recombination of the *his3$^{\beta}$::INV* system in different *rad* backgrounds (Figure 2A). Recombination frequencies were reduced only 10- and 2-fold below wild-type levels in *rad51*Δ and *rad59*Δ cells, respectively, but 40-fold in the double *rad51*Δ *rad59*Δ, consistent with previous reports (Bai and Symington 1996; Shinohara *et al.* 1998; Malagon and Aguilera

[1]*Present address:* Frederick Cancer Research Center, National Institutes of Health, Frederick, MD 21702-1201.

[2]*Corresponding author:* Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Avd. Reina Mercedes 6, 41012 Sevilla, Spain. E-mail: aguilo@us.es

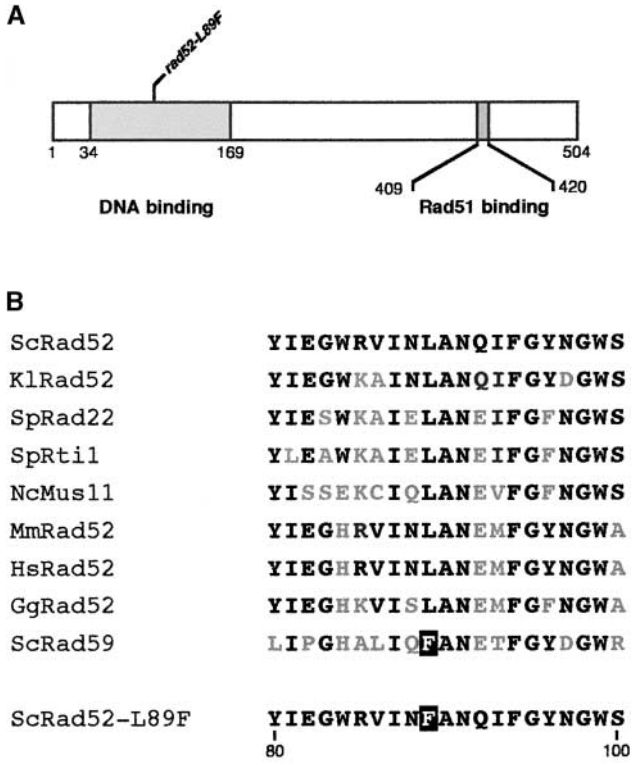

FIGURE 1.—Primary structure of the Rad52-L89F protein. (A) Functional domains of Rad52 and localization of the *rad52-L89F* mutation. (B) Comparative alignment of Rad52 and Rad59 orthologs (Sc, *S. cerevisiae*; Kl, *Kluyveromyces lactis*; Sp, *Schizosaccharomyces pombe*; Nc, *Neurospora crassa*; Gg, chicken; Mm, mouse; Hs, human) and Rad52-L89F. Residues identical to those of ScRad52 are shown in black and the single substitution of Rad52-L89F is indicated as a solid box. The *rad52-L89F* mutant was obtained from the hyperrecombinant strain M137-11Ar51k (*MAT*α *ade2 can1-100 his3$^{\beta}$::INV leu2 lys2-128*α *rad51*Δ*::KanMX4 spt6-140 trp1 ura3*) by UV mutagenesis. Cells (50 μl) grown in YEPD to an OD_{660nm} of 0.9 were diluted in 6 ml of water and poured into a glass petri dish for irradiation with 45 J/m^2 of UV light (λ = 254 nm). After 5 hr of recovery in YEPD in the dark, cells were plated with the appropriate dilutions. Cell survival was 30% and 10,600 clones were screened for low His$^+$ recombination.

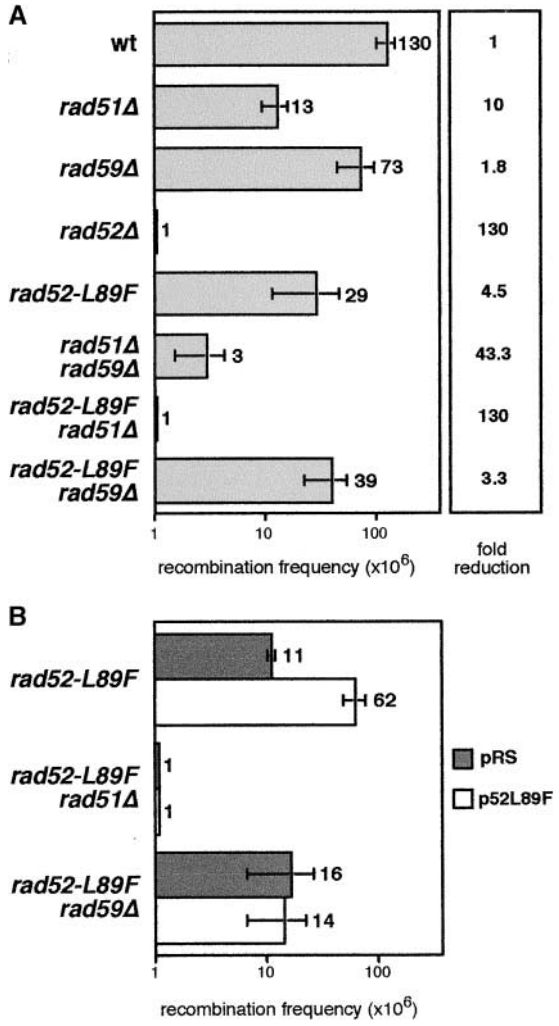

FIGURE 2.—Recombination frequencies of the chromosomal inverted repeat system *his3$^{\beta}$::INV* in different *rad* mutants. (A) Recombination in wild-type, *rad51*Δ, *rad59*Δ, *rad52*Δ, *rad52-L89F*, *rad51*Δ *rad59*Δ, *rad52-L89F rad51*Δ, and *rad52-L89F rad59*Δ strains. (B) Recombination in *rad52-L89F*, *rad52-L89F rad51*Δ, and *rad52-L89F rad59*Δ mutants carrying either pRS424 (pRS, negative control) or pRS424-rad52L89F (p52L89F, overexpressing rad52-L89F) plasmids. Recombination frequencies were determined as the median frequency of six independent colonies isolated from YEPD at 30° (PRADO *et al.* 1997). Frequencies are the average and standard deviation (shown in bars) of two to four median values. The *rad52-L89F* mutation was transferred to other genetic backgrounds by genetic crosses. All *rad*Δ strains used for the recombination assays were derived from M137-11A (*MAT*α *ade2 can1-100 his3$^{\beta}$::INV leu2 lys2-128*α *rad51*Δ*::KanMX4 trp1 ura3*) and were constructed by transformation with the corresponding deletion cassettes or by genetic crosses (MALAGON and AGUILERA 2001).

2001). Nevertheless, whereas in *rad52*Δ cells the reduction was >100-fold, *rad52-L89F* shows, as do *rad51*Δ and *rad59*Δ, only a slight decrease (<5-fold). Interestingly, *rad52-L89F rad51*Δ mutants showed a synergistic decrease and had the same recombination levels as *rad52*Δ. In contrast, deletion of *RAD59* had no effect in *rad52-L89F*.

To determine whether the effect of *rad52-L89F* on recombination was due to a leaky activity of Rad52-L89F, we wondered if its overexpression could reestablish wild-type recombination. As can be seen in Figure 2B, multicopy *rad52-L89F* partially suppressed the recombination defect of *rad52-L89F* up to levels of *rad59*Δ, but had no effect in *rad59*Δ or *rad51*Δ backgrounds (Figure 2B). Therefore, *rad52-L89F* causes the same recombination phenotype as *rad59*Δ, regardless of a putative leakiness of the Rad52-L89F activity.

The recombination phenotypes of *rad52-L89F* are indeed similar to those of the previously characterized *rad52-R70K* allele in *RAD* and *rad51*Δ backgrounds, although they differ when Rad59 is not present (BAI *et al.* 1999). This suggests that a Rad52 amino-terminal domain covering at least the residues from 70 to 89 is essential for recombination in the absence of Rad51. Interestingly, both residues 89 and 70 are conserved in

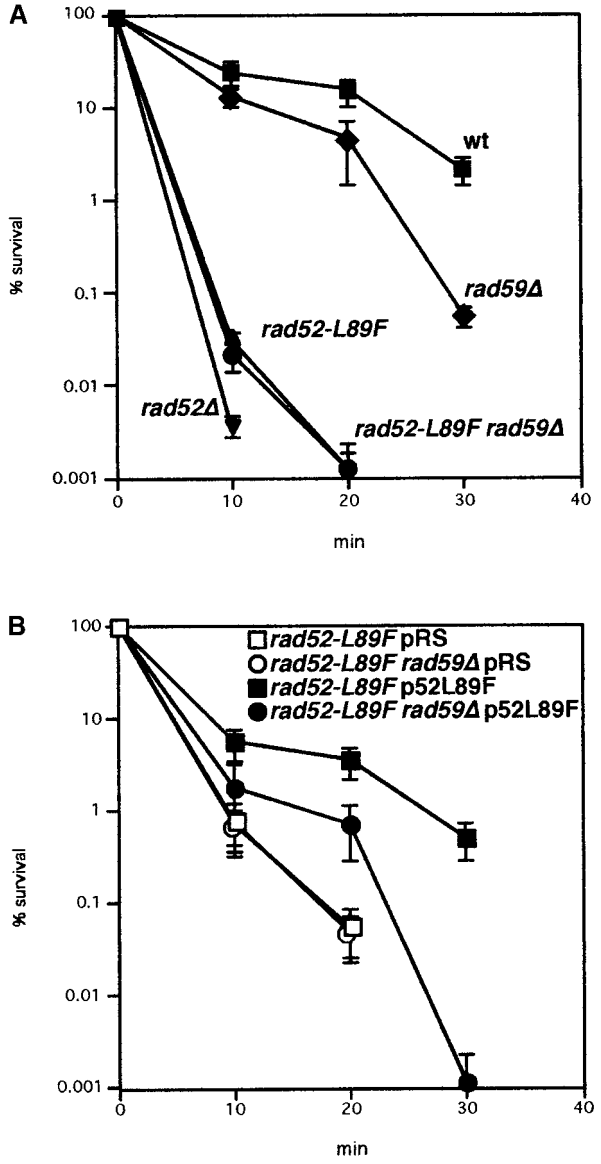

FIGURE 3.—MMS sensitivity of different *rad* strains. (A) Sensitivity of wild-type, *rad59*Δ, *rad52*Δ, *rad52-L89F*, and *rad52-L89F rad59*Δ mutants. (B) Sensitivity of *rad52-L89F* and *rad52-L89F rad59*Δ mutants carrying either pRS424 (pRS, negative control) or pRS424-rad52L89F (p52L89F, overexpressing rad52-L89F) plasmids. Cells were exposed to 0.5% MMS for 0, 10, 20, and 30 min before plating onto YEPD. For each strain, the percentage of survival is referred to the value of cells not exposed to MMS (0 min), taken as 100%. Data are the average and standard deviation (shown in bars) of two to four independent experiments.

all known Rad52 orthologs and the L89F and R70K changes make the terminal domain of the mutant Rad52 proteins more similar to Rad59 (Figure 1B).

Repair of MMS damage in *rad52-L89F*: To further characterize genetic interaction between *rad52-L89F* and *RAD59*, methyl methanesulfonate (MMS) sensitivity was determined by cell survival after different time exposures to 0.5% MMS. The *rad52-L89F* mutant showed a weaker MMS sensitivity than *rad52*Δ, but much stronger than *rad59*Δ (Figure 3A), in contrast to its low recombination defect. Nevertheless, as in recombination, *rad52-L89F* sensitivity was not affected in *rad59*Δ background. Therefore, these data support the idea that *rad52-L89F* behaves like *rad59*Δ. In any case, Rad52-L89F was leaky, as suggested from the observation that overexpression of Rad52-L89F enhanced MMS resistance of *rad52-L89F* strains (Figure 3B).

Rad52-L89F is affected in its ability to interact with Rad59: The similarity of phenotypes between *rad59*Δ and *rad52-L89F* could be explained if in the *rad52-L89F* mutant the levels of Rad59 protein were reduced, as reported for *rad52*Δ mutants (DAVIS and SYMINGTON 2001). Nevertheless, this was not the case because the levels of Rad59 protein in *rad52-L89F* were similar to those of wild-type cells (Figure 4A).

We tested the possibility that Rad52-L89F was impaired in its ability to interact with Rad59. For this purpose, we purified Rad59 fused to the glutathione *S*-transferase (Rad59::GST) from wild-type, *rad52*Δ, and *rad52-L89F* strains overexpressing the GST-fusion protein. Rad59::GST is functional, as it rescues the MMS sensitivity of *rad59*Δ. As negative control we purified GST from a wild-type strain overexpressing GST. As can be seen in Figure 4B, Rad52-L89F protein was present in cell extracts at levels lower than those of Rad52. Indeed, other missense mutations in the amino terminus reduce the levels of Rad52 (ASLESON and LIVINGSTON 2003). As expected, wild-type Rad52 copurified with Rad59::GST, but not with GST. However, no Rad52-L89F protein was detected in the purified Rad59::GST fraction. Since Rad52-L89F protein levels are reduced, we cannot rule out the possibility of a nondetected weak interaction with Rad59 even though our results suggest that Rad52-L89F is affected in its ability to interact with Rad59.

Biological significance of the *in vivo* Rad52-Rad59 interaction: Both human and yeast Rad52 proteins form multimeric ring structures (SHINOHARA *et al.* 1998; STASIAK *et al.* 2000; RANATUNGA *et al.* 2001), and Rad59 has also been reported to self-associate (DAVIS and SYMINGTON 2003). It would be interesting to know whether Rad52 and Rad59 could form heteromeric ring structures (SYMINGTON 2002). This is supported by the fact that the Rad52 regions necessary and sufficient for self-interaction and Rad59 binding coincide (DAVIS and SYMINGTON 2003).

Our study confirms that the amino terminus of Rad52 is important for its interaction with Rad59. The reduced ability of Rad52-L89F to interact with Rad59 could at least partially explain the *rad59*Δ-like recombination phenotype of the *rad52-L89F* mutant. It could cause a reduction of the presence of Rad59 at recombination centers, leading to a *rad59* phenocopy. As Rad59 is essential in recombination occurring in the absence of Rad51-dependent strand exchange, this would explain why the recombination phenotype of *rad52-L89F* is specifically observed in a *rad51* background.

The MMS sensitivity of *rad52-L89F* is much more severe than its recombination defect. Other mutations in

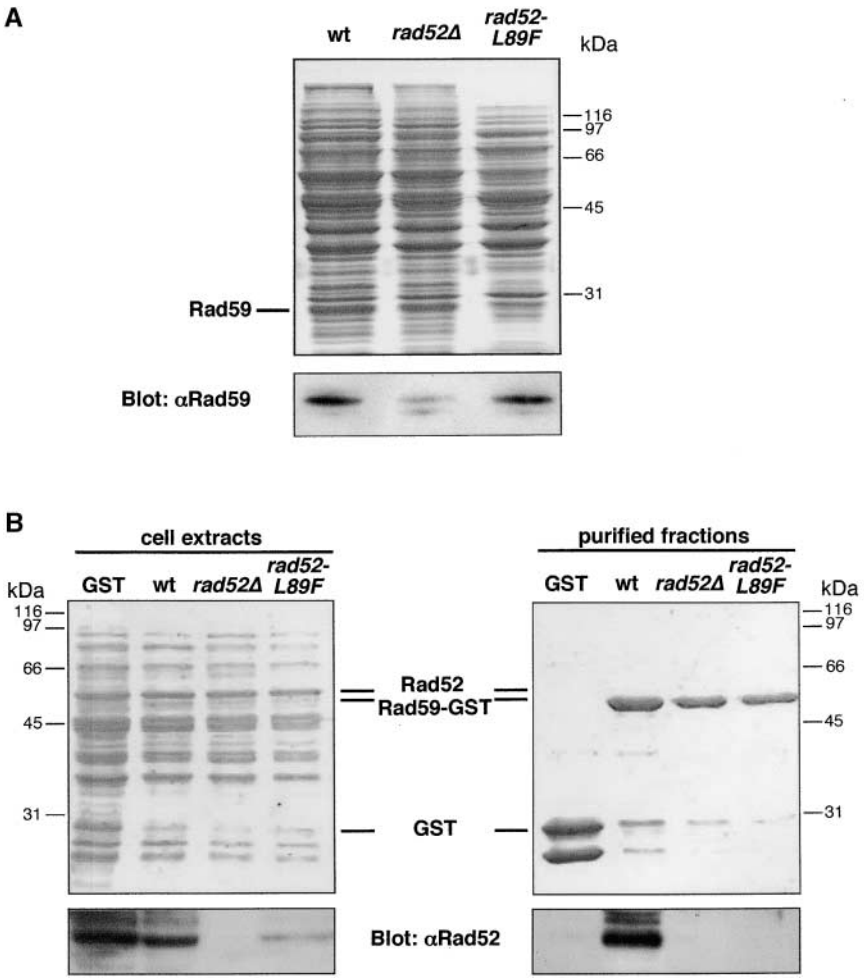

FIGURE 4.—Rad52-L89F-Rad59 interaction. (A) SDS-PAGE analysis of Rad59 protein in wild-type, *rad52*Δ, and *rad52-L89F* strains. Total protein extract (5 μg) was loaded for each strain. Coomassie staining (top) and Western blot using αRad59 polyclonal antibody (bottom) are shown. The position in the gel at which Rad59 migrates is indicated. (B) Purification of Rad59-GST fusion protein in wild-type, *rad52*Δ, and *rad52-L89F* strains. Purification of GST in the wild type was included as a negative control. Protein expression was under the control of the *CUP1* promoter and was induced by addition of $CuSO_4$ to a final concentration of 0.5 mM. Coomassie staining (top) and Western blot using αRad52 polyclonal antibody (bottom) of total cell extracts (left) and purified fractions (right) are shown. The positions in the gel at which Rad59-GST, Rad52, and GST migrate are indicated. For the preparation of extracts, cells were grown in 500 ml of SC-Leu to an OD_{660nm} of 0.8. Cells were harvested, washed with water, and resuspended in one pellet volume of 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 4 mM $MgCl_2$, 5 mM DTT, 10% glycerol, 1 M NaCl. Leupeptin and pepstatin A were added to a final concentration of 2 μg/ml and 1 μg/ml, respectively. Extracts were made with glass beads (McCRAITH and PHIZICKY 1990), followed by supplementation with 1 mM PMSF and 30 min centrifugation at 14,000 rpm in a JA-20 Beckman rotor. For Rad59::GST protein purification, supernatant was incubated with 1/100 volume of glutathione-Sepharose 4B (A. P. Biotech) previously equilibrated in 50 mM potassium phosphate pH 7.2, 1 mM DTT, 0.5 mM EDTA, 10% glycerol, 1% Triton X-100, 1 M NaCl. Samples were washed twice with 500 bead volume of 50 mM potassium phosphate pH 7.2, 1 mM DTT, 0.5 mM EDTA, 10% glycerol, 1% Triton X-100, 0.5 M NaCl. Proteins were eluted by boiling in 1× loading buffer for 5 min.

RAD52 and other *RAD* genes have been reported to separate recombinational and DNA repair phenotypes (MORTENSEN *et al.* 2002; SYMINGTON 2002). In our case, the lower amount of stable Rad52-L89F protein present in the cell (Figure 4B) is sufficient for spontaneous recombination, but not for the repair of MMS-induced damage. Consistently, overexpression of Rad52-L89F significantly suppresses the MMS sensitivity phenotype (Figure 3B).

Concluding remarks: A novel *rad52* mutation (*rad52-L89F*), identified by its specific effect in recombination occurring in the absence of Rad51, encodes a mutant Rad52 protein impaired in its ability to interact with Rad59. This, together with the strong similarity of recombination and repair phenotypes of *rad52-L89F* and *rad59*Δ, suggests that Rad59-Rad52 interaction is essential for the role of Rad59 in recombination in the absence of Rad51-mediated strand exchange.

We are grateful to L. Symington for yeast strain gifts, P. Sung and W. Heyer for antibodies, F. Prado for critical reading of the manuscript, and D. Haun for style supervision. This work was supported by grants from the Spanish Ministry of Science and Technology (BMC2000-0439 and SAF2003-00204) and the Regional Government of Andalusia (CVI0102). F.C.-L. was the recipient of predoctoral training grants from the Spanish Ministry of Education and Ministry of Health.

LITERATURE CITED

ADZUMA, K., T. OGAWA and H. OGAWA, 1984 Primary structure of the *RAD52* gene in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:** 2735–2744.

AGUILERA, A., and H. L. KLEIN, 1988 Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. Isolation and genetic characterization of hyper-recombination mutations. Genetics **119:** 779–790.

ASLESON, E. N., and D. M. LIVINGSTON, 2003 Investigation of the stability of yeast *rad52* mutant proteins uncovers post-translational and transcriptional regulation of Rad52p. Genetics **163:** 91–101.

BAI, Y., and L. S. SYMINGTON, 1996 A Rad52 homolog is required for *RAD51*-independent mitotic recombination in *Saccharomyces cerevisiae*. Genes Dev. **10:** 2025–2037.

BAI, Y., A. P. DAVIS and L. S. SYMINGTON, 1999 A novel allele of *RAD52* that causes severe DNA repair and recombination deficiencies only in the absence of *RAD51* or *RAD59*. Genetics **153:** 1117–1130.

Bartsch, S., L. E. Kang and L. S. Symington, 2000 *RAD51* is required for the repair of plasmid double-stranded DNA gaps from either plasmid or chromosomal templates. Mol. Cell. Biol. **20:** 1194–1205.

Davis, A. P., and L. S. Symington, 2001 The yeast recombinational repair protein Rad59 interacts with Rad52 and stimulates single-strand annealing. Genetics **159:** 515–525.

Davis, A. P., and L. S. Symington, 2003 The Rad52-Rad59 complex interacts with Rad51 and replication protein A. DNA Repair **2:** 1127–1134.

Davis, A. P., and L. S. Symington, 2004 *RAD51*-dependent break-induced replication in yeast. Mol. Cell. Biol. **24:** 2344–2351.

Lin, F. L., K. Sperle and N. Sternberg, 1984 Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:** 1020–1034.

Malagon, F., and A. Aguilera, 2001 Yeast *spt6-140* mutation, affecting chromatin and transcription, preferentially increases recombination in which Rad51p-mediated strand exchange is dispensable. Genetics **158:** 597–611.

Malkova, A., E. L. Ivanov and J. E. Haber, 1996 Double-strand break repair in the absence of *RAD51* in yeast: a possible role for break-induced DNA replication. Proc. Natl. Acad. Sci. USA **93:** 7131–7136.

McCraith, S. M., and E. M. Phizicky, 1990 A highly specific phosphatase from *Saccharomyces cerevisiae* implicated in tRNA splicing. Mol. Cell. Biol. **10:** 1049–1055.

Mortensen, U. H., C. Bendixen, I. Sunjevaric and R. Rothstein, 1996 DNA strand annealing is promoted by the yeast Rad52 protein. Proc. Natl. Acad. Sci. USA **93:** 10729–10734.

Mortensen, U. H., N. Erdeniz, Q. Feng and R. Rothstein, 2002 A molecular genetic dissection of the evolutionarily conserved N terminus of yeast Rad52. Genetics **161:** 549–562.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Petukhova, G., S. A. Stratton and P. Sung, 1999 Single strand DNA binding and annealing activities in the yeast recombination factor Rad59. J. Biol. Chem. **274:** 33839–33842.

Prado, F., J. I. Piruat and A. Aguilera, 1997 Recombination between DNA repeats in yeast *hpr1*Δ cells is linked to transcription elongation. EMBO J. **16:** 2826–2835.

Prado, F., F. Cortes-Ledesma, P. Huertas and A. Aguilera, 2003 Mitotic recombination in *Saccharomyces cerevisiae*. Curr. Genet. **42:** 185–198.

Ranatunga, W., D. Jackson, J. A. Lloyd, A. L. Forget, K. L. Knight *et al.*, 2001 Human *RAD52* exhibits two modes of self-association. J. Biol. Chem. **276:** 15876–15880.

Shinohara, A., M. Shinohara, T. Ohta, S. Matsuda and T. Ogawa, 1998 Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. Genes Cells **3:** 145–156.

Stasiak, A. Z., E. Larquet, A. Stasiak, S. Muller, A. Engel *et al.*, 2000 The human Rad52 protein exists as a heptameric ring. Curr. Biol. **10:** 337–340.

Symington, L. S., 2002 Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. **66:** 630–670.

Communicating editor: L. S. Symington