A Core Activity Associated with the N Terminus of the Yeast *RAD52* Protein Is Revealed by *RAD51* Overexpression Suppression of C-Terminal *rad52* Truncation Alleles

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ABSTRACT

C-terminal *rad52* truncation and internal deletion mutants were characterized for their ability to repair MMS-induced double-strand breaks and to produce viable spores during meiosis. The *rad52-Δ251* allele, encoding the N-terminal 251 amino acids of the predicted 504-amino-acid polypeptide, supports partial activity for both functions. Furthermore, *RAD51* overexpression completely suppresses the MMS sensitivity of a *rad52-Δ251* mutant. The absence of the C terminus in the truncated protein makes it likely that suppression occurs by bypassing the C-terminal functions of Rad52p. *RAD51* overexpression does not suppress the low level of spore viability that the *rad52-Δ251* allele causes and only partially suppresses the defect in *rad52* alleles encoding the N-terminal 292 or 327 amino acids. The results of this study also show that intragenic complementation between *rad52* alleles is governed by a complex relationship that depends heavily on the two alleles involved and their relative dosage. In heteroallelic *rad52* diploids, the *rad52-Δ251* allele does not complement *rad52* missense mutations altering residues 61 or 64 in the N terminus. However, complementation is achieved with each of these missense alleles when the *rad52-Δ251* allele is overexpressed. Complementation also occurs between *rad52-Δ327* and an internal deletion allele missing residues 210 through 327. We suggest that the first 251 amino acids of Rad52p constitute a core domain that provides critical *RAD52* activities.

RAD52 plays a critical role in recombinational repair of double-strand breaks in yeast. Previous work on *RAD52* suggests that it may have multiple functions in this process. Yeast Rad52p has been found to bind DNA and to promote annealing of complementary single-stranded DNA (Mortensen *et al.* 1996). Other work has detected binding between Rad52p and the strand exchange protein, Rad51p (Milne and Weaver 1993; Sung 1997), as well as the heterotrimeric single-stranded DNA binding protein, replication protein A (RPA; Firmenich *et al.* 1995; Hays *et al.* 1998). Studies using the human Rad52p homolog, hRad52p, show that it binds to itself (Shen *et al.* 1996) suggesting that the yeast protein may also self-associate and function as a dimer or as a larger aggregate. Besides the identification of these interactions, recent *in vitro* studies have shown that Rad52p works with RPA to promote Rad51p-mediated strand exchange (Sung 1997; New *et al.* 1998; Shinohara and Ogawa 1998), and similar results have been obtained with the human Rad52p homolog (Benson *et al.* 1998). More recent studies on human Rad52p demonstrate that the protein forms a multimeric structure (Shinohara *et al.* 1998; Van Dyck *et al.* 1998; Passy *et al.* 1999) that binds to the ends of a DNA double-strand break (Van Dyck *et al.* 1999). This indicates that Rad52p may be required to mediate access of recombinational repair proteins to the site of double-strand breaks. Still unknown however are the roles that the domains in Rad52p have in integrating the functions of Rad51p and RPA during double-strand break repair.

Our laboratory has created *rad52* mutations and has characterized their effects on mitosis and meiosis. One study from our laboratory examined two C-terminal truncation alleles, *rad52-Δ327* and *rad52-Δ169* (Boundy-Mills and Livingston 1993). The *rad52-Δ327* allele encodes the first 327 amino acids of the predicted 504-amino-acid Rad52p polypeptide, and it supports partial double-strand break repair activity. The *rad52-Δ169* allele contains the first 167 amino acids of Rad52p, and mutants carrying this allele behave in a manner similar to that of a mutant with a deletion of the coding region. Characterization of the *rad52-Δ327* allele also found that the intact N-terminal end of Rad52p provided by this truncation allele could be fully complemented by providing a second allele with a wild-type C-terminal end like the N-terminal missense mutation *rad52-2* (P64L). Because a *rad52-Δ169*/*rad52-2* diploid did not exhibit intragenic complementation, this study defined a region between residues 169 and 327 needed for complementation. Further examination demonstrated that the *rad52-Δ327* allele could be suppressed by overexpression of

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RAD51 while the *rad52-Δ169* allele could not (Milne and Weaver 1993; Kaytor and Livingston 1996). The results of the intragenic complementation and *RAD51* suppression studies are indicative of the partial activity of the *rad52-Δ327* peptide that is no longer present in the *rad52-Δ169* peptide. This suggests that a region between amino acids 169 and 327 is vital to Rad52p function.

To better understand these phenomena, a set of *rad52* alleles with C-terminal truncations and in-frame deletions that eliminate portions of the region between residues 169 and 327 have been created to answer three questions. First, how much of the N terminus is required for partial activity? Second, are there domains in Rad52p that must be intact for *RAD51* overexpression to effectively suppress mutant *rad52* alleles? Third, what domains of Rad52p must be on the same polypeptide to achieve intragenic complementation between two mutant *rad52* alleles?

MATERIALS AND METHODS

Yeast strains and growth conditions: All yeast strains in this study are isogenic with SSL204 (Dornfeld and Livingston 1991) and are listed in Table 1. Cells were grown on YPD agar plates and in YM-1 liquid medium or, for strains carrying plasmids, on Ura omission plates and in SC-Ura liquid medium.

MMS survival: Sensitivity to methyl methanesulfonate (MMS)-induced double-strand breaks was measured by the method of Prakash and Prakash (1977). Cells were grown at 30° to saturation and treated with 0.5% MMS at room temperature for varying lengths of time. Viability was determined by plating aliquots of cells onto YPD or Ura omission plates and incubating them at either 30° or 33°. The Ura omission medium was used for *RAD51* overexpression studies when strains were transformed with the 2μ vector YEp24 or YEp24-*RAD51*, or for intragenic complementation studies when *rad52* alleles were overexpressed from the 2μ vector pRS426 (Christianson *et al.* 1992).

Spore viability: Diploid cells were patched onto YPD plates and then replica plated to 1% potassium acetate plates supplemented with required amino acids and nucleic acid bases and incubated at 30°. After 3–5 days tetrads were dissected on YPD plates and allowed to germinate at 30°. Spore colonies were counted after 3 days.

Construction of *rad52* alleles: C-terminal truncation alleles were constructed by using PCR to introduce a stop codon and a *Bam*HI site at specific positions in *RAD52*. Fragments of *RAD52* were amplified using primer Rad52-2 (5′-ggctttggtgtgttgttgat-3′) with primer Rad52-1597s (5′-aggatcctaatccgttggcctaaacaaa-3′), Rad52-1721s (5′-aggatcctacaggttcttcgtcgagtcg-3′), or Rad52-1840s (5′-aggatcctaatcctgtttggaagcatcg-3′). Conditions for PCR were 94° for 4 min followed by 30 cycles of 1 min at 94°, 1 min at 57°, and 2.5 min at 72°. This was followed by a 4-min incubation at 72°. The resulting fragments were subcloned into YIp5-*RAD52* using restriction endonuclease digestion by *Bst*EII and *Bam*HI. New *rad52* alleles were introduced into strains SSL204 and SSL204A (Dornfeld and Livingston 1991) or SSL231 (Kaytor and Livingston 1994) by the two-step gene replacement method (Scherer and Davis 1979). The presence of the deletions was confirmed by PCR using primer pairs Rad52-2 and Rad52-10 (5′-gaatatgcttggacgtagtctg-3′) or Rad52-1 (5′-cccgttagtgattctcgatg-3′) and Rad52-8 (5′-tttcggccaggaagcgtttc-3′).

The internal deletion *rad52-iΔ210B* was constructed by using primers Rad52-1 (5′-cccgttagtgattctcgatg-3′) and Rad52-1597 (5′-aggatccgttggcctaaacaaattg-3′) to amplify a fragment that was subcloned into *RAD52* using the *Bst*EII and *Bam*HI sites. This fused amino acid 210 to downstream sequences at the *Bam*HI site. *rad52-iΔ251B* and *rad52-iΔ292B* were constructed in the same manner except that primers Rad52-1721 (5′-aggatccaggttcttcgtcgagtcgg-3′) and Rad52-1840 (5′-aggatcctgtttggaagcatcgag-3′) were used.

The *rad52-2:iΔ210B* allele contains two lesions, the *rad52-2* missense mutation and the in-frame deletion from the *rad52-iΔ210B* allele. It was constructed by subcloning the *Bgl*II-*Sal*I fragment from *rad52-iΔ210B* into *rad52-2* carried in the vector YCp50.

rad52-96A is a missense mutation that was recovered from a library of mutagenized *rad52* plasmids (Kaytor and Livingston 1994). The causative mutation in this allele was localized and found to be an amino acid substitution of glycine for arginine 235.

Several *rad52* alleles were maintained on either the CEN plasmid, YCp50, or on the 2μ plasmid pRS426 (Christianson *et al.* 1992). *rad52* alleles were overexpressed by subcloning *Eco*RI-*Sal*I fragments from YCp50 plasmids containing *rad52-2*, *rad52-301A*, *rad52-Δ327*, *rad52-Δ292*, and *rad52-Δ251* into pRS426.

Visualization of mutant *rad52* proteins: To place an epitope tag at the C terminus of Rad52p, the stop codon and adjacent sequence were mutagenized to extend the coding sequence to include a *Not*I restriction site. The sequence bearing three copies of the epitope for hemagglutinin (HA1; Tyers *et al.* 1992, 1993) was cloned into the *Not*I site. The extended gene confers MMS resistance to a strain deleted for *RAD52*, suggesting that the tag does not interfere drastically with *RAD52* activity. The tag was also placed on mutant alleles, and all were cloned between the *Eco*RI and *Sal*I sites of the vectors pRS316 and pRS426 (Christianson *et al.* 1992). Protein extracts were prepared from log-phase yeast cultures using a glass bead breakage method (Katz and Solomon 1988). The proteins were separated by electrophoresis in a SDS gel and then electroblotted to nitrocellulose (Schleicher & Schuell, Keene, NH). HA-tagged polypeptides were detected by incubating the filter with a mouse anti-HA monoclonal antibody (Roche Molecular Biochemicals, Indianapolis), followed by incubation with a goat anti-mouse IgG conjugated to horseradish peroxidase (HRP; Bio-Rad, Hercules, CA). Proteins were visualized using HRP substrate reagents followed by exposure to Hyperfilm ECL according to the manufacturer's instructions (Amersham Pharmacia, Buckingham, UK).

RESULTS

Partial activities conferred by *rad52* truncation alleles: We had shown previously that a strain harboring a *rad52* allele (*rad52-Δ327*) retaining the first 327 amino acids of the 504-amino-acid open reading frame maintains partial resistance to MMS exposure, while a strain with a shorter truncation allele (*rad52-Δ169*) retaining only 167 amino acids is as sensitive as a strain with a deletion allele (Boundy-Mills and Livingston 1993). To more precisely define how little of Rad52p is required for this activity, three additional truncation alleles were assessed for the ability to promote survival to MMS exposure. These new alleles retain the first 292, 251, and 210

TABLE 1

Strains

Strain	Genotype	Source or Reference
SSL204	*RAD52 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Dornfeld and Livingston (1991)
SSL204A	*RAD52 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Dornfeld and Livingston (1991)
SSL209A	*rad52-1MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Boundy-Mills and Livingston (1993)
SSL212	*rad52-ΔHS MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Dornfeld and Livingston (1991)
SSL212A	*rad52-ΔHS MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Dornfeld and Livingston (1991)
SSL231	*RAD52 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101 can1::BYA112*	Kaytor and Livingston (1994)
SSL242	*rad52-Δ169 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Boundy-Mills and Livingston (1993)
SSL243	*rad52-2 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Boundy-Mills and Livingston (1993)
SSL245	*rad52-2 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Boundy-Mills and Livingston (1993)
SSL741	*rad52-22B MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL742	*rad52-23A MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL748	*rad52-76A MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL754	*rad52-Δ210 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL755	*rad52-Δ251 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL756	*rad52-Δ251 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL757	*rad52-Δ292 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL758	*rad52-Δ292 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL759	*rad52-Δ210 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL760	*rad52-96A MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL793	*rad52-Δ169 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL794	*rad52-Δ327 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL795	*rad52-Δ327 MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL802	*rad52-301A MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Nguyen and Livingston (1997)
SSL802A	*rad52-301A MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	Nguyen and Livingston (1997)
SSL851	*rad52-ιΔ251B MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL851A	*rad52-ιΔ251B MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101 can1::BYA112*	This study
SSL852	*rad52-ιΔ210B MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101 can1::BYA112*	This study
SSL852A	*rad52-ιΔ210B MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study
SSL853	*rad52-ιΔ292B MAT*α *his3-Δ200 leu2 trp1 ura3-52 ade2-101 can1::BYA112*	This study
SSL853A	*rad52-ιΔ292B MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101*	This study

Diploids: Genotype	Cross
*RAD52 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *RAD52 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL204 × SSL204A
*rad52 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL243 × SSL245
*rad52-301A MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-301A MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL802 × SSL802A
*rad52-Δ327 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-Δ327 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL794 × SSL795
*rad52-Δ292 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-Δ292 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL757 × SSL758
*rad52-Δ251 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-Δ251 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL755 × SSL756
*rad52-Δ210 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-Δ210 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL754 × SSL759
*rad52-Δ169 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-Δ169 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL242 × SSL793
*rad52-ΔHS MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-ΔHS MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL212 × SSL212A
*rad52-Δ327 MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-2 MAT*α *ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL241 × SSL243

(*continued*)

TABLE 1

(Continued)

Diploids: Genotype	Cross
rad52-Δ292 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL243 × SSL757
rad52-Δ251 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL243 × SSL755
rad52-Δ210 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL243 × SSL759
rad52-Δ169 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL242 × SSL243
rad52-Δ327 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-301A MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL241 × SSL802
rad52-Δ292 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-301A MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL757 × SSL802
rad52-Δ251 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-301A MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL755 × SSL802
rad52-Δ210 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-301A MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL759 × SSL802
rad52-Δ169 *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-301A MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL242 × SSL802
rad52-ιΔ292B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA::112* *rad52-ιΔ292B MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL853 × SSL853A
rad52-ιΔ251B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-ιΔ251B MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL851 × SSL851A
rad52-ιΔ210B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-ιΔ210B MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL852 × SSL852A
rad52-ιΔ292B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL853 × SSL245
rad52-ιΔ251B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52*	SSL851 × SSL245
rad52-ιΔ210B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-2* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL852 × SSL245
rad52-ιΔ292B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-Δ327* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL853 × SSL794
rad52-ιΔ251B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-Δ327* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL851A × SSL795
rad52-ιΔ210B *MAT***a** *ade2-101 his3-Δ200 leu2 trp1 ura3-52 can1::BYA112* *rad52-Δ327* *MATα ade2-101 his3-Δ200 leu2 trp1 ura3-52 CAN1*	SSL851A × SSL795

amino acids and are designated *rad52-Δ292*, *rad52-Δ251*, and *rad52-Δ210*, respectively (Figure 1).

The sensitivity of these strains to MMS exposure was determined by incubating stationary phase cells in a MMS solution and withdrawing aliquots at various times. Figure 2 depicts the time course of survival for the truncation mutants. The *rad52-Δ327* strain is ~200-fold more resistant to MMS exposure than the *rad52-Δ169* strain after 30 min of exposure to MMS. The three new truncation mutants are all more resistant than the *rad52-Δ169* strain after 30 min of exposure to MMS. The *rad52-Δ292* and the *rad52-Δ251* strains are 100- and 80-fold more resistant, respectively, than the *rad52-Δ169* strain, while the *rad52-Δ210* strain is at best 2-fold more resistant than the control strain. Thus, partial resistance to MMS exposure persists when only 251 amino acids are present and may even be found in a protein with 210 amino acids.

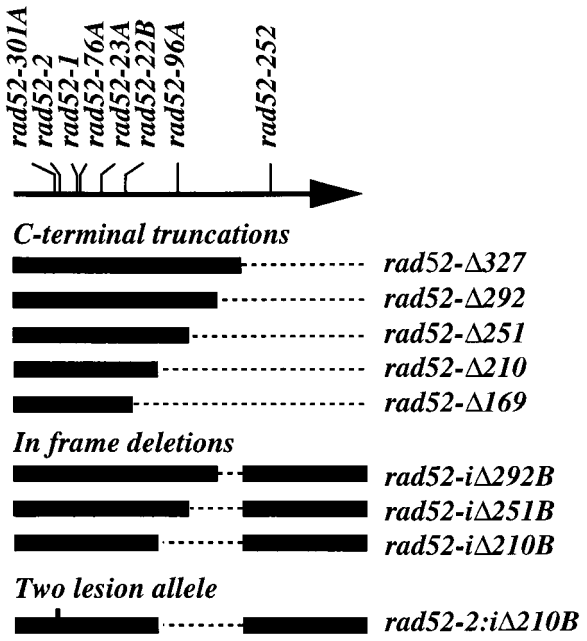

Figure 1.—*rad52* alleles used in this study. The *RAD52* transcription unit is represented by the arrow, and the relative locations of eight missense mutations are indicated above the arrow. *rad52* truncation and internal deletion alleles are shown below. The solid boxes denote the regions of *RAD52* present in each allele, and dotted lines denote regions deleted.

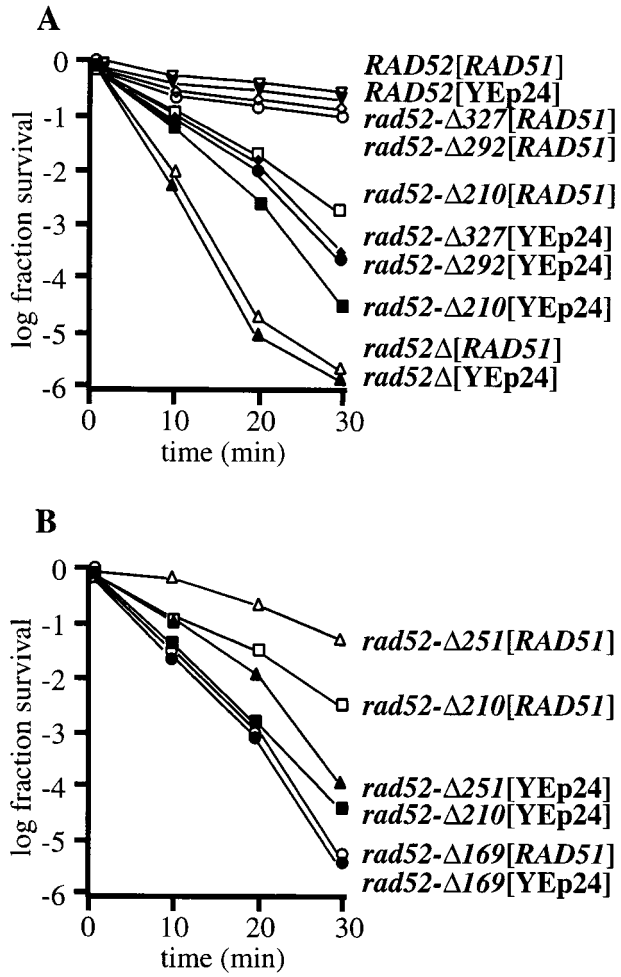

Figure 2.—MMS sensitivity of *rad52* truncation alleles and the effect of *RAD51* overexpression. (A) The log fraction of surviving cells after exposure to MMS from strains with the *rad52* C-terminal truncation alleles *rad52-Δ327*, *rad52-Δ292*, and *rad52-Δ210*, as well as from a wild-type strain (*RAD52*) and from a deletion strain (*rad52-ΔHS*), was plotted *vs.* time of exposure. (B) The log fraction of surviving cells after exposure to MMS from strains with the *rad52* C-terminal truncation alleles *rad52-Δ251*, *rad52-Δ210*, and *rad52-Δ169* was plotted *vs.* time of exposure. The solid symbols are the strains containing the vector control. The open symbols are the strains transformed with YEp24::*RAD51*. These studies were done at 33°.

As an independent measure of retention of function, we also assessed the ability of homoallelic truncation diploids to produce viable spores. We previously determined that homoallelic diploids of *rad52-Δ327* yield viable spores, the percentage being a function of strain background and of sporulation conditions (Boundy-Mills and Livingston 1993; Nguyen and Livingston 1997). In the current study the homoallelic *rad52-Δ327* diploid yielded ~40% spore viability in contrast to the >90% viability of the wild-type diploid (Table 2). The *rad52-Δ251* and *rad52-Δ292* homoallelic diploids also yielded nearly 40% viable spores, while the *rad52-Δ210* diploid did not sporulate. Thus, the intermediate spore viability correlates with the partial retention of resistance to MMS exposure. Together, these phenotypic assays suggest that a Rad52 polypeptide chain with as little as 251 amino acids can promote a low level of MMS damage repair and partially succeed at sporulation.

N-terminal truncation alleles that retain partial activities can also be suppressed by high-level expression of *RAD51*: Work from our laboratory and from other laboratories has shown that high-level expression of *RAD51* can suppress some *rad52* alleles (Milne and Weaver 1993; Schild 1995; Kaytor and Livingston 1996). In our studies we found that both the truncation allele *rad52-Δ327* and a ts allele, *rad52-252* (D371G), containing a missense mutation in the C-terminal region can be suppressed to near wild-type levels in tests of resistance to MMS treatment (Kaytor and Livingston 1996). Five other alleles [*rad52-1* (A90V), *rad52-2* (P64L), *rad52-76A* (N97T), *rad52-23A* (V128I), *rad52-22B* (V162A)], all missense mutations mapping to the N terminus of the protein, are not suppressed to nearly the same extent as *rad52-Δ327* or *rad52-252*.

To further these studies, we attempted to suppress the shorter truncation alleles by overexpression of *RAD51*. The results (Figure 2, A and B) show that, like the *rad52-Δ327* allele, both the *rad52-Δ292* and *rad52-Δ251* alleles are nearly completely suppressed by overexpression of *RAD51*. For example, the *rad52-Δ251* strain is 400-fold more resistant to MMS treatment (at 30 min)

TABLE 2

Spore viability

Genotype	Spore viability	
	Vector control (%)	*RAD51* plasmid (%)
RAD52/RAD52	42/44 (95.5)	41/44 (93.2)
rad52-Δ327/rad52-Δ327	59/132 (44.7)[a]	105/132 (79.5)[a]
rad52-Δ292/rad52-Δ292	52/132 (39.4)[a]	82/132 (62.1)[a]
rad52-Δ251/rad52-Δ251	60/132 (45.5)	67/132 (50.8)
rad52-Δ210/rad52-Δ210	SD	SD
rad52-Δ169/rad52-Δ169	SD	SD
rad52-iΔ292B/rad52-iΔ292B	72/124 (58.1)	ND
rad52-iΔ251B/rad52-iΔ251B	66/132 (50.0)	ND
rad52-iΔ210B/rad52-iΔ210B	39/108 (36.1)	ND
rad52-ΔHS/rad52-ΔHS	SD	SD

SD, sporulation deficient; ND, not determined.

[a] The *RAD51* plasmid value is significantly higher than the plasmid control value for these diploids ($P < 0.005$).

when *RAD51* is overexpressed. This resistance level is similar to that of a *RAD52* strain used as a control. The *rad52-Δ210* strain is partially suppressed by *RAD51* overexpression and the *rad52-Δ169* strain is not suppressed.

In addition we retested the six missense alleles, *rad52-1*, *rad52-2*, *rad52-22B*, *rad52-23A*, *rad52-76A*, and *rad52-252*, that we had previously examined (Kaytor and Livingston 1996) along with another missense allele, *rad52-96A* (R235G). These tests were all done using liquid treatment of a cell suspension and a complete time course. Only the *rad52-252* and *rad52-96A* alleles are suppressed to near wild-type levels; none of the five missense mutations in the N-terminal portion are suppressed (data not shown). These results confirm the positional allele specificity for *RAD51* suppression that we previously observed.

Suppression by *RAD51* overexpression also works during meiosis. The results in Table 2 show that the partial spore viability of a homoallelic diploid of *rad52-Δ327* is raised from 45 to 80% and the value for a homoallelic diploid of *rad52-Δ292* is raised from 39 to 62% when *RAD51* is overexpressed. Significant suppression does not occur for a homoallelic diploid of *rad52-Δ251*, and the *rad52-Δ210* and *rad52-Δ169* homoallelic diploids do not sporulate even when *RAD51* is overexpressed. The results show that while truncation alleles can be suppressed during meiosis, the level of suppression is less than that for MMS resistance in mitotic cells. In addition the suppression does not extend to alleles *rad52-Δ251* or *rad52-Δ210* in meiosis, whereas it does in measurements of MMS resistance in mitosis.

Taken together, these results define three characteristics about suppression by *RAD51* overexpression. First, suppression by *RAD51* overexpression requires an intact N-terminal sequence because none of the missense mutations mapping to the N terminus can be suppressed to the same level as the truncation alleles that have the wild-type sequence in this region. Second, these results show that *RAD51* overexpression most likely suppresses MMS sensitivity by bypassing the need for the C terminus of Rad52p because truncations that retain only the first 251 residues and are missing the putative Rad51p binding site (Milne and Weaver 1993; Mortensen *et al.* 1996) are nearly completely suppressed. Third, *RAD51* high copy suppression does not work as efficiently during meiosis.

***rad52* alleles containing in-frame deletions:** To learn whether the properties of the truncation alleles could be altered by including the C-terminal third of Rad52p, we constructed internal deletion alleles. These alleles, *rad52-iΔ210B*, *rad52-iΔ251B*, and *rad52-iΔ292B*, fuse amino acids 210, 251, and 292, respectively, of Rad52p in frame to residue 328. The truncation alleles were tested for their MMS resistance, with and without overexpression of *RAD51*, and for their ability to sporulate as homoallelic diploids. For the most part the internal deletion alleles have phenotypes much like those of the truncation alleles.

First, while the internal deletion mutants are sensitive to exposure to MMS, like the truncation alleles, they retain partial activity to resist MMS killing (Figure 3). After 30 min exposure, *rad52-iΔ210B* is as sensitive to MMS exposure as *rad52-Δ327*. *rad52-iΔ251B* and *rad52-iΔ292B* are more resistant than *rad52-Δ327* at this time. (After 45 min exposure all mutants lost viability.) Second, diploids homoallelic for the internal deletion alleles sporulate and yield approximately half the number of viable spores of a wild-type diploid (Table 2). In this case the phenotypes of *rad52-iΔ251B* and *rad52-iΔ292B* are similar to the phenotypes of the corresponding truncation alleles *rad52-Δ251* and *rad52-Δ292*, respectively.

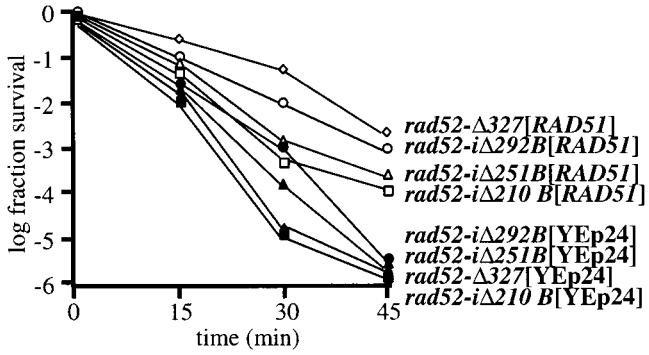

Figure 3.—MMS sensitivity of *rad52* internal deletion alleles and the effect of *RAD51* overexpression. The log fraction of surviving cells after exposure to MMS from strains with the *rad52* internal deletion alleles *rad52-iΔ292B*, *rad52-iΔ251B*, and *rad52-iΔ210B* were compared to the survival of a strain with the truncation allele *rad52-Δ327*. The solid symbols are the strains containing the vector control. The open symbols are the strains transformed with YEp24::*RAD51*. These studies were done at 33°.

A major difference is that a homoallelic diploid of *rad52-iΔ210B* sporulates and yields viable spores. The corresponding truncation allele *rad52-Δ210* does not sporulate. A possible reason for this difference could be that residues extending from 328 to the C terminus are sufficient to support a reduced level of successful sporulation. Third, like the truncation alleles, the MMS sensitivity of the internal deletion mutants can be suppressed by high-level expression of *RAD51* (Figure 3). Interestingly, while high level of expression of *RAD51* suppresses *rad52-Δ251* and *rad52-Δ292* nearly to wild-type resistance (Figure 2), the three internal deletion alleles are not completely suppressed. The lack of complete suppression is evidenced by the result that after 45 min none of the three internal deletion mutants are as resistant as a suppressed *rad52-Δ327* mutant (Figure 3). We suspect that the inclusion of the C-terminal residues containing the Rad51p binding domain (Milne and Weaver 1993; Mortensen *et al.* 1996) interferes and inhibits the bypass suppression observed for the truncation alleles. The effect of high-level *RAD51* expression on the sporulation capacity of the internal deletion mutants was not examined.

The phenotypes of the internal deletion alleles again show that partial activities are retained in the absence of the full primary sequence of Rad52p. While the retention of partial activities of the internal deletions most likely results from the preservation of the N-terminal domain, other possibilities are plausible. In the case of the internal deletion alleles, the partial activities might result not from the loss of a functional domain but rather from the inhibitory effect that the N- and C-terminal domains exert on each other by being brought closer together than they are in their normal arrangement.

Intragenic complementation between mutant forms of Rad52p appears to require a continuous wild-type sequence of the first 327 amino acids: Earlier work in our laboratory found that N-terminal and C-terminal functions may be provided *in trans* by the *rad52-Δ327* and *rad52-2* (L64P) alleles (Boundy-Mills and Livingston 1993). To ascertain whether any of the shorter truncation alleles could also complement the *rad52-2* mutation, heteroallelic diploids containing the shorter truncation alleles and the *rad52-2* allele were made and subjected to MMS treatment. Surprisingly, none of the shorter truncation alleles, *rad52-Δ292*, *rad52-Δ251*, or *rad52-Δ210*, complement the *rad52-2* mutation under these conditions (Figure 4A). Consistent with this observation is the result that the *rad52-2* / *rad52-Δ327* heteroallelic diploid is also the only strain to demonstrate complementation in meiosis. This diploid yields a wild-type level of spore viability, while heteroallelic diploids containing *rad52-2* with either the *rad52-Δ292* or the *rad52-Δ251* truncation allele yield the partial spore viability of the respective homoallelic truncation diploids (data not shown). As expected, heteroallelic diploids of *rad52-Δ210* or *rad52-Δ169* with *rad52-2* do not sporulate.

To assess whether the failure to achieve complementation was a peculiarity of the *rad52-2* allele, a second N-terminal missense allele, *rad52-301A* (K61N), was also tested. Again, the *rad52-301A* allele can be complemented for MMS resistance by the *rad52-Δ327* allele, but not by any of the shorter truncation alleles (Figure 4B). These results suggest that complementation by mutant protein chains may require more than the core defined by the partial activity and *RAD51* suppression results.

The set of three internal deletion alleles, *rad52-iΔ210B*, *rad52-iΔ251B*, and *rad52-iΔ292B*, corroborated the need for more than the first 251 amino acid residues to achieve complementation in heteroallelic diploids. None of these internal deletion alleles are capable of complementing the MMS sensitivity of a *rad52-2* allele in a heteroallelic diploid (Figure 4C). At this point we had yet to find complementation with any of the truncation or internal deletion alleles that deleted amino acid residues between 210 and 327.

Additional experiments revealed that truncation and internal deletion alleles can be complemented under certain conditions. First, all three internal deletion alleles, the most severe (*rad52-iΔ210B*) removing residues 210 through 327, complement the *rad52-Δ327* allele (Figure 4D). This result dispelled the possibility that previous failures to achieve complementation between the internal deletion alleles and the *rad52-2* allele resulted from instability of the internal deletion peptides. Rather, the results implied that to achieve complementation, one of the complementing peptides has to contain wild-type sequences that include residues between amino acids 251 and 327. Consistent with this interpretation is the failure of the *rad52-Δ292* allele to complement any of the internal deletion alleles (data not

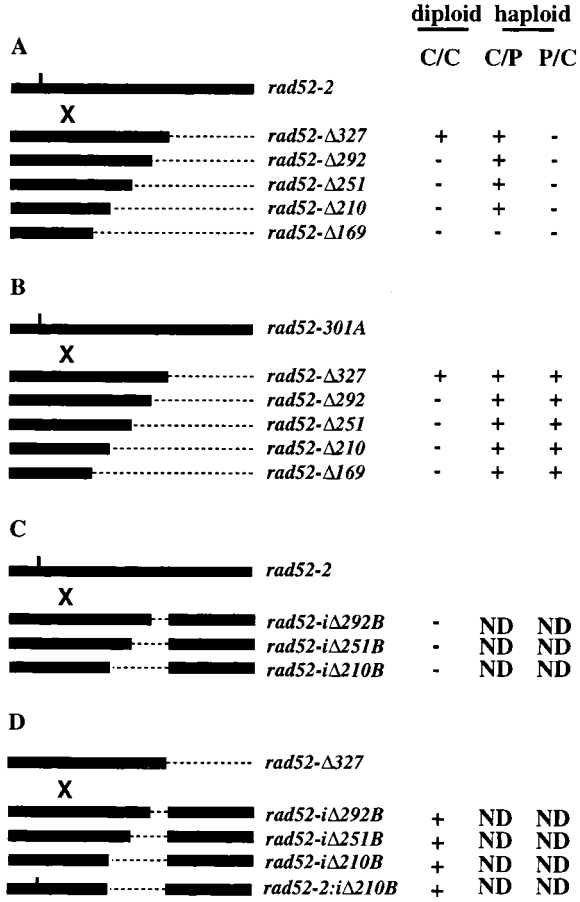

Figure 4.—Summary of intragenic complementation and overexpression results. The figure represents a compilation of the relative resistance to MMS exposure of heteroallelic *rad52* diploids or, in the case of overexpression, *rad52* haploids carrying a second *rad52* allele on a high copy plasmid. +, either wild-type levels of survival or a deficit of less than an order of magnitude relative to a wild-type control after a 45-min exposure. −, either a decrease of three to four orders of magnitude relative to wild-type or no significant increase compared to an appropriate negative control after a 45-min exposure. The positive control for complementation experiments was either a homoallelic wild-type diploid or a heteroallelic diploid known to demonstrate complementation, *e.g.*, *rad52-2*/*rad52-Δ327*. The negative control was either a homoallelic *rad52* deletion diploid or a heteroallelic diploid known to be incapable of complementation, *e.g.*, *rad52-2*/*rad52-Δ169*. The positive control for the overexpression studies was a *rad52-2* strain transformed with *rad52-Δ327* on a high copy plasmid. This strain has wild-type levels of resistance to MMS. The negative control was a *rad52-Δ327* strain with *rad52-2* overexpressed. This strain is no more resistant than either haploid strain by itself. For the diploids, C/C refers to the chromosomal expression of both alleles. For the haploids, C/P and P/C refer to the expression of one of the two alleles from a 2μ vector, the first letter designating the allele depicted at the top of each section heading. (A) Heteroallelic diploids with *rad52-2* crossed to the truncation alleles. (B) Heteroallelic diploids with *rad52-301A* crossed to the truncation alleles. (C) Heteroallelic diploids with *rad52-2* crossed to the internal deletion alleles. (D) Heteroallelic diploids with *rad52-Δ327* crossed to the internal deletion alleles. In the case of the *rad52-2:iΔ210B* allele, complementation was judged in a *rad52-Δ327* haploid transformed with the two-lesion allele expressed from a CEN vector. All experiments were done at 30°.

shown), but the success of the *rad52-Δ327* allele in complementing a complex allele containing both the *rad52-2* missense mutation and the internal deletion of the *rad52-iΔ210B* allele (Figure 4D). This latter success illustrates well that nearly wild-type *RAD52* activity can be achieved between two peptides, one wild type for the first 327 residues and one wild type for the residues beyond position 327.

We could also achieve complementation under a second condition in which one of the two mutant alleles is expressed on a high copy plasmid. In these experiments haploids containing one *rad52* allele were transformed with a 2μ plasmid-based vector containing a second allele. We found that when the truncation alleles *rad52-Δ292*, *rad52-Δ251*, and *rad52-Δ210* are expressed from high copy plasmids, they complement the missense alleles *rad52-2*(P64L) and *rad52-301A*(K61N) (Figure 4, A and B). The shortest truncation allele, *rad52-Δ169*, also complements *rad52-301A* under these conditions. Control experiments (data not shown) revealed that high-level expression of any truncation allele in a mutant bearing a chromosomal copy of the same mutation does not enhance the mutant's MMS resistance. Thus, the truncated Rad52 polypeptides can be complemented by full-length missense mutations having a wild-type C-terminal sequence as long as the truncation alleles are expressed on a high copy plasmid.

We also performed studies to learn whether the reverse configuration of high copy expression of the missense alleles within mutants bearing the truncation alleles would lead to complementation. The *rad52-2* allele does not complement any of the truncation alleles (Figure 4A). This result is consistent with our previous observation (repeated in this study) that the same relationship holds for the *rad52-2* and *rad52-Δ327* alleles, two alleles that, unlike the shorter truncations, complement in heteroallelic diploids (Boundy-Mills and Livingston 1993). While high copy expression of *rad52-2* does not lead to complementation, high copy of *rad52-301A*, when expressed in any of the truncation alleles, does lead to complementation (Figure 4B). Again, we stress that control experiments show that MMS resistance cannot be achieved simply by overexpressing a mutant allele in a strain bearing a chromosomal copy of the same mutation, indicating that the complementation that is observed is dependent on the interaction or sequential function of both mutant alleles.

To investigate whether the differential expression levels of the mutant proteins might contribute to some of the differences in the complementation patterns, we analyzed relative protein levels by epitope-tagging mutant proteins at their C-terminal end. Blotting and probing revealed that all mutant forms could be detected when the alleles were expressed on a high copy plasmid except for the product of *rad52-2* that was seen only in small amounts (Figure 5). The detection of all mutant protein forms, save for the low level of the *rad52-2* prod-

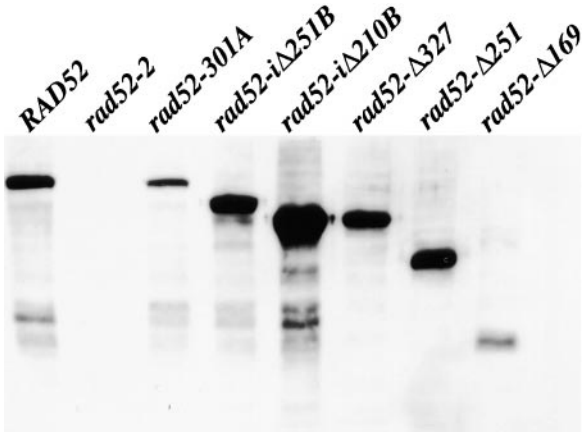

Figure 5.—Visualization of mutant proteins. The procedures used to produce this blot are described in materials and methods. The film was exposed for 5 min. The full-length product of the *rad52-2* allele was detectable after exposure for 2 hr.

uct, shows that all mutant forms are expressed. We note that the products of the truncation alleles and internal deletion alleles appear to be present in greater amounts than the full-length, wild-type, and *rad52-301A* products (Figure 5). Whether or not the greater amounts represent greater stability of these truncated forms or simply greater survival during extraction or more efficient blotting is unknown. Nevertheless, the study suggests that the diminished capacity of the truncation and internal deletion alleles in functional tests is unlikely to result from unstable protein products. Furthermore, while we could detect the epitope-tagged proteins expressed from the high copy plasmids, the signal from the same tagged proteins expressed from low copy plasmids was significantly less detectable (data not shown). The diminished signal from proteins expressed from low copy plasmids suggests that each mutant form is present in lower amounts when expressed from a single chromosomal copy than from a high copy plasmid.

Thus, these measurements indicate that most of our complementation data is not complicated by gross instability of mutant forms. The exception is the very low level of the *rad52-2* product we can detect. If this failure is indicative of a severe cellular instability for this mutant form, it would partially explain why high-level expression of *rad52-2* does not complement the truncation alleles. The possibility that the truncation products stabilize the *rad52-2* product when the truncation products are expressed from a high copy plasmid is being investigated.

Our overall conclusion is that two mutant forms of Rad52p can complement each other under two conditions. The first is when both alleles are expressed from chromosomal copies in a heteroallelic diploid, and one allele encodes wild-type sequences containing the first 327 residues of the N terminus and the other allele encodes wild-type sequences for the remainder of the C terminus. The second is that the requirement for the first 327 residues can be relaxed to 210 residues, and even to 169 residues, in some cases, if one of the complementing proteins is expressed at high levels.

DISCUSSION

The *RAD52* core domain: Our study aimed to define the minimal requirements for functionality of truncated and internally deleted *RAD52* products. The allele *rad52-Δ251*, encoding a peptide that is less than half of the longest possible open reading frame, retains a remarkable amount of activity in tests of resistance to MMS exposure and in spore viability. In these tests that rely on expression of a single chromosomal copy, no shorter truncation allele could function as well, nor could the truncation alleles encoding 292 and 327 residues greatly exceed *rad52-Δ251* in these capacities. On the basis of functionality of single copy sequences we consider the first 251 residues to contain the core of *RAD52*.

In our studies we have also created situations where shorter protein products can exhibit core properties or where a longer protein product exceeds the ability of the *rad52-Δ251* product. These situations rely on the presence of complementing *rad52* alleles, high copy expression of *rad52* alleles, or high copy expression of *RAD51*. For example, an exception to the generalization that *rad52-Δ251* meets the functionality of longer protein products is the ability of *rad52-Δ327*, but not *rad52-Δ251*, to complement missense mutations in a heteroallelic diploid. In this situation, functionality is achieved by the interaction of two partially active mutant forms of *RAD52*. There are many examples of the shorter truncation alleles exhibiting substantial activity. One example is the *rad52-Δ210* product that is partially rescued by high-level expression of *RAD51*. However, we note that while the *rad52-Δ210* allele is partially rescued by high copy expression of *RAD51*, the *rad52-Δ251* allele is brought to near wild-type functionality in tests of MMS resistance by excess amounts of Rad51p. Another striking example is the ability of *rad52-Δ169* to complement *rad52-301A* when the truncated polypeptide is expressed in high copy. This is the first experimental evidence that the *rad52-Δ169* product retains any activity *in vivo*. Indeed, its ability to complement a missense mutation, in the absence of any other measurable activity, suggests that it may rescue the protein product of the missense mutation by binding to it. In turn, this would place the putative self-interaction domain within the first 169 residues. Thus, while *rad52-Δ251* neither outperforms all other alleles in every test nor performs as well as *rad52-Δ327* in some tests, its partial function in mitosis and meiosis and its ability to be brought to near wild-type MMS resistance by high copy expression of *RAD51* demonstrate its retention of core function.

The size of this domain is interesting because it is approximately the size of *RAD59*, a *RAD52* family member that is needed for *RAD51*-independent recombination events (Bai and Symington 1996). The first 251 residues of *RAD52* are 28% identical and 50% similar in amino acid sequence to *RAD59*. Functionally, the two genes must have some common activity because overexpression of *RAD52* can suppress the γ-ray sensitivity of a *rad59* mutant. Curiously, the experiment does not work in reverse; high copy expression of *RAD59* does not suppress a *rad52* mutant. One explanation for this failure might be that Rad59p lacks the C-terminal functions of Rad52p. If this were so, then overexpression of *RAD51* might be expected to suppress the *rad59* mutant as it does the *rad52-Δ251* allele. This experiment failed as well (Bai and Symington 1996). The failure of *RAD51* to suppress a *rad59* mutant while being able to suppress the *rad52-Δ251* mutant implies that the truncated *RAD52* protein product possesses some unique activity in addition to a Rad51p binding site that distinguishes it from Rad59p. Accordingly, we attempted to suppress a *rad59Δ* mutant by overexpressing *rad52-Δ251* either by itself or by simultaneous overexpression of *RAD51* from the same plasmid. In both cases we failed to achieve suppression as judged by growth on agar plates containing MMS (data not shown). [Control experiments revealed that we could duplicate the original observation that overexpression of *RAD52* suppresses the MMS sensitivity of a *rad59Δ* mutant (Bai and Symington 1996) and that we could also show that the plasmid simultaneously overexpressing both *rad52-Δ251* and *RAD51* confers MMS resistance to a *rad52* null strain.] The results suggest that the N termini of Rad52p and Rad59p are not interchangeable.

Suppression of *rad52* mutations by *RAD51* overexpression: Our studies on suppression of *rad52* mutations by overexpression of *RAD51* show that this most likely occurs by bypassing the C-terminal function of Rad52p. A corollary of this conclusion is that the core of *RAD52* must be intact in order for high-level expression of *RAD51* to suppress the MMS sensitivity of a *rad52* mutant allele. The conclusion that *RAD51* overexpression works by bypassing the C-terminal half of Rad52p comes directly from its suppressive effects on truncation alleles as small as *rad52-Δ210*. The protein products of these truncation alleles have eliminated the putative Rad51p binding site on Rad52p (Milne and Weaver 1993; Mortensen *et al.* 1996). Whether *RAD51* overexpression suppresses the missense alleles *rad52-96A* (R235G) and *rad52-252* (D371G) that have changes in the vicinity of the Rad51p binding domain by direct contact, rather than through a bypass mechanism, is not known. The observation of a strict positional specificity of *RAD51* suppression of the MMS sensitivity of missense mutants, however, demonstrates the need for an intact core to achieve suppression. None of five N-terminal missense mutations that change residues in the region from position 61 to position 162 are suppressed, while the missense mutations with changes at positions 235 and 371 are. The suppressive effect on the *rad52-96A* allele is interesting because the amino acid change (R235G) lies in the residues that distinguish *rad52-Δ251* from *rad52-Δ210*. As *rad52-Δ251* is nearly completely suppressible by overexpression and *rad52-Δ210* is not as completely suppressible, the suppressibility of a mutation at residue 235 may signal that core functions might be found in truncation alleles shorter than *rad52-Δ251*.

RAD51 overexpression suppression of meiotic functions is interesting for two reasons. First, while the truncation alleles *rad52-Δ327* and *rad52-Δ291* are partially suppressed for the production of viable spores, they are not fully suppressed. In the case of mitotic suppression of MMS sensitivity, the suppression brings the mutants to near the resistance of a wild-type strain. One explanation for the difference in degree of suppression between mitotic and meiotic functions would be that Rad52p and Rad51p interact differently, or are present in different stoichiometry, during meiosis. Another possibility is that the truncation alleles remove not only the Rad51p binding domain of Rad52p but destroy a Rad52p function that is more important for meiotic division than for MMS resistance during mitotic growth. For example, the RPA interaction domain of yeast Rad52p has been inferred to lie in this region (Hays *et al.* 1998), and the action of this protein may be different in meiosis than mitosis. The second interesting observation about the meiotic suppression results is that both the *rad52-Δ251* and *rad52-Δ210* alleles can be suppressed during mitosis, but neither can be suppressed in meiosis. While this might have been expected for the *rad52-Δ210* allele that does not sporulate, the failure to achieve meiotic suppression is somewhat surprising for the *rad52-Δ251* allele because its unsuppressed level of sporulation is not significantly different from the two longer alleles, *rad52-Δ291* and *rad52-Δ327*, that can be partially suppressed. The drop-off in meiotic suppressibility again suggests that the region between residues 210 and 327 may hold a function that is more important for the meiotic function of Rad52p than for its mitotic function.

Intragenic complementation among *rad52* alleles: While the *rad52-Δ251* and the *rad52-Δ291* mutants expressing the core amino acids can be rendered nearly completely resistant to MMS exposure by *RAD51* overexpression, these mutations cannot be complemented in a heteroallelic diploid with the *rad52-2* allele. We have shown previously, and confirmed in this study, that a heteroallelic diploid of *rad52-Δ327* and *rad52-2* has near wild-type resistance to MMS exposure (Boundy-Mills and Livingston 1993 and this article). Further exploration of the intragenic complementation between mutant Rad52 protein chains showed that the rules governing complementation are complex. Nevertheless, we point out two features of the complementation that are clear from our results.

We first note that *rad52-Δ251* can complement *rad52-2* but only if the expression of the truncation allele is altered by expressing it from a high copy plasmid in a strain with a chromosomal copy of the *rad52-2* allele. The *rad52-Δ251* allele also fails to complement a second missense allele, *rad52-301A*, in a heteroallelic diploid but again does so when the quantity of the truncation protein is elevated above that of the *rad52-301A* protein. In both cases we suspect that the *rad52-Δ251* allele is providing the core function because *rad52-2* and *rad52-301A* mutants have little activity by themselves or when *RAD51* is overexpressed in them. Thus, while the *rad52-Δ251* allele does not behave like the *rad52-Δ327* allele when these alleles are expressed as chromosomal copies in heteroallelic diploids with a chromosomal copy of the *rad52-2* or *rad52-301A* missense alleles, *rad52-Δ251* nonetheless can be brought to near wild-type levels of MMS resistance by expressing it from a high copy plasmid in a cell with either of these two missense *rad52* alleles.

Second, we conclude that the rules of intragenic complementation show that while the N- and C-terminal portions of Rad52p can be supplied *in trans*, wild-type N-terminal sequences must extend to residue 327 for successful complementation in cells where both mutant alleles are expressed as single chromosomal copies. Three experiments argue for this conclusion. First, only the *rad52-Δ327* allele and none of the shorter truncation alleles or internal deletion alleles complement the *rad52-2* allele when present in heteroallelic diploids. Second, the *rad52-Δ327* allele complements internal deletion alleles, including *rad52-iΔ210B*, encoding a Rad52 protein missing residues 210 through 327. Finally, the *rad52-Δ327* allele complements an allele containing both the missense mutation of *rad52-2* and the internal deletion removed from the *rad52-iΔ210B* allele. Thus, complementation is achieved with the *rad52-Δ327* peptide and numerous other mutant peptides that have wild-type sequences extending from residue 328 to the C terminus. The complementation of the internal deletion alleles by the *rad52-Δ327* allele shows that the residues from 210 to 327 need not be present in both mutant protein molecules. This means either that a putative Rad52p self-association domain cannot reside in this region or that the complementation really works by the independent action of the two mutant proteins, one supplying the N-terminal functions and the other the C-terminal functions.

Conclusion: These studies have shown how few of the amino acid residues of the open reading frame of *RAD52* need to be present to achieve partial activity in tests of resistance to MMS exposure during mitotic growth and of spore viability after meiosis. By virtue of the differences in the degree to which the truncation alleles can be rescued by *RAD51* overexpression and by intragenic complementation with *rad52* missense alleles, the results also suggest that the portion of Rad52p between residues 210 and 327 contains domains necessary for fully successful sporulation and intragenic complementation.

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