# A New Recombinational DNA Repair Gene From *Schizosaccharomyces pombe* With Homology to *Escherichia coli* RecA

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ABSTRACT

A new DNA repair gene from *Schizosaccharomyces pombe* with homology to RecA was identified and characterized. Comparative analysis showed highest similarity to *Saccharomyces cerevisiae* Rad55p. *rhp55*$^+$ (*r*ad *h*omologue *p*ombe *55*) encodes a predicted 350-amino-acid protein with an $M_r$ of 38,000. The *rhp55*Δ mutant was highly sensitive to methyl methanesulfonate (MMS), ionizing radiation (IR), and, to a lesser degree, UV. These phenotypes were enhanced at low temperatures, similar to deletions in the *S. cerevisiae RAD55* and *RAD57* genes. Many *rhp55*Δ cells were elongated with aberrant nuclei and an increased DNA content. The *rhp55* mutant showed minor deficiencies in meiotic intra- and intergenic recombination. Sporulation efficiency and spore viability were significantly reduced. Double-mutant analysis showed that *rhp55*$^+$ acts in one DNA repair pathway with *rhp51*$^+$ and *rhp54*$^+$, homologs of the budding yeast *RAD51* and *RAD54* genes, respectively. However, *rhp55*$^+$ is in a different epistasis group for repair of UV-, MMS-, or γ-ray-induced DNA damage than is *rad22*$^+$, a putative *RAD52* homolog of fission yeast. The structural and functional similarity suggests that *rhp55*$^+$ is a homolog of the *S. cerevisiae RAD55* gene and we propose that the functional diversification of RecA-like genes in budding yeast is evolutionarily conserved.

DOUBLE-strand breaks (DSBs) are an important genotoxic lesion caused by free radicals during cellular metabolism, by various chemical agents, and by ionizing radiation (IR). Moreover, DSBs appear as an intermediate during meiotic recombination in *Saccharomyces cerevisiae* (Friedberg *et al.* 1995). These lesions can be healed by several DNA repair pathways that evolved in eukaryotes and were found in yeasts and mammals. Among these, homologous recombination, single-strand annealing (SSA), and nonhomologous DNA end-joining provide the main mechanisms for DSB repair (Haber 1992; Kanaar *et al.* 1998). Homologous recombination uses the information from the undamaged sister chromatid or homolog to repair the DSB. It constitutes the only intrinsically error-free mechanism for DSB repair (Resnick 1976; Szostak *et al.* 1983). In contrast, SSA and DNA end-joining repair DNA ends in an intrinsically error-prone manner, inducing mutations during the process (Lin *et al.* 1984; Roth and Wilson 1988). The impact of the different mechanisms in DNA repair could differ between yeasts and mammals (Kanaar *et al.* 1998). Yeasts such as *Schizosaccharomyces pombe* or *S. cerevisiae* preferentially use homologous recombination for DSB repair to achieve high fidelity (Haber 1992). One reason could be that these organisms have genomes densely packed with genes that would result in a direct negative impact of error-prone repair (Kanaar *et al.* 1998).

The study of DSB repair in the budding yeast *S. cerevisiae* established that the *RAD52* epistasis group of genes is involved in DNA repair by homologous recombination. The products of the *RAD52* group genes are concomitantly involved in DSB repair as well as in mitotic and meiotic recombination (Petes *et al.* 1991; Game 1993). At present 10 genes have been identified in this group: *RAD50*, *MRE11*, *XRS2*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, and *RFA1* (Petes *et al.* 1991; Game 1993; Friedberg *et al.* 1995; Bai and Symington 1996; Hays *et al.* 1998). Among them, *RAD50*, *MRE11*, and *XRS2* were shown to be involved both in recombinational repair and DNA end-joining (Schiestl *et al.* 1994; Milne *et al.* 1996; Moore and Haber 1996). *RAD51*, *RAD52*, and *RAD54* are the most important genes of the group as judged by the extreme sensitivity to IR of the respective mutants (Game 1993). Genetic and biochemical experiments indicated that Rad51p can interact with both proteins (Shinohara *et al.* 1992; Milne and Weaver 1993; Donovan *et al.* 1994; Schild 1995; Jiang *et al.* 1996; Clever *et al.* 1997).

The *RAD51*, *RAD55*, and *RAD57* genes encode proteins with sequence similarity to the *E. coli* key recombination protein RecA (Kans and Mortimer 1991; Aboussekhra *et al.* 1992; Basile *et al.* 1992; Shinohara

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*et al.* 1992; Lovett 1994). However, the three proteins do not perform redundant functions, because the individual mutation in any of the three genes causes overlapping but distinct phenotypes (Game 1993). Rad51p has been shown to form a nucleoprotein filament similar to that formed by RecA protein (Ogawa *et al.* 1993) and to catalyze homologous pairing and DNA strand exchange *in vitro* (Sung 1994). This strongly suggests that it is a functional budding yeast homolog of bacterial RecA. Null mutants of both *RAD55* and *RAD57* exhibit a curious enhancement of DNA damage sensitivity at lower temperatures (Lovett and Mortimer 1987; Hays *et al.* 1995; Johnson and Symington 1995). Both proteins interact (Hays *et al.* 1995; Johnson and Symington 1995) and form a stable heterodimer (Sung 1997), which is consistent with the largely identical phenotypes of the respective mutants. An interaction between Rad55p and Rad51p was identified in the two-hybrid system (Hays *et al.* 1995; Johnson and Symington 1995). Biochemical experiments revealed that the Rad55p:Rad57p heterodimer stimulates Rad51p in the strand exchange reaction *in vitro* (Sung 1997). Unlike Rad51p, the Rad55p:Rad57p dimer cannot promote DNA pairing and strand exchange in the presence of RPA on its own (Sung 1997). This suggests a unique function of the Rad55p:Rad57p heterodimer as an accessory protein to Rad51p.

Recent studies established the evolutionary conservation of some components of the budding yeast recombinational DNA repair machinery. Homologs of the *RAD52* group genes, *RAD51*, *RAD52*, *RAD54*, *RAD50*, and *MRE11* were identified in several eukaryotic organisms including humans (Kanaar *et al.* 1998). The isolation of some of these genes was greatly aided by the isolation of recombinational repair genes from the fission yeast *S. pombe*. As fission yeast and budding yeast show great primary sequence divergence (Russell and Nurse 1986), the existence of homologs in both organisms makes it highly likely that the respective gene is conserved in eukaryotes. Homologs for *RAD51* and *RAD54* have been isolated from *S. pombe* and been named *rhp51* and *rhp54*, respectively (Muris *et al.* 1993, 1996; Shinohara *et al.* 1993; Jang *et al.* 1994). Mutations in both genes confer extreme sensitivity to IR in fission yeast similar to the situation in budding yeast. However, the *S. pombe* mutants confer a degree of genomic instability not noted for the respective mutations in *S. cerevisiae*. The fission yeast *rad22* gene product has been proposed to be a Rad52p homolog (Ostermann *et al.* 1993). However, the sequence homology is more limited than that of *rhp51* or *rhp54* with the *S. cerevisiae* counterpart, and the *rad22* phenotypes do not match the severity of the *S. cerevisiae rad52* mutant. In addition, the fission yeast *rad32* gene was recognized to be a homolog of *MRE11* (Tavassoli *et al.* 1995).

In *Escherichia coli*, a single RecA protein performs the central recombinational repair function (Kowalczykowski *et al.* 1994). In the bacterium *Myxococcus xanthus*, two *RecA* genes have been identified, suggesting functional diversification (Norioka *et al.* 1995). This diversification is extended in the budding yeast with three RecA-like proteins for DNA repair (Rad51p, Rad55p, Rad57p) and the meiosis-specific Dmc1 protein (Bishop *et al.* 1992). Not unexpectedly the situation in mammals is even more complex. Seven genes encoding proteins with homology to RecA have already been identified. Besides the rather strong homologies to the yeast Rad51 and Dmc1 proteins by the mammalian homologs (Shinohara *et al.* 1993; Habu *et al.* 1996), it is difficult to classify the remaining RecA-like proteins, R51H3, Rad51C, Xrcc2, Xrcc3, and hRec2 (Thompson 1996; Rice *et al.* 1997; Cartwright *et al.* 1998; Dosanjh *et al.* 1998) in the absence of information other than the primary sequence. Thus it is not known whether the mammalian genes represent homologs of the Rad51 protein or some of them might represent mammalian homologs to the budding yeast Rad55 and Rad57 proteins. Therefore, it remains unclear whether the functional diversification of RecA-like proteins seen in budding yeast is unique to this organism or a basic feature of the mechanisms of recombinational DNA repair in eukaryotes.

We have taken the fission yeast *S. pombe* as a model system to identify additional RecA-like proteins in this organism. The functional analysis possible in this organism provides additional criteria to the primary sequence information to classify new genes. Here we report the identification of a new RecA-like DSB DNA repair gene whose protein product has only slightly higher homology to the Rad55 protein of *S. cerevisiae* than to other RecA-like proteins. However, the analysis of the gene deletion provided strong evidence that this gene is a *RAD55* homolog. Therefore, it was named *rhp55*$^+$ for *r*ad *h*omolog *S. pombe* 55. Rhp55 protein acts in one DNA repair pathway together with Rhp51 and Rhp54 proteins, but in a different pathway than the putative Rad52p homolog Rad22p of fission yeast.

## MATERIALS AND METHODS

**Strains, media, and growth conditions:** The *S. pombe* strains used in this study are listed in Table 1. The *S. pombe* strains used for the characterization of the *rhp55*$^+$ gene had the mutations *smt-0* or *mat1PΔ17::LEU2* representing deletions of the DSB-related site in the *mat1* locus. The reason for this is discussed in the text. *E. coli* strain DH5α was used for recombinant DNA procedures in *E. coli*. *S. pombe* media malt extract agar (MEA), yeast extract agar (YEA), yeast extract liquid (YEL), and minimal medium (MMA), as well as the general genetic manipulations, have been described elsewhere (Gutz *et al.* 1974; Alfa *et al.* 1993). When necessary, 0.01% (w/v) of supplements (amino acids and nucleosides) were added to the media. For meiotic time-course experiments the synthetic minimal medium *S. pombe* minimal (PM; Beach *et al.* 1985) and PM without $NH_4Cl$ (PM-N; Watanabe *et al.* 1988) were used. Methyl methanesulfonate (MMS) was added as a liquid to solid media cooled to 50° before pouring, and plates were

**TABLE 1**

***S. pombe* strains used in this study**

| Name | Genotype | Source |
|---|---|---|
| PA39 | *h⁻ ade6-M210/h⁺ ade6-M216* | Collection Bern |
| | *h⁻ smt-0 ura4-D18* | Collection Bern |
| | *h⁻ smt-0 ura4-D18 ade7-152* | Collection Bern |
| RK3 | *h⁻ smt-0 ade7-152* | Collection Bern |
| RK4 | *h⁺ mat1PΔ17::LEU2 leu1-32 arg6-1* | Collection Bern |
| | *h⁻ arg3-D4 ura4-D18* | S. Waddell |
| HE683 | *h⁺ rad22Δ::ura4⁺ ade6-M216 ura4-D18 leu1-32* | H. Schmidt |
| *rhp51Δ* | *h⁺ rhp51Δ::ura4⁺ ade6-704 ura4-D18 leu1-32* | K. Vreeken |
| *rhp54Δ* | *h⁺ rhp54Δ::ura4⁺ ade6-704 ura4-D18 leu1-32* | K. Vreeken |
| IBGY5 | *h⁺ ura4-D18 ade6-469* | This study |
| IBGY12 | *h⁺ mat1PΔ17::LEU2 leu1-32 ade7-150* | This study |
| IBGY13 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp55Δ::ura4⁺ ura4-D18 ade7-150* | This study |
| IBGY18 | *h⁻ smt-0 arg3-D4 ura4-D18 leu1-32 ade7-152* | This study |
| IBGY19 | *h⁻ smt-0 rhp55Δ::ura4⁺ ura4-D18* | This study |
| IBGY20 | *h⁻ smt-0 rhp51Δ::ura4⁺ ura4-D18* | This study |
| IBGY21 | *h⁻ smt-0 rhp54Δ::ura4⁺ ura4-D18* | This study |
| IBGY22 | *h⁻ smt-0 rad22Δ::ura4⁺ ura4-D18* | This study |
| IBGY23 | *h⁻ smt-0 rhp55Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32 ade7-152* | This study |
| IBGY27 | *h⁻ smt-0 ade6-469* | This study |
| IBGY30 | *h⁻ smt-0 rhp55Δ::arg3⁺ arg4-D4 ura4-D18 leu1-32* | This study |
| IBGY36 | *h⁻ smt-0 rhp55Δ::ura4⁺ ura4-D18 ade6-469* | This study |
| IBGY37 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp55Δ::ura4⁺ ura4-D18 lys7-2* | This study |
| IBGY38 | *h⁺ mat1PΔ17::LEU2 leu1-32 lys7-2* | This study |
| IBGY39 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp55Δ::ura4⁺ ura4-D18 ade6-M26* | This study |
| IBGY40 | *h⁺ mat1PΔ17::LEU2 leu1-32 ade6-M26* | This study |
| IBGY41 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp55Δ::ura4⁺ ura4-D18 arg1-230* | This study |
| IBGY42 | *h⁻ smt-0 his1-102* | This study |
| IBGY43 | *h⁻ smt-0 rhp55Δ::arg3⁺ arg3-D4 rad22Δ::ura4⁺ ura4-D18 leu1-32* | This study |
| IBGY44 | *h⁻ smt-0 rhp55Δ::arg3⁺ arg3-D4 rhp51Δ::ura4⁺ ura4-D18 leu1-32* | This study |
| IBGY45 | *h⁻ smt-0 rhp55Δ::arg3⁺ arg3-D4 rhp54Δ::ura4⁺ ura4-D18 leu1-32* | This study |
| IBGY46 | *h⁻ smt-0 rhp55Δ::ura4⁺ ura4-D18 his1-102* | This study |
| IBGY47 | *h⁺ mat1PΔ17::LEU2 leu1-32 arg1-230* | This study |
| IBGY77 | *h⁺ mat1PΔ17::LEU2 leu1-32 ade6-M375* | This study |
| IBGY78 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp55Δ::ura4⁺ ura4-D18 ade6-M375* | This study |
| IBGY83 | *h⁻ smt-0 rhp55Δ::ura4⁺ ura4-D18 ade7-152* | This study |
| IBGY84 | *h⁻ smt-0 rhp55Δ::ura4⁺ ura4-D18 leu1-32* | This study |
| IBGY85 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp55Δ::ura4⁺ ura4-D18 arg6-1* | This study |
| IBGY86 | *h⁺ rhp55Δ::ura4⁺ ura4-D18 leu1-32* | This study |
| IBGY187 | *h⁺ mat1PΔ17::LEU2 leu1-32 rhp51Δ::ura4⁺ ura4-D18* | This study |
| IBGY188 | *h⁺ mat1PΔ17::LEU2 leu1-32 rad22Δ::ura4⁺ ura4-D18* | This study |
| IBGY190 | *h⁺ mat1PΔ17::LEU2 leu1-32 rad22Δ::ura4⁺ ura4-D18 rhp55Δ::arg3⁺ arg3-D4* | This study |
| IBGY200 | *h⁻ smt-0 rad22Δ::ura4⁺ rhp51Δ::ura4⁺ ura4-D18 ade6-M216* | This study |
| IBGY201 | *h⁻ smt-0 rad22Δ::ura4⁺ rhp54Δ::ura4⁺ ura4-D18 ade6-M216* | This study |

used at the same day. *S. pombe* cells were transformed by the lithium acetate procedure of Schiestl *et al.* (1993). *S. pombe* strains were grown at 30°, except when 18° or 20° was used as specified. All meiosis experiments were performed at 25°, the standard temperature for *S. pombe* meiosis.

**DNA libraries and plasmids:** The *S. pombe* cDNA library in pDB20 (Fikes *et al.* 1990) and a *S. pombe* genomic DNA library in phage λ-ZAP (kind gift from T. Enoch) were used to clone the *rhp55⁺* cDNA and genomic DNA, respectively. Several recombinant clones were isolated and the plasmids with DNA inserts were excised from phage DNA using ExAssist helper phage (Stratagene, La Jolla, CA). The oligonucleotides used for screening the cDNA library were oligo.rhp55#1 5′-AGCAC CTGGGATGGAAAAAC-3′ and oligo.rhp55#2 5′-GAATAGG CATTGATAGGTTGTC-3′. Two plasmids, pZAP-8 and pZAP-11 were found to contain ~3.9-kbp inserts encompassing the *rhp55* open reading frame (ORF) as judged by DNA sequencing. The *S. pombe* complementation vector pIBG81 carrying the *rhp55⁺* promoter and coding sequence was constructed by cloning the 1836-bp *Sal*GI-*Eco*RV from pZAP-8 in the *Sal*GI- and *Sma*I-cleaved *LEU2* vector pIRT2 (Hindley *et al.* 1987). Plasmid pIBG48 with the *rhp55* cDNA under the control of the *adh* promoter was constructed by recloning of the *Bam*HI-*Kpn*I cDNA fragment from pIBG40 into the pART1 plasmid. pART1 is a derivative of pIRT2, having the *adh* promoter. pIBG40 is the pBlueScript KS with *Hin*dIII insert of *rhp55⁺* cDNA.

**Construction of the *rhp55* deletion:** Construction of the

*rhp55*Δ*::ura4*$^+$ deletion mutant was performed by replacement of the complete ORF by the *S. pombe ura4*$^+$ gene. Two PCR fragments representing the adjacent sequences flanking the *rhp55*$^+$ ORF were generated. Two sets of primers were used to amplify 314- and 337-bp fragments flanking the *rhp55*$^+$ ORF: 5′-GGCTCTAGAGAGCTTACTCTTGCATTCCTG-3′ and 5′-GGCGGATCCTCCAGCACAGTGCGACCTAC-3′; and 5′-GGCATCGATTTTGGAATGTGAGTCCTAGG-3′ and 5′-GGCGTCGACGTCCGTACACTGAATGCGAC-3′. Restriction digestion resulted in two fragments with cohesive ends: *Xba*I-*Bam*HI and *Cla*I-*Sal*I. Recloning of these fragments into the plasmid pBS (±) carrying a 1764-bp *Hin*dIII insert of *ura4*$^+$ gene resulted in a plasmid pIBG62-8, where the *ura4*$^+$ gene is flanked by regions of homology to sequences surrounding the *rhp55*$^+$ ORF in the genome. The gene replacement module was excised with *Sal*I/*Xba*I and used to transform the haploid strain *h*$^-$ *smt-0 ura4-D18 ade7-152* by selection for ura$^+$ transformants. To construct the *rhp55*Δ*::arg3*$^+$ deletion mutant the new gene replacement plasmid pIBG62-9 was constructed as follows. The *ura4*$^+$ gene was excised from pIBG62-8 by *Hin*dIII digestion and replaced by the 1783-bp *Sma*I-*Pst*I fragment carrying the *arg3*$^+$ gene from plasmid paR3 (Waddell and Jenkins 1995). The gene replacement module was excised by *Kpn*I/*Sac*I digestion and transformed in *S. pombe* IBGY18 selecting for arg$^+$ transformants. The resultant ura$^+$ and arg$^+$ transformant were analyzed by Southern hybridization to confirm the deletion of the entire *rhp55*$^+$ gene.

**DNA manipulation:** Standard procedures were used for DNA sequencing, DNA hybridization, screening of DNA libraries, and mapping of transcription initiation sites by primer extension. DNA primer 5′-TCCAGCACAGTGCGACCTAC-3′, complementary to base pairs −21 to −40 of the *rhp55*$^+$ cDNA, was used for primer extension analysis. Chromosomal DNA from *S. pombe* was isolated as described (Wright *et al.* 1986).

**MMS-, UV-, and γ-ray-sensitivity tests:** MMS and UV sensitivity were tested by drop assays. Sequential 10-fold dilutions of exponentially growing cells were spotted on the appropriate plates with or without MMS. To test for UV sensitivity, cells were irradiated on plates after spotting. Plates were incubated at 30° or 18°. To examine IR survival, exponentially growing cells were washed, resuspended in saline, and irradiated with γ-rays using a $^{60}$Co source with a dose rate of 70 Gy/min. Appropriate dilutions were plated on complete media to determine the survival at 30° or 20°. All irradiation experiments were repeated at least twice.

**Sporulation efficiency and spore viability:** To evaluate sporulation efficiency the number of spores, asci, and vegetative cells was scored microscopically. The sporulation efficiency (percentage sporulation) was calculated as $(0.25\,S + A)/(0.25\,S + A + 0.5\,C)$, where $S$ is the number of spores, $A$ the number of asci, and $C$ the number of vegetative cells. To determine the spore viability, tetrads were dissected on YEA plates.

**Meiotic time course and Northern analysis:** Synchronized meiosis was induced by shifting vegetatively growing cells of the diploid strains PA39 from PM to PM-N medium (time point, 0 hr). Meiotic prophase in this time course started ~5 hr after induction, as determined by the amount of "horse tail" nuclei after staining with DAPI (Bähler *et al.* 1993). The first meiotic division started ~9 hr after induction as determined by the amount of cells with more than one nucleus (Bähler *et al.* 1993). Total RNA was isolated from aliquots withdrawn at various time points as described elsewhere (Grimm *et al.* 1991). A total of 20 μg of RNA was transferred to Zeta-Probe membrane (BioRad, Richmond, CA) after agarose gel electrophoresis denaturing and used for hybridization with $^{32}$P-labeled *rhp55*$^+$, *byr1*$^+$ (Nadin-Davis and Nasim 1990), and *ura4*$^+$ DNA probes under conditions recommended by the membrane supplier. The 314-bp PCR-generated fragment of *rhp55*$^+$ cDNA and a 0.4-kb *byr1*$^+$ PCR fragment were used as hybridization probes. Quantitation was performed using ImageQuant software after exposure in a phosphoimager.

**Meiotic recombination:** Frequencies of meiotic intra- and intergenic recombination were determined by random spore analysis. For each interval crosses were performed in triplicate on MEA plates. For intragenic recombination analysis the spores were microscopically counted and plated on MMA and YEA with appropriate supplements. The amount of recombinant spores on MMA was counted and normalized to the amount of viable spores. For intergenic recombination analysis spore clones were randomly picked, grown on YEA master plates, and then replicated on MMA with appropriate supplements to determine the amount of recombinant spores. Spontaneously generated diploids were identified by replica plating on MEA plates and excluded from the quantitation.

**Cytology and flow cytometry:** DAPI and Calcofluor staining of the exponentially growing cells was performed as described (Alfa *et al.* 1993). Slides with stained cells were analyzed using an epifluorescence microscope (Zeiss Axiovert) and the images were recorded with a monochrome cooled CCD camera (Kappa). To determine the cellular DNA content, propidium-iodide-stained cells were analyzed using a Becton-Dickinson (Franklin Lakes, NJ) FACScan as described (Alfa *et al.* 1993).

## RESULTS

***rhp55*$^+$ encodes a new *S. pombe* protein with homology to RecA:** By DNA data bank analysis we identified on *S. pombe* cosmids 3C7 and 25A8 an ORF encoding a polypeptide with significant homology to bacterial RecA proteins. These cosmids had been mapped on chromosome I of *S. pombe* near the *ras1*$^+$ gene (Hoheisel *et al.* 1993). The greatest homology was detected between the predicted new protein and Rad55p from *S. cerevisiae* (Figure 1). The gene was named *rhp55*$^+$, for *r*ad *h*omolog *p*ombe *55*, not only because of the sequence homology, but also because of the functional analysis discussed below. Analysis of this ORF revealed the presence of NTP-binding motifs (Walker boxes A and B) in the predicted protein typical for RecA-like proteins. To identify the complete coding sequence of the new gene we screened a *S. pombe* cDNA library using as a probe the 314-bp PCR fragment encompassing both Walker boxes, which was generated from cDNA. The identified recombinant plasmid contained a cDNA insert of ~1.4 kb. The insert was recloned as a *Hin*dIII fragment in pBlueScriptKS(±) and sequenced. The cDNA sequence data are available from GenBank under accession no. AF053410. The cloned cDNA contained the complete ORF for *rhp55*$^+$ as well as 5′- and 3′-untranslated regions. A run of $(T)_{24}$, likely to represent the poly(A) tail of the mRNA, was found to be attached to the 3′ end of cDNA (position 1303; the numbering system refers to the DNA sequence starting at the first ATG as +1). The 1443-bp cDNA contained an ORF of 1050 bp, which can be translated into a protein of 350 amino acids (aa) with a predicted molecular mass of 38,900. Because the ATG initiation codon is preceded by two in-frame stop codons, and the cloned cDNA exhibited biological ac-

tivity (Figure 4B), the use of this ATG for initiation of translation is highly likely. By comparison of the cDNA sequence and the genomic DNA sequence from cosmid 3C7, one 44-bp intron close to the ATG start codon was identified (Figure 1A). The first exon comprises nucleotides 1–16 of the coding sequence and the intron/exon junctions perfectly match the *S. pombe* consensus sequence for 3′ and 5′ splice and branch sites: GTA(A/T)GT (5′ intron site) . . . CTAAPy (branch site) . . . PyAG (3′ intron site), where Py is pyrimidines (Prabhala *et al.* 1992). In the 5′ region of the gene, three putative transcription starts were mapped to positions −100, −153, and −182 by primer extension (data not shown). Upstream of these transcription initiation sites, two potential TATA-boxes, TAAAATAA (position −235) and TATAAA (position −283), can be identified. The spacing of the TATA boxes in relation to the transcription starts is in agreement with the reported 35–120 nucleotide (nt) distance for known *S. pombe* genes (Russell 1989). In the 3′-untranslated region a possible polyadenylation signal was found at position 1116 (AT TAAT), as well as possible termination signals for mRNA synthesis (TTTTTA) at positions 1181 and 1253.

Amino acid comparison of Rhp55p with *E. coli* RecA and related proteins from budding and fission yeast revealed a significant level of identity (Figure 1B). The highest level of homology among the five proteins exists in the central region encompassing the NTP-binding motifs A and B (from ∼aa 24–157 in the Rhp55p sequence). The N-terminal regions of Rhp55p and Rad55p are shorter than the N termini of ScRad51p, SpDmc1p, and ScRad57p. Pairwise alignment using ClustalW (version 1.74) showed the highest overall homology between Rhp55p and *S. cerevisiae* Rad55p (27.9% of identical and 52.5% of similar aa). The homologies to the *S. cerevisiae* Rad57 and Rad51 proteins were 26.5% identity and 52.1% similarity as well as 21.2% identity and 40.8% similarity, respectively. Despite the moderate overall homology between the two proteins, the majority of the critical aa positions responsible for ATP-binding and conformational changes in the RecA protein have been conserved (see Figure 1B). The dendrogram shows closer similarity of Rhp55p to *S. cerevisiae* Rad55p than to Rad57p, Dmc1p, or Rad51p (Figure 1C). The sequence homology of Rhp55p to RecA and RecA-like proteins from the *RAD52* group implicated the protein in recombinational repair of DNA damage.

***rhp55*$^+$ is transcribed in vegetatively growing cells and is induced during meiosis:** Expression of *rhp55*$^+$ was examined in vegetatively growing cells and during meiosis. In a synchronized meiotic time course, total RNA was analyzed for the presence of *rhp55*-specific mRNAs. The result of this experiment (Figure 2) revealed that *rhp55*$^+$ was expressed both in mitotically dividing cells before entering meiosis (time point, 0 hr) and throughout meiosis (time, 2–10 hr). Only one mRNA species of ∼1.4 kb was detected on Northern blots, in accordance with the size of the isolated cDNA. During meiosis the expression of the gene gradually increased with a maximum occurring at 8 hr, which corresponded in this time course to meiosis I prophase. The level of *rhp55*$^+$ transcript was normalized at each time point using *byr1*$^+$ mRNA as a control. The maximal level of meiotic induction of *rhp55*$^+$ transcript levels (8 hr) was found to be fourfold above its expression in mitotically dividing cells (0 hr). A similar increase was obtained when the *ura4*$^+$ gene transcript was used for normalization (data not shown). Both controls, *ura4*$^+$ and byr1$^+$, exhibit essentially unchanged transcription levels during meiosis (Parisi *et al.* 1999). The expression pattern of the *rhp55*$^+$ gene suggested that it has a role during vegetative growth and during meiosis.

***rhp55*Δ cells contain aberrant nuclei and exhibit an increased DNA content:** Mutants of the *S. pombe* recombinational repair genes *rhp51*$^+$ and *rhp54*$^+$ were shown to have slow growth and cell elongation phenotypes (Muris *et al.* 1996). The *rhp55* mutant did not exhibit a clear slow-growth phenotype: at 30° the mutant cells grew normally, whereas at 18° slightly slower growth was observed (see Figure 4A and data not shown). However, the previous studies with *rhp51*Δ and *rhp54*Δ are difficult to interpret because the strains analyzed were not deficient for the DSB related to mating-type switching in fission yeast. The DSB associated with mating-type switching in *S. pombe* (Gutz and Schmidt 1985; Egel 1989; Arcangioli 1998) causes problems in experiments with DSB repair mutants that have not been fully appreciated in previous studies with *S. pombe*. The resulting bias against loss-of-function mutants in DSB repair genes is reflected in the results of screens for DNA repair mutants (Lehmann 1996). In *S. cerevisiae*, this problem usually does not arise because standard laboratory strains contain a mutation in the *HO* gene, encoding the endonuclease delivering the DSB at *MAT*. Although a similar mutation is not available in *S. pombe*, the use of *cis* mutations (*smt-0* or *mat1P*Δ*17::LEU2*) affecting DSB formation at *mat1* fulfill the same purpose.

Throughout this study we used strains containing these mutations to avoid compound effect between the DSB repair mutant and the DSB at *mat1*. The double DAPI-Calcofluor staining of *rhp55* mutant cells in exponential growth at 30° revealed that ∼17% of the cells were elongated at least more than twofold compared to wild type and that these cells contained aberrant nuclei (Figure 3A). This observation was further substantiated by fluorescence analysis of *rhp55* mutants (Figure 3B). Wild-type cells growing under conditions of limited nitrogen accumulate in the G1 phase (Figure 3B, top). This served to visualize the two peaks, corresponding to G1 with a 1n DNA content and to G2 cells with a 2n content. Exponentially growing fission yeast wild-type cells in full medium contain mostly a 2n or higher DNA content. This is because fission yeast spends most of the time in the G2 phase of the cycle and, be-

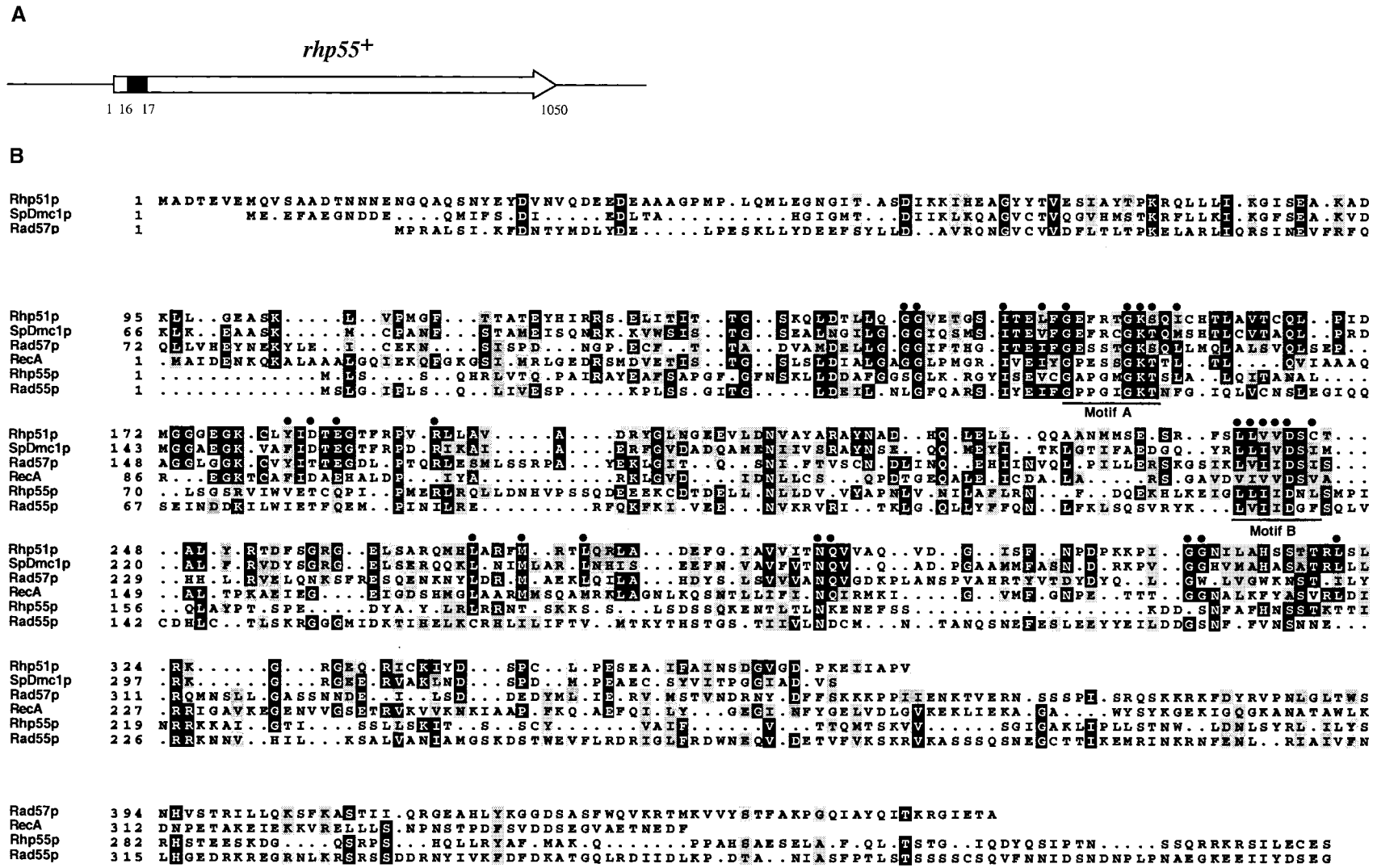


Figure 1.

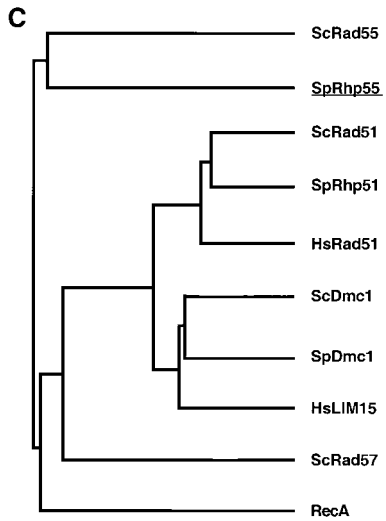


Figure 1 (*Continued*).—Structure of the *rhp55*$^+$ gene and the relationship of Rhp55p to other RecA-like proteins. (A) Exon-intron structure of the fission yeast *rhp55*$^+$ gene. The open reading frame of the *rhp55*$^+$ gene is shown as an open arrow. The black box represents the intron. The numbering refers to the nucleotides of the *rhp55*$^+$ protein-coding sequence. (B) Amino acid (aa) alignment of *S. pombe* Rhp55p with other RecA-like proteins from *S. cerevisiae* and *S. pombe*. The alignment was generated using Pile-Up (GCG software package; Devereux *et al.* 1984) and viewed with Boxshade (Bioinformatics Group, ISREC). Identical aa are highlighted in black, similar aa are shaded as follows: P, A, G, S, and T; E, D, N, and Q; V, I, L, and M; F, W, and Y; K, R, and H. Dots indicate highly conserved residues in the RecA sequence important for protein structure and function. (C) Dendrogram of clustering relationship between eukaryotic RecA-like proteins. The dendrogram was generated by Pile-Up (GCG software; Devereux *et al.* 1984). Sc, *S. cerevisiae*; Sp, *S. pombe*; Hs, *Homo sapiens*.

cause after the completion of DNA synthesis (S phase), cells are still unseparated (Figure 3B, middle). This is in accordance with previous observations and typical for fission yeast (Forsburg and Nurse 1991). However, in *rhp55* mutants the peak of intensity of propidium iodide fluorescence shifted and broadened in the direction of greater DNA content. The distribution of cells (Figure 3B, bottom) also indicated a greater heterogeneity of cell size and DNA content in the mutant compared to wild type. Approximately 48% of mutant cells

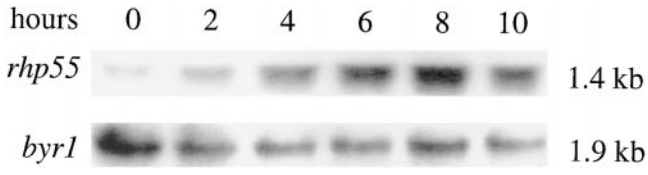


Figure 2.—Induction of *rhp55*$^+$ expression during meiosis. Total RNA was analyzed by Northern hybridization at the indicated time points after induction of meiosis. The signals were quantified on a phosphoimager using ImageQuant. The level of *rhp55* transcript was normalized to *byr1* as an internal control.

contained DNA exceeding 2n, in contrast to 26% in the wild-type cells. From these data we conclude that, even in the complete absence of a mating-type switch-associated DSB, *rhp55* cells exhibit defects in nuclear morphology and DNA content. This may indicate a role of Rhp55p in processes other than DNA repair of exogenously induced DNA damage.

***rhp55*$^+$ is a DNA-damage-repair gene:** To determine whether the *rhp55*$^+$ gene is involved in DNA damage repair, we constructed a null allele by homologous recombination. The entire ORF of the *rhp55* was substituted by the *ura4*$^+$ or *arg3*$^+$ gene. The deletion mutant was viable in haploid cells, indicating that *rhp55*$^+$ is not essential for mitotic growth. We tested the sensitivity of the deletion strain *rhp55*Δ*::ura4*$^+$ to the alkylating agent MMS and to UV light in drop assays. For comparison we included in this test other mutants of *S. pombe*-DNA-repair genes: *rhp51*, *rhp54*, and *rad22*. The first two are homologs of the *S. cerevisiae RAD51* and *RAD54* genes (Muris *et al.* 1993, 1996; Shinohara *et al.* 1993); the latter has been proposed to be a homolog of the *RAD52* gene (Ostermann *et al.* 1993). As shown in Figure 4A, the *rhp55* mutant was sensitive to MMS and to a lesser extent to UV at 30°. The sensitivity to MMS was less extreme than in *rhp51* and *rhp54* mutants. The sensitivity of the *rhp55* mutant to UV light at 30° was more pronounced at higher UV doses (see also Figure 4B). In contrast, the *rad22* mutant was almost fully resistant to these DNA-damaging agents. Interestingly, at 18° the *rhp55* mutant exhibited a much higher sensitivity to MMS and UV. Under these conditions the survival of *rhp55* mutants was indistinguishable from that of *rhp51* and *rhp54* mutants. This unusual property of cold-enhanced sensitivity to DNA-damaging agents is a unique feature of the *S. cerevisiae rad55* and *rad57* mutants (Lovett and Mortimer 1987; Hays *et al.* 1995; Johnson and Symington 1995). Because the damage to cells caused by MMS is rather complex and DSBs constitute only a part of the lesions induced by this drug (Friedberg *et al.* 1995), we examined the effect of IR. In Figure 5 the γ-ray survival curves of the *rhp55* mutant at two temperatures (30° and 20°) are shown along with those of the *rhp51*, *rad22*, and *rhp54* single mutants. These data demonstrate that the *rhp55*$^+$ gene is involved in the repair of DNA damage induced by γ-rays. Moreover, the γ-ray sensitivity is also enhanced at lower temperatures as was found for MMS and UV damage (Figure 5A). At 20° the *rhp55* mutant is as sensitive to IR as the single *rhp51* or *rhp54* mutants. Again, the *rad22* mutant was only slightly sensitive to γ-irradiation (Figure 5A). From this data we conclude that *rhp55*$^+$ is a new DNA-damage-repair gene in fission yeast.

**The *rhp55*$^+$ cDNA complements the DNA-damage-repair defect caused by the *rhp55*Δ mutation:** To further ascertain that we have identified the full extent of the *rhp55*$^+$ gene and not missed an intron, we constructed two complementation plasmids. Previous analysis of

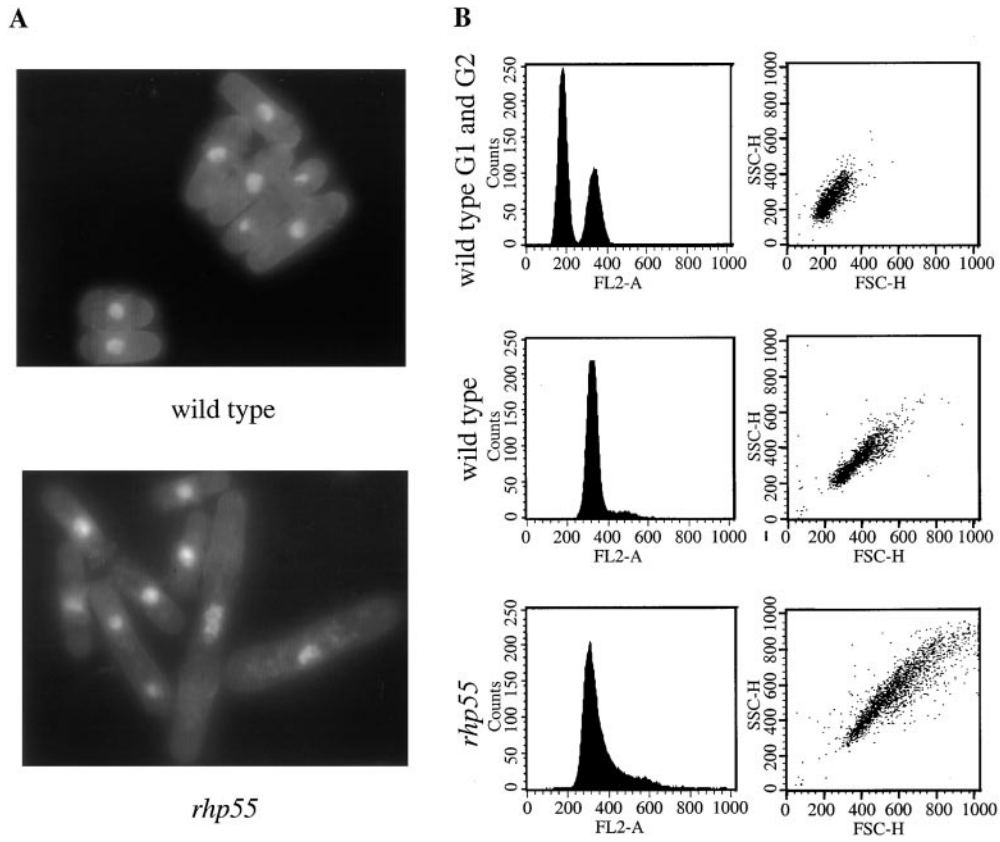


Figure 3.—Fluorescence microscopy and FACS analysis of the *rhp55*Δ mutant. (A) DAPI and Calcofluor double staining of wild-type and *rhp55*Δ cells. (B) Results from FACS analysis. Left, plots of cell counts over propidium iodide staining; right, distributions of cells plotting the peak of intensity of propidium iodide fluorescence and the total intensity. This gives an indication of the heterogeneous nature of the cells and the nuclear organization. Control wild-type cells (*h*$^-$ *smt-0 ura4-D18* strain) grown in minimal media with limiting nitrogen source were used to demonstrate the DNA contents of cells in G1 and G2 (top row). Cells of wild-type strain (middle row) and *rhp55*Δ mutant strain IBGY19 (bottom row) were used during exponential growth in full medium (YEL medium).

genes that control meiotic recombination in fission was complicated by the occurrence of multiple introns, *e.g.*, *rec12* (Lin and Smith 1994; Bergerat *et al.* 1997; Fox and Smith 1998). In pIBG48 the *rhp55*$^+$ cDNA was placed under the control of *S. pombe adh* promoter, and pIBG81 contained the *rhp55*$^+$ gene with its authentic promoter. These constructs were transformed into the *rhp55*Δ strain IBGY84 and the resulting clones were analyzed for the DNA repair phenotype of the mutant. The data presented in Figure 4B demonstrate that both the *rhp55*$^+$ cDNA and the cognate gene complement the MMS and UV sensitivity of the mutant equally efficiently,

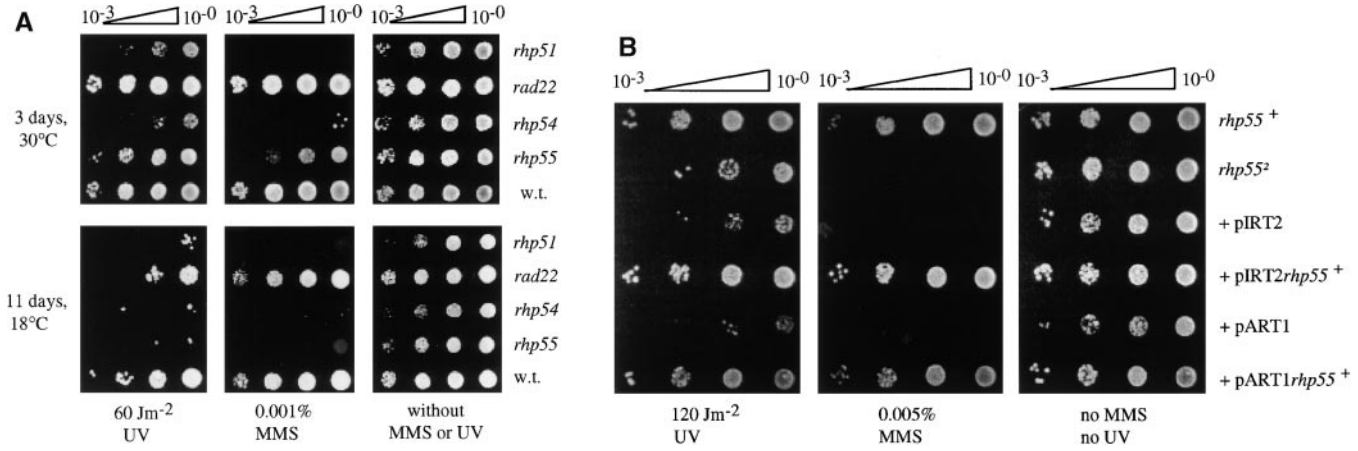


Figure 4.—MMS and UV sensitivity of the *rhp55*Δ mutant. (A) MMS and UV survival test of the *rhp55* mutant. MMS and UV sensitivity was tested by drop assay (see materials and methods). The following isogenic strains were used: *h*$^-$ *smt-0 ura4-D18* (wild type), IBGY19 (*rhp55*Δ), IBGY20 (*rhp51*Δ), IBGY21 (*rhp54*Δ), and IBGY22 (*rad22*Δ). (B) Complementation of MMS and UV sensitivity of the *rhp55*Δ mutant by cloned cDNA and cognate gene. Complementation was tested by drop-assays (see materials and methods). The strains were grown at the indicated temperatures and plates were photographed after incubation for the indicated times. *rhp*$^+$, wild-type strain *h*$^-$ *smt-0 ura4-D18*; *rhp55*$^2$, *rhp55*Δ strain IBGY84; +pIRT2 and +pIRT2*rhp55*$^+$, IBGY84 transformed with either empty vector or pIBG81; +pART1 and +pART1*rhp55*$^+$, IBGY84 transformed with an empty vector and pIBG48.

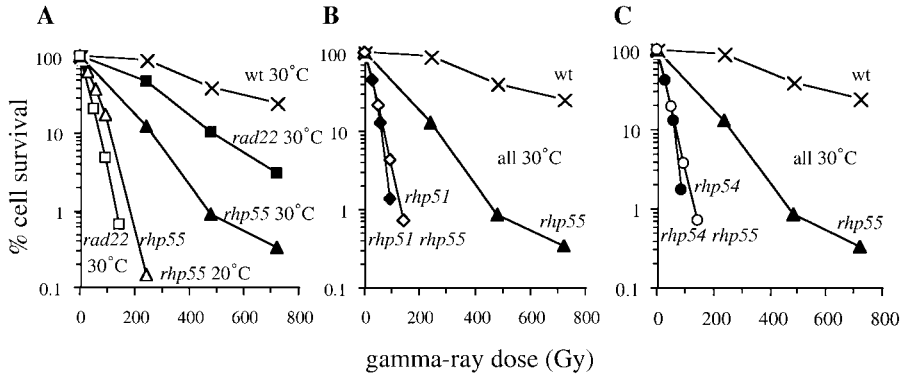


Figure 5.—γ-ray survival curves of single and double mutants of the *S. pombe* DSB repair genes. (A) Cell survival curves of *rad22*, *rhp55*, and *rad22 rhp55* double mutants. (B) Survival curves of *rhp51*, *rhp55*, and *rhp51 rhp55* double mutants. (C) Survival curves of *rhp54*, *rhp55*, and *rhp54 rhp55* double mutants. The following strains were used: x, wild type (*h⁻ smt-0 ura4-D18*); ■, *rad22*Δ (IBGY-22); ▲, *rhp55*Δ (IBGY19); △, *rhp55*Δ at 20°; □, *rad22*Δ *rhp55*Δ (IBGY43); ◆, *rhp51*Δ (IBGY20); ◇, *rhp51*Δ *rhp55*Δ (IBGY44); ●, *rhp54*Δ (IBGY21); ○, *rhp54*Δ *rhp55*Δ (IBGY45). All survival curves were determined at 30° except when indicated at 20° for *rhp55*Δ.

restoring the wild-type level of damage resistance. This indicated that the cloned cDNA encodes a functional protein.

To explore the functional relation of *rhp55*$^+$ to known *S. cerevisiae* homologs we also examined the ability of the *rhp55*$^+$ cDNA to complement the MMS sensitivity of *S. cerevisiae rad51*, *rad55*, and *rad57* mutants by placing the cDNA onto centromeric plasmids under the control of a set of *S. cerevisiae* promoters of different strengths (Mumberg *et al.* 1995). All constructs, when transformed in *S. cerevisiae*, failed to complement the MMS sensitivity of *rad51*, *rad55*, or *rad57* mutants (data not shown). The absence of interspecies complementation between budding and fission yeasts is quite common and reflects the evolutionary divergence between both organisms (Russell and Nurse 1986).

**Rhp55p acts in one pathway with Rhp51 and Rhp54 proteins, but in a different pathway than the putative Rad52p homolog, Rad22p:** Double-mutant analysis allows the assignment of genes to one or different epistasis groups in DNA repair, which are believed to represent different pathways for the repair of a specific DNA lesion (Game 1993; Friedberg *et al.* 1995). In *S. cerevisiae RAD51*, *RAD52*, *RAD54*, and *RAD55* have been assigned to one epistasis group, the recombinational repair group. The double mutants *rhp55 rhp51* and *rhp55 rhp54* showed the same γ-ray sensitivity at 30° as the *rhp51* and *rhp54* single mutants (Figure 5, B and C). This indicates that *rhp55* is epistatic to *rhp51* and *rhp54* and functions in the same DNA repair pathway. However, the *rhp55 rad22* double mutant exerted a synergistic sensitivity to γ-rays at 30° if compared to the single mutants (Figure 5A). This indicates that these genes act in different DNA repair pathways competing for the same substrate. We extended the epistatic analysis of putative recombinational repair genes by testing *rad22 rhp55*, *rad22 rhp51*, *rad22 rhp54*, *rhp51 rhp55*, and *rhp54 rhp55* double mutants for sensitivity to MMS and UV. The data presented in Figure 6 demonstrated that *rad22 rhp51*, *rad22 rhp54*, and *rad22 rhp55* double mutants were significantly more sensitive to low doses of DNA-damaging agents (0.0001% MMS and 40 J m$^{-2}$ UV) than the respective

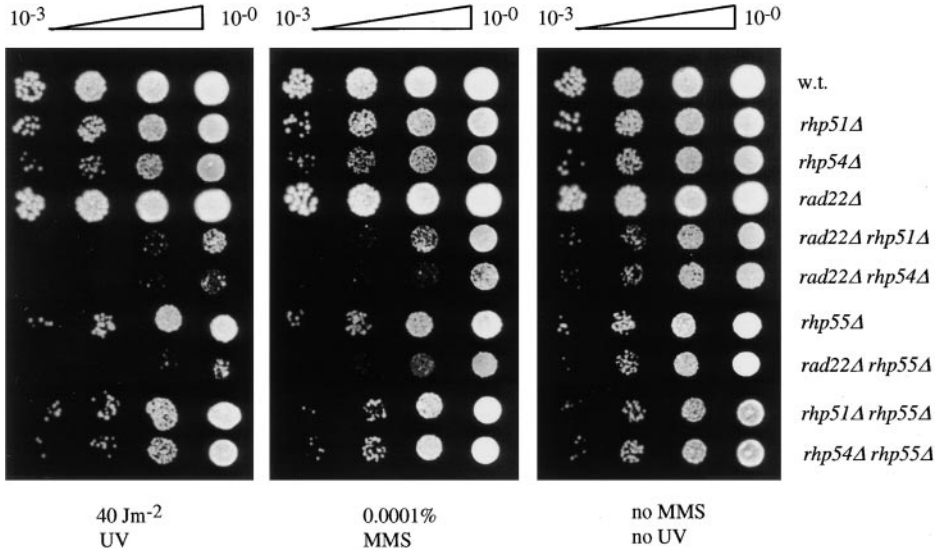


Figure 6.—Epistatic analysis of *rad22*$^+$, *rhp51*$^+$, *rhp54*$^+$ and *rhp55*$^+$ genes for repair of MMS and UV damage. MMS and UV sensitivity were tested by drop assay as described in materials and methods. Isogenic strains used were wild type (*h⁻ smt-0 ura4-D18*), *rhp51*Δ (IBGY20), *rhp54*Δ (IBGY21), *rhp55*Δ (IBGY19), *rad22*Δ (IBGY22), *rhp51*Δ *rad22*Δ (IBGY200), *rhp54*Δ *rad22*Δ (IBGY-201), *rhp55*Δ *rad22*Δ (IBGY43), *rhp-51*Δ *rhp55*Δ (IBGY44), and *rhp54*Δ *rhp55*Δ (IBGY45). Plates were incubated at 30° for 3 days and photographed.

**TABLE 2**

**Sporulation in *rhp55* mutants**

| Strain[a] | Spores | Asci | Vegetative cells | Sporulation efficiency (%)[b] | Fold reduction[c] |
|---|---|---|---|---|---|
| Wild type | 19.3 ± 3.3 | 9.2 ± 2.9 | 69.9 ± 4.1 | 26.9 ± 4.7 | |
| *rhp55* | 4.6 ± 0.6 | 4.0 ± 0.7 | 90.8 ± 1.0 | 10.2 ± 3.0 | 2.6 |
| *rad22* | 14.4 ± 1.5 | 1.4 ± 0.6 | 84.1 ± 0.8 | 11.0 ± 0.2 | 2.4 |
| *rad22 rhp55* | 15.3 ± 0.9 | 3.0 ± 0.6 | 81.7 ± 1.4 | 14.4 ± 0.8 | 1.9 |
| *rhp51* | 2.7 ± 0.1 | 1.1 ± 0.2 | 96.2 ± 0.1 | 3.6 ± 0.4 | 10.3 |

[a] Strains RK3 × IBGY12 (wild type), IBGY13 × IBGY83 (*rhp55*Δ), IBGY22 × IBGY188 (*rad22*Δ), IBGY20 × IBGY187 (*rhp51*Δ), and IBGY43 × IBGY190 (*rhp55*Δ) were crossed in triplicate.
[b] The sporulation efficiency was calculated as described in materials and methods.
[c] The reduction is given relative to the wild-type crosses.

single-mutant strains. This synergistic behavior suggests that Rad22p acts in a different pathway than Rhp51p, Rhp54p, and Rhp55 for the repair of this type of DNA damage. Moreover, the *rhp51 rhp55* and *rhp54 rhp55* double mutants are as resistant to these doses as single *rhp51* and *rhp54* mutants, confirming the epistatic interaction identified previously for γ-ray sensitivity (Figure 5). Therefore, the double-mutant analysis shows the existence of an epistasis group for the repair of γ-ray, MMS, and UV damage in fission yeast similar to the *RAD52* group in budding yeast consisting presently of *rhp51*$^+$, *rhp54*$^+$, and *rhp55*$^+$. However, *rad22*$^+$, a gene proposed to be the fission yeast *RAD52* homolog, acts in a different pathway than the Rhp51, Rhp54, and Rhp55 proteins.

***rhp55*Δ affects meiosis and causes a small reduction in meiotic recombination:** The *S. cerevisiae rad51*, *rad55*, and *rad57* mutants are characterized by strong meiotic defects such as reduced sporulation efficiency and gross spore inviability (Petes *et al.* 1991; Game 1993). Therefore, we examined the sporulation of *rhp55* crosses in comparison with wild-type crosses at 25°, the standard temperature for meiosis experiments in fission yeast. We found that the sporulation efficiency was reduced 2.6-fold in the mutant (Table 2). The sporulation efficiency of wild-type crosses was consistent with previous determinations (Bähler *et al.* 1993). Moreover, only about half of the mutant spores (54.4%) were found to be viable, whereas wild-type crosses resulted in 92.4% viable spores (Table 3). This difference was statistically significant with a probability of $<0.01$ ($\chi^2$ analysis). Analyzing the distribution of inviable spores among tetrads revealed that more tetrads with 0 viable spores were observed than anticipated from a random process in the mutant (9 found *vs.* 3.73 expected). This difference was significant with a probability of $<0.01$. The reason for the increase in this particular class might be related to premeiotic events in the mutant. Vegetatively growing cells were found to contain aberrant nuclei and aberrant DNA content (see Figure 3). Mating of such cells may lead to the complete meiotic inviability observed in this class. However, mitotic events cannot account alone for the low-spore viability in *rhp55* cells, because the classes with 4 and 3 viable spores are also significantly reduced in the mutant compared to wild type. Because fission yeast contains only three chromosomes, random chromosome segregation results in considerable viability among the meiotic products. The assumption of random MI segregation and equational MII segregation predicts 22% of the spores to be viable, including haploid, diploid, and chromosome III disomic spores (G. R. Smith, personal communication). Fission yeast chromosome I and II disomes are not viable (Niwa and Yanagida 1985). The *rhp55* data suggest that this pro-

**TABLE 3**

**Spore viability in *rhp55* mutants**

| | Tetrads with viable spores | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain[a] | 4 | 3 | 2 | 1 | 0 | Total tetrads | % viable | Fold reduction |
| Wild type | 64 | 13 | 3 | 2 | 0 | 82 | 92.4 | |
| *rhp55* | 11 | 26 | 25 | 15 | 9 | 68 | 54.4 | 1.7 |
| *rad22* | 0 | 4 | 9 | 18 | 15 | 46 | 26.1 | 3.5 |
| *rad22 rhp55* | 13 | 11 | 10 | 2 | 0 | 36 | 74.3 | 0.8 |
| *rhp51* | 0 | 0 | 0 | 5 | 11 | 16 | 7.8 | 11.8 |

[a] Strains RK3 × IBGY12 (wild type), IBGY13 × IBGY83 (*rhp55*Δ), IBGY22 × IBGY188 (*rad22*Δ), IBGY20 × IBGY187 (*rhp51*Δ), and IBGY43 × IBGY190 (*rhp55*Δ *rad22*Δ) were crossed in triplicate.

**TABLE 4**

**Meiotic intragenic recombination in *rhp55* mutants**

| Chromosome and alleles | | Prototrophs/$10^6$ viable spores[a] | | Fold reduction[b] |
|---|---|---|---|---|
| | | Wild type | *rhp55*Δ | |
| II L | *ade7-150* × *ade7-152* | 443 ± 119 | 405 ± 92 | 1.1 |
| III R | *ade6-469* × *ade6-M375* | 474 ± 53 | 164 ± 42 | 2.9 |
| III R | *ade6-469* × *ade6-M26* | 8920 ± 1454 | 5772 ± 227 | 1.6 |

Strains crossed were as follows: RK3 × IBGY12 and IBGY83 × IBGY13 (*ade7-150* × *ade7-152* alleles); IBGY27 × IBGY77 and IBGY36 × IBGY78 (*ade6-469* × *ade6-M375* alleles); IBGY27 × IBGY40 and IBGY36 × IBGY39 (*ade6-469* × *ade6-M26* alleles). (L) Left and (R) right arms of chromosomes II and III, respectively.

[a] Mean and standard deviation from three independent crosses.

[b] The reduction is given relative to the wild-type crosses.

tein is important for full spore viability, but not essential, because the spore viability is significantly above the expected level for random segregation. The reduction in spore viability found in the *rhp55* mutant is less than found previously with *rhp51*, for which a spore viability of 1.7% has been reported (Muris *et al.* 1997). However, that value was not determined by tetrad analysis but by determining the colony-forming units of a spore suspension. Moreover, it relates to a wild-type level that was artificially set to 100%. As shown in Table 3, the spore viability of the *rhp51* mutant was 7.8% as determined by tetrad analysis, which is significantly below the expected 22% ($P < 5\%$). As *rhp55*$^+$ and *rad22*$^+$ were found to act in different DNA repair pathways (see above), it was of interest to determine if the double mutant will show synergism for meiosis. Tables 2 and 3 show the sporulation efficiencies and spore viability of *rad22* and *rad22 rhp55* mutant crosses. The *rad22* mutant showed the reduction in sporulation efficiency similar to *rhp55* mutant (2.4- and 2.6-fold, respectively); however, spore viability was two times lower (26.1 and 54.4%, respectively), which was significant ($P < 0.01$). The *rhp55 rad22* double mutant showed less reduction in sporulation efficiency and spore viability in comparison with the single mutants. However, the difference with the *rhp55* single mutant was statistically not significant. This indicates the absence of a synergistic effect of the two mutations in meiosis in fission yeast.

**TABLE 5**

**Meiotic intergenic recombination in *rhp55* mutants**

| Chromosome and interval | Recombinants (%)[a] | | Fold reduction[b] |
|---|---|---|---|
| | Wild type | *rhp55*Δ | |
| I R *his1-lys7* | 20.3 ± 3.1 | 11.1 ± 0.7 | 1.8 |
| II L *ade7-arg6* | 10.1 ± 1.5 | 5.9 ± 0.4 | 1.7 |
| III R *ade6-arg1* | 36.0 ± 0.1 | 25.7 ± 1.8 | 1.4 |

Strains crossed were as follows: IBGY42 × IBGY38 and IBGY46 × IBGY37 (*his1-lys7* interval); RK3 × RK4 and IBGY83 × IBGY85 (*ade7-arg6* interval); IBGY27 × IBGY47 and IBGY36 × IBGY41 (*ade6-arg1* interval). (L) Left and (R) right arms of chromosomes I, II, and III, respectively.

[a] Mean and standard deviation from three independent crosses. For each interval 1000–1100 random spores were analyzed.

[b] The reduction is given relative to the wild-type crosses.

To determine to what extent *rhp55*Δ mutants were affected in meiotic recombination, we analyzed meiotic intra- and intergenic recombination. Intragenic recombination was examined in three intervals, two in the *ade6* locus on chromosome III (*ade6-469* × *ade6-M26* and *ade6-469* × *ade6-M375* crosses) and one in the *ade7* locus on chromosome II (*ade7-150* × *ade7-152* cross; Table 4). In the *ade6* locus we observed a small reduction (1.6- and 2.9-fold) in intragenic recombination. This reduction was significant as indicated by the nonoverlapping standard deviations. No reduction of intragenic recombination was found in the *ade7* locus. Intergenic recombination (Table 5) was analyzed in three intervals placed on the three fission yeast chromosomes. In the *his1-lys7* (chromosome I), *ade7-arg6* (chromosome II), and *ade6-arg1* (chromosome III) intervals, meiotic intergenic recombination was reduced 1.8-, 1.7-, and 1.4-fold, respectively, in the mutant when compared to wild-type crosses. As indicated by the nonoverlapping standard deviations, these reductions were significant. Similarly, only small reductions in meiotic intergenic recombination were found in *rhp51* and *rhp54* mutants of *S. pombe* (Muris *et al.* 1997). No region specificity was observed in intergenic recombination, as the reduction in the mutant was similar in all intervals tested. From this data we conclude that Rhp55p is required for full meiotic recombination in fission yeast.

## DISCUSSION

In this study we report the isolation of *rhp55*$^+$, a new recombinational DNA repair gene, from *S. pombe* encoding the likely fission yeast homolog of the *S. cerevisiae* Rad55 protein. Rhp55p acts in one DSB repair pathway with Rhp51 and Rhp54 proteins, which is different from

the pathway in which the putative Rad52p homolog, Rad22p, acts. Rhp55 protein is important for recombinational DNA repair of exogenously induced DNA damage by MMS, UV, and IR. Moreover, Rhp55p is required for genomic stability, efficient sporulation, and full meiotic recombination in fission yeast. Throughout this study we used strains deficient for the mating-type switching-related DSB to avoid possible compound effects with the mutations in DSB repair.

**Rhp55p is a new recombinational repair protein in *S. pombe*:** Recombinational repair is a major pathway for the repair of MMS-induced and γ-ray-induced DNA lesions in yeasts (Petes *et al.* 1991; Game 1993; Friedberg *et al.* 1995; Lehmann 1996). Moreover, it appears that in *S. pombe*, an organism lacking photolyase for the repair of UV damage, recombinational repair constitutes a major pathway also for the repair of UV-induced DNA damage (Lehmann 1996). Based on the following evidence, Rhp55p is a new recombinational repair protein in fission yeast. First, the severity and the spectrum of the sensitivities of *rhp55* mutants toward MMS, γ-ray, and UV are consistent with a role of Rhp55p in recombinational repair. Second, the phenotypes of the *rhp55* mutant are similar and, at lower temperatures, almost identical to that of two other recombinational repair mutants in fission yeast, *rhp51* and *rhp54* (Muris *et al.* 1993, 1996; Shinohara *et al.* 1993). Third, double-mutant analysis established that Rhp55, Rhp51, and Rhp54 proteins act in the same DNA repair pathway. Fourth, analysis of meiotic recombination in *rhp55* mutants demonstrated a mild but significant defect in intra- and intergenic homologous recombination.

The cold-enhanced sensitivity of the *rhp55* deletion mutant to UV, MMS, and γ-ray is unique among the recombinational repair mutants in fission yeast, but similar to the phenotype of deletions in the *S. cerevisiae RAD55* and *RAD57* genes (Lovett and Mortimer 1987; Hays *et al.* 1995; Johnson and Symington 1995; see below). Protein:protein interaction leads to a net gain of entropy, and temperature sensitivity is often a consequence of impaired hydrophobic interactions within a protein (Shortle 1989) or between subunits of a complex (Cantor and Schimmel 1980). An example for an intrinsically cold-sensitive heteromultimeric protein complex is the microtubule (Mandelkow and Mandelkow 1994). Also the assembly of functional α- and γ-tubulin is a cold-sensitive process in deletion mutants of the *GIM* genes (Geissler *et al.* 1998). Cold-sensitive assembly of a protein complex can also be found in mutants affecting ribosome assembly (Guthrie *et al.* 1969; Lhoest and Colson 1981). The cold-sensitive phenotype of the *rhp55* deletion suggests that it either stabilizes a heteromultimeric complex or is involved in the assembly of a protein complex important for the repair of DNA damage. The biochemical properties of the Rad55p:Rad57p heterodimer of budding yeast (Sung 1997) are compatible with both models.

**Rhp55p is the homolog of the *S. cerevisiae* Rad55 protein:** On the basis of the specific evidence discussed below, we suggest that Rhp55p is a homolog of the *S. cerevisiae* Rad55 protein. First, pairwise sequence comparisons and the clustering relationship show higher homology of Rhp55p to Rad55 protein than to any other RecA-like protein in budding yeast. Second, the overall protein sequence of Rhp55 protein resembles that of Rad55p more than that of any other RecA-like protein in *S. cerevisiae*. All RecA-like proteins are characterized by a central core containing the highest homology and the two consensus sequences for nucleotide binding. In addition, they have N- and C-terminal extensions of different lengths (Heyer 1994). Both Rhp55 and Rad55 proteins are characterized by rather short, almost identical length N-terminal extensions (see Figure 1B). Taking the conserved glycine at position 26 (G24 of Rhp55p equivalent to G44 in RecA; see Figure 1B) as a reference point, the N-terminal region of Rhp55p and Rad55p is significantly smaller than that of any other RecA-like protein in the two yeasts (Rhp55p, 25 aa; Rad55p, 23 aa). This is clearly distinct from the much longer N-terminal regions of Rad51-like proteins (126 aa for Rhp51p and 162 aa for Rad51p), Dmc1-like proteins (101 aa for SpDmc1p, 98 aa for ScDmc1p), or Rad57p-like proteins (102 aa). The C-terminal extension of Rhp55p is clearly different from Rad51p and Dmc1p in length and sequence, but similar to Rad55p and Rad57p (see Figure 1B and Heyer 1994). Third, the phenotypes of the *rhp55* mutant in DNA damage repair are comparable to those of *rad55* and *rad57* mutants. Fourth, *rhp51*$^+$ has been isolated as a high-copy suppressor of the *rhp55* deletion mutation (G. V. Savchenko, unpublished observations); similar high-copy suppression of the *rad55* and the *rad57* deletion by *RAD51* has been identified in budding yeast (Hays *et al.* 1995; Johnson and Symington 1995). Fifth, the cold-enhanced DNA-damage sensitivity of *rhp55* mutants is the most compelling argument for its similarity to *RAD55*. The cold-enhanced phenotypes of the *rad55* and *rad57* deletion mutants are a unique feature for these DNA-damage-repair genes (see above; Petes *et al.* 1991; Game 1993). The absence of interspecies complementation is not an argument against this interpretation as this is quite commonly found because of the evolutionary divergence between both organisms (Russell and Nurse 1986). Failure of interspecies complementation between the two yeasts has been reported even when the mammalian homolog showed complementation in one of the yeast species. One example of this effect is the Rad54 protein (Kanaar *et al.* 1996; Muris *et al.* 1997). Other examples can be found with components of the transcriptional apparatus, another evolutionarily conserved process (Shpakovski *et al.* 1995).

Therefore, the occurrence of a Rad55p homolog in fission yeast suggests that the functional diversification of RecA-like genes in *S. cerevisiae* with one homolog,

Rad51p, performing a highly similar function to RecA (Sung 1994) and two additional proteins, Rad55 and Rad57, performing a different accessory role (Sung 1997), may not be a peculiarity of budding yeast but rather a general feature of the molecular mechanisms of recombinational repair in eukaryotes. Future biochemical work with the *S. pombe* protein will have to substantiate this notion. This leads to the prediction of the occurrence of a Rad57-like protein in fission yeast and a strong candidate for this has been recently identified (H. Shinagawa, personal communication). Thus, some of the mammalian RecA-like proteins (see Introduction) may turn out to perform a accessory role to the human Rad51 protein similar to that of the Rad55: Rad57 heterodimer to Rad51p in *S. cerevisiae*.

**Rhp51, Rhp54, and Rhp55 proteins form a recombinational repair epistasis group that does not include the putative Rad52p homolog Rad22p:** Double-mutant analysis can be used to define epistasis groups that are likely to reflect an organization in pathways (Friedberg *et al.* 1995). If a double mutant exhibits a sensitivity no greater than the most sensitive single mutant, the two genes in question are considered epistatic, *i.e.*, being in one pathway. If, however, the double mutant exhibits an additive sensitivity, the two genes are likely to act in different pathways. Synergistic enhancement of the sensitivity in the double mutant not only suggests that both genes act in different pathways, but also that these pathways compete for the same substrate. DNA-damage-repair epistasis groups have been extensively defined in *S. cerevisiae* (Friedberg 1988; Game 1993; Friedberg *et al.* 1995) but only to a much smaller extent in *S. pombe*. The mutant phenotypes of *rad32* (Tavassoli *et al.* 1995), *rad21* (Birkenbihl and Subramani 1992), *rad22* (Ostermann *et al.* 1993), *rhp55* (this article), *rhp51*, and *rhp54* (Muris *et al.* 1993, 1996, 1997) had suggested an involvement of the respective genes in a recombinational repair pathway similar to the *RAD52* pathway of budding yeast. Here we greatly extend the limited epistasis analysis in *S. pombe*, which showed that *rad32*$^+$ and *rhp51*$^+$ act in one pathway for the repair of UV damage (Tavassoli *et al.* 1995), by demonstrating that this epistasis group also includes *rhp55*$^+$ and *rhp54*$^+$, but not *rad22*$^+$, for the repair of γ-ray-, MMS-, and UV-induced damage (Figure 6). This strongly suggests that *rad22*$^+$ is not a *functional* homolog of the *S. cerevisiae RAD52* gene as previously suggested (Ostermann *et al.* 1993). This interpretation is also more consistent with the much weaker phenotypes of the *rad22* mutant for UV, MMS, and γ-ray survival (this article; Ostermann *et al.* 1993; Muris *et al.* 1997) than those reported for the *S. cerevisiae rad52* mutant (Petes *et al.* 1991; Game 1993; Friedberg *et al.* 1995). In *S. cerevisiae*, Rad52p is central for homologous recombination and SSA in contrast to Rad51p, Rad55p, and Rad57p, which have no role in SSA (Ivanov *et al.* 1996).

The existence of Rad59p in *S. cerevisiae*, a Rad52p-related protein that functions in *RAD51*-independent recombination (Bai and Symington 1996), poses the question of whether *rad22*$^+$ may be the fission yeast homolog of *RAD59*. This appears unlikely because, in *S. cerevisiae*, *RAD59* and *RAD51* are epistatic for repair of DNA damage (L. Symington, personal communication), unlike *rhp51* and *rad22* in fission yeast.

The question of whether there is a functional *RAD52* and/or *RAD59* homolog in fission yeast can at present not be answered. Database searches revealed the presence of a second ORF besides *rad22* in the fission yeast genome, which may encode a protein with homology to Rad52p (V. I. Bashkirov, unpublished observations). The function of this putative gene is unknown. Similar to the *S. pombe rad22* mutant, homozygous mutations in genes with homology to *RAD52* in mouse ES cells or chicken DT40 cells were found to lack obvious DNA repair defects. Moreover, such cells exhibited only minor deficiency in targeted recombination (Rijkers *et al.* 1998; Yamaguchi-Iwai *et al.* 1998). Thus, also in chicken and mouse these proteins do not have the same important function Rad52p has in budding yeast.

**Rhp55 protein is involved in maintaining genomic stability and in meiosis:** Previous work in the *rhp51* and *rhp54* mutants suggested a high degree of genomic instability in these mutants reflected by an accumulation of aberrantly long cells with a higher than normal DNA content (Muris *et al.* 1996). However, it is difficult to fully interpret these results because these studies have been conducted in strains where the *mat1*-related DSB occurs, which may lead to compound effects in DSB repair mutants. In this study, we have used DSB-negative strains that harbor *cis*-acting mutation (*smt-0*, *mat1P-Δ17::LEU2*), preventing the formation of a mating-type associated DSB. Thus, the observed increase in elongated cells with greater than normal DNA content found in *rhp55* mutants was not caused by a compound effect with the *mat1*-related DSB. Similar effects of mutations in recombinational repair genes in budding yeast have not been noticed. Therefore, DSB repair proteins exhibit a more visible role in genomic stability in fission yeast than in budding yeast. For a full discussion of this point and the reasoning for a possible involvement of DSB repair proteins in DNA replication see Muris *et al.* (1996). In conclusion, *rhp55*$^+$ and the recombinational DNA repair pathway are required for genomic stability in fission yeast in the absence of exogenously added genotoxic stress. Thus, fission yeast might present an informative model system to understand the inviability of vertebrate cells lacking Rad51 protein (Lim and Hasty 1996; Tsuzuki *et al.* 1996; Sonoda *et al.* 1998).

The Rhp55 protein is suggested to have a role in meiosis based on the following evidence. First, the mutant exhibited reduced sporulation efficiency and spore viability. Some of the observed meiotic lethality is probably a result of the accumulation of genomic aberrations before meiosis as suggested by the spore viability pattern

and the cytological analysis of vegetatively growing cells. Aneuploid cells are exceedingly unstable in fission yeast (Niwa *et al.* 1986) and a majority of the *rhp55* mutant cells appeared to have a normal DNA content, strongly suggesting that not all of the meiotic lethality is due to premeiotic events. This is supported by the spore viability pattern in the mutant. The spore viability of *rhp55* mutants in fission yeast is significantly higher than that of *rad55* mutants in budding yeast, which was reported to be <2.5% (Lovett and Mortimer 1987). One possible reason for this difference could be that fission yeast has only 3 chromosomes, which, with random chromosome segregation, still is expected to lead to 22% spore viability (G. R. Smith, personal communication). Budding yeast, however, has 16 chromosomes resulting in <1% expected viable spores with random segregation. Second, the *rhp55* gene is transcriptionally induced in meiosis. Third, meiotic recombination is reduced in *rhp55* mutants. The observed reduction may be an underestimate as only the viable progeny could be analyzed. This reduction is similar in extent to mutations in *rhp54* and *rhp51*, which were reported to be 1.7- and 2.5-fold, respectively, in the *his1-leu2* interval (Muris *et al.* 1997). Moreover, the analysis of meiotic recombination selects for viable meiotic products, which are less likely to result from a meiosis of mating partners with aberrant DNA content. The effect of the *rhp55* mutation on meiotic recombination in fission yeast is probably significantly lower than that of a *RAD55* mutation in budding yeast. Although a role of Rad55p in meiotic recombination was suggested (Game 1993), no quantitative data are available for *rad55* strains. As Rad55p and Rad57p act as a heterodimer (Sung 1997), one may extrapolate from the available *rad57* data, which suggest at least a 10-fold reduction in viable intragenic recombinants (Borts *et al.* 1986). The reason for this difference between fission and budding yeast is unknown.

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