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RESEARCH ARTICLE

Genetic analysis reveals different roles of *Schizosaccharomyces pombe sfr1/dds20* in meiotic and mitotic DNA recombination and repair

Fuat K. Khasanov · Albina F. Salakhova · Olga S. Khasanova · Alexandra L. Grishchuk · Olga V. Chepurnaja · Vladimir G. Korolev · Juerg Kohli · Vladimir I. Bashkirov

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Abstract DNA double-strand break (DSB) repair mediated by the Rad51 pathway of homologous recombination is conserved in eukaryotes. In yeast, Rad51 paralogs, *Saccharomyces cerevisiae* Rad55–Rad57 and *Schizosaccharomyces pombe* Rhp55–Rhp57, are mediators of Rad51 nucleoprotein formation. The recently discovered *S. pombe* Sfr1/Dds20 protein has been shown to interact with Rad51 and to operate in the Rad51-dependent DSB repair pathway in parallel to the paralog-mediated pathway. Here we show that Sfr1 is a nuclear protein and acts downstream of Rad50 in DSB processing. *sfr1Δ* is epistatic to *rad18*$^{-}$ and *rad60*$^{-}$, and Sfr1 is a high-copy suppressor of the replication and repair defects of a *rad60* mutant. Sfr1 functions in a Cds1-independent UV damage tolerance mechanism. In contrast to mitotic recombination, meiotic recombination is significantly reduced in *sfr1Δ* strains. Our data indicate that Sfr1 acts in DSB repair mainly outside of S-phase, and is required for wild-type levels of meiotic recombination. We suggest that Sfr1 acts early in recombination and has a specific role in Rad51 filament assembly, distinct from that of the Rad51 paralogs.

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F. K. Khasanov · A. F. Salakhova · O. S. Khasanova · V. I. Bashkirov
Institute of Gene Biology, Russian Academy of Sciences, 119334 Moscow, Russia

A. L. Grishchuk · J. Kohli (✉)
Institute of Cell Biology, University of Berne, Baltzer-Strasse 4, 3012 Berne, Switzerland
e-mail: juerg.kohli@izb.unibe.ch

O. V. Chepurnaja · V. G. Korolev
St. Petersburg Nuclear Physics Institute, Russian Academy of Sciences, 188350 Gatchina, Russia

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Introduction

The repair of double-strand breaks (DSBs), either programmed in meiosis, occurring spontaneously, or induced by genotoxic factors, is crucial for cell survival. From the three major DSB repair mechanisms non-homologous DNA-end joining (NHEJ), single-strand annealing (Savitsky et al. 1995), and homologous recombination (HR), only the last is error-free. Repair by HR, also called recombinational repair, is evolutionarily conserved and catalyzed by members of the *RAD52* group of genes first identified in the budding yeast *Saccharomyces cerevisiae* for their involvement in ionizing radiation (IR) resistance (Symington 2002). Initially, these genes were grouped into one DNA repair pathway on the basis of epistasis interactions of loss of function alleles in IR survival experiments. Further studies on biochemical properties and mutual interactions among the encoded proteins supported this grouping. The *RAD52* group in budding yeast consists of 11 genes: *MRE11*, *RAD50*, *XRS2*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *RDH54/TID1* and *RFA1* (Symington 2002). The products of the first three genes, Mre11, Rad50 and Xrs2, form the so-called MRX complex implicated in the processing of DSBs, telomere maintenance, NHEJ, and the DNA damage checkpoint, while the other eight genes comprise the RAD51 subgroup, with functions at the later stages of recombinational repair. Both subgroups are also implicated in homologous recombination during meiosis and mating-type switching. Within the Rad51 subgroup, Rad51, Rad55, and Rad57 are proteins with significant homology to the key bacterial recombination protein RecA.

Rad51 is a eukaryotic functional homolog of RecA, and forms a nucleoprotein filament on ssDNA active in homologous DNA pairing and strand-exchange reactions in vitro (Murayama et al. 2008; Ogawa et al. 1993; Sung 1994; Sung and Robberson 1995). Rad55 and Rad57, usually referred to as Rad51 paralogs, form a stable heterodimer, which is thought to play an accessory role to Rad51 by mediating the assembly of the Rad51 pre-synaptic filament (Sung 1997). The Rad55–Rad57 complex interacts with the Rad51 protein, and was shown in vitro to help Rad51 overcome the inhibitory binding of RPA to single-stranded DNA in strand-exchange reactions (Sung 1997).

The detection of four Rad51 paralogs in *Schizosaccharomyces pombe* [Rhp55, Rhp57, Rlp1 (Khasanov et al. 2004), and Rdl1 (Martin et al. 2006)] and of five paralogs in vertebrates: Rad51B, Rad51C, Rad51D, XRCC2 and XRCC3 (Symington 2002; Thompson and Schild 2001), indicates a functional diversification of RecA-like proteins during evolution. The exact mechanism of Rad51 paralog function is not known, but it is believed that they act by enhancing the nucleation or stabilization of the Rad51 nucleoprotein filament. Further homologs of the *RAD52* group have been identified in *S. pombe* as well, including *rad32* ($MRE11^{Sc}$), *rad50*, *nbs1* ($XRS2^{Sc}$), *rad51/rhp51* ($RAD51^{Sc}$), *rhp54* ($RAD54^{Sc}$), *rhp55* ($RAD55^{Sc}$), *rhp57* ($RAD57^{Sc}$), *rlp1* (novel *rad51* paralog), *rad22A* and *rti1/rad22B* ($RAD52^{Sc}$ and $RAD59^{Sc}$) (Hartsuiker et al. 2001; Khasanov et al. 2004; Ueno et al. 2003); Khasanov and Bashkirov 2001; Pastink et al. 2001). Although the biochemistry of the corresponding proteins, including the RecA-like proteins Rad51, Rhp55, Rhp57, and Rlp1 has not been systematically studied, they are presumed to have similar properties like their *S. cerevisiae* counterparts. However, there are some important differences in phenotypes of the corresponding null mutants in *S. pombe*. Although the *S. pombe RAD52* group proteins are not essential for cell viability, they have a more visible role in DNA replication than their *S. cerevisiae* homologs. In addition, the hypersensitivity of *S. pombe* mutants to UV damage indicates a more important role in response to UV compared to their budding yeast counterparts (Lehmann et al. 1995; Pastink et al. 2001).

Several additional genes can be assigned to the *S. pombe RAD52* group based on epistasis in IR sensitivity (Lehmann et al. 1995; Martin et al. 2006; Morikawa et al. 2004; Morishita et al. 2002). Rad18 (Smc6) is an evolutionarily conserved protein, and the founding member of a new subgroup of the SMC superfamily involved in DNA repair. Like other SMC proteins such as condensins and cohesins, Rad18 is part of a high-molecular-weight complex together with its Smc partner Spr18 (Smc5) (Fousteri and Lehmann 2000). Rad60 is a protein with a C-terminal ubiquitin-like motif related to SUMO-1. It is likely to be a homolog of *S. cerevisiae* Esc2 and human Nip45. Rad60 has been suggested to be required for the recombinational rescue of stalled replication forks, and is under control of the replication checkpoint kinase Cds1 (Boddy et al. 2003). Rad62, like Rad60, is a novel member of the *RAD52* epistasis group that is essential for DSB repair in fission yeast (Morikawa et al. 2004). The phenotypic similarity of *rad60*, *rad62*, *rad18* and *spr18* mutants, plus physical and genetic interactions identified between these genes, suggest that they function in a single pathway (Boddy et al. 2003; Morikawa et al. 2004; Morishita et al. 2002). The *fbh1* gene encoding the F-box DNA helicase with a role in processing of recombination intermediates acts downstream of *rad51* and *rhp57* in a pathway of recombinational repair (Morishita et al. 2005). The *rlp1* (Khasanov et al. 2004) and *rdl1* (Martin et al. 2006) genes encode two RecA-like proteins as nearest homologs of human *XRCC2* and *RAD51D*. These proteins together with Sws1 form a complex that controls an early step of homologous recombination (Martin et al. 2006).

Recently, an additional member of the *S. pombe RAD52* group has been identified independently as *sfr1* (Akamatsu et al. 2003), and as *dds20* (Salakhova et al. 2005). Throughout this publication, we are using the designation *sfr1*. Sfr1 was identified as a protein with homology to the C-terminal region of Swi2, a protein involved in mating-type switching (Akamatsu et al. 2003). Dds20 was discovered as a specific high-copy suppressor of defects in DSB repair of an *rhp55Δ* mutant (Salakhova et al. 2005). Deletion of the gene confers sensitivity to DNA damaging agents (MMS, ionizing radiation, and UV), but no major role in replication restart and mating-type switching has been found (Akamatsu et al. 2003; Salakhova et al. 2005). Epistasis analysis of the mutants for damage sensitivity revealed that Dds20/Sfr1 defines a novel Rad51-dependent sub-pathway of recombinational repair in *S. pombe*, which operates in parallel to the sub-pathway mediated by the Rad51 paralogs Rhp55 and Rhp57 (Akamatsu et al. 2003; Salakhova et al. 2005). Yeast two-hybrid analysis and co-immunoprecipitation experiments revealed that, similar to the Rhp55–Rhp57 heterodimer, the Sfr1 protein interacts with Rad51 (Akamatsu et al. 2003; Salakhova et al. 2005). Moreover, it was found that this novel protein is part of a complex containing Swi5 and Rad51, and functions specifically in DNA recombinational repair, while another Swi5- and Rad51-containing complex, Swi5–Swi2–Rad51 may function in mating-type switching together with the chromodomain protein Swi6 (Akamatsu et al. 2003). Interestingly, a deletion mutant of *swi5*, originally discovered as mating-type switching gene, had DNA repair defects almost indistinguishable to those of an *sfr1Δ* mutant (Akamatsu et al. 2003). The lack of synthetic lethality and checkpoint-dependent S-phase delay in an *sfr1Δ rad2Δ* double mutant, in contrast to *rhp55Δ rad2Δ* and *rhp57Δ rad2Δ* strains,

suggests that two Rad51-dependent sub-pathways of repair are using different mechanisms to mediate the assembly of the Rad51 nucleoprotein filament in *S. pombe* (Akamatsu et al. 2003; Haruta et al. 2008; Salakhova et al. 2005).

In this publication, we further characterize the phenotypes of an *sfr1Δ* mutant both in mitosis and meiosis, along with epistasis relationships of *srf1* with other genes involved in recombinational repair. We show that in contrast to *rhp55Δ* strains, chromosome maintenance and mitotic recombination is not affected in an *sfr1Δ* mutant. However, Sfr1 plays an important role in meiotic recombination and in UV damage-tolerance.

Materials and methods

Strains, plasmids and genetic manipulations

Strains used in this study are listed in Table 1. The plasmid pUR19:*sfr1*$^+$ was isolated by screening of an *S. pombe* genomic DNA library for plasmids able to suppress the repair defects of a *rhp55Δ* strain (Salakhova et al. 2005). Media and general genetic manipulations with *S. pombe* have been described elsewhere (Gutz et al. 1974). The tagging of *sfr1* with 13 × Myc was performed as described in Bähler et al. (1998).

Assays for genotoxic stress response

Sensitivity to MMS was tested in chronic and/or acute exposure to the drug. For the chronic exposure assay (spot test), sequential 10- or 5-fold dilutions of exponentially growing cells were spotted on the appropriate rich or minimal media with or without drug, and plates were incubated at the indicated temperature. The acute exposure assay was performed with exponentially growing cells by the addition of the indicated amount of MMS. Aliquots were then withdrawn at different times thereafter; MMS was neutralized by addition of an equal volume of 10% sodium thiosulfate; cells were plated after appropriate dilution, and grown for 4–6 days at the indicated temperature for survival determination. For UV survival assays, serial dilutions of exponentially growing cultures were plated on full media, irradiated with the indicated UV doses with a germicidal lamp, and the fraction of surviving cells was scored and compared to the non-irradiated control (colonies after 5 days). To examine IR survival, exponentially growing cells were washed, re-suspended in saline and irradiated with a ^{60}Co γ-ray source at a dose rate of 35 Gy/min. Appropriate dilutions were plated on full media to determine survival at 30°C. All genotoxic stress experiments were repeated at least three times and the standard errors calculated. For a series of experiments with a given set of strains for epistasis analysis, the conditions for all strains were kept identical. But for different series the parameters were not all kept identical (growth conditions, geometry of exposure). This is the likely reason for the differences in sensitivity of a specific strain in different series of experiments.

Mitotic and meiotic recombination, spore viability

Random spore analysis was used to determine the frequencies of meiotic intra- and intergenic recombination, as described in Khasanov et al. (1999). Triplicate crosses were performed for each interval tested. A chromosome loss and inter-homolog recombination assay was performed using the non-sporulating diploids IBGY592/594 (wild type), IBGY597/IBGY598 (*rhp55Δ*) and IBGY599/IBGY604 (*sfr1Δ*) essentially as described (Hartsuiker et al. 2001). To determine sister chromatid recombination frequency, we used the system described in Schuchert and Kohli (1988) using strains IBGY606 (wild type), IBGY608 (*sfr1Δ*), and IBGY609 (*rhp55Δ*).

The sporulation efficiency was evaluated by microscopic scoring of the numbers of spores, asci and vegetative cells. The sporulation efficiency (% sporulation) was calculated as $(0.25S + A)/(0.25S + A + 0.5C)$, where S is the number of spores, A the number of asci, and C the number of vegetative cells. To determine spore viability, tetrads were dissected and the number of colony-forming spores was expressed as the percentage of the number obtained in a wild type cross. In all, 30 tetrads from wild type and *sfr1Δ* crosses were dissected.

Immunofluorescence microscopy

Indirect immunofluorescence microscopy was performed with established methods (Hagan and Hyams 1988). 4′,6-Diamino-2-phenylindole (DAPI) was used for DNA staining at 0.5 μg/ml. Primary mouse anti-cMyc antibody (9E10 at 1:1,000 dilution) and secondary antibody (Alexa Fluor® 555-conjugated goat anti-mouse IgG at 1:2000) were used for cell staining. The slides were analyzed with an epi-fluorescence (Zeiss Axiovert) microscope. Analysis of Rad51 foci formation in irradiated *S. pombe* cells was performed as described (Caspari et al. 2002).

Results

Synergistic interaction of *sfr1Δ* with a *rad50* null mutant defective in DSB processing

Previously, we suggested that Sfr1 protein operates at early stages of DSB repair in a Rad51-dependent sub-pathway parallel to the sub-pathway defined by the Rad51 paralogs

Table 1 Strains

Strain	Genotype
RK3	*h⁻ smt-0 ade7-152*
3-99	*h⁻ lys4-95*
EH65	*h⁻ smt-0 rad50::KnMX6 ura4-D18*
BVY6	*h⁻ smt-0 ura4-D18 leu1-32 ade7-152*
IBGY10	*h⁻ smt-0 ura4-D18*
IBGY12*	*h⁺ mat1PΔ17::LEU2 leu1-32 ade7-150*
IBGY20*	*h⁻ smt-0 rad51Δ::ura4⁺ura4-D18*
IBGY21*	*h⁻ smt-0 rhp54Δ::ura4⁺ura4-D18*
IBGY27*	*h⁻ smt-0 ade6-469*
IBGY30	*h⁻ smt-0 rhp55Δ::ura4⁺ arg3-D4 ura4-D18 leu1-32*
IBGY32	*h⁺ rad51Δ::ura4⁺ arg3-D4 ura4-D18 leu1-32*
IBGY38*	*h⁺ mat1PΔ17::LEU2 leu1-32 lys7-2*
IBGY40*	*h⁺ mat1PΔ17::LEU2 leu1-32 ade6-M26*
BGY42*	*h⁻ smt-0 his1-102*
IBGY77*	*h⁺ mat1PΔ17::LEU2 leu1-32 ade6-M375*
IBGY84	*h⁻ smt-0 rhp55::ura4⁺ ura4-D18 leu1-32*
IBGY225	*h⁻ smt-0 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY226	*h⁻ smt-0 sfr1Δ::arg3⁺ rhp55Δ::ura4⁺ arg3-D4 ura4-D18 leu1-32*
IBGY227	*h⁻ smt-0 sfr1Δ::arg3⁺ rad51Δ::ura4⁺ arg3-D4 ura4-D18 leu1-32*
IBGY228	*h⁻ smt-0 sfr1Δ::arg3⁺ rhp54Δ::ura4⁺ arg3-D4 ura4-D18 leu1-32*
IBGY233	*h⁺ mat1PΔ17::LEU2 leu1-32 ura4-D18*
IBGY249	*h⁻ smt-0 arg3-D4 ura4-D18 leu1-32*
IBGY251	*h⁻ smt-0 uve1Δ::LEU2 arg3-D4 ura4-D18 leu1-32*
IBGY252	*h⁻ smt-0 rad13Δ::ura4⁺ arg3-D4 ura4-D18 leu1-32*
IBGY253	*h⁻ smt-0 uve1Δ::LEU2 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY257	*h⁻ smt-0 rad13Δ::ura4⁺ sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY261	*h⁺ uve1Δ::LEU2 rad13Δ::ura4⁺ ura4-D18 leu1-32 ade6-M26*
IBGY267	*h⁺ cds1:: ura4⁺ ura4-D18 leu1-32 arg3-D4*
IBGY271	*h⁻ smt-0 uve1Δ::LEU2 rad13Δ::ura4⁺ sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY272	*h⁺ cds1:: ura4⁺ ura4-D18 sfr1Δ::arg3⁺ arg3-D4 leu1-32*
IBGY277	*h⁺ mat1PΔ17::LEU2 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY447	*h⁻ lys4-95 sfr1-13Myc*
IBGY472	*h⁺ rqh1::ura4⁺ ura4-D18 leu1-32 arg3-D4*
IBGY474	*h⁻ arg3-D4 ura4-D18 leu1-32*
IBGY475	*h⁺ srs2::LEU2 ura4-D18 leu1-32 arg3-D4*
IBGY479	*h⁺ rqh1::ura4⁺ sfr1::arg3⁺ ura4-D18 arg3-D4 leu1-32*
IBGY480	*h⁺ srs2::LEU2 sfr1::arg3⁺ ura4-D18 arg3-D4 leu1-32*
IBGY502	*h⁺ rad18-na74 arg3-D4 ura4-D18 leu1-32*
IBGY506	*h⁺ rad60-1 arg3-D4 ura4-D18 leu1-32*
IBGY510	*h⁺ sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY512	*h⁺ rad60-1 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY513	*h⁺ rad18-na74 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 leu1-32*
IBGY546	*h⁻ smt-0 sfr1Δ::arg3⁺ arg3-D4 leu1-32 ura4-D18 ade7-152*
IBGY548	*h⁺ mat1PΔ17::LEU2 leu1-32 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 ade7-150*
IBGY550	*h⁻ smt-0 sfr1Δ::arg3⁺ arg3-D4 leu1-32 ura4-D18 ade6-469*
IBGY552	*h⁺ mat1PΔ17::LEU2 leu1-32 sfr1Δ::arg3⁺ arg3-D4 ura4-D18 ade6-M26*

Table 1 continued

Strain	Genotype
IBGY554	*h*$^+$ *mat1PΔ17::LEU2 leu1-32 sfr1Δ::arg3*$^+$ *arg3-D4 ura4-D18 ade6-M375*
IBGY557	*h*$^+$ *mat1PΔ17::LEU2 leu1-32 sfr1Δ::arg3*$^+$ *arg3-D4 ura4-D18 lys7-2*
IBGY559	*h*$^-$ *smt-0 sfr1Δ::arg3*$^+$ *arg3-D4 leu1-32 ura4-D18 his1-102*
IBGY560	*h*$^-$ *smt-0 arg3-D4 leu1-32 ura4-D18 ade1-40*
IBGY561	*h*$^+$ *mat1PΔ17::LEU2 leu1-32 sfr1Δ::arg3*$^+$ *arg3-D4 ura4-D18 ade1-40*
IBGY564	*h*$^+$ *mat1PΔ17::LEU2 leu1-32 arg3-D4 ura4-D18 lys4-95*
IBGY565	*h*$^-$ *smt-0 arg3-D4 leu1-32 ura4-D18 sfr1Δ::arg3*$^+$ *lys4-95*
IBGY592/594	*h*$^-$ *smt-0/smt-0 ade6-M210/ade6-M216 arg3-D4/arg3-D4 ura4-D18/ura4*
IBGY597/598	*h*$^-$ *smt-0/smt-0 ade6-M210/ade6-M216 arg3-D4/arg3-D4 ura4-D18/ura4*$^+$ *rhp55Δ::ura4*$^+$*/rhp55Δ::ura4*$^+$
IBGY599/604	*h*$^-$ *smt-0/smt-0 ade6-M210/ade6-M216 arg3-D4/arg3-D4 ura4-D18/ura4*$^+$ *sfr1Δ::ura4*$^+$*/sfr1Δ::ura4*$^+$
IBGY606	*h*$^-$ *smt-0 ura4-D18 ade6 M375-int::pUC18/ura4*$^+$*/ade6-469 arg3-D4*
IBGY608	*h*$^-$ *smt-0 ura4-D18 ade6-M375 int::pUC18/ura4*$^+$*/ade6-469 arg3-D4 sfr1::arg3*$^+$
IBGY609	*h*$^-$ *smt-0 ura4-D18 ade6-M375 int::pUC18/ura4*$^+$*/ade6-469 arg3-D4 rhp55::arg3*$^+$
IBGY722	*h*$^-$ *smt-0 rad50::KnMX6 ura4-D18 sfr1::ura4*

Most strains were constructed for this study with exception of EH65 obtained from Hartsuiker, Brighton. For strains marked with * see Khasanov et. al. (1999)

(Rhp55, Rhp57), and has a specific mediator role in Rad51 nucleoprotein assembly, which differs from that of the Rad51 paralogs (Salakhova et al. 2005). It is thought that the Rad51 filament is formed on single-stranded DNA after processing of DSBs by 5′–3′ exonuclease activity. DSB end processing in *S. cerevisiae* is dependent on two partially redundant pathways: one defined by the 5′–3′ exonuclease activity of the MRN complex (Mre11/Rad50/Xrs2[Nbs1]) and another by additional exonucleases. Here, we address the relationship of the Sfr1-mediated sub-pathway to the pathways of DSB processing acting upstream of Rad51 filament assembly. Epistasis analysis in repair of MMS-induced damage is presented in Fig. 1. The *sfr1Δ rad50Δ* double mutant showed synergistic enhancement of MMS-sensitivity compared to the single mutants.

Epistasis analysis of *sfr1Δ* with *rqh1Δ* and *srs2Δ* helicase mutants

Deletion of *rhp55* or *rhp57* was shown to suppress the sensitivity of *rqh1Δ* cells to DNA damaging agents (Hope et al. 2005). Rqh1 is a DNA helicase involved in DNA repair, recombination, and UV tolerance (Murray et al. 1997). Since Sfr1 was postulated to operate in parallel to the sub-pathway mediated by the Rad51 paralogs Rhp55 and Rhp57 (Akamatsu et al. 2003; Salakhova et al. 2005), we investigated the genetic relationship between *rqh1Δ* and *sfr1Δ* mutants in epistasis experiments. Treatment of the *rqh1Δ sfr1Δ* double mutant with MMS indicated partial suppression of the repair defect of the *rqh1* mutant (Fig. 2a). A similar effect was observed, when the *rqh1Δ sfr1Δ* double mutant was exposed to UV and gamma-rays (Fig. 2b, c). Thus, these data show that Rqh1 acts downstream of Sfr1 during repair of UV damage.

The deletion of *S. pombe srs2*, encoding another DNA-helicase in fission yeast, resulted in moderate sensitivity to UV and MMS (Fig. 2d, e). Epistasis analysis showed that the double mutant *sfr1Δ srs2Δ* was more sensitive to the DNA damaging action of MMS and UV than the single mutants (Fig. 2d, e). This indicated that Sfr1 and Srs2 participate in different pathways for MMS and UV damage repair.

Sfr1 interaction with Rad18 and Rad60

The *S. pombe* Rad18 (Smc protein) has been shown to act in the same ionizing radiation repair pathway like Rad51 in response to ionizing radiation (Lehmann et al. 1995). The *rad18* gene is essential, and it was also implicated in a DNA damage checkpoint (Verkade et al. 1999). The recently discovered *rad60* gene is also essential and involved in DSB repair. It functions in the same pathway as *rad51* (Morishita et al. 2002). We tested the epistasis interactions between *sfr1Δ* and *rad60-1*, and also *rad18-na74*

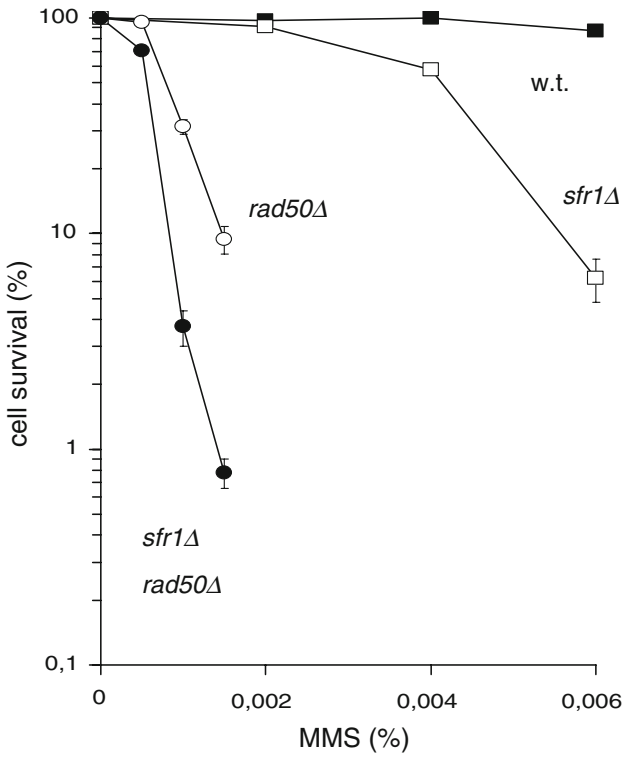


Fig. 1 Epistasis analysis of *sfr1Δ* with *rad50Δ*. *sfr1Δ* is synergistic with the deletion mutant of *rad50* which is involved in DSB processing during DNA recombination and repair. MMS survival curves of *sfr1Δ*, *rad50Δ*, and the double mutant are shown. The following strains were used: IBGY474 (wild-type), IBGY277 (*sfr1Δ*), IBGY434 (*exo1-1Δ*), EH65 (*rad50Δ*), and IBGY722 (*rad50Δ sfr1Δ*). All survival curves were determined at 30°C

for repair of γ-ray (Fig. 3a) and MMS-induced damage (data not shown). The double mutants *sfr1Δ rad18-na74* and *sfr1Δ rad60-1* were no more sensitive than the *sfr1Δ* mutant, which suggests that all three genes act in the same DSB repair pathway.

When combined, the hypomorphic mutations *rad18-na74* and *rad60-1* exhibited synthetic lethality. Overexpression of *rad60* partially suppressed the MMS sensitivity of *rad18-na74* cells (Morishita et al. 2002). We found that high-copy *sfr1* could partially suppress the MMS hyper-sensitivity of the *rad60-1* mutant (Fig. 3b), as well as its low plating efficiency at 30°C (Fig. 3c). However, no suppression of the MMS hypersensitivity of *rad18-na74* was observed (data not shown). Since Rad60 and Rad18 proteins interact, and are thought to have partially overlapping roles in DSB repair, the overexpression of Sfr1 seems to suppress the Rad18-independent function of the Rad60 protein.

Sfr1 acts in a Cds1-independent UV damage tolerance pathway

Pyrimidine dimers and pyrimidine 6-4 photoproducts are the main lesions produced by UV light, and can stall replication forks. Two mechanisms are used by *S. pombe* cells to repair UV lesions: nucleotide excision repair (NER) and UV damage excision (UVDE) repair (Yasui and McCready 1998). Recombinational repair genes are also important for cell survival upon UV damage. In addition to a poorly understood role in the UVDE pathway, they are involved in a UV damage tolerance pathway controlled by the Cds1 checkpoint kinase (Murray et al. 1997). *S. pombe* mutants defective in DSB repair show significant hypersensitivity to UV light (Hartsuiker et al. 2001; Khasanov et al. 1999; Muris et al. 1993; Murray et al. 1997; Tavassoli et al. 1995; Tsutsui et al. 2001).

We found that the *sfr1Δ* mutant is sensitive to UV and hypersensitive at low temperature (Fig. 4a). MMS sensitivity is also more pronounced at low temperature (Fig. 4b). Thus, Sfr1 may be involved in the assembly or function of a multiprotein complex active in the UV and MMS damage response. Epistatis analysis indicates that *sfr1* is involved neither in NER, nor in UVDE repair, since the double mutants with *rad13Δ* and *uve1Δ* were more sensitive than the single mutants (Fig. 4c). The *rad13* gene encodes the *S. pombe* homolog of human XPG, and *uve1* the UV damage endonuclease. As with MMS, the double *sfr1Δ rhp55Δ* mutant showed synergism compared to the single mutants after UV irradiation (Fig. 4c). However, the sensitivity of the *sfr1Δ rad51Δ* and *sfr1Δ rhp54Δ* double mutants indicated epistasis (Fig. 4d). In addition, as with IR, *sfr1Δ* showed an epistasis interaction with the *rad60-1* and *rad18-na74* mutations for MMS (data not shown). This suggests that, as with its response to MMS and γ-ray-induced damage, Sfr1 operates in a Rad51-dependent sub-pathway in response to UV irradiation, parallel to the Rhp55 sub-pathway.

When both the NER and the UVDE repair pathways were inactivated in a *rad13Δ uve1Δ* mutant, further addition of the *sfr1Δ* mutation substantially enhanced UV sensitivity (Fig. 4e). This indicates that Sfr1 acts in a UV damage tolerance mechanism, allowing replication of UV-damaged DNA in the absence of damage removal by NER and UVDE. A UV damage tolerance pathway involving the recombinational repair group genes *rad51*, *rhp54*, *mus81*, and *rad18* has been identified, and was shown to be controlled by the Cds1 kinase operating during the intra-S phase checkpoint (Boddy et al. 2000; Murray et al. 1997). Since *sfr1Δ* is epistatic to *rad51Δ* and *rhp54Δ*, we tested whether *sfr1Δ* and *cds1Δ* also show an epistasis relation. As shown in Fig. 4f, the *sfr1Δ cds1Δ* double mutant was more sensitive than either single mutant. Considering the indication of the existence of two subpathways responsible for the repair of IR- and MMS-induced DNA damage (see above), and synergism between *sfr1Δ* and *chk1Δ* or *rad3Δ* (data not shown), an explanation could be that Sfr1 acts in a checkpoint-independent recombinational sub-pathway of DNA damage tolerance.

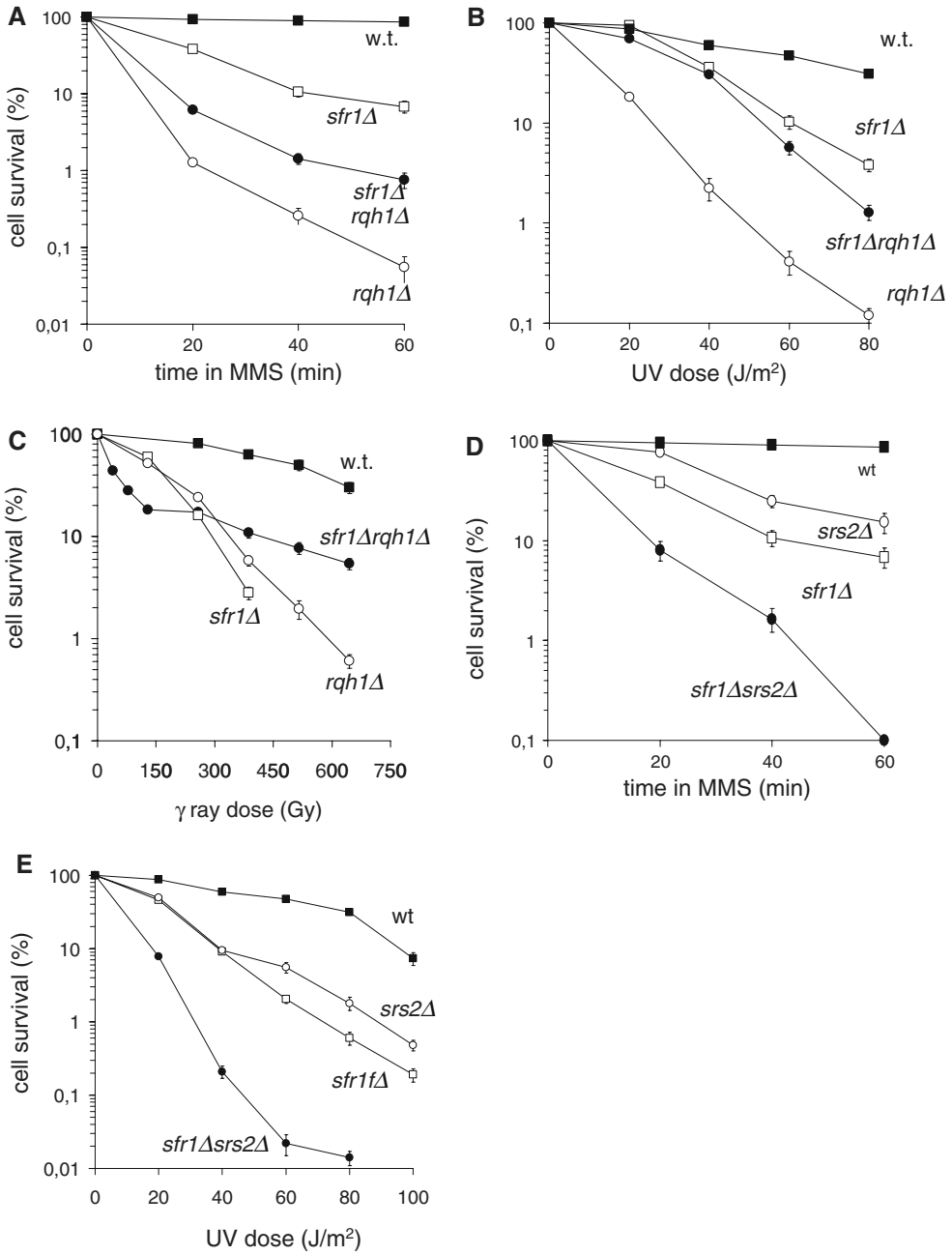
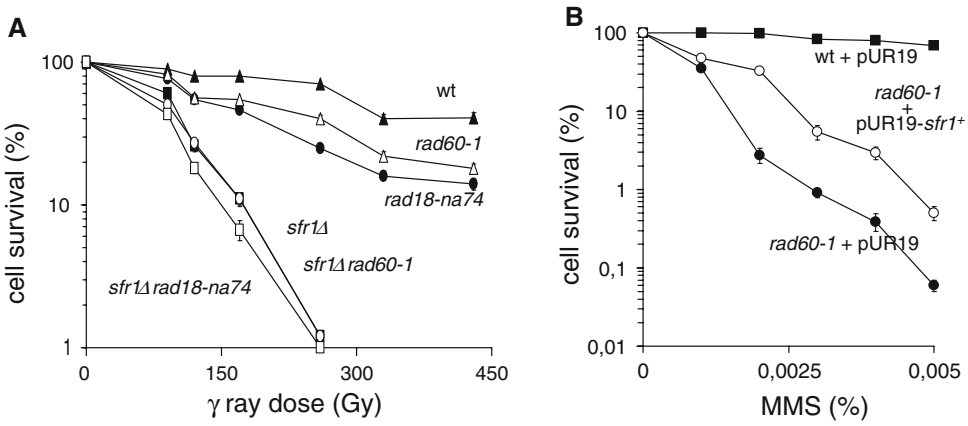


Fig. 2 Epistasis analysis of *sfr1Δ* with helicase mutants. *sfr1Δ* showed partial suppression of the sensitivity of *rqh1Δ* cells to MMS (**a**), UV-light (**b**), and γ-rays (**c**). A synergistic interaction was found for *sfr1Δ* and *srs2Δ* for cell survival after MMS (**d**) and UV treatment (**e**). The following strains were used: IBGY474 (wild type), IBGY277 (*sfr1Δ*), IBGY472 (*rqh1Δ*), IBGY479 (*rqh1Δ sfr1Δ*) IBGY475 (*srs2Δ*), and IBGY480 (*srs2Δ sfr1Δ*). All survival curves were determined at 30°C

Sfr1 is not involved in chromosome maintenance and mitotic recombination

Increased frequencies of minichromosome loss have been reported for *rad51Δ* and *rhp54Δ* (Muris et al. 1996). In an assay measuring both the loss of chromosome III and inter-homolog recombination, *rad50Δ* was shown to have a strong defect in chromosome maintenance (Hartsuiker et al. 2001). We employed this assay for investigation of chromosome loss and/or inter-homolog recombination in the *sfr1Δ* mutant, in comparison with wild type and *rhp55Δ*. As shown in Table 2, *sfr1Δ* had a wild-type rate of chromosome loss, whereas *rhp55Δ* lost chromosomes at a rate 2,300-fold higher than wild type. Similarly, no defect in mitotic crossing-over was found in *sfr1Δ* cells (Table 3). In contrast, the *rhp55Δ* showed a hyper-recombination phenotype: 11-fold increase of the rate of mitotic crossing-over.



C

Strain	Plating efficiency (%)
wild type + pUR19	100
rad60-1 + pUR19	7.5 ± 0.23
rad60-1 + pUR19:*sfr1*$^+$	36.2 ± 1.14

Fig. 3 Interactions of *sfr1Δ* with the *rad18-na74* and *rad60-1* mutants. **a** γ-ray survival curves of *sfr1Δ*, *rad60-1*, *rad18-na74*, and the double mutants. The *sfr1* deletion was epistatic to the *rad60-1* and *rad18-na74* mutants. **b** High level expression of *sfr1*$^+$ partially suppressed the MMS sensitivity of *rad60-1*. Quantitative tests were done for survival at conditions of chronic exposure to MMS. The *rad60-1* strain was transformed with empty vector pUR19 or with pUR19::*sfr1*$^+$. **c** Overexpression of *sfr1*$^+$ partially suppressed the low viability of the hypomorphic *rad60-1* mutant. The following strains were used: IBGY474 (wild type), IBGY502 (*rad18-na74*), IBGY506 (*rad60-1*), IBGY512 (*rad60-1 sfr1Δ*), IBGY513 (*rad18-na74 sfr1Δ*), and IBGY510 (*sfr1Δ*)

It has been shown that *S. pombe RAD52* group mutants exhibit either a hyper- (*rad51Δ*, *rhp54Δ*, and *rti1Δ*), or a hypo-recombination (*rad50Δ*) phenotype in an assay for spontaneous intrachromosomal recombination (Hartsuiker et al. 2001; Osman et al. 2000, 2003; van den Bosch et al. 2002). Thus, this assay was applied to the study of *sfr1Δ*. In a haploid strain with the *ura4*$^+$ marker flanked by direct repeats of the *ade6*$^-$ alleles *ade6-469* and *ade6-M375*, formation of Ade$^+$ recombinants results from homologous recombination between the *ade*$^-$ alleles on one chromatid (intra-chromatid recombination), or between gene copies on sister chromatids (unequal sister-chromatid recombination). We found that the frequency of intrachromosomal recombination was slightly increased (1.2-fold, not statistically significant) in *sfr1Δ* when compared to wild type strain (Table 4). In contrast, in *rhp55Δ* the frequency of intrachromosomal recombination was elevated 4.1-fold, which translates into a 3.2-fold increase in recombination rate. These results indicate that Sfr1 has no role in homologous intrachromosomal recombination in vegetative cells. Alternatively, an increase of unequal sister-recombination might have been masked by a decrease of intrachromatid recombination, or vice versa.

Meiotic recombination is reduced in the *sfr1Δ* mutant

Deletion mutants of the *S. pombe RAD52* group genes, *rad51*, *rhp54*, and *rhp55* show defects in meiotic recombination to various extents (Grishchuk and Kohli 2003; Khasanov et al. 1999; Muris et al. 1997; van den Bosch et al. 2001). Moreover, the microarray-based meiosis transcription profile of *sfr1* (ORF SPBC28F2.07) showed that transcription of the gene increased during wild-type meiosis about 6-fold with a peak at the end of S-phase (Mata et al. 2002). This argues for a role of Sfr1 in meiosis. Thus, we tested the *sfr1Δ* mutant for meiosis-related phenotypes. We found that spore viability in an *sfr1Δ* cross (IBGY225 × IBGY277) was almost the same as in the wild-type cross (IBGY10 × IBGY233), 88 and 92%, respectively. Sporulation efficiency was slightly reduced: 32% in wild type and 25% in the *sfr1Δ* mutant. Then we analyzed meiotic intra- and intergenic recombination. Reduction in the frequency of intragenic recombination, 5- to 20-fold, was observed depending on which of three intervals was tested (Table 5). Intergenic recombination (Table 6) was analyzed in two intervals: *his1-lys7* (chromosome I) and *ade1-lys4* (chromosome II). It was found to be reduced 15- and 19-fold, respectively, in the *sfr1Δ* crosses. From these data we conclude that Sfr1 is required for wild-type levels of meiotic recombination in fission yeast.

The *rhp55Δ* mutant is defective in meiosis and meiotic recombination, showing a 2.6-fold reduction in sporulation efficiency, 1.7-fold reduction in spore viability, and 1.3- to 2.9-fold and 1.4- to 2.6-fold decrease in intragenic and intergenic recombination, dependent on alleles and intervals used (Grishchuk and Kohli 2003; Khasanov et al.

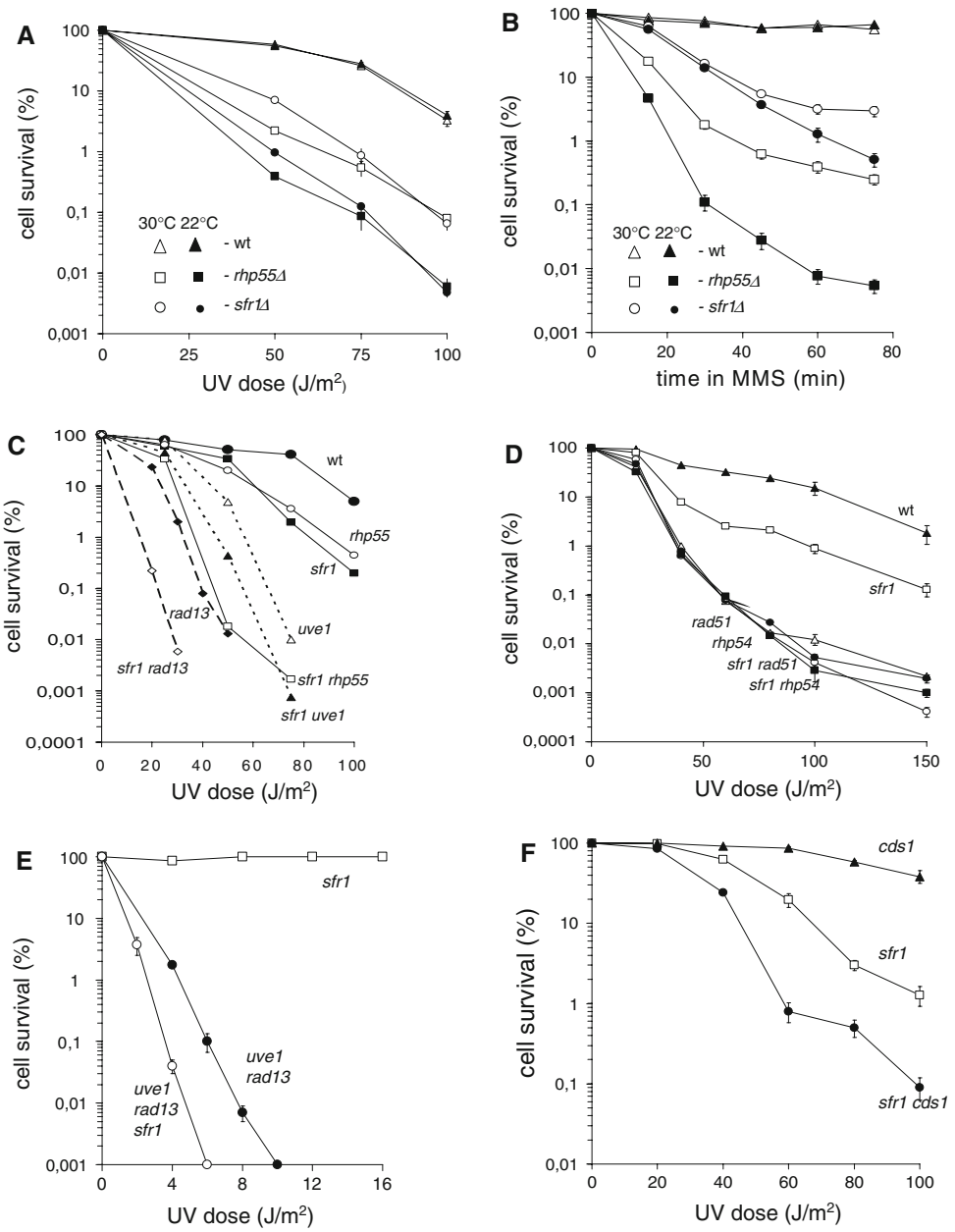


Fig. 4 Analysis of the UV and MMS damage response in the *sfr1Δ* mutant. All survival curves were determined at 30°C or, when indicated, at 20°C. **a**, **b** Cold-sensitivity of the *sfr1Δ* mutant to UV light and MMS. Strains used were BVY6 (wild type), IBGY84 (*rhp55Δ*), IBGY225 (*sfr1Δ*); **c** epistasis analysis between *sfr1Δ* and deletions of *S. pombe* genes involved in the NER and UVDE pathways of UV-damage repair, and in *rhp55Δ*. Strains used were IBGY251 (*uve1Δ*), IBGY252 (*rad13Δ*), IBGY30 (*rhp55Δ*), IBGY226 (*rhp55Δ dds20Δ*), IBGY253 (*sfr1Δ uve1Δ*), IBGY257 (*sfr1Δ rad13Δ*), IBGY225 (*sfr1Δ*), and IBGY249 (wild-type). **d** Epistasis analysis between *sfr1Δ* and deletions of the recombination repair genes *rad51* and *rhp54*. The following strains were employed: BVY6 (wild type), IBGY20 (*rad51Δ*), IBGY21 (*rhp54Δ*), IBGY225 (*sfr1Δ*), IBGY227 (*sfr1Δ rad51Δ*), and IBGY228 (*sfr1Δ rhp54Δ*). **e** Synergism between *sfr1Δ* and an NER-UVDE double mutant. Strains used were IBGY277 (*sfr1Δ*), IBGY261 (*uve1Δ rad13Δ*), and IBGY271 (*uve1Δ rad13Δ sfr1Δ*). **f** Synergism was found for *sfr1Δ* and *cds1Δ*, mutants of genes involved in UV-damage tolerance. Strains used were: IBGY267 (*cds1Δ*), IBGY510 (*sfr1Δ*), and IBGY272 (*cds1Δ sfr1Δ*). All the mutants used for the experiments reported in **c** to **f** carried deletions of the respective genes

Table 2 Loss of chromosome III in *sfr1Δ* and *rhp55Δ* diploids

Haploids per 10^6 cells[a] (fold increase in mutants)			Haploidization rate per cell division[b] (fold increase in mutants)		
Wild type	*sfr1Δ*	*rhp55Δ*	Wild type	*sfr1Δ*	*rhp55Δ*
0.27×10^2	0.3×10^2 (1.1) $P \geq 0.05$[c]	1.1×10^5 (4,140) $P < 0.001$[c]	4.34×10^{-6}	4.9×10^{-6} (1.1)	1.0×10^{-2} (2,300)

[a] Based on 10 independent cultures

[b] Calculated with the Lea–Coulson method of the median (Lea and Coulson 1949)

[c] Mann–Whitney *U* test: probability of equality to wild type

1999). Overexpression of *sfr1*$^+$ on pUR19 fully suppressed the defect in sporulation efficiency, partially rescued spore viability, but was unable to suppress the impaired intragenic and intergenic recombination defects of the *rhp55Δ* mutant (same intervals as in Tables 5 and 6, data not shown). This may indicate that Rhp55 and Sfr1 have

Table 3 Mitotic crossover frequency between centromere and *ura4* locus on chromosome III in *sfr1Δ* and *rhp55Δ* diploids

Recombinants per 10^6 cells[a] (fold increase in mutants)			Rate of recombination per cell division[b] (fold increase in mutants)		
Wild type	*sfr1Δ*	*rhp55Δ*	Wild type	*sfr1Δ*	*rhp55Δ*
0.8×10^3	0.6×10^3 (0.75) $P > 0.05$[c]	1.4×10^4 (17) $P < 0.001$[c]	7.95×10^{-5}	6.15×10^{-5} (0.77)	8.68×10^{-4} (11)

[a] Based on 10 independent cultures

[b] Calculated with the Lea–Coulson method of the median (Lea and Coulson 1949)

[c] Mann–Whitney *U* test: probability of equality to wild type

Table 4 Intrachromosomal recombination in *sfr1Δ* and *rhp55Δ*

Ade^+ recombinants per 10^6 cells[a] (fold increase in mutants)			Rate per cell division[b] (fold increase in mutants)		
Wild type	*sfr1Δ*	*rhp55Δ*	Wild type	*sfr1Δ*	*rhp55Δ*
4.4×10^4	5.4×10^4 (1.2) $P \geq 0.05$[c]	18.2×10^4 (4.1) $P < 0.001$[c]	3.25×10^{-3}	3.86×10^{-3} (1.2)	10.5×10^{-3} (3.2)

[a] Based on 10 independent cultures. The median is given

[b] Calculated with the Lea–Coulson method of the median (Lea and Coulson 1949)

[c] Mann–Whitney *U* test: probability of equality to wild type

Table 5 Meiotic intragenic recombination in *sfr1Δ*

Chromosome and alleles[a]	Total of Ade^+ colonies	Prototrophs/10^6 viable spores[a]		
		Wild-type	*sfr1Δ*	Fold reduction
II L *ade7-150* × *ade7-152*	10^3	420 ± 85	21 ± 5	20.0
III R *ade6-469* × *ade6-M375*	10^3	635 ± 17	68 ± 7	9.3
III R *ade6-469* × *ade6-M26*	10^4	10,850 ± 42	2,080 ± 389	5.2

[a] Strains crossed were as follows: RK3 × IBGY12 and IBGY548 × IBGY546 (*ade7-150* × *ade7-152* alleles); IBGY27 × IBGY77 and IBGY550 × IBGY554 (*ade6-469* × *ade6-M375* alleles); IBGY27 × IBGY40 and IBGY550 × IBGY552 (*ade6-469* × *ade6-M26* alleles). *L* Left and *R* right arms of chromosomes II and III, respectively

[b] Mean and standard deviations from three independent crosses

Table 6 Meiotic intergenic recombination in *sfr1Δ*

Chromosome and interval[a]	Genetic distance (cM)[b]		Fold reduction
	Wild type	*sfr1Δ*	
IR *his1-lys7*	38.2 ± 3.7	2.5 ± 0.45	15
IIR *ade1-lys4*	42.7 ± 3.0	2.2 ± 0.7	19

[a] Mean and standard errors from three independent crosses are shown. For each interval 1,000–1,200 random spores were analyzed. The following strains were crossed: IBGY42 × IBGY38 and IBGY559 × IBGY557 (*his1-lys7* interval), IBGY560 × IBGY564 and IBGY561 × IBGY565 (*ade1-lys4* interval). R indicates the right arms of chromosomes I and II

[b] Genetic distances (cM) were determined according to the standard method for *S. pombe* (Munz 1994) with the formula $-50 \ln(1 - 2R)$ with *R* indicating the frequency of recombinants

different functions in meiotic recombination. The spore viability defect of the *rhp55Δ* mutant may be a compound of Rhp55 deficiency in meiotic DNA replication and, less likely, meiotic recombination and segregation defects (Khasanov et al. 1999). The ability of high-copy Sfr1 to partially suppress spore lethality of *rhp55Δ* may be due to suppression of only one pathway defect in meiotic DNA replication. In parallel, high-copy Sfr1 also suppresses the mitotic cell elongation phenotype of *rhp55Δ* only partially (data not shown).

Sfr1 is a nuclear protein

To study the cellular localization of the Sfr1 protein, we tagged the gene with 13 Myc epitopes, and examined immunofluorescence in mitotically dividing cells using anti-Myc antibodies. The *sfr1*-Myc13 cells produced a functional protein, as they were as resistant to MMS and UV as the wild type strain (data not shown). Fluorescence was detected in the nuclei of mitotic cells, and co-localized with DAPI-stained chromosomal DNA (Fig. 5). Small unspecific background staining for wild type cells was

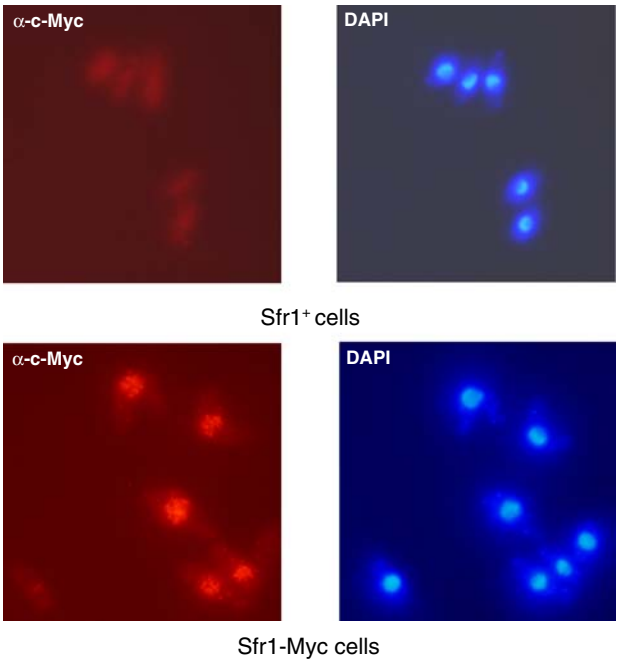


Fig. 5 Cellular localization of the Sfr1 protein. Fluorescence images for Sfr1-Myc13 and DNA (DAPI) are shown. Mitotic cells of strains 3–99 without the tag (*upper panels*), and of IBGY447 with the tag (*lower panels*) were stained

observed. Close inspection of images revealed that Sfr1-Myc13 occurred in approximately 30–50 foci per nucleus.

Discussion

Rad51-dependent sub-pathways of recombinational repair

It has been proposed before that in *S. pombe* the Rad51 paralogs Rhp55–Rhp57, and Sfr1 operate in parallel pathways upstream of Rad51 at an early stage of recombination (Akamatsu et al. 2003; Salakhova et al. 2005). Recently, it was shown that Sfr1 enhances the DNA-strand transfer activity of Rhp51 and Dmc1 in vitro (Kurokawa et al. 2008; Murayama et al. 2008). These findings are supported by the in vivo data presented. The similarity between Sfr1 and Rhp55–Rhp57 functions include interaction between Sfr1 and Rad51 as demonstrated by two-hybrid analysis and co-immunoprecipitation (Akamatsu et al. 2003; Salakhova et al. 2005), the rescue of *sfr1Δ* phenotypes by Rad51 overexpression (Salakhova et al. 2005), higher sensitivity of *sfr1Δ* to UV and MMS at low temperatures (Fig. 4b, c), and decrease of ionizing radiation-induced Rad51 foci in *sfr1Δ* cells (data not shown). Moreover, *sfr1Δ* specifically suppresses repair defects of cells lacking the Rad51 paralogs (Salakhova et al. 2005).

In eukaryotes, double-strand breaks are initially processed by two partially redundant sub-pathways, one mediated by the MRN complex (Mre11/Rad50/Nbs1), the other by additional exonucleases including the 5′–3′ exonuclease Exo1. In agreement, the epistasis analysis for *rad50Δ* in *S. pombe* showed synergism for repair of IR- and MMS-induced damage and the formation of IR-induced Rad51 foci (Tomita et al. 2003). After 3′-protruding single-strand ends are formed, two parallel sub-pathways, mediated by the Rad51-paralogs and Sfr1, facilitate Rad51 filament formation (Kurokawa et al. 2008; Murayama et al. 2008). This order of events at early stages of recombination (Fig. 6) is supported by our genetic analysis of mutants in the proposed sub-pathways. Two stages of DSB processing and Rad51 filament assembly are proposed: Two upstream sub-pathways of DSB processing feed into two downstream sub-pathways for Rad51 nucleoprotein assembly. If the DSBs processed by the MRN and Exo1 sub-pathways are utilized by both filament-mediator sub-pathways, the Rhp55/57- and the Sfr1-dependent one, any combination of mutations from two subsequent stages of repair would result in synergistic enhancement of the repair defects. This predicted synergisms in MMS- and IR-induced damage repair was demonstrated for *sfr1Δ* and *rad50Δ* (Fig. 1), and *rhp55Δ* and *exo1Δ* (Salakhova et al. 2005), and data not shown. Synergistic enhancement of sensitivity to IR and MMS for *rhp55Δ* and *rad50Δ* has been demonstrated before (Hartsuiker et al. 2001).

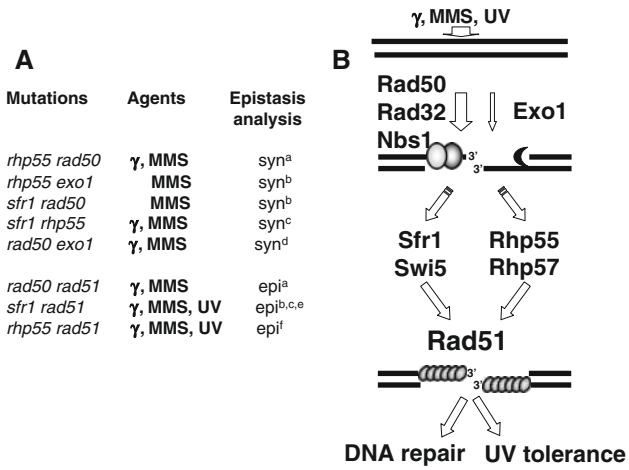


Fig. 6 Model on recombinational DNA repair pathways in *S. pombe* mitotic cells. **a** Review of epistasis analysis of repair genes of the *RAD52* group. The data were taken from (*a*) (Hartsuiker et al. 2001), (*b*) this study, (*c*) (Salakhova et al. 2005), (*d*) (Tomita et al. 2003), (*e*) (Akamatsu et al. 2003), and (*f*) (Khasanov et al. 1999). **b** After DNA damage leading to a double-strand break, the ends are processed by nuclease activities of partially redundant pathways, the Rad50/Rad32/Nbs1 complex, and by Exo1. The Rad51 nucleoprotein filament is assembled on 3′-protruding ssDNA ends concomitant with RPA displacement via two parallel mechanisms, the Rad51 paralog- and the Sfr1-mediated reactions. Strand invasion initiated by the Rad51 filament, and further processing lead to DNA repair or to UV tolerance

The two Rad51-filament assembly pathways in *S. pombe* seem to be partially redundant, as overexpression of *sfr1* partially rescues repair and cell-proliferation defects of the *rhp55Δ* mutant (Salakhova et al. 2005). However, while overexpression also partially rescues the reduced sporulation and spore viability of *rhp55Δ*, the defect in meiotic recombination cannot be rescued (data not shown), implying that Sfr1 and Rhp55 also have distinct functions in meiotic DSB repair. In agreement with our model (Fig. 6) for two subpathways operating upstream of mitotic Rad51, the high-copy *sfr1*$^{+}$ cannot rescue the absence of downstream functions, namely of Rad51 and Rhp54 (Salakhova et al. 2005). We found that *sfr1Δ* was epistatic with point mutations in the Smc gene *rad18* and in *rad60*, additional components of the *RAD52* pathway in *S. pombe* (Fig. 3a).

While overexpression of *sfr1* partially improved the defect in repair of MMS-induced lesions and low plating efficiency of the hypomorphic *rad60-1* mutant (Fig. 3b, c), the repair defect of the *rad18-na74* mutant was not alleviated (data not shown). Rad60 was primarily implicated in repair of stalled or collapsed replication forks by recombination (Boddy et al. 2003; Morishita et al. 2002) together with other *RAD52* group members, including Rad51 and Rhp55. Moreover, Rad60 physically interacts with and is functionally related to the structural maintenance of chromosomes protein complex SMC5/6 (Boddy et al. 2003). The primary role of Smc5/6 may be to hold DNA duplexes together at collapsed replication forks (Ampatzidou et al. 2006). Our speculation that overexpression of Sfr1 may improve the efficacy of Rad51-DNA filament formation is in line with in vitro results showing stimulation of Rad51 by Sfr1 (Kurokawa et al. 2008). This may then increase the probability of strand invasion into the homologous duplex, when proximity of duplexes is crippled by the *rad60-1* mutation. It is not yet known, whether overexpression of the Rhp55–Rhp57 hetero-dimer can suppress the defects of the *rad60-1* mutant.

Although the two *S. pombe* sub-pathways for Rad51 filament formation are partially redundant, and the severity of DNA repair defects in *sfr1Δ* and *rhp55Δ* is similar, many of the mitotic and meiotic phenotypes of the mutants differ significantly. Firstly, unlike *rhp55Δ* (which exhibits slow growth, cell elongation, aberrant nuclear morphology (Khasanov et al. 1999), deletion of *sfr1* has no effects on cell morphology and proliferation. Moreover, the *sfr1Δ rad2Δ* double mutant is viable, unlike *rhp55Δ rad2Δ*, and all other combinations of presently known *S. pombe RAD52* group mutants with *rad2Δ* (Hartsuiker et al. 2001; Muris et al. 1996; Tsutsui et al. 2000). This indicates that Sfr1 is not directly involved in repair and restart of collapsed replication forks, when a replisome reaches a nick in the DNA of *rad2Δ* cells as a result of unligated Okazaki fragments (Waga and Stillman 1998). However, despite the lack of an obvious defect in replication, the *sfr1Δ* mutant exhibits a moderate sensitivity to high doses of HU and CPT (Salakhova et al. 2005). HU blocks replication by depleting dNTPs (stalled replication forks) and CPT inhibits topoisomerase I, respectively. This suggests only a minor, non-essential role of Sfr1 in replication restart by recombination in mitotic cells. However, when the repair of DSBs during replication was compromised by *rhp55Δ*, and the S-phase checkpoint was triggered (as manifested by cell elongation), the overexpression of Sfr1 could partially rescue this defect. Secondly, deletion of *sfr1* had no significant effect on mitotic inter-homolog and sister-chromatid recombination, while elimination of the parallel subpathway by *rhp55* mutation resulted in an 11-fold and 3.2-fold increase of mitotic recombination rates, respectively (Tables 3, 4). In contrast, while the *rhp55* mutant showed only a 2-fold reduction in meiotic intra- and intergenic recombination (Grishchuk and Kohli 2003; Khasanov et al. 1999), *sfr1* deletion reduced intragenic recombination (conversion) 5- to 20-fold (Table 5), and intergenic recombination (crossover) 15- to 19-fold (Table 6). Moreover, while *sfr1Δ* cells showed wild-type level of chromosome III loss in diploids, *rhp55Δ* cells lost chromosomes 2,300-fold more frequently (Table 2). Finally, in contrast to *rhp55Δ*, the *sfr1Δ* mutant showed no defects in mating-type switching, a mechanism that involves mitotic homologous recombination at the *mat* locus (Akamatsu et al. 2003; Salakhova et al. 2005).

Sfr1 and UV damage tolerance

In fission yeast DNA damage results in a cascade of signal transduction mediated largely by protein phosphorylation. It is initiated by the checkpoint kinase Rad3 (Martinho et al. 1998). As a result, the two downstream kinases, Chk1 and Cds1 are activated to relay the signal to downstream effectors acting in cell cycle control, transcriptional regulation, replication, and recombination. Chk1 activation is required for G_2 cell cycle arrest, but Cds1 is activated only in S-phase (Martinho et al. 1998). Pyrimidine dimers and pyrimidine 6–4 photoproducts generated by UV during S-phase represent a serious obstacle to the replication machinery, leading to replication fork stalling or collapse (McCready et al. 2000). Besides the two major UV-damage repair mechanisms, NER and UVDE, fission yeast also has a tolerance pathway that allows survival with unrepaired or unrepairable DNA damage. The Cds1-dependent UV damage tolerance pathway includes at least 6 genes: *rqh1*, *mus81*, *rad51*, *rhp54*, *rad18*, and *rad22A* (Boddy et al. 2000; Murray et al. 1997). The epistasis analysis of UV sensitivity (Fig. 4) suggested that Sfr1 operates in a Rad51-dependent subpathway in parallel to the Rad51 paralogs. In addition, it showed that Sfr1 is also involved in a UV-damage

tolerance pathway, since *sfr1Δ* further sensitized the *uve1Δ rad13Δ* double mutant, which was completely unable to repair UV lesions (Fig. 4d). However, Sfr1 contributed to DNA damage tolerance synergistically with the Cds1-dependent UV damage tolerance pathway. These data and the synergism with other checkpoint kinase mutants, *rad3Δ* and *chk1Δ* (data not shown), suggested a unique checkpoint-independent function for Sfr1 in UV damage tolerance.

Thus, the existence of two Rad51-dependent UV damage tolerance pathways is proposed: the Rad51 paralog-mediated one is suggested to be activated by the intra-S checkpoint, and the other, Sfr1-mediated, is thought to be permanently active. This model is consistent with epistasis analysis of *sfr1Δ*, *rhp55Δ*, and *rqh1Δ*, the latter gene encoding the RecQ-like helicase with roles in homologous recombination-related mechanisms, particularly in the processing of UV-damage by recombination. The UV-sensitivity of the *rqh1Δ* mutant is partially rescued by loss of either *rhp55* (Laursen et al. 2003) or *sfr1* (Fig. 2b). This was also the case for the *rad51Δ rqh1Δ* double mutant, but not for the *rhp54Δ rqh1Δ* double mutant (Laursen et al. 2003). This may imply that the Rqh1 helicase is important for the resolution of the intermediates for recombinational UV damage tolerance. In the absence of Rqh1, the intermediates formed via recombination initiation cannot be resolved, which leads to significant lethality of the *rqh1Δ* mutant upon UV treatment (Hope et al. 2005). When recombination is abolished completely, as in *rad51Δ rqh1Δ* cells, or partially by elimination of one the Rad51-dependent sub-pathways (in *rhp55Δ rqh1Δ* and *sfr1Δ rqh1Δ* cells), the damage processing may be channeled to other pathways, and lethal recombination intermediates may not form, or be formed in reduced amount via one of the remaining sub-pathways. This is consistent with suppression of the UV sensitivity of the *rqh1Δ* mutant by *sfr1Δ* (Fig. 2b) (Hope et al. 2005). As Rqh1 has a dual function in UV-damage repair, acting both in S-phase and in G_2 (Caspari et al. 2002; Laursen et al. 2003), it is not clear which function is suppressed by deletion of *sfr1*. Considering the minor role of Sfr1 in mitotic S-phase, where UV response is regulated by the checkpoints, we speculate that Sfr1 may act predominantly in the G_2 phase of the cell cycle.

Conclusion

What is the precise role of Sfr1 in DNA repair and recombination? We propose that Sfr1 acts downstream of DSB-end processing factors as a mediator of Rad51-nucleoprotein formation, as also indicated by recent in vitro data (Kurokawa et al. 2008; Murayama et al. 2008). We propose that Sfr1 functions in parallel to that of the Rad51 paralogs Rhp55 and Rhp57 (Fig. 6). This hypothesis is supported by our genetic and molecular data pointing to important functional similarities between Sfr1 and the Rad51 paralogs. These data include the physical interaction of Sfr1 with Rad51 protein, and the rescue of the *sfr1* mutant phenotype by overexpression of Rad51 (Akamatsu et al. 2003; Salakhova et al. 2005). Further genetic and biochemical studies are required to understand the precise role of Sfr1 in DNA recombination and repair. It will also be important to determine, whether a similar function is present in budding yeast, another well-studied model organism, and in higher eukaryotes.

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