Srs2 and RecQ homologs cooperate in *mei-3*-mediated homologous recombination repair of *Neurospora crassa*

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ABSTRACT

Homologous recombination and post-replication repair facilitate restart of stalled or collapsed replication forks. The *SRS2* gene of *Saccharomyces cerevisiae* encodes a 3′–5′ DNA helicase that functions both in homologous recombination repair and in post-replication repair. This study identifies and characterizes the *SRS2* homolog in *Neurospora crassa*, which we call *mus-50*. A knockout mutant of *N.crassa*, *mus-50*, is sensitive to several DNA-damaging agents and genetic analyses indicate that it is epistatic with *mei-3* (*RAD51* homolog), *mus-11* (*RAD52* homolog), *mus-48* (*RAD55* homolog) and *mus-49* (*RAD57* homolog), suggesting a role for *mus-50* in homologous recombination repair. However, epistasis evidence has presented that MUS50 does not participate in post-replication repair in *N.crassa*. Also, the *N.crassa mus-25* (*RAD54* homolog) *mus-50* double mutant is viable, which is in contrast to the lethal phenotype of the equivalent *rad54 srs2* mutant in *S.cerevisiae*. Tetrad analysis revealed that *mus-50* in combination with mutations in two RecQ homologs, *qde-3* and *recQ2*, is lethal, and this lethality is suppressed by mutation in *mei-3*, *mus-11* or *mus-25*. Evidence is also presented for the two independent pathways for recovery from camptothecin-induced replication fork arrest: one pathway is dependent on QDE3 and MUS50 and the other pathway is dependent on MUS25 and RECQ2.

INTRODUCTION

Mechanisms of DNA repair are highly conserved from bacteria to higher eukaryotes. Studies in the model organism *Saccharomyces cerevisiae* have characterized several prototypical DNA repair pathways responsible for repairing specific kinds of damage (1–3). For example, proteins in the *RAD3* epistasis group participate in the nucleotide excision repair pathway, which removes ultraviolet (UV)-radiation-induced pyrimidine dimers and other bulky DNA lesions. Proteins in the *RAD52* epistasis group participate in homologous recombination (HR) repair of DNA double-strand breaks; and proteins in the *RAD6* epistasis group are involved in post-replication repair (PRR), a mechanism for enhancing survival of cells with persistent unrepaired DNA damage.

The *S.cerevisiae SRS2* gene encodes a structural homolog of bacterial 3′–5′ helicases UvrD and Rep. An *srs2* mutation was first identified as a suppressor of the trimethoprim sensitivity of *rad6* and *rad18* mutants (4). UV-sensitivity of *rad6* and *rad18* mutants is also suppressed by *srs2* in strains that are proficient in the *RAD52* pathway (i.e. recombination repair) (4,5). In addition, *srs2* mutations suppress methyl methanesulfonate (MMS)-sensitivity of *rad5* and *pol30-46* mutants that are deficient in the error-free *RAD6*- and *RAD18*-dependent repair pathway (6). A function of the wild-type *SRS2* allele may be to suppress HR, because *srs2* mutants have a hyper-recombination phenotype (7–9). It has been proposed on the basis of genetic studies that Srs2 facilitates switching from HR to PRR at stalled replication forks. This idea is supported by biochemical data indicating that Srs2 causes the dissociation of Rad51-nucleoprotein filaments from single-stranded DNA, thereby destroying strand-exchange intermediates during HR (10,11). The ability of the Srs2 protein to dislodge Rad51 from DNA appears to be especially important in Rad54- or Sgs1-deficient strains that may produce aberrant HR intermediates; thus, *rad54Δ srs2Δ* or *sgs1Δ srs2Δ* mutants are either not viable or grow extremely poorly (12,13). Rad54 is a protein of the SWI2/SNF2 family that promotes Rad51-mediated strand invasion and D-loop formation (14,15), and *SGS1* encodes a DNA helicase with the same polarity as Srs2 (16–19). The double mutants *rad54Δ srs2Δ* or *sgs1Δ srs2Δ* are inviable. It is likely that this is due to aberrant or inappropriate HR, because they can be suppressed by mutations

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that block an early step in HR, such as *rad51*, *rad52*, *rad55* and *rad57* (20,21).

The *Schizosaccharomyces pombe* homolog of *SRS2* shares a number of characteristics with *S.cerevisiae SRS2*, including similar mutant phenotype (i.e. mutagen sensitivity and hyper-recombination) and genetic interactions with *rhp54* and *rqh1* (homologs of *S.cerevisiae RAD54* and *SGS1*, respectively) (22–24). However, in contrast to *S.cerevisiae srs2*, *S.pombe srs2* does not suppress the UV-sensitivity of PRR mutants and is epistatic with mutants in PRR (24). Although *SRS2* homologs have been identified in a number of organisms, they have not yet been reported in higher eukaryotes.

The RecQ helicase family is another conserved group of helicases (25). These helicases have 3′–5′ helicase activity, and RecQ homologs have been identified in numerous species. There is only one RecQ homolog in *S.cerevisiae*, but unlike Srs2 helicases, multiple RecQ homologs have been identified in higher eukaryotes. For example, four RecQ homologs were predicted from analysis of the complete *Caenorhabditis elegans* genomic sequence (26), and five human RecQ homologs have been identified, including BLM, WRN and RTS, which are associated with the genetic diseases Bloom syndrome, Werner syndrome and Rothmund–Thomson syndrome, respectively (27–29). These results suggest that RecQ genes have undergone several duplication events during the course of evolution.

The filamentous fungus *Neurospora crassa* is also a well-characterized model organism that has been used extensively for genetic studies of DNA repair. The DNA sequence of the entire ∼40 Mb *N.crassa* genome was completed recently (30). The *N.crassa* genome is approximately three times larger than that of the *S.cerevisiae* or *S.pombe* genomes (∼12 Mb each), and it may encode up to twice as many proteins as *S.cerevisiae* (∼6300) and *S.pombe* (∼4800). These characteristics suggest that *N.crassa* may be evolutionarily older than yeast.

Many repair-deficient mutants have been isolated and characterized in *N.crassa*. Genetic and molecular analyses of these mutants revealed that the *uvs*-6, *mei-3*, *mus-11*, *mus-25*, *mus-48*, *mus-49*, *mus-51* and *mus-52* genes are involved in recombinational repair and are homologs of *S.cerevisiae RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *YKU70* and *YKU80*, respectively [(31–34); Y. Murayama *et al*., unpublished data] (Table 1). The *N.crassa mus-8*, *uvs-2* and *mus-41* genes participate in PRR and are homologs of *S.cerevisiae RAD6*, *RAD18* and *RAD5*, respectively [(35,36); A. Kato *et al*., unpublished data] (Table 1). Database searching identified two putative *N.crassa* RecQ homologs (*qde-3* and *recQ2*), which were subsequently found to play roles in gene silencing and DNA repair (37–39). QDE3 is a large RecQ helicase with a molecular weight of 216.6 kDa, and RECQ2 is a small RecQ helicase of molecular weight 55.3 kDa. The *qde-3 recQ2* double mutant is viable but hypersensitive to DNA-damaging agents. These results suggest that QDE3 and RECQ2 may play complementary roles in DNA repair (39).

This study characterizes a *N.crassa* homolog of yeast *SRS2* called *mus-50* and its interactions with *N.crassa* RecQ helicases. Mutants of *mus-50* are mutagen-sensitive and epistatic to HR proteins including *mei-3*, *mus-11*, *mus-48* and *mus-49*. The MMS-sensitivity of PRR-deficient mutants is not suppressed by *mus-50*. Genetic analysis showed that *mus-50* is

Table 1. Genes related to recombination repair and post-replication repair in *N.crassa* and *S.cerevisiae*

S.cerevisiae	*N.crassa*
Recombination repair genes	
RAD50	*uvs-6*
RAD51	*mei-3*
RAD52	*mus-11*
RAD54	*mus-25*
RAD55	*mus-48*
RAD57	*mus-49*
YKU70	*mus-51*
YKU80	*mus-52*
SGS1	*qde-3*
	recQ2
SRS2	*mus-50*
Post-replication repair genes	
RAD6	*mus-8*
RAD18	*uvs-2*
RAD5	*mus-41*

a synthetic lethal in combination with *N.crassa qde-3* and *recQ2* owing to aberrant HR. Evidence is also presented for the two independent subpathways for recovery from camptothecin (CPT)-induced replication fork arrest. One pathway is dependent on QDE3 and MUS50, and the other is dependent on MUS25 and RECQ2.

MATERIALS AND METHODS

Strains and plasmids

Neurospora crassa strains used in this study are listed in Table 2. C1-T10-37A and C1-T10-28a are wild-type strains closely related to the standard Oak Ridge wild type (40). *S.cerevisiae* W303-1A (*MATa ade2-1 can1-100 his3-11*, *15 leu2-3*, *112 trp1-1 ura3-1*) was used as a wild type and TH110-1A (*MATa ade2-1 can1-100 his3-11*, *15 leu2-3*, *112 trp1-1 ura3-1 Δsrs2*::HIS3) was used as an *srs2* mutant. *Escherichia coli* strains DH-1 and XL-1 Blue were used for the amplification of plasmids. pBluescript SK⁺ (Stratagene) was used for general DNA manipulation. pCB1003 (41), carrying the *E.coli* hygromycin B resistance gene driven by the *Aspergillus nidulans trpC* promoter, was used as a vector in the transformation of *N.crassa* spheroplasts. pAS2-1 (Clontech) was a yeast expression vector.

DNA and RNA manipulation

Standard molecular techniques were carried out according to Sambrook *et al*. (42). DNA sequencing was achieved using the ALF*express* sequencer (Amersham Pharmacia Biotech). *N.crassa* genomic DNA was prepared from mycelia using a procedure described by Irelan *et al*. (43). *N.crassa* total RNA was extracted using RNAwiz (Ambion). PCR amplification was carried out with the Expand™ High-Fidelity PCR system (Roche Diagnostics Corp., Basel, Switzerland) according to the manufacturer's protocol. cDNA of *mus-50* was cloned by PCR from a *N.crassa* cDNA library from the Fungal Genetics Stock Center (FGSC).

Gene disruption by Repeat Induced Point mutation

The Repeat Induced Point (RIP) mutation technique was used to disrupt the *mus-50* gene (44). DNA fragments of *mus-50*

Table 2. Strains of *N.crassa* used in this study

Strain/FGSC number	Genotype (allele number or isolation number)	Source/reference
C1-T10-37A	*A*	(40)
C1-T10-28a	*a*	(40)
FGSC4105A	*A pan-2* (B36)	FGSC
C2-S2-8a	*a pan-2* (OGW1)	Laboratory stock
K16-CR4-31A	*A mus-50* (RIP1)	This study
K16-CR6-2a	*a mus-50* (RIP1)	This study
K16-CR7-2A	*A mus-50* (RIP1) *pan-2* (B36)	This study
K16-CR12-2a	*a mus-50* (RIP1) *pan-2* (OGW1)	This study
FGSC2764A	*A mei-3*	FGSC
FGSC6410a	*a mus-11*	FGSC
YST-F1-5A	*A mus-48::Hyg^r*	Laboratory stock
YST-S5-9A	*A mus-49::Hyg^r*	Laboratory stock
FGSC6425A	*A mus-25*	FGSC
74OR-270-104a	*a uvs-6 al-2 pan-2 cot-1*	(61)
FGSC5145a	*a mus-8*	FGSC
K16-CR49-32a	*a uvs-2* (RIP1)	Laboratory stock
KTO-R5-O4A	*A mus-41* (RIP1) *pan-2* (OGW1)	Laboratory stock
KTO-r-17A	*A qde-3* (RIP1)	(38)
KTO-Q2-2A	*A recQ2::Hyg^r*	Laboratory stock
K16-CT6-2-6a	*a mei-3 mus-50* (RIP1)	This study
K16-CT11-1-5 a	*a mus-11 mus-50* (RIP1)	This study
K16-CT27-2-1A	*A mus-48::Hyg^r mus-50* (RIP1)	This study
K16-CR33-8a	*a mus-49::Hyg^r mus-50* (RIP1)	This study
K16-CR15-12a	*a mus-25 mus-50* (RIP1)	This study
K16-CT20-2-4a	*a uvs-6 mus-50* (RIP1) *al-2 pan-2 cot-1*	This study
K16-CT3-1-1A	*A mus-8 mus-50* (RIP1)	This study
K16-CR50-7a	*a uvs-2* (RIP1) *mus-50* (RIP1) *pan-2* (B36)	This study
K16-CR9-46A	*A mus-41* (RIP1) *mus-50* (RIP1) *pan-2* (OGW1)	This study
K16-CT7-1-1A	*A qde-3* (RIP1) *mus-50* (RIP1)	This study
K16-CB83-5a	*a qde-3* (RIP1) *mus-50* (RIP1)	This study
K16-CT13-1-1a	*a recQ2::Hyg^r mus-50* (RIP1)	This study
K16-CR43-12A	*A mei3 mus-25*	This study
K16-CR42-2A	*A mei3 mus-25 mus-50* (RIP1)	This study
K16-CR35-11A	*A mei-3 recQ2::Hyg^r mus-50* (RIP1)	This study
K16-CR34-17a	*a mus-11 recQ2::Hyg^r mus-50* (RIP1)	This study
K16-CR36-10a	*a mus-25 recQ2::Hyg^r mus-50* (RIP1)	This study

FGSC, Fugal Genetics Stock Center.

were generated by PCR using *N.crassa* genomic DNA as a template and the following two primers: *mus50-5′* (5′-GGCTCAATATAGCTGCTCTTGC-3′) and *mus50-3′* (5′-CTGCAGCCTCTTCCATCTTC-3′). PCR cycling conditions were as follows: initial denaturation at 94°C for 2 min, subsequent steps at 94°C for 15 s, annealing at 59°C for 30 s, extension at 72°C for 1.5 min, 10 cycles. Then, 94°C for 15 s, 59°C for 30 s, 72°C for 1.5 min (each cycle at 72°C for 5 s added), 25 cycles, final extension at 72°C for 5 min, hold at 4°C. The PCR product was integrated into pT7Blue (Novagen) to give pT7-*mus50*. pT7-*mus50* was digested with BamHI and XbaI, and ligated to the BamHI- and XbaI-digested pCB1003 to yield the plasmid p1003-*mus50*. This plasmid was introduced into *N.crassa* wild-type strain and a hygromycin-resistant transformant was crossed to the wild-type strain to induce RIP mutation. Sequence analysis confirmed numerous GC-to-AT transitions at the *mus50* gene locus.

Northern blots

RNA was isolated from germinating conidia, which were cultured at 30°C for 6 h with shaking. To induce gene expression, germinating conidia (1×10^7 ml^{-1}) were irradiated with UV (100 J/m^2) and cultured for the indicated time period. The BamHI–XbaI DNA fragment from pT7-*mus50* was labeled with ^{32}P using the Multiprime DNA Labeling system (Amersham Pharmacia Biotech) and used as a probe.

General genetic manipulation in *N.crassa*

Genetic procedures including cross and tetrad dissection were carried out according to the methods of Davis and de Serres (45). Transformation was performed as described by Vollmer and Yanofsky (46) and Tomita *et al*. (36), except that *Trichoderma harzianum* lysing enzyme (Sigma) was used as a substitute for Novozym.

Mutagen sensitivity

The survival of UV-irradiated *N.crassa* cells was measured as described previously (47). To measure the survival rate of hydroxyurea (HU)-, MMS-, CPT- or bleomycin (BLM)-treated *N.crassa* cells, 1×10^3 conidia were directly mixed with melted agar medium containing various concentrations of HU, MMS, CPT or BLM, and plated on Petri dishes. They were incubated at 30°C for 3 days, and the number of colonies were counted. The survival of mutagen-treated yeast cells was measured by a modification of the method of Prakash and Prakash (48). Stationary cells were washed and resuspended in sterile distilled water. To assay UV-sensitivity, yeast cell suspensions (1×10^6 cells/ml in 67 mM phosphate buffer,

pH 7.0) were diluted and spread on the agar medium at a concentration of 300 cells per plate. These plates were UV-irradiated at various doses, and incubated at 30°C for 2 days. To assay MMS-sensitivity, 30 μl of MMS was added to 20 ml cell suspension (1×10^6 cells/ml in 67 mM phosphate buffer, pH 7.0) and incubated at 30°C with mild shaking. At 15 min intervals an aliquot was removed, diluted and plated at a concentration of 300 cells per plate. Plates were incubated at 30°C for 2 days and the number of colonies were counted. All the experiments were repeated at least three times, and standard deviation at each point was calculated.

UV- and MMS-induced reversion assays

For reversion assay, two pantothenic acid-requiring strains, B36 and OGW1, were crossed with *mus-50* to make *pan-2* (B36) *mus-50* and *pan-2* (OGW1) *mus-50* strains. The *pan-2* (B36) and *pan-2* (OGW1) alleles carry base substitution and frameshift mutations, respectively. Conidial suspensions (1×10^7 conidia/ml) were irradiated at various doses of UV and cell survival and reversion were measured as follows: (i) a portion of the conidial suspension was diluted to 10^4 conidia/ml and 0.1 ml samples of the diluted suspension were mixed with the medium supplemented with panthotenate (10 μg/ml) and plated on Petri dishes. Each plate was incubated at 30°C for 3 days and the number of colonies were counted. (ii) To determine the number of *pan-2* revertants, 1 ml aliquot of conidial suspensions were mixed with the medium supplemented with panthotenate (0.04 μg/ml) and plated on Petri dishes. The number of revertants was counted and reversion frequency was calculated as the number of revertants per 1×10^7 survivors. MMS-induced reversion was carried out using a similar procedure as follows: conidial suspension (1×10^7 conidia/ml) was diluted to 10^4 conidia/ml,

and 0.03 ml samples were mixed with medium containing various concentrations of MMS supplemented with panthotenate (10 μg/ml). Cells were plated, incubated at 30°C for 3 days and the number of colonies was counted. *pan-2* revertants were identified by mixing 1 ml aliquot of the conidial suspensions in medium containing various concentrations of MMS supplemented with panthotenate (0.04 μg/ml). Cells were plated and revertants were counted after 3 days at 30°C. Reversion frequency was calculated as described above.

RESULTS

Identification and characterization of *mus-50* mutant

A putative homolog of *S.cerevisiae SRS2* was identified by searching the *Neurospora* genome database (http://www.broad.mit.edu/annotation/fungi/neurospora/). One candidate *Neurospora* gene, *NCU04733.1*, was identified, which encodes an 1105 amino acid protein (DDBJ accession no. AB193440) with the seven consensus DNA helicase motifs that are characteristic of DNA helicases, including *S.cerevisiae* and *S.pombe* Srs2 (Figure 1A). The mRNA from this gene, which we tentatively call *ncsrs2*, was expressed at a low level in untreated cells, but induced significantly by UV or MMS (Figure 2A). The *ncsrs2* gene was cloned into yeast expression vector pAS2-1 and the resulting plasmid, pAS2-NCSRS2, was introduced and expressed in *S.cerevisiae srs2Δ* mutant cells. The *srs2Δ* mutants with vector pAS2-1 or pAS2-NCSRS2 were exposed to UV or MMS and cell survival was evaluated (Figure 2B). The results show that the NCSRS2 expression vector fully complements the DNA damage sensitivity of *S.cerevisiae srs2Δ*, suggesting that NCSRS2 is functionally similar to yeast Srs2 *in vivo*.
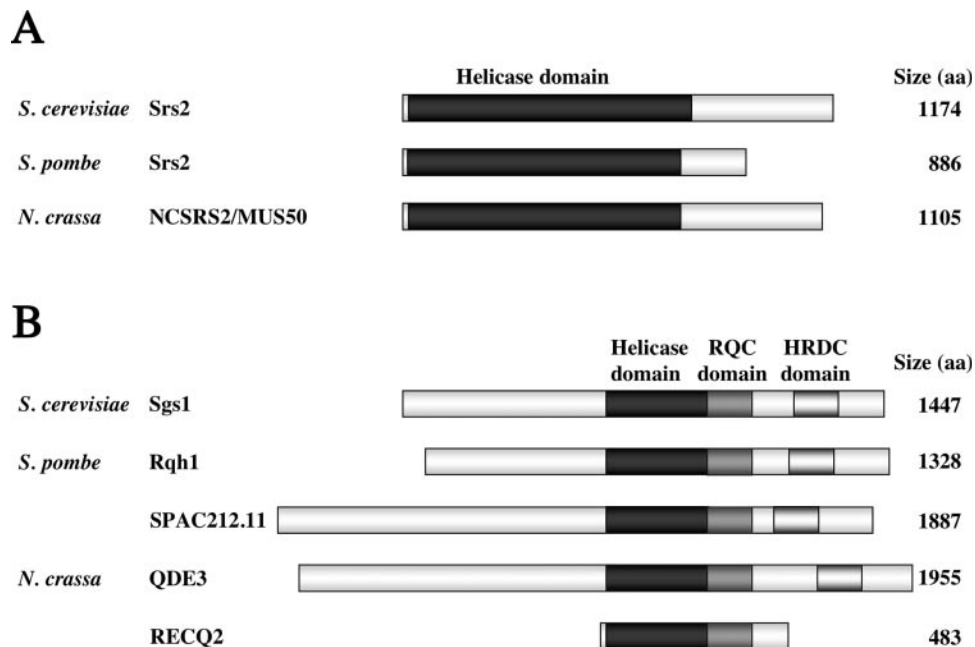

Figure 1. Schematic alignment of Srs2 and RecQ helicases. The names of species and gene products are shown on the left-hand side, while protein size (number of amino acid residues) is shown on the right-hand side. (**A**) Schematic alignment of Srs2 helicases. Their conserved helicase domains are shown in black. (**B**) Schematic alignment of RecQ helicases. Their conserved helicase, RQC and HRDC domains are shown in black, dark gray and light gray, respectively.
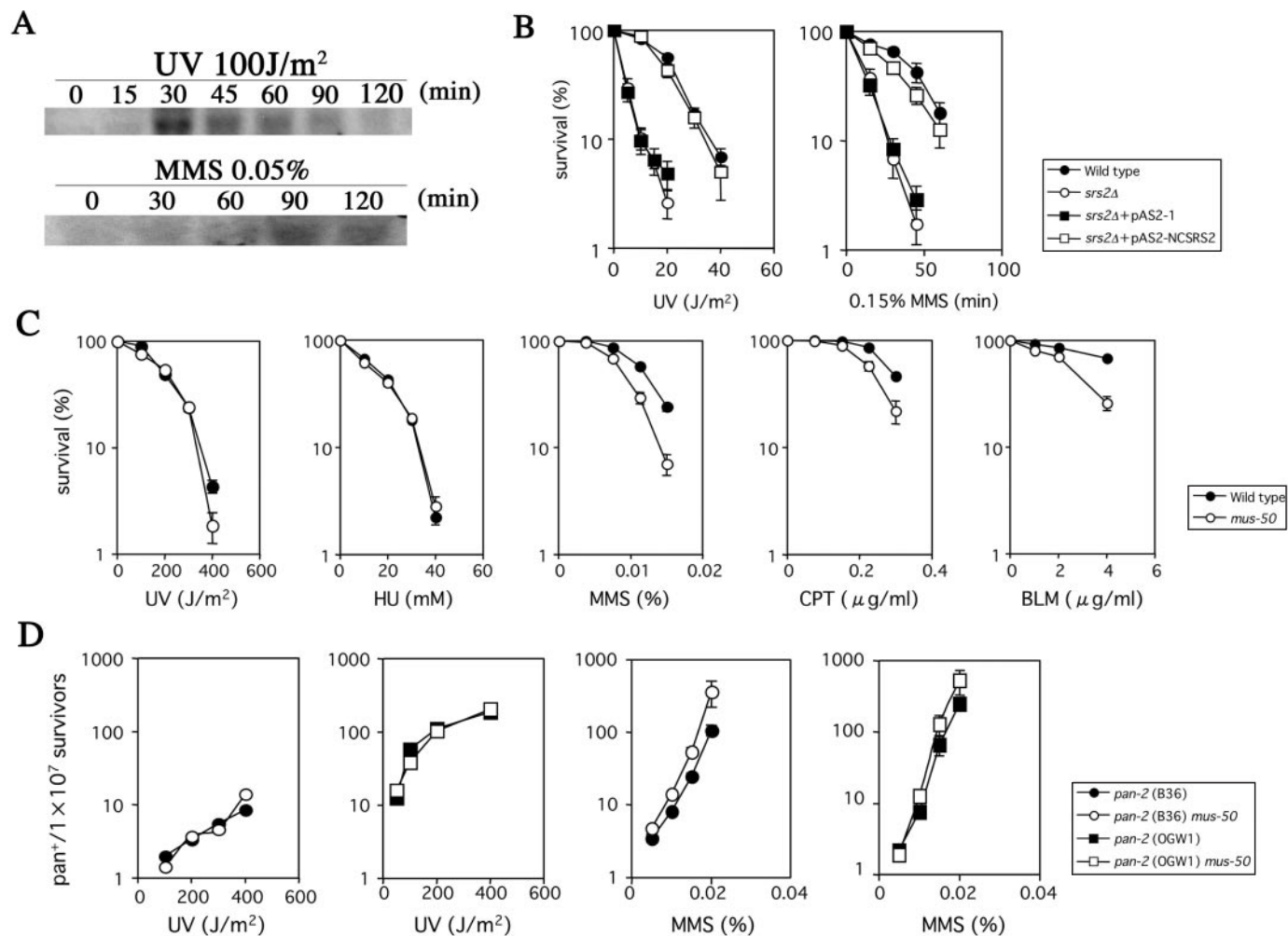
Figure 2. Characterization of *mus-50*. (**A**) Northern-blot analyses of *mus-50* transcripts. Upper panel: germinating mycelia were irradiated with UV at a dose of 100 J/m^2. At indicated time intervals after irradiation, a sample was collected and the level of *mus-50* transcripts was analyzed by northern-blot hybridization. Lower panel: germinating mycelia were treated with 0.05% MMS. At indicated intervals after MMS treatment, a sample was collected and the level of *mus-50* transcripts was analyzed by northern-blot hybridization. The 28S rRNA was estimated by ethidium bromide staining to confirm equal loading of all samples (data not shown). (**B**) Complementation of the UV- and MMS-sensitivities of the *S.cerevisiae srs2Δ* mutant by the *N.crassa mus-50* gene. Stationary cells were UV-irradiated at the indicated dose. UV-irradiated cells were diluted and plated at 300 colonies per plate. Plates were incubated at 30°C for 2 days. To assay MMS-sensitivity, 30 μl of MMS was added to 20 ml of yeast suspension. Yeast suspensions were shaken continuously at 30°C for the indicated time period. Dilution, plating and incubation were as described above. Closed circles, wild type; open circles, *srs2Δ*; closed squares, *srs2Δ+pAS2-1*; and open squares, *srs2Δ+pAS2-NCSRS2*. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**C**) Sensitivity to UV, HU, MMS, CPT and BLM of the wild-type and *mus-50* strains. A conidial suspension was irradiated with UV at indicated dose or mixed with medium containing HU, MMS, CPT or BLM at indicated concentration. Colonies were counted after incubation at 30°C for 3 days. Closed circles, wild type and open circles, *mus-50*. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**D**) Comparison of the UV- and MMS-induced *pan2* reversion frequency. The B36 and OGW1 alleles of *pan-2* have base substitution and frameshift mutations in the *pan-2* locus, respectively. The *y*-axis indicates the number of revertants per 1×10^7 survivors. The *x*-axis indicates UV dose or MMS concentration. Closed circles, *pan-2* (B36); closed squares, *pan-2* (OGW1); open squares, *pan-2* (B36) *mus-50*; and open squares, *pan-2* (OGW1) *mus-50*. The error bar of each point shows the standard deviation calculated from the data of those independent experiments.

The function of the *ncsrs2* gene in *N.crassa* was examined using targeted gene disruption by RIP mutation (44). The RIP mutation procedure effectively inactivated *ncsrs2* by introducing numerous GC-to-AT transition mutations, as confirmed by DNA sequencing (data not shown). Furthermore, *ncsrs2* mRNA was not detected by northern-blot analysis of mRNA from the RIPed strain even in cells exposed to UV irradiation (data not shown). These results indicate that the RIP mutation procedure effectively generated a null allele of *ncsrs2*. The mutagen sensitivity of this null *ncsrs2* mutant was examined using quantitative

dose–response survival curves (Figures 2C). The *ncsrs2* mutant is sensitive to MMS, CPT and BLM, but it is not sensitive to UV or HU. In contrast, mutants in *S.cerevisiae srs2* and *S.pombe srs2* are sensitive to all these agents including UV and HU (24,49,50). Following established rules of genetic nomenclature in *Neurospora* (51), *ncsrs2* was renamed *mus-50*, because it is differentially sensitive to some types of DNA damage. The mutation frequency of the *mus-50* mutant is similar to wild-type *N.crassa* (Figure 2D). In contrast, *srs2* mutants suppress UV-induced mutagenesis in *S.cerevisiae* (49).

Epistasis analyses of *mus-50* with recombination repair genes

Genetic interactions between *mus-50* and recombination repair genes were examined by measuring MMS-sensitivity of single or double mutants carrying *mus-50* and *mei-3*, *mus-11*, *mus-48*, *mus-49*, *mus-25* or *uvs-6* (Table 1). The MMS-sensitivity of *mei-3* and *mus-11* was the same in *mus-50* wild-type and *mus-50* mutant backgrounds (Figure 3A and B). The same genetic effects were seen between *mus-50* and *mus-48* or *mus-49* (data not shown). These results indicate that *mus-50* is epistatic with these genes and may participate in the same biological functions as these genes.

S.cerevisiae Rad54 belongs to the SWI2/SNF2 family of chromatin remodeling proteins (14,15). One possible function of Rad54 might be to alter accessibility to the Rad51-DNA nucleofilament. *rad54* is a synthetic lethal with *S.cerevisiae* and *S.pombe srs2*, such that *S.cerevisiae rad54 srs2* and *S.pombe rhp54 srs2* are not viable. However, the *N.crassa* homolog of *rad54*, *mus-25*, is viable as a double mutant with *mus-50*, and *N.crassa mus-25 mus-50* is more sensitive to MMS than either parental single mutant (Figure 3C). This
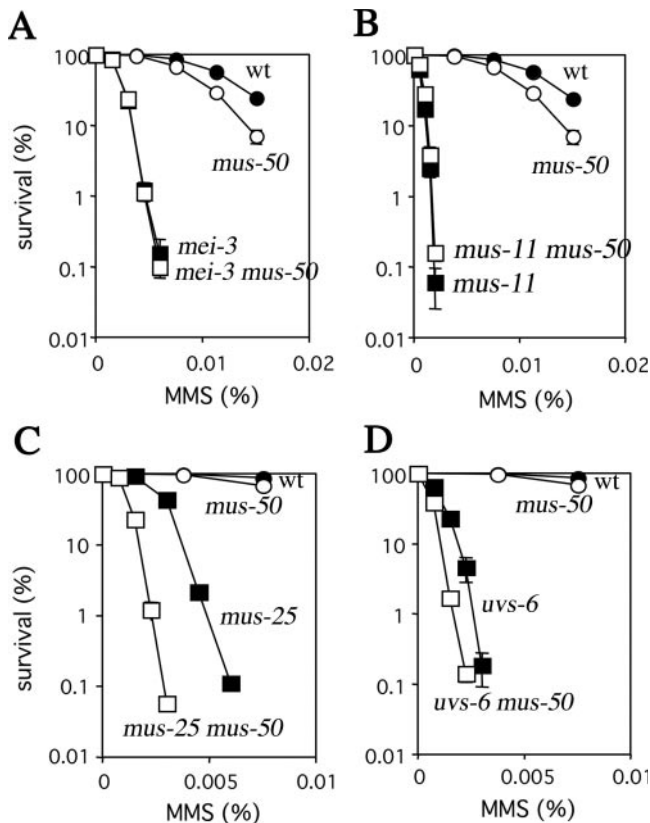

Figure 3. Epistasis analysis of *mus-50* and recombination repair defective. Cells were scored for MMS-sensitivity as described in the legend to Figure 2C. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**A**) Closed circles, wild type; open circles, *mus-50*; closed squares, *mei-3*; and open squares, *mei-3 mus-50*. (**B**) Closed circles, wild type; open circles, *mus-50*; closed squares, *mus-11*; and open squares, *mus-11 mus-50*. (**C**) Closed circles, wild type; open circles, *mus-50*; closed squares, *mus-25*; and open squares, *mus-25 mus-50*. (**D**) Closed circles, wild type; open circles, *mus-50*; closed squares, *uvs-6*; and open squares, *uvs-6 mus-50*.

suggests that *mus-50* is not epistatic with *mus-25* in *N.crassa*, and that *N.crassa* may express a novel recombination repair pathway that is independent of MUS25 and MUS50.

Saccharomyces cerevisiae Rad50, Xrs2 and Mre11 proteins function in a complex that protects and processes the termini of DNA breaks and which is required for recombination between sister chromatids (52,53). Mutants in *rad50*, *xrs2* and *mre11* grow extremely poorly when combined with *srs2Δ* (9,21). In contrast, the *N.crassa uvs-6 mus-50* double mutant is not defective for growth and is more sensitive to MMS than either parental single mutant (Figure 3D), suggesting that *mus-50* is not epistatic with *uvs-6*.

N.crassa mus-50 does not suppress the mutagen sensitivity of PRR-deficient mutants

Mutations in *srs2* suppress the UV- and MMS-sensitivity of PRR-deficient mutants such as *S.cerevisiae rad18* or *rad6*. In contrast, *S.pombe srs2* does not suppress or exacerbate the UV-sensitivity of PRR-deficient mutants (i.e. double mutants have same sensitivity as parental single mutants) (24). *N.crassa mus-8* is the homolog of *S.cerevisiae RAD6*, so the role of *mus-50* in PRR was determined by comparing the MMS-sensitivity of *N.crassa mus-8*, *mus-50* and *mus-8 mus-50* (Figure 4A). The results showed that double mutant is more sensitive than the *mus-8* single mutant, indicating that *mus-8* is not epistatic to *mus-50*. Similarly, two other *N.crassa* PRR genes, *uvs-2* (*RAD18* homolog) and *mus-41* (*RAD5* homolog) are not epistatic to *mus-50* (Figure 4B and C). These results suggest that MUS50 is not directly involved in PRR in *N.crassa*.

mus-50 is a synthetic lethal as a triple mutant with two *N.crassa* RecQ homologs

Double mutants *sgs1 srs2* in *S.cerevisiae* and *rqh1 srs2* in *S.pombe* are lethal or poorly viable (13,22). Analogous interaction between the corresponding genes in *N.crassa* was investigated in this study. In *N.crassa*, there are two RecQ helicase homologs, *qde-3* and *recQ2* (37–39) (Figure 1B), and double mutants *qde-3 mus-50* and *recQ2 mus-50* were constructed and characterized. These double mutants grow normally (data not shown) and are more sensitive to MMS than either parental single mutant (Figure 5A and B). However, *qde-3 recQ2 mus-50* triple mutants are lethal, as shown by tetrad dissection after crossing the double mutant strains with each other (Figure 5C). This result suggests that MUS50, QDE3 and RECQ2 play redundant roles in repairing spontaneous DNA damage during vegetative growth.

Mutation of *mei-3*, *mus-11* or *mus-25* suppresses the lethality of *qde-3 recQ2 mus-50*

In *S.cerevisiae*, the lethality of the *sgs1Δ srs2Δ* double mutant is suppressed by the deletion of *RAD51*, *RAD55* or *RAD57*, whose functions are in the early stage of HR (20). The possibility of a similar effect in *N.crassa* was examined by crossing a *qde-3 mus-50* mutant to one of the following triple mutants: *mei-3 recQ2 mus-50*, *mus-11 recQ2 mus-50* or *mus-25 recQ2 mus-50*. Tetrad analyses revealed that *mei-3 qde-3 recQ2 mus-50* and *mus-11 qde-3 recQ2 mus-50* quadruple mutants are viable, indicating that *mei-3* and *mus-11* (homologs of *S.cerevisiae RAD51* and *RAD52*, respectively)
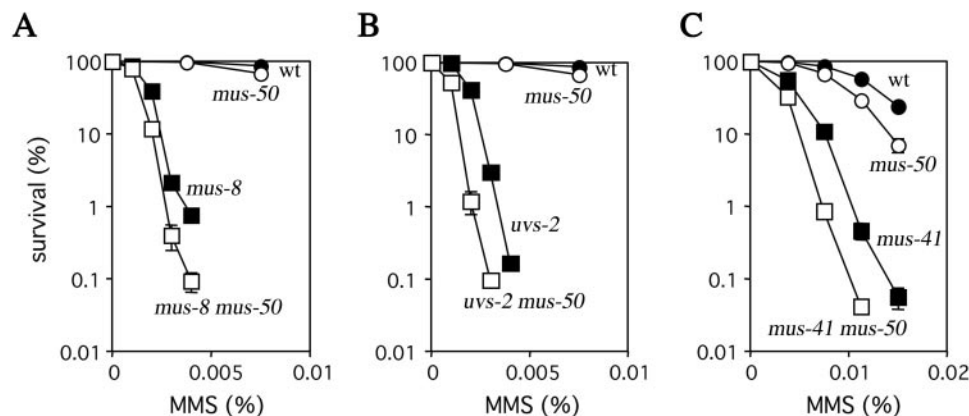
Figure 4. Epistasis analysis of *mus-50* and PRR defective. Cells were scored for MMS-sensitivity as described in the legend to Figure 2C. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**A**) Closed circles, wild type; open circles, *mus-50*; closed squares, *mus-8*; and open squares, *mus-8 mus-50*. (**B**) Closed circles, wild type; open circles, *mus-50*; closed squares, *uvs-2*; and open squares, *uvs-2 mus-50*. (**C**) Closed circles, wild type; open circles, *mus-50*; closed squares, *mus-41*; and open squares, *mus-41 mus-50*.

suppress the synthetic lethality of *qde-3 recQ2 mus-50* (Figure 6A and B). Surprisingly, mutations in *mus-25* (homolog of *S.cerevisiae RAD54*), which acts in the later stage of HR, also suppress the synthetic lethality of the *qde-3 recQ2 mus-50* triple mutant (Figure 6C). These results suggest that the lethality of *qde-3 recQ2 mus-50* triple mutant is due to the formation of irreversible recombination intermediates.

MEI3-dependent HR pathway requires MUS50, QDE3, RECQ2 and MUS25

The above results suggest that MUS50 is involved in MEI3-dependent homologous recombination and that MUS50 is functionally redundant to QDE3 and RECQ2. Previous studies also show that *mei-3* is epistatic to *mus-25* (33). To investigate whether these helicase proteins function in MEI3-dependent homologous recombination, the genetic relationship between these genes was analyzed in greater detail. Epistasis analysis based on MMS-sensitivity revealed that *mei-3* is epistatic to *mus-50* but not to *qde-3* and *recQ2* (Figure 7A). On the other hand, there is no epistatic relationship between *mus-25* and *mus-50*, *qde-3* or *recQ2* (Figure 7B). In addition, the synergistic effect of *mus-25* and *mus-50* on MMS-sensitivity was suppressed by a mutation in *mei-3*, suggesting that MEI3 acts upstream of MUS25 and MUS50 in the repair of MMS-induced DNA damage (Figure 7C). The complexity of these results and epistatic relationships may reflect the fact that MMS induces a number of different biologically relevant types of cellular damage/stress.

CPT causes type I topoisomerase-mediated single-strand nicks that lead to replication fork collapse (54,55). We investigated genetic interactions between recombination repair genes in the repair of CPT-induced DNA damage. Epistasis analysis revealed that *qde-3* but not *recQ2* is epistatic to *mus-50*, suggesting that MUS50 and QDE3 cooperate in the repair of CPT-induced replication fork collapse (Figure 8A). Epistasis analysis between *mei-3* and *mus-50*, *qde-3*, *recQ2* or *mus-25* revealed that *mei-3* is epistatic to *mus-50*, *qde-3*, *recQ2* and *mus-25*, indicating that MUS50, QDE3, RECQ2 and MUS25 function together in a MEI3-dependent pathway for the repair of CPT-induced DNA damage (Figure 8B). The

results also show that *mus-25* is epistatic to *recQ2* but not *mus-50* and *qde-3* (Figure 8C). Furthermore, the CPT sensitivities of *mus-25 mus-50* and *mus-25 qde-3* were similar to the *mei-3* single mutant (Figure 8C). These observations suggest that MEI3-mediated repair of CPT-induced replication fork collapse has two downstream subpathways: one pathway utilizes MUS50 and QDE3 and the other pathway utilizes RECQ2 and MUS25. This hypothesis is also supported by the observation that the *mei-3 mus-25 mus-50* triple mutant is as sensitive to CPT as the *mei-3* single mutant (Figure 8C).

DISCUSSION

This report demonstrates that the *N.crassa SRS2* homolog *mus-50* is functionally redundant to two RecQ family helicases, QDE3 and RECQ2, and that these two RecQ family helicases play different roles in HR in *N.crassa*. In contrast, *S.cerevisiae* has only one RecQ helicase, Sgs1. Although Rqh1 has been known as a RecQ homolog in *S.pombe*, a second RecQ homolog (SPAC212.11) was found and characterized recently (56). However, it has not been analyzed whether the SPAC212.11 is involved in the repair pathway of *S.pombe*. Therefore, this is the first report of multiple RecQ helicases with diverse recombination functions in a lower eukaryote.

Why is the phenotype of *N.crassa mus-50* milder than the phenotype of yeast *srs2*?

In *S.cerevisiae*, *srs2* mutations suppress the UV- and MMS-sensitivity of PRR-deficient mutants, such as *rad6*, *rad18*, *rad5*, *ubc13* and *mms2* (4,5,57), in strains that are proficient in recombination repair. The following explanation for these results was proposed: Srs2 interacts with collapsed replication forks and channels them from the HR repair pathway to the PRR pathway, thus preventing completion of aberrant HR events. In *S.pombe*, Srs2 may play a different role, because it is epistatic with PRR genes based on complementation of UV-sensitivity (24).

N.crassa mus-50 is functionally distinct from *S.cerevisiae* and *S.pombe srs2*. In particular, *mus-50* is not sensitive to UV or HU and has different genetic interactions with PRR genes in
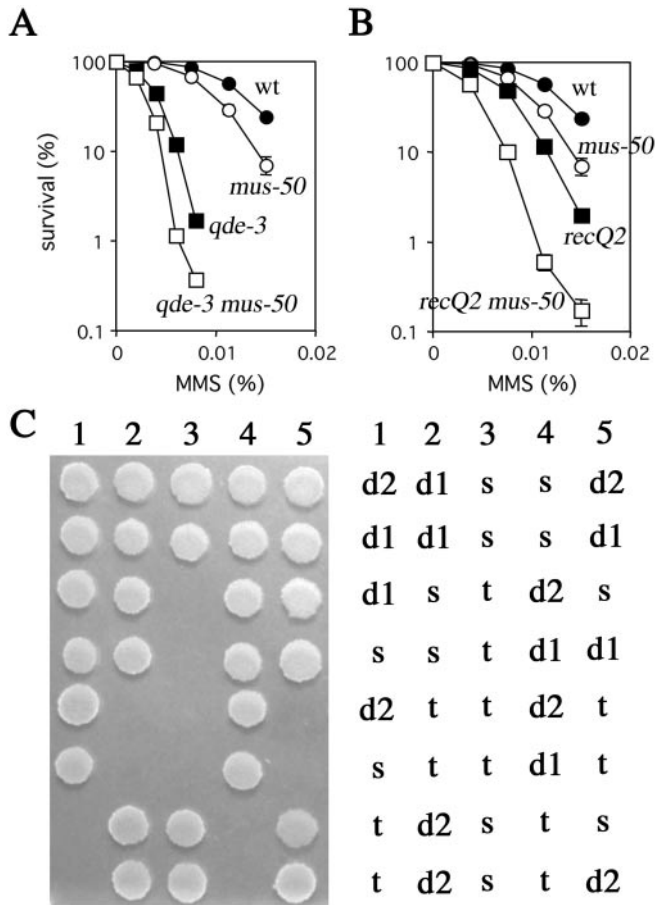
Figure 5. Genetic interactions between *mus-50* and RecQ helicase mutants. (**A**) Epistasis analysis of *mus-50* and *qde-3* mutant. Cells were scored for MMS-sensitivity as described in the legend to Figure 2C. Closed circles, wild type; open circles, *mus-50*; closed squares, *qde-3*; and open squares, *qde-3 mus-50*. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**B**) Epistasis analysis of *mus-50* mutant and *recQ2* mutant. Cells were scored for MMS-sensitivity as described in the legend to Figure 2C. Closed circles, wild type; open circles, *mus-50*; closed squares, *recQ2*; and open squares, *recQ2 mus-50*. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**C**) Tetrad analysis of spores from a cross between *qde-3 mus-50* and *recQ2 mus-50* double mutant strains. In the *N.crassa* eight ascospores are produced in an ascus. After dissected spores were incubated for 1 week at 25°C, spot test was carried out to identify the genotypes of the progeny. *mus-50* single mutant (s), *qde-3 mus-50* double mutant (d1), *recQ2 mus-50* double mutant (d2) and *qde-3 recQ2 mus-50* triple mutant (t).

Figure 6. The synthetic lethality of the *qde-3 recQ2 mus-50* triple mutant strain is rescued by the deletion of *mei-3*, *mus-11* or *mus-25*. (**A**) Tetrad analysis of spores from a cross between *qde-3 mus-50* and *mei-3 recQ2 mus-50* mutant strains. *mus-50* single mutant (s), *qde-3 mus-50* double mutant (d1), *recQ2 mus-50* double mutant (d2), *mei-3 mus-50* double mutant (d3), *qde-3 recQ2 mus-50* triple mutant (t), *mei-3 qde-3 mus-50* triple mutant (t1), *mei-3 recQ2 mus-50* triple mutant (t2) and *mei-3 qde-3 recQ2 mus-50* quadruple mutant (q). (**B**) Tetrad analysis of spores from a cross between *qde-3 mus-50* and *mus-11 recQ2 mus-50* mutant strains. *mus-50* single mutant (s), *qde-3 mus-50* double mutant (d1), *recQ2 mus-50* double mutant (d2), *mus-11 mus-50* double mutant (d3), *qde-3 recQ2 mus-50* triple mutant (t), *mus-11 qde-3 mus-50* triple mutant (t1), *mus-11 recQ2 mus-50* triple mutant (t2) and *mus-11 qde-3 recQ2 mus-50* quadruple mutant (q). (**C**) Tetrad analysis of spores from a cross between *qde-3 mus-50* and *mus-25 recQ2 mus-50* mutant strains. *mus-50* single mutant (s), *recQ2 mus-50* double mutant (d2), *mus-25 mus-50* double mutant (d3), *qde-3 recQ2 mus-50* triple mutant (t), *mus-25 qde-3 mus-50* triple mutant (t1) and *mus-25 qde-3 recQ2 mus-50* quadruple mutant (q).

N.crassa. *N.crassa mus-50* is not epistatic with *mus-8*, *uvs-2* or *mus-41* based on MMS-sensitivity. These results suggest that *N.crassa* MUS50 is redundant to or has overlapping functions with another *N.crassa* protein. Since overexpression of Sgs1 partially suppresses some defects of *srs2* mutants in *S.cerevisiae* (58), it seems possible that one of the *N.crassa* RecQ family helicases might be at least partially redundant in function to *N.crassa* MUS50. This hypothesis is supported by the following observations: (i) there are two RecQ homologs (*qde-3* and *recQ2*) in *N.crassa*; (ii) *qde-3 recQ2*, *qde-3 mus-50* and *recQ2 mus-50* double mutants are viable; (iii) the *qde-3 recQ2 mus-50* triple mutant is not viable; and (iv) the lethality of the triple mutant is suppressed by the mutation of *mei-3*. These data suggest that QDE3, RECQ2 and MUS50 share
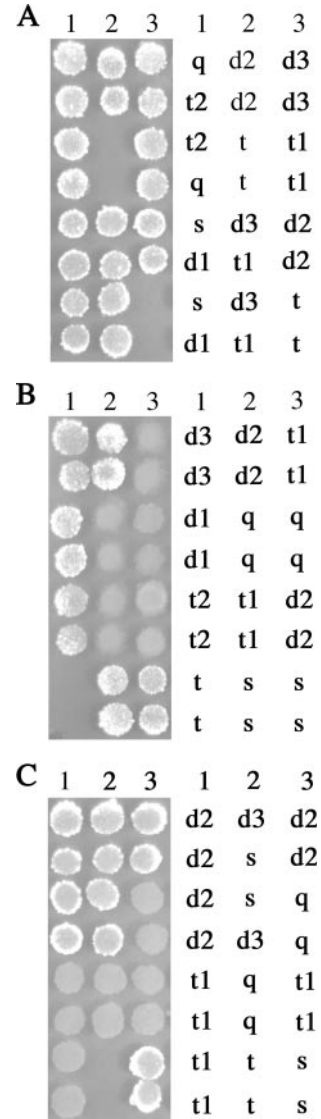
some level of redundancy and complement each other for cell viability. It appears likely that the redundant function of these helicases is to resolve collapsed recombination intermediates. If correct, this hypothesis explains the apparent dispensability of *mus-50* for PRR in *N.crassa*, because *qde-3* participates in both HR and PRR (38). If Srs2 helicase function
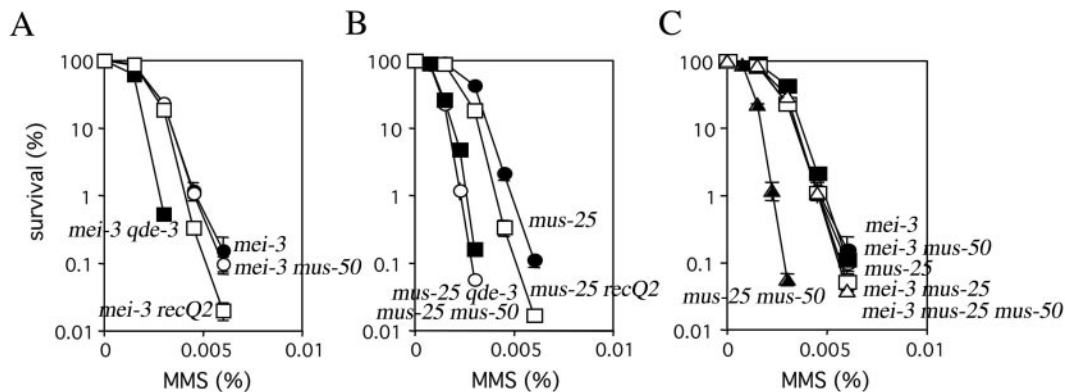
Figure 7. Epistasis analysis of *mei-3*, *mus-25*, *mus-50*, *qde-3* and *recQ2* for MMS-sensitivity. Cells were scored for MMS-sensitivity as described in the legend to Figure 2C. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**A**) Epistasis analysis of *mei-3* and *mus-50*, *qde-3* and *recQ2*. Closed circles, *mei-3*; open circles, *mei-3 mus-50*; closed squares, *mei-3 qde-3*; and open squares, *mei-3 recQ2*. (**B**) Epistasis analysis of *mus-25* and *mus-50*, *qde-3* and *recQ2*. Closed circles, *mus-25*; open circles, *mus-25 mus-50*; closed squares, *mus-25 qde-3*; and open squares, *mus-25 recQ2*. (**C**) Epistasis analysis of *mei-3*, *mus-50* and *mus-25*. Closed circles, *mei-3*; open circles, *mei-3 mus-50*; closed squares, *mus-25*; open squares, *mei-3 mus-25*; closed triangles, *mus-25 mus-50*; and open triangles, *mei-3 mus-25 mus-50*.
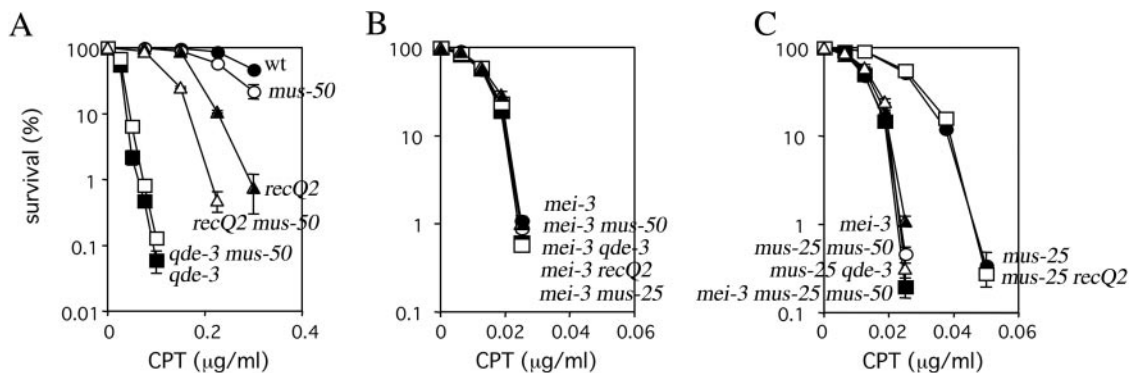

Figure 8. Epistasis analysis of *mei-3*, *mus-25*, *mus-50*, *qde-3* and *recQ2* for CPT-sensitivity. Cells were scored for CPT-sensitivity as described in the legend to Figure 2C. The error bar of each point shows the standard deviation calculated from the data of those independent experiments. (**A**) Epistasis analysis of *mus-50*, *qde-3* and *recQ2*. Closed circles, wild type; open circles, *mus-50*; closed squares, *qde-3*; open squares, *qde-3 mus-50*; closed triangles, *recQ2*; and open triangles, *recQ2 mus-50*. (**B**) Epistasis analysis between *mei-3* and *mus-50*, *qde-3*, *recQ2*, *mus-25*. Closed circles, *mei-3*; open circles, *mei-3 mus-50*; closed squares, *mei-3 qde-3*; open squares, *mei-3 recQ2*; and open triangles, *mei-3 mus-25*. (**C**) Epistasis analysis between *mus-25* and *mus-50*, *qde-3*, *recQ2* or between *mei-3* and *mus-25 mus-50*. Closed circles, *mus-25*; open circles, *mus-25 mus-50*; closed squares, *mus-25 qde-3*; open squares, *mus-25 recQ2*; closed triangles, *mei-3*; and open triangles, *mei-3 mus-25 mus-50*.

is redundant to RecQ helicase activity, this also explains why Srs2 helicases are not conserved in higher eukaryotes that express multiple RecQ helicases.

Two subpathways for MEI3-mediated HR facilitate repair of CPT-induced replication fork collapse

The topoisomerase I inhibitor CPT inhibits topoisomerase I leading to single-strand nicks and eventual replication fork collapse (54,55). The sensitivity of *mus-50* mutants to CPT indicates that MUS50 promotes the repair of CPT-induced collapsed replication forks. Epistasis analysis based on CPT-sensitivity demonstrated that there are two downstream subpathways involved in this process: one pathway requires MUS50 and QDE3 and the other pathway requires RECQ2 and MUS25. These results imply that (i) two RecQ helicases function in these two different recombination subpathways and (ii) multiple helicases participate in the MEI3-mediated recombination repair of collapsed replication forks.

The RecQ family is defined by the presence of seven highly conserved amino acid sequence motifs in the so-called helicase domain (25). Just C-terminal to this domain is the RQC (RecQ family C-terminal) domain, which is apparently unique to RecQ helicases and might have a role in directing specific protein–protein interactions. The C-terminal region of RecQ members also contains the HRDC (helicase RNase D C-terminal) domain, which has a probable role in DNA binding (59). QDE3 is a large RecQ helicase, which contains the highly conserved helicase domains, a RQC domain and an HRDC domain. In contrast, RECQ2 is smaller and does not contain an HRDC domain. This structural difference may provide variation in the binding affinities to DNA or protein of QDE3 and RECQ2. Thus, we can propose the hypothesis that QDE3 act in the early stage of MEI3-mediated HR, while RECQ2 may act in a later stage of HR (i.e. during MUS25-promoted D-loop formation and after MEI3 filament-mediated strand invasion). This hypothesis is supported by the fact that *mus-25* is epistatic to *recQ2* but not to *mus-50* and *qde-3* and

that the mutation of *mus-25* suppresses the synthetic lethality of the *qde-3 recQ2 mus-50* triple mutant. Differences in the structure and binding activities of QDE3 and RECQ2 may determine their differential functions in MEI3-dependent HR.

Saccharomyces cerevisiae Sgs1 and Srs2 are thought to restrict or suppress HR, because *sgs1* and *srs2* mutants have a hyper-recombination phenotype and because Srs2 promotes dissociation of the Rad51-nucleoprotein filament (7–11,60). Thus, it is possible that MUS50 and QDE3 share this role in suppressing HR in its early stages and promoting alternate processing of recombination intermediates by other repair pathways. RECQ2 is not likely to function in this manner, since it is thought to act during the later stages of HR. Further analysis of these mutants and proteins will be required to confirm this hypothesis and to explore how the activities of these three helicases are regulated and coordinated.

REFERENCES

1. Friedberg,E.C. (1988) Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. *Microbiol. Rev*., **52**, 70–102.
2. Prakash,S., Sung,P. and Prakash,L. (1993) DNA repair genes and proteins of *Saccharomyces cerevisiae*. *Annu. Rev. Genet*., **27**, 33–70.
3. Friedberg,E.C., Bardwell,A.J., Bardwell,L., Feaver,W.J., Kornberg,R.D., Svejstrup,J.Q., Tomkinson,A.E. and Wang,Z. (1995) Nucleotide excision repair in the yeast *Saccharomyces cerevisiae*: its relationship to specialized mitotic recombination and RNA polymerase II basal transcription. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **347**, 63–68.
4. Lawrence,C.W. and Christensen,R.B. (1979) Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of *Saccharomyces cerevisiae rad6* mutants. *J. Bacteriol.*, **139**, 866–876.
5. Schiestl,R.H., Prakash,S. and Prakash,L. (1990) The *SRS2* suppressor of *rad6* mutations of *Saccharomyces cerevisiae* acts by channeling DNA lesions into the *RAD52* DNA repair pathway. *Genetics*, **124**, 817–831.
6. Broomfield,S. and Xiao,W. (2002) Suppression of genetic defects within the *RAD6* pathway by *srs2* is specific for error-free post-replication repair but not for damage-induced mutagenesis. *Nucleic Acids Res.*, **30**, 732–739.
7. Aguilera,A. and Klein,H.L. (1988) Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. *Genetics*, **119**, 779–790.
8. Aguilera,A. and Klein,H.L. (1989) Genetic and molecular analysis of recombination events in *Saccharomyces cerevisiae* occurring in the presence of the hyper-recombination mutation *hpr1*. *Genetics*, **122**, 503–517.
9. Rong,L., Palladino,F., Aguilera,A. and Klein,H.L. (1991) The hyper-gene conversion *hpr5-1* mutation of *Saccharomyces cerevisiae* is an allele of the *SRS2/RADH* gene. *Genetics*, **127**, 75–85.
10. Krejci,L., Van Komen,S., Li,Y., Villemain,J., Reddy,M.S., Klein,H., Ellenberger,T. and Sung,P. (2003) DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature*, **423**, 305–309.
11. Veaute,X., Jeusset,J., Soustelle,C., Kowalczykowski,S.C., Le Cam,E. and Fabre,F. (2003) The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature*, **423**, 309–312.
12. Palladino,F. and Klein,H.L. (1992) Analysis of mitotic and meiotic defects in *Saccharomyces cerevisiae SRS2* DNA helicase mutants. *Genetics*, **132**, 23–37.
13. Lee,S.K., Johnson,R.E., Yu,S.L., Prakash,L. and Prakash,S. (1999) Requirement of yeast *SGS1* and *SRS2* genes for replication and transcription. *Science*, **286**, 2339–2342.
14. Eisen,J.A., Sweder,K.S. and Hanawalt,P.C. (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.*, **23**, 2715–2723.
15. Tan,T.L., Kanaar,R. and Wyman,C. (2003) Rad54, a Jack of all trades in homologous recombination. *DNA Repair (Amst.)*, **2**, 787–794.
16. Gangloff,S., McDonald,J.P., Bendixen,C., Arthur,L. and Rothstein,R. (1994) The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.*, **14**, 8391–8398.
17. Watt,P.M., Louis,E.J., Borts,R.H. and Hickson,I.D. (1995) Sgs1: a eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II *in vivo* and is required for faithful chromosome segregation. *Cell*, **81**, 253–260.
18. Lu,J., Mullen,J.R., Brill,S.J., Kleff,S., Romeo,A.M. and Sternglanz,R. (1996) Human homologues of yeast helicase. *Nature*, **383**, 678–679.
19. Bennett,R.J., Keck,J.L. and Wang,J.C. (1999) Binding specificity determines polarity of DNA unwinding by the Sgs1 protein of *S. cerevisiae*. *J. Mol. Biol.*, **289**, 235–248.
20. Gangloff,S., Soustelle,C. and Fabre,F. (2000) Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. *Nature Genet.*, **25**, 192–194.
21. Klein,H.L. (2001) Mutations in recombinational repair and in checkpoint control genes suppress the lethal combination of *srs2Δ with other DNA repair genes in Saccharomyces cerevisiae*. *Genetics*, **157**, 557–565.
22. Wang,S.W., Goodwin,A., Hickson,I.D. and Norbury,C.J. (2001) Involvement of *Schizosaccharomyces pombe* Srs2 in cellular responses to DNA damage. *Nucleic Acids Res.*, **29**, 2963–2972.
23. Maftahi,M., Hope,J.C., Delgado-Cruzata,L., Han,C.S. and Freyer,G.A. (2002) The severe slow growth of *Δsrs2 Δrqh1* in *Schizosaccharomyces pombe* is suppressed by loss of recombination and checkpoint genes. *Nucleic Acids Res.*, **30**, 4781–4792.
24. Doe,C.L. and Whitby,M.C. (2004) The involvement of Srs2 in post-replication repair and homologous recombination in fission yeast. *Nucleic Acids Res.*, **32**, 1480–1491.
25. Bachrati,C.Z. and Hickson,I.D. (2003) RecQ helicases: suppressors of tumorigenesis and premature aging. *Biochem. J.*, **374**, 577–606.
26. Jeong,Y.S., Kang,Y., Lim,K.H., Lee,M.H., Lee,J. and Koo,H.S. (2003) Deficiency of *Caenorhabditis elegans* RecQ5 homologue reduces life span and increases sensitivity to ionizing radiation. *DNA Repair (Amst.)*, **2**, 1309–1319.
27. Ellis,N.A., Groden,J., Ye,T.Z., Straughen,J., Lennon,D.J., Ciocci,S., Proytcheva,M. and German,J. (1995) The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell*, **83**, 655–666.
28. Yu,C.E., Oshima,J., Fu,Y.H., Wijsman,E.M., Hisama,F., Alisch,R., Matthews,S., Nakura,J., Miki,T., Ouais,S. *et al*. (1996) Positional cloning of the Werner's syndrome gene. *Science*, **272**, 258–262.
29. Kitao,S., Shimamoto,A., Goto,M., Miller,R.W., Smithson,W.A., Lindor,N.M. and Furuichi,Y. (1999) Mutations in *RECQL4* cause a subset of cases of Rothmund–Thomson syndrome. *Nature Genet.*, **22**, 82–84.
30. Galagan,J.E., Calvo,S.E., Borkovich,K.A., Selker,E.U., Read,N.D., Jaffe,D., FitzHugh,W., Ma,L.J., Smirnov,S., Purcell,S. *et al*. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*, **422**, 859–868.
31. Hatakeyama,S., Ishii,C. and Inoue,H. (1995) Identification and expression of the *Neurospora crassa mei-3* gene which encodes a protein homologous to Rad51 of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.*, **249**, 439–446.
32. Sakuraba,Y., Schroeder,A.L., Ishii,C. and Inoue,H. (2000) A Neurospora double-strand-break repair gene, *mus-11*, encodes a *RAD52* homologue and is inducible by mutagens. *Mol. Gen. Genet.*, **264**, 392–401.
33. Handa,N., Noguchi,Y., Sakuraba,Y., Ballario,P., Macino,G., Fujimoto,N., Ishii,C. and Inoue,H. (2000) Characterization of the

Neurospora crassa mus-25 mutant: the gene encodes a protein which is homologous to the *Saccharomyces cerevisiae* Rad54 protein. *Mol. Gen. Genet.*, **264**, 154–163.

34. Ninomiya,Y., Suzuki,K., Ishii,C. and Inoue,H. (2004) Highly efficient gene replacements in Neurospora strains deficient for nonhomologous end-joining. *Proc. Natl Acad. Sci. USA*, **101**, 12248–12253.

35. Soshi,T., Sakuraba,Y., Kafer,E. and Inoue,H. (1996) The *mus-8* gene of *Neurospora crassa* encodes a structural and functional homolog of the Rad6 protein of *Saccharomyces cerevisiae*. *Curr. Genet.*, **30**, 224–231.

36. Tomita,H., Soshi,T. and Inoue,H. (1993) The Neurospora *uvs-2* gene encodes a protein which has homology to yeast *RAD18*, with unique zinc finger motifs. *Mol. Gen. Genet.*, **238**, 225–233.

37. Cogoni,C. and Macino,G. (1999) Posttranscriptional gene silencing in Neurospora by a RecQ DNA helicase. *Science*, **286**, 2342–2344.

38. Kato,A., Akamatsu,Y., Sakuraba,Y. and Inoue,H. (2004) The *Neurospora crassa mus-19* gene is identical to the *qde-3* gene, which encodes a RecQ homologue and is involved in recombination repair and postreplication repair. *Curr. Genet.*, **45**, 37–44.

39. Pickford,A., Braccini,L., Macino,G. and Cogoni,C. (2003) The QDE-3 homologue RecQ-2 co-operates with QDE-3 in DNA repair in *Neurospora crassa*. *Curr. Genet.*, **42**, 220–227.

40. Tamaru,H. and Inoue,H. (1989) Isolation and characterization of a laccase-derepressed mutant of *Neurospora crassa*. *J. Bacteriol.*, **171**, 6288–6293.

41. Carroll,A.M., Sweigard,J.A. and Valent,B. (1994) Improved vectors for selecting resistance to hygromycin. *Fungal Genet. Newslett.*, **41**, 22.

42. Sambrook,J., Fritsch,E.F. and Maniatis,T.M. (1989) *Molecular Cloning: A Laboratory Manual, 2nd edn*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

43. Irelan,J., Miao,V. and Selker,E.U. (1993) Small scale DNA preps for *Neurospora crassa*. *Fungal Genet. Newslett.*, **40**, 24.

44. Selker,E.U., Cambareri,E.B., Jensen,B.C. and Haack,K.R. (1987) Rearrangement of duplicated DNA in specialized cells of Neurospora. *Cell*, **51**, 741–752.

45. Davis,R.H. and de Serres,F.J. (1970) Genetic and microbiological research techniques for *Neurospora crassa*. *Methods Enzymol.*, **17**, 79–143.

46. Vollmer,S.J. and Yanofsky,C. (1986) Efficient cloning of *Neurospora crassa*. *Proc. Natl Acad. Sci. USA*, **83**, 4869–4873.

47. Inoue,H. and Ishii,C. (1984) Isolation and characterization of MMS-sensitive mutants of *Neurospora crassa*. *Mutat. Res.*, **125**, 185–194.

48. Prakash,L. and Prakash,S. (1977) Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. *Genetics*, **86**, 33–55.

49. Aboussekhra,A., Chanet,R., Zgaga,Z., Cassier-Chauvat,C., Heude,M. and Fabre,F. (1989) *RADH*, a gene of *Saccharomyces cerevisiae* encoding a putative DNA helicase involved in DNA repair. Characteristics of radH mutants and sequence of the gene. *Nucleic Acids Res.*, **17**, 7211–7219.

50. Huang,M.E., de Calignon,A., Nicolas,A. and Galibert,F. (2000) *POL32*, a subunit of the *Saccharomyces cerevisiae* DNA polymerase delta, defines a link between DNA replication and the mutagenic bypass repair pathway. *Curr. Genet.*, **38**, 178–187.

51. Perkins,D.D. (1999) Neurospora genetic nomenclature. *Fungal Genet. Newslett.*, **46**, 34–41.

52. Haber,J.E. (1998) The many interfaces of Mre11. *Cell*, **95**, 583–586.

53. Saeki,T., Machida,I. and Nakai,S. (1980) Genetic control of diploid recovery after gamma-irradiation in the yeast *Saccharomyces cerevisiae*. *Mutat. Res.*, **73**, 251–265.

54. Liu,L.F., Duann,P., Lin,C.T., D'Arpa,P. and Wu,J. (1996) Mechanism of action of camptothecin. *Ann. N Y Acad. Sci.*, **803**, 44–49.

55. Nitiss,J.L. and Wang,J.C. (1996) Mechanisms of cell killing by drugs that trap covalent complexes between DNA topoisomerases and DNA. *Mol. Pharmacol.*, **50**, 1095–1102.

56. Mandell,J.G., Goodrich,K.J., Bahler,J. and Cech,T.R. (2005) Expression of a RecQ helicase homolog affects progression through crisis in fission yeast lacking telomerase. *J. Biol. Chem.*, **280**, 5249–5257.

57. Ulrich,H.D. (2001) The *srs2* suppressor of UV sensitivity acts specifically on the *RAD5-* and *MMS2*-dependent branch of the *RAD6* pathway. *Nucleic Acids Res.*, **29**, 3487–3494.

58. Mankouri,H.W., Craig,T.J. and Morgan,A. (2002) *SGS1* is a multicopy suppressor of *srs2*: functional overlap between DNA helicases. *Nucleic Acids Res.*, **30**, 1103–1113.

59. Janscak,P., Garcia,P.L., Hamburger,F., Makuta,Y., Shiraishi,K., Imai,Y., Ikeda,H. and Bickle,T.A. (2003) Characterization and mutational analysis of the RecQ core of the bloom syndrome protein. *J. Mol. Biol.*, **330**, 29–42.

60. Watt,P.M., Hickson,I.D., Borts,R.H. and Louis,E.J. (1996) *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. *Genetics*, **144**, 935–945.

61. de Serres,F.J., Inoue,H. and Schupbach,M.E. (1980) Mutagenesis at the *ad-3A* and *ad-3B* loci in haploid UV-sensitive strains of *Neurospora crassa*. I. Development of isogenic strains and spontaneous mutability. *Mutat. Res.*, **71**, 53–65.