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Mrc1 Is Required for Sister Chromatid Cohesion To Aid in Recombination Repair of Spontaneous Damage

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The *SRS2* gene of *Saccharomyces cerevisiae* encoding a 3′→5′ DNA helicase is part of the postreplication repair pathway and functions to ensure proper repair of DNA damage arising during DNA replication through pathways that do not involve homologous recombination. Through a synthetic gene array analysis, genes that are essential when Srs2 is absent have been identified. Among these are *MRC1*, *TOF1*, and *CSM3*, which mediate the intra-S checkpoint response. *srs2*Δ *mrc1*Δ synthetic lethality is due to inappropriate recombination, as the lethality can be suppressed by genetic elimination of homologous recombination. *srs2*Δ *mrc1*Δ synthetic lethality is dependent on the role of Mrc1 in DNA replication but independent of the role of Mrc1 in a DNA damage checkpoint response. *mrc1*Δ, *tof1*Δ and *csm3*Δ mutants have sister chromatid cohesion defects, implicating sister chromatid cohesion established at the replication fork as an important factor in promoting repair of stalled replication forks through gap repair.

The *SRS2* gene of *Saccharomyces cerevisiae* encodes a DNA helicase with a 3′→5′ polarity (36). Genetic studies have placed *SRS2* in the postreplication DNA repair pathway (1, 25, 37). *SRS2* is not an essential gene but is required for complete meiotic viability (1, 33). Mitotically, *srs2*Δ mutants have increased spontaneous gene conversion rates but are only modestly sensitive to DNA-damaging agents (1, 25, 37). These phenotypes suggest that Srs2 is involved in regulating a cellular response to spontaneous DNA damage. Indeed, recent in vitro studies have confirmed a proposed negative regulation of recombination by Srs2 (23, 53). Srs2 can destabilize Rad51 nucleoprotein filaments, thereby destroying homologous recombination strand exchange intermediates and allowing other repair pathways such as translesion synthesis or template switching to repair DNA gaps.

Although the Srs2 protein is not essential for wild-type yeast cells, in certain mutant strains the *SRS2* gene is very important or essential. The synthetic lethalities known for the *srs2*Δ mutant include *sgs1*Δ (9, 20), *rad54*Δ (1, 37), *rad50*Δ, *mre11*Δ, *xrs2*Δ (1, 20, 37), *top3*Δ (6, 20), *rad27*Δ (5), and *pol32*Δ (13). In addition, there are haploid *srs2*Δ synthetic sickness interactions which are enhanced to lethality in the diploid state. The most notable of these is the interaction with the *rdh54*Δ mutation (21, 38). Most of these lethalities are due to inappropriate or aberrant homologous recombination, as they can be suppressed by mutation of genes that encode products required for the early steps of homologous recombination. The exception to the list above is the *rad27*Δ *srs2*Δ synthetic lethality, but as the *rad27*Δ single mutant requires an intact homologous recombination pathway for survival, suppression cannot be tested (5).

SRS2 expression begins in late G_1 and peaks during S phase (12). Expression can be induced by DNA-damaging agents but only in the G_2 phase of the cell cycle (12). These observations, along with genetic studies of the *srs2*Δ mutant and the biochemical studies of the protein, have led to the proposal that the Srs2 protein functions during DNA replication to aid in the repair of single-strand gaps caused by replication fork stalling and to prevent the formation of toxic recombination intermediates that initiate at the sites of the single-strand gaps. Indeed, Srs2 protein is phosphorylated by DNA damage checkpoint kinases in response to intra-S DNA damage (27). Moreover, *srs2*Δ mutant strains do not activate the Rad53 checkpoint kinase in response to intra-S DNA damage (27).

The mechanisms of gap filling and replication restart are not entirely known. Gap filling is proposed to occur through the action of the error-free branch of the postreplication repair pathway (14, 41) and Srs2 is proposed to aid in promoting use of this pathway by antagonizing homologous recombination initiation. Gap filling may occur through translesion synthesis, template switching, or fork reversal or through homologous recombination with the sister chromatid. The last three mechanisms all propose intermediates, which involve pairing of the newly replicated DNA strands, with one newly replicated strand used as a template for replication of the blocked nascent strand. Since sister strands are involved, it might be expected that a structure may be assembled at the site of the damage or stalled replication fork to promote the sister chromatid interactions.

Sister chromatid cohesion is established during S phase as replication passes through a chromosomal region (18, 29, 40, 47, 49). Although factors such as hemicatenes of the nascent sister strands (24, 28, 42) and full intercatenation of sister chromatids may be involved in promoting sister chromatid

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cohesion, the actual cohesion is achieved through the binding of a protein cohesion complex containing Scc1, Scc3, Smc1, and Smc3 (10, 48). Timely deposition of sister cohesion complex is essential for proper chromatid cohesion and chromosome segregation in M phase (49). If cohesions are expressed in G_2, they may associate with the chromatids but do not provide the proper support for sister chromatid cohesion and accurate segregation at mitosis (49). In addition to ensuring correct chromatid segregation in mitosis, cohesions are necessary for postreplication repair and genomic stability (43).

To further understand the role of Srs2 in promoting postreplication repair and replication fork restart, we have undertaken a large-scale screen for novel *srs2*Δ synthetic lethal interactions (46). Among the synthetic lethal interactions that we identified were *mrc1*Δ, *tof1*Δ and *csm3*Δ plus mutations in the Ctf18 RFC-like complex encompassing the *ctf18*Δ, *ctf8*Δ, *ctf4*Δ, and *dcc1*Δ mutants. A recent paper with a microarray-based screen for *srs2*Δ synthetic lethal interactions also reported on the *mrc1*Δ, *tof1*Δ and *cms3*Δ group of mutants and the *ctf4*Δ mutant (31). The Ctf18 RFC-like complex loads PCNA onto DNA (3). The Ctf18 RFC-like complex is proposed to have a specialized role in DNA replication during some type of polymerase switch to activate cohesion complexes associated with unreplicated DNA to form sister chromatid cohesion interactions as the replication fork passes through the associated cohesion complex (29).

Mrc1 and Tof1 are checkpoint proteins involved in transmitting the DNA replication arrest signal to downstream effectors (2, 8, 44). Mrc1 activates the Rad53 kinase in response to replication stress and is itself phosphorylated by the Mec1 kinase. A second role for Mrc1 has recently been described. Mrc1 and Tof1 are associated with undamaged replication forks and move with the forks (17, 32). The association is independent of the checkpoint activity but is thought to be the first sensor for stalled replication forks to recruit Mec1 to sites of stalled replication. Tof1 and Csm3 interact in a two-hybrid assay and by coimmunoprecipitation (15, 30), and *csm3*Δ mutants have mitotic phenotypes similar to that of a *tof1*Δ mutant regarding activation of Rad53 kinase and cell cycle arrest following replication fork stalling (46). Thus, it is likely that Csm3 forms a complex with Tof1 at the replication fork and that Csm3-Tof1 collaborates with Mrc1 to mediate Rad53 signaling.

In this paper we show that the *srs2*Δ *mrc1*Δ/*tof1*Δ/*csm3*Δ lethality is due to aberrant homologous recombination and that this is also the case for the *srs2*Δ lethality with the Ctf18 complex components. Additionally, we show that *mrc1*Δ strains have a defect in sister chromatid cohesion and propose that attempted repair of replication fork damage through homologous recombination requires sister chromatid cohesion at the site of damage. These findings suggest a model in which Mrc1 with Csm3-Tof1 sets up the establishment of a cohesion complex at the site of damage on a stalled replication fork.

MATERIALS AND METHODS

Synthetic genetic array analysis. Synthetic genetic array analysis was carried out as described previously (45). Briefly, a *MAT*α synthetic gene array starting strain containing *srs2*Δ::*natR* (*MAT*α *srs2*Δ::*natR can*Δ*1*::*MFA1pr-HIS3 his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0*) was used to identify viable gene deletions that show synthetic genetic interactions with *srs2*Δ. Each synthetic gene array screen was conducted three times, and the resultant double mutants were scored for synthetic genetic interactions by both visual inspection and computer-based image analysis. The putative synthetic genetic interactions were confirmed by random spore analysis first, and the interactions that appeared to be inconclusive were tested by tetrad dissection as described previously (46).

Strains. The strains used for genetic crosses were in the W303 *RAD5* background and carried the *leu2-3,112 his3-11,15 trp1-1 ura3-1 ade2-1 can1-100* markers. The strains used for recombination assays have been described previously (22, 35). The *mrc1*Δ mutant was made by *KanMX4* disruption of the *MRC1* coding region with a PCR product derived with primers 5′-GCTCTGTCGCCG AAAATTCCTATAATTCCAACGGAACTTATTGGACGTACGCTGCAGG TCGAC-3′ and 5′-AAAGGATTTGATTATTGATGGATGTTTGAAAACGC CAACTAGTGAATCGATGAATTCGAGCTCG-3′. All strains are isogenic to W303 with the exception of some of the sister chromatid cohesion mutant strains. All strains were grown at 30°C. The *mrc1-AQ* strain was a kind gift from Steven Elledge.

Genetic methods. Methylmethane sulfonate (MMS) sensitivity was determined with freshly made YPD plates containing 0.016% MMS. Overnight cultures of strains to be tested were serially diluted, and 3-μl aliquots of each dilution were applied to YPD and YPD-MMS plates. Growth was assessed after 1, 2, and 3 days at 30°C. Fluctuation tests were done by the median method (26) and were repeated three to five times for each genotype. Recombination rates and chromosome loss rates were determined by fluctuation tests as previously described (22, 35). All media were prepared as described previously (22).

Assessing sister chromatid cohesion. *yko*Δ::*kanMX* alleles were amplified by PCR and introduced into strains YPH1477 (Tet operator array located 35 kb from *CEN5*) (*MAT***a** *ade2-1 trp1-1 can1-100 his3-11,15 leu2*::*LEU2tetR-GFP ura3*::*3xURA3tetO112 PDS1-13Myc-TRP1*), Y819 (Tet operator array located 12.7 kb from *CEN4*) (*MAT***a** *ade2-1 leu2-3,112 can1-100 ura3-1 trp1-1*::*lacO-TRP*::*lacO-LEU2 his3-11,15*::*GFP-lacI-HIS3*), and YPH1444 (Tet operator array located 1.8 kb from *CEN15*) (*MAT***a** *ade2 his3 trp1 ura3 leu2 can1 lacI-NLS-GFP*::*HIS3 lacO*::*URA3*::*CEN15*) by transformation (30). All mutants were verified by PCR assays.

YPH1477 Tet repressor-GFP/Tet operator repeat strains. Strains were grown logarithmically overnight in YPD, collected by centrifugation, and resuspended in pH 3.9 YPD medium with 2 μg of α-factor per ml for 2 h at 30°C to arrest cells in G_1. Cells were then collected by centrifugation and washed with normal-pH YPD medium once before being arrested in G_2/M with 15 μg of nocodazole per ml for 1 h at 30°C. Cell pellets were then collected by centrifugation and fixed in 4% paraformaldehyde for 5 min, washed once with SK buffer (1 M sorbitol, 0.05 M K_2PO_4), and resuspended in SK for cohesion assessment. Cells were briefly sonicated prior to microscopic examination for a cohesion defect. For the strains showing a cohesion defect, the frequency of cells containing two green fluorescent protein (GFP) dots was assayed in G_1-synchronized cells in order to rule out the possibility of aneuploidy.

Y819 and YPH1444 Lac repressor-GFP/Lac operator repeat strains. Strains were grown logarithmically overnight in YPD, collected by centrifugation, and resuspended in pH 3.9 YPD medium with 2 μg of α-factor per ml in G_1 for 1.5 h at 30°C. Cells were then collected by centrifugation and washed with synthetic medium lacking histidine and resuspended in pH 3.9 SC lacking histidine with 2 μg of α-factor per ml and 25 mM 3-aminotriazole for 30 min at 30°C to induce the Lac repressor-GFP fusion protein that is expressed from the *HIS3* promoter. Cells were then collected by centrifugation, washed, and resuspended in YPD with 15 μg of nocodazole per ml for 1 h at 30°C to arrest cells in G_2/M. Cells were then fixed and processed for the cohesion assay as described above.

Pds1 assay. Pds1 tagged with 13 Myc epitopes (Pds1-13Myc) was detected by indirect immunofluorescence in nocodazole-arrested and paraformaldehyde-fixed cells with anti-Myc antibodies (Roche) as described previously (30). The presence or absence of the Pds1-13Myc signal was determined in cells containing two separated GFP dots to ensure that cells had not initiated anaphase. Between 80 and 90% of cells with two GFP dots had high levels of Pds1 by this assay. At least 20 two-dot cells were examined for each strain with the chromosome V cohesion reporter (YPH1477 derivatives).

RESULTS

Synthetic lethality interactions with *srs2*Δ. To identify additional genes requiring *SRS2* for viability, we performed a synthetic genetic array analysis on an *srs2* mutant (45, 46). In total, 26 genes were confirmed as positive by random spore analysis and by tetrad analysis (Fig. 1). Most genes in this list

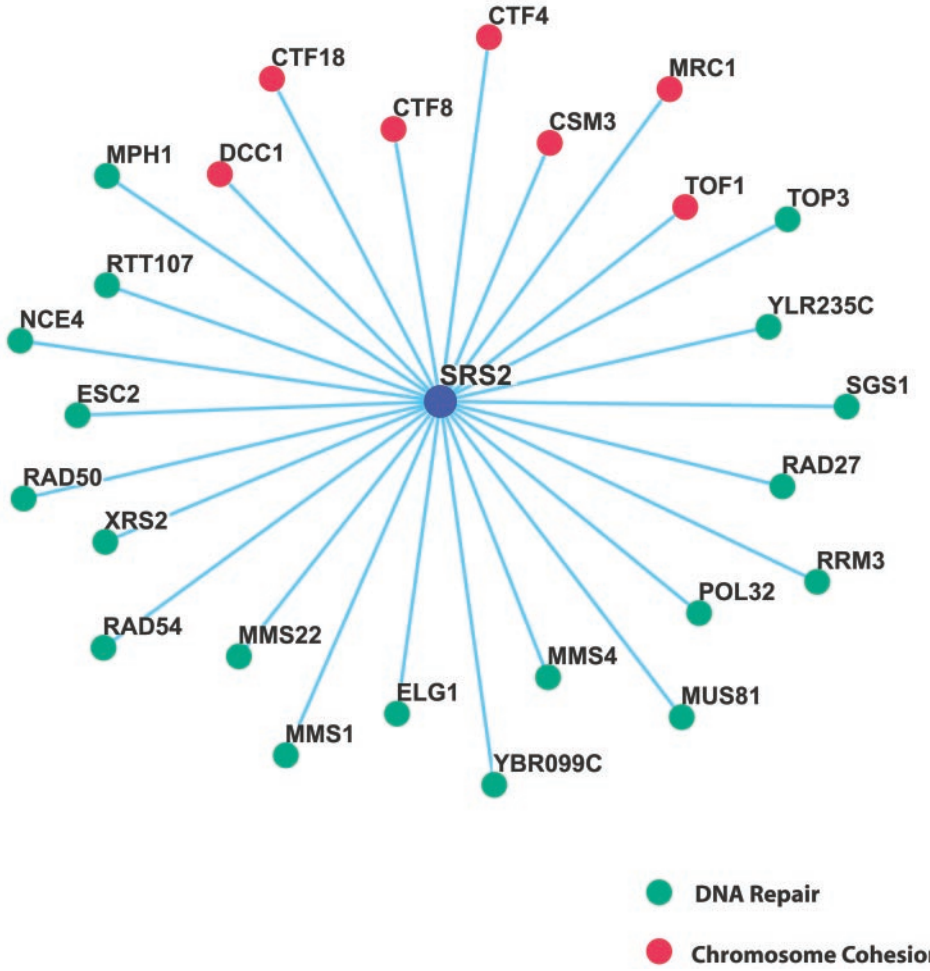

FIG. 1. *SRS2* genetic interactions. Genome-wide synthetic genetic screens with *srs2*Δ identify genes involved in DNA repair and sister chromatid cohesion. The results of synthetic genetic array analysis with *srs2*Δ are presented as a genetic interaction map. Lines connecting genes represent synthetic lethality or synthetic slow growth. Colored circles designate the cellular role of the interacting genes.

are involved in different DNA repair pathways in processing replication fork stalling, consistent with *SRS2*'s cellular role in regulating the cellular response to spontaneous DNA damage (4). Strikingly, many genes involved in chromosome cohesions were identified: *MRC1*, *TOF1*, *CSM3*, *DCC1*, *CTF8*, *CTF18*, and *CTF4*. Dcc1, Ctf8, and Ctf18 form an alternative RFC complex and function in the establishment of chromosome cohesion (3). Mrc1 andTof1 are associated with complexes at the replication fork, which may participate in both replication and the S-phase checkpoint (17, 32). The cohesion roles of *MRC1* and *TOF1* have recently been identified (30, 54). *CSM3* is a newly discovered gene required for efficient cohesion. Csm3 interacts physically with Tof1 (30) and also participates in the S-phase checkpoint (46).

Since Mrc1, Tof1, and Csm3 have multiple roles, it is important to identify why they are essential when Srs2 is missing. Ctf4 is another cohesion establishment factor, being essential in cohesion and having some role in DNA replication (11). These synthetic interactions suggest that spontaneous repair events occurring in the absence of the Srs2 protein require sister chromatid cohesion. Since homologous recombination is increased in the absence of Srs due to the loss of the Srs2 Rad51 filament-destabilizing activity, one possibility is that appropriate homologous recombination requires sister chromatid cohesion, even in haploid cells. The cohesion roles of Mrc1, Tof1, and Csm3 could be linked to S-phase checkpoint function in that they may aid in establishment of cohesion at sites of replication stalling.

***mrc1*Δ/*tof1*Δ/*csm3*Δ lethality with *srs2*Δ is due to inappropriate homologous recombination.** Since most of the *srs2*Δ synthetic lethal interactions are due to excess or inappropriate homologous recombination, we tested whether the *srs2*Δ synthetic lethality with *mrc1*Δ, *tof1*Δ, or *csm3*Δ arose from a similar homologous recombination problem by asking if loss of homologous recombination could suppress the synthetic lethality. As shown in Fig. 2A, *rad51*Δ suppresses *srs2*Δ in all three mutant situations. Similar results were obtained with a *rad52*Δ mutation (data not shown). It can also be seen that the *srs2*Δ *tof1*Δ and *srs2*Δ *csm3*Δ double mutants are able to form a microcolony, while the *srs2*Δ *mrc1*Δ double mutant rarely grew for more than eight generations. To test whether the *srs2*Δ *mrc1*Δ synthetic lethality was due to loss of the Srs2 DNA

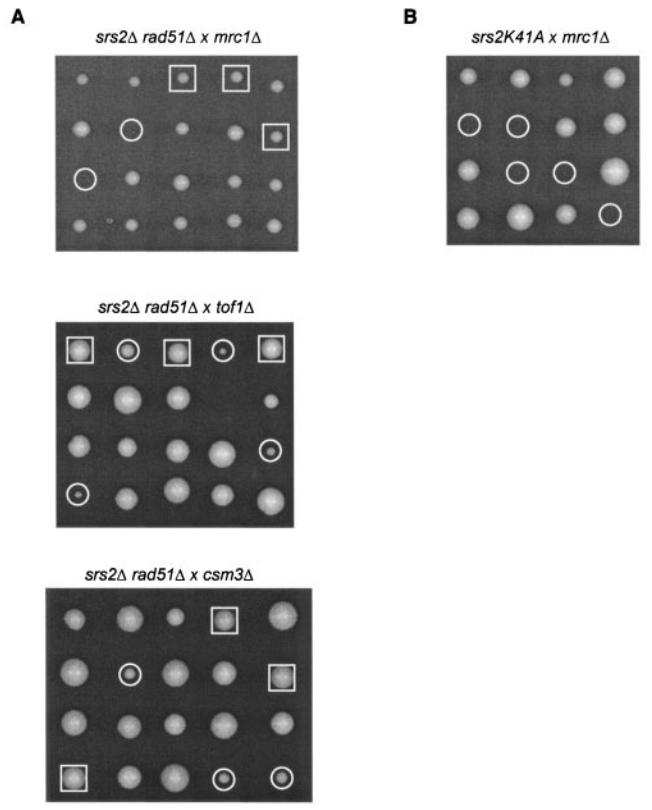

FIG. 2. *srs2*Δ synthetic interaction with *mrc1*Δ, *tof1*Δ, and *csm3*Δ. (A) The *srs2*Δ synthetic interactions are suppressed by loss of homologous recombination with a *rad51*Δ mutation. Five tetrads from each cross are shown. The circles mark the double mutants *srs2*Δ *mrc1*Δ, *srs2*Δ *tof1*Δ, and *srs2*Δ *csm3*Δ. The squares mark the triple mutants *srs2*Δ *mrc1*Δ *rad51*Δ, *srs2*Δ *tof1*Δ *rad51*Δ, and *srs2*Δ *csm3*Δ *rad51*Δ. In each cross, the triple mutant grew better than the double mutant. The strains used were HKY1307-12C (*srs2*Δ *rad51*Δ), HKY1253-15C (*mrc1*Δ), HKY1375-1D (*tof1*Δ), and HKY1374-2A (*csm3*Δ). (B) The circles mark the double mutant *srs2K41A mrc1*Δ. The *srs2K41A* mutant is mutated in the Walker A box, is defective in ATP binding, and has no DNA helicase activity or Rad51 filament removal activity (52). The strains used were HKY1435 (*srs2K41A*) and HKY1336-9B (*mrc1*Δ).

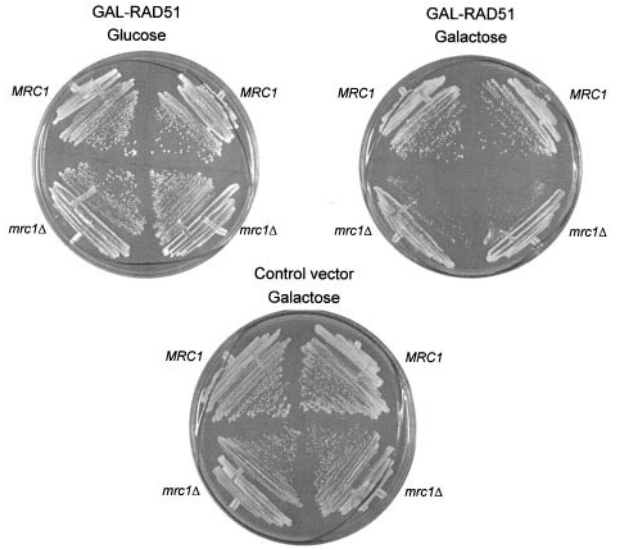

FIG. 3. High-copy-number expression of Rad51 reduces the viability of an *mrc1*Δ strain. Transformants carrying a *GAL1-RAD51* plasmid were streaked on SC-glucose or SC-galactose plates lacking leucine. The control galactose plate shows transformants with an empty *GAL1* vector to verify that the *mrc1*Δ mutant was defective in growth on galactose-containing medium. The strains used were HKY1093-5A (*MRC1*) and HKY1235-15C (*mrc1*Δ).

helicase activity, we examined an *srs2* allele that is mutated at the Walker A box and is specifically defective in the helicase activity (22a, 23). The *srs2K41A mrc1*Δ mutations are lethal (Fig. 2B), showing that a strain deficient in Mrc1 requires the Srs2 DNA helicase activity for viability. Thus, some Srs2 function requiring ATP hydrolysis becomes essential when Mrc1, Tof1, or Csm3 is missing. We propose that this function is the Srs2 antirecombinase activity of Rad51 filament destabilization.

Recombination and genomic instability in the *mrc1*Δ mutant. Srs2 DNA helicase has been shown to reverse Rad51 filaments (23, 53). The helicase is thought to regulate the repair of spontaneous damage by antagonizing Rad51 filaments and preventing homologous recombination events. The observation that the *srs2*Δ *mrc1*Δ lethality is due to homologous recombination suggests that excess homologous recombination, even in a strain containing functional Srs2, would be deleterious. To test this, we induced overexpression of Rad51 from a *GAL1* promoter, reasoning that if there is excess Rad51 protein, the endogenous Srs2 protein would not be able to reverse all of the Rad51 filaments. Indeed, we observed that the *mrc1*Δ *SRS2* strain showed reduced growth when *RAD51* expression was increased (Fig. 3). This finding supports the hypothesis that recombination in an *mrc1*Δ strain can be detrimental to cell growth. We suspect that Mrc1 has no role in the recombination process per se, as *mrc1*Δ mutants show no increase in MMS sensitivity (data not shown), a phenotype associated with all known recombination-defective mutants.

The *mrc1*Δ mutant grows slightly more slowly than an *MRC1* strain (2). This could be the result of elevated recombination levels, which we have shown above to be deleterious. Therefore, we determined the recombination rate in a *mrc1*Δ haploid strain with a recombination reporter for intrachromosomal gene conversion and for deletions between direct repeats, which result primarily from single-strand annealing. *mrc1*Δ has wild-type levels of gene conversion, but deletion events are increased sixfold (Fig. 4). These may reflect an increased occurrence of spontaneous double-strand breaks. We also determined genomic instability rates in a *mrc1*Δ diploid strain, measuring spontaneous chromosome loss and recombination (22). We found little increase in the *mrc1*Δ mutant, with chromosome loss being marginally increased 2.8-fold over that of the wild type (from 2.0×10^{-6} to 5.6×10^{-6}) and recombination by mitotic crossing over and break-induced replication increased 2.4-fold over that of the wild type (from 1.4×10^{-5} to 3.4×10^{-5}). Since chromosome loss was not increased in the *mrc1*Δ mutant, the loss of Mrc1 at the replication fork does not lead to chromosome missegregation. Nonetheless, the increase in single-strand annealing suggests that there is a significant increase in spontaneous double-strand breaks in the *mrc1*Δ mutant. Since Mrc1 has been reported to act at replication

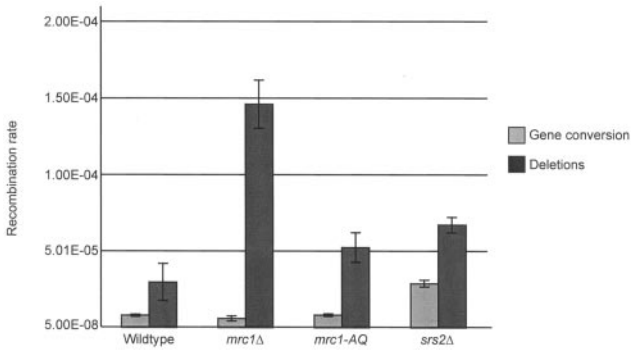

FIG. 4. Intrachromasomal recombination assay. Each strain carried the recombination reporter *leu2-ri*::*URA3*::*leu2-bsteii*, which has a heteroallelic duplication of *LEU2*, with *URA3* between the *LEU2* genes. Gene conversion was determined by fluctuation tests, measuring Leu^+ Ura^+ rates. The deletion rate was determined by fluctuation tests, measuring fluoroorotic acid resistance rates. Each test was performed with nine colonies and done three times for each genotype, with three spore segregants for each genotype. The strains used were HKY1538-2C, HKY1538-6A, and HKY1538-10A for the wild type, HKY1505-1C, HKY1505-6B, and HKY1505-7B for *mrc1*Δ, HKY1538-1A, HKY1538-5C, and HKY1538-12A for *mrc1-AQ*, and HKY1323-1B and HKY1323-12D for *srs2*Δ.

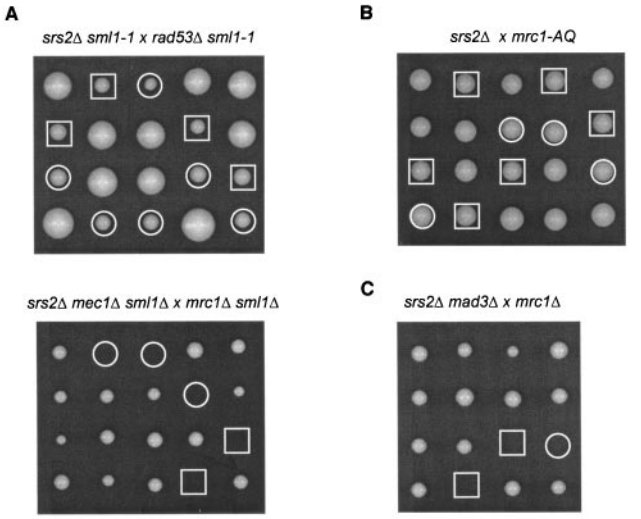

FIG. 5. *srs2*Δ interactions with replication checkpoint defective mutants. (A) Five tetrads are shown from each cross. The circles in the top panel, *srs2*Δ *sml1-1* (HKY1369-2D) × *rad53*Δ *sml1-1* (HKY987-2B), mark *rad53*Δ *sml1-1* spore segregants. The squares in the top panel mark *srs2*Δ *rad53*Δ *sml1-1* spore segregants. The circles in the lower panel, *srs2*Δ *mec1*Δ *sml1*Δ (HKY1494-7A) × *mrc1*Δ *sml1*Δ (HKY1239-26A), mark *srs2*Δ *mrc1*Δ *sml1*Δ spore segregants. The squares in the lower panel mark *srs2*Δ *mrc1*Δ *mec1*Δ *sml1*Δ spore segregants. (B) Five tetrads are shown from the cross *srs2*Δ (HKY1303-1B) × *mrc1-AQ* (HKY1534-3C). The circles mark *mrc1-AQ* segregants. The squares mark *srs2*Δ *mrc1-AQ* spore segregants. (C) Four tetrads are shown from the cross *srs2*Δ *mad3*Δ (HKY1371-7D) × *mrc1*Δ (HKY1235-21D). The circle marks an *srs2*Δ *mrc1*Δ spore segregant. The squares mark *srs2*Δ *mrc1*Δ *mad3*Δ spore segregants.

forks (17, 32), the implication is that replication forks are more prone to breakage in the absence of Mrc1.

***mrc1*Δ *srs2*Δ synthetic lethality is not due to a replication checkpoint defect.** *MRC1* has been proposed to function during DNA replication arrest to transmit the replication arrest signal to downstream effectors (17, 32). *mrc1*Δ mutants have a replication checkpoint defect in that Rad53 kinase is not fully activated in an *mrc1*Δ mutant when replication is inhibited by hydroxyurea treatment. To test if the replication checkpoint defect associated with *mrc1*Δ contributes to *mrc1*Δ *srs2*Δ lethality, we analyzed three different checkpoint-defective strains. First, we examined the phenotype of the *srs2*Δ *rad53*Δ *sml1*Δ mutant, reasoning that Rad53 is an essential factor in the replication stress checkpoint response and acts downstream of Mrc1 (2). We found that the *srs2*Δ *rad53*Δ *sml1*Δ mutant had no growth defect (Fig. 5A). Second, we tested whether the Mec1 kinase was required for *srs2*Δ viability by asking if an *srs2*Δ *mec1*Δ *sml1*Δ strain was viable. We found that this strain had no growth defect (data not shown), further reinforcing the idea that loss of Srs2 and the associated increase in recombination are not in themselves lethal and do not elicit a DNA damage checkpoint response.

Third, we examined an allele of *MRC1*, *mrc1-AQ*, in which all the TQ and SQ motifs that are phosphorylation targets of the Mec1 kinase have been mutated to AQ residues. The Mrc1-AQ protein still associates with the replication fork but is deficient in the replication checkpoint response (32). The *srs2*Δ *mrc1-AQ* double mutant had normal viability (Fig. 5B), showing that the essential function of Mrc1 in an *srs2*Δ strain is not the replication checkpoint response. Thus, Mrc1 must have another function that is essential in an *srs2*Δ mutant. Even though Mrc1 and Srs2 have been implicated in the intra-S checkpoint response for induced damage (2, 27, 44), these do not appear to be required under conditions of spontaneous damage. We suggest that it is the processing of damage intermediates through homologous recombination that requires the Mrc1 protein.

We then tested whether elimination of the Mec1 DNA damage checkpoint factor would rescue the *srs2*Δ *mrc1*Δ lethality. We observed that the *srs2*Δ *mrc1*Δ *mec1*Δ *sml1*Δ mutations were lethal (Fig. 5A), showing that the *srs2*Δ *mrc1*Δ double mutant growth defect was not due to a checkpoint arrest. Microscopic inspection of the apparently nongrowing colonies showed that the *srs2*Δ *mrc1*Δ *mec1*Δ *sml1*Δ segregants have approximately two- to threefold more cells in the microscopic colonies than the *srs2*Δ *mrc1*Δ *sml1*Δ segregants.

***mrc1*Δ *srs2*Δ synthetic lethality is not due to a spindle checkpoint defect.** We were concerned that the *srs2*Δ *mrc1*Δ lethality might actually result from a mitosis spindle checkpoint arrest. Therefore, we mutated the spindle checkpoint by introducing an *mad3*Δ mutation into the *srs2*Δ *mrc1*Δ mutant and asked if this would relieve the lethal phenotype. As shown in Fig. 5C, loss of the spindle checkpoint does not suppress the *srs2*Δ *mrc1*Δ lethality, suggesting that this lethality arises from a different defect.

***mrc1*Δ has a sister chromatid cohesion defect independent of checkpoint function.** *mrc1*Δ has been identified as a synthetic lethal partner in screens for synthetic lethal interactions with sister chromatid cohesion mutants (46, 54). Therefore, we examined the *mrc1*Δ and *mrc1-AQ* mutants for sister chromatid cohesion. We observed that the *mrc1*Δ mutant has a sister chromatid cohesion defect, while the *mrc1-AQ* has no cohesion

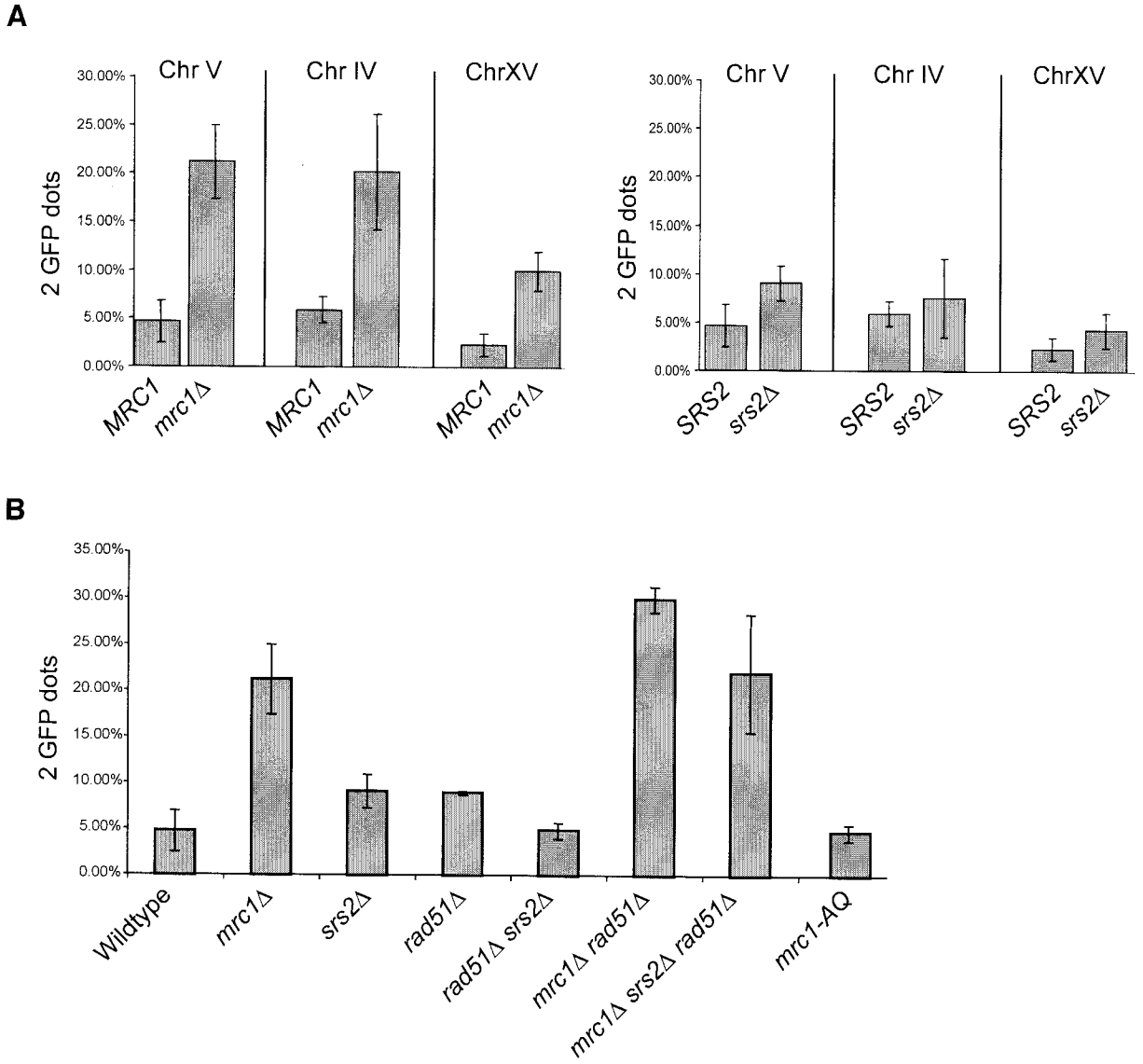

FIG. 6. Sister chromatid cohesion assays in *mrc1* mutants. (A) Sister chromatid cohesion assays were performed for three separate chromosomal locations as marked in wild-type, *mrc1*Δ, and *srs2*Δ strains. The mean percentage of two GFP dots from 200 cells is shown. Each experiment was repeated three to four times with different transformants for each genotype. The strains used were YPH1477 (wild type), Y4986 and Y4987 (*mrc1*Δ) and Y5168 (*srs2*Δ) for chromosome V, Y819 (wt), Y4989 and Y4990 (*mrc1*Δ), and Y5165 (*srs2*Δ) for chromosome IV, and YPH1444 (wild type), Y4992 and Y4993 (*mrc1*Δ), and Y5247 (*sr2*Δ) for chromosome XV. (B) Sister chromatid cohesion on chromosome V was performed on the indicated strains, counting 200 cells each time in two independent experiments and for each genotype. The strains used were YPH1477 (wild type), Y4986 and Y4987 (*mrc1*Δ), Y5168 (*srs2*Δ), Y6234 (*rad51*Δ), Y6344 (*rad51*Δ *srs2*Δ), Y6187 (*mrc1*Δ *rad51*Δ), Y6219 (*mrc1*Δ *srs2*Δ *rad51*Δ), and Y6346 and Y6355 (*mrc1-AQ*).

defect (Fig. 6). Moreover, the *srs2*Δ mutant does not have a sister chromatid cohesion defect in our assays, nor does the *rad51*Δ mutant. Another group recently found that *srs2*Δ has a slight sister chromatid cohesion defect (54), which suggests that loss of Srs2 may make cells more susceptible to improper cohesion complex assembly but that this effect may depend on other genetic background effects.

Suppression of the *mrc1*Δ *srs2*Δ lethality does not suppress the sister chromatid cohesion defect. Since a *rad51*Δ mutation suppresses the *srs2*Δ *mrc1*Δ defect, it was important to determine if the sister chromatid cohesion defect is also suppressed in this triple mutant. We found no effect of the *rad51*Δ mutation on sister chromatid cohesion in any background, including the *srs2*Δ *mrc1*Δ double mutant. Thus, suppression of the lethal phenotype is due to elimination of homologous recombination in a cohesion-defective situation, and cells are viable when sister chromatid cohesion is reduced as long as homologous recombination is controlled.

***srs2*Δ synthetic lethality with Ctf18-RFC complex genes is due to inappropriate recombination.** Given the *srs2*Δ *mrc1*Δ synthetic lethality and the sister chromatid cohesion defect in the *mrc1*Δ mutant, we further examined *srs2*Δ synthetic lethality with a collection of viable cohesion-defective mutants. We tested each gene by crossing the mutant to an *srs2*Δ *rad51*Δ

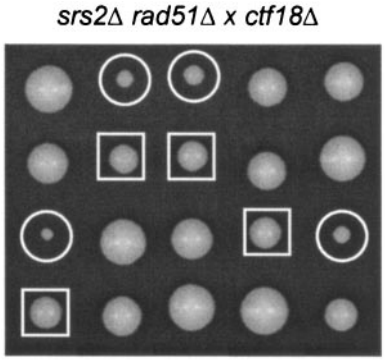

FIG. 7. *srs2*Δ synthetic sickness with *ctf18*Δ is relieved by loss of *RAD51* function. Five tetrads are shown from the cross *srs2*Δ *rad51*Δ (HKY1403-1B) × *rad18*Δ. The circles mark *srs2*Δ *ctf18*Δ spore segregants. The squares mark *srs2*Δ *ctf18*Δ *rad51*Δ spore segregants.

strain so that we could determine if any observed synthetic lethality was suppressible by loss of homologous recombination. We confirmed that *srs2*Δ was synthetically lethal or sick with *ctf4*Δ, *ctf8*Δ, *ctf18*Δ, and *dcc1*Δ and found that the *ctf4*Δ, *ctf8*Δ, *ctf18*Δ, and *dcc1*Δ synthetic interactions are suppressed by a *rad51*Δ mutation. An example of this suppression is shown for *ctf18*Δ in Fig. 7. Since the Ctf18-RFC complex has been linked to the establishment of sister chromatid cohesion during replication (30), the synthetic lethality reinforces the proposal that an important aspect of regulating homologous recombination in mitosis is to have the recombination occur between or within cohesed sister chromatids. Cohesion may prevent nonsister chromatids from engaging in promiscuous homologous recombination and thus inhibit deleterious recombination in the *srs2*Δ mutant.

DISCUSSION

We found that the *srs2*Δ mutant has several novel synthetic lethal interactions, which include the *mrc1*Δ, *tof1*Δ, and *csm3*Δ mutations; these genes encode proteins that are proposed to form a complex that acts during DNA replication at the replication fork. Mrc1 and Tof1 have been shown directly to be associated with replication forks (17, 32), and Csm3 has been shown to interact with Tof1 by yeast two-hybrid analysis and by coimmunoprecipitation (15, 46). Mrc1, Tof1, and Csm3 have been shown to be required for DNA damage checkpoint activation in response to replication blocks (2, 8, 44, 46). Although Srs2 is required for full Rad53 activation and regulation of S-phase progression in response to induced intra-S damage, Srs2 is not essential for a normal S phase, and there is no spontaneous activation of Rad53 (27). Thus, it is unlikely that the *srs2*Δ *mrc1*Δ synthetic lethality is due to a failure to activate Rad53 under normal growth conditions.

Moreover, an *srs2*Δ mutant does not require Rad53 for survival in undamaged conditions and the *mrc1*Δ lethality with *srs2*Δ can be separated from the function of Mrc1 in the checkpoint DNA damage signaling. The role of Srs2 during S phase appears to be the promotion of gap repair by the *RAD18* postreplication repair pathway, through mechanisms that do not involve homologous recombination and double-strand break formation. In the absence of Srs2, more gaps are repaired by the homologous recombination pathway. We do not know if the homologous recombination events are promoted by double-strand breaks or initiated by single-strand gaps. It is also possible that single-strand gaps are processed to double-strand breaks once a commitment to homologous recombination is made by having a Rad51 filament form on the single-strand gap. Whatever the case, we believe that double-strand breaks are not formed in the *srs2*Δ *mrc1*Δ *rad51*Δ mutant, as this mutant does not show any cell cycle arrest or reduced growth compared to *mrc1*Δ, *srs2*Δ, or *rad51*Δ single mutants.

We propose that the spontaneous lesions in the *srs2*Δ *mrc1*Δ mutant that stall replication forks are channeled into a homologous recombination repair pathway instead of a gap-filling pathway due to loss of the Srs2 antirecombinase action against Rad51. Normally, this would be tolerated, but when Mrc1 is also absent, this becomes a lethal situation. The lethality arises from attempting homologous recombination without the correct molecular scaffold set up at the point of replication stalling. We suggest that this scaffold is sister chromatid cohesion that is established specifically at a point of fork stalling. Sister chromatid cohesion may stabilize the fork and may promote sister chromatid recombination, which would be necessary to reestablish a replication fork if it becomes collapsed by a double-strand break. The increased spontaneous deletion rate that we observed in the *mrc1*Δ mutant but not the *mrc1-AQ* mutant (Fig. 4) indicates that more double-strand breaks form in the absence of the Mrc1 protein. Although this level of double-strand breaks can be tolerated when Srs2 is present (although it probably accounts for the slower growth of the *mrc1*Δ deletion mutant), in its absence this becomes lethal.

We suggest that the role of Mrc1 and its functionally associated partners Tof1 and Csm3 is to recruit the Ctf18-RFC-like complex to the site of fork stalling. The Ctf18-RFC-like complex would then recruit or activate a cohesion complex as replication occurs (29), even if the newly replicated DNA is gapped. This model would account for the *srs2*Δ synthetic lethality with *ctf18*Δ and components of the Ctf18-RFC-like complex as well as synthetic sickness with structural maintenance of chromosome (SMC) mutants defective in condensin and cohesin subunits (31, 46). In this model, the double-strand breaks would not inhibit S-phase progression, and indeed double-strand breaks are not inhibitory to S-phase progression (A. Pellicioli and M. Foiani, personal communication). Rather, when they are engaged by the homologous recombination pathway in G_2, sister chromatid cohesion is required for their repair (39, 49).

Inactivating homologous recombination rescues the *srs2*Δ *mrc1*Δ synthetic lethality because the homologous recombination pathway is no longer active and cells can now use the gap-filling pathways to repair the single-strand gaps. Thus, the postreplication repair pathway becomes a mechanism to avoid inappropriate homologous recombination events that may result in genome instability.

Srs2 and Mrc1 are involved in the S-phase checkpoint pathway (2, 27, 44). The sister chromatid cohesion factor SMC1 has also been linked to a branch of the S-phase checkpoint pathway involving ATM and NBS1 in mammalian cells (19, 55). Indeed, the yeast *smc1-259* mutation has been shown to affect DNA damage response, causing sensitivity to ionizing radiation, UV, and MMS treatment (19). However, the mammalian

ATM serine target sites in SMC1 are not present in the yeast Smc1 protein, so it is not known if Smc1 of *S. cerevisiae* is involved in the S-phase checkpoint pathway.

It is possible that the *srs2*Δ *mrc1*Δ synthetic lethality arises from loss of two checkpoint signaling effectors that converge on Smc1. We do not believe that this is the explanation for the synthetic lethality. First, *mrc1*Δ shows a synthetic sickness, not lethality, with *rad50*Δ and *xrs2*Δ but not with *mre11*Δ (46). Rad50, Xrs2, and Mre11 form a complex in *S. cerevisiae* (16, 51), and Mre11 is the key player in checkpoint signaling (7, 34, 50). Second, in mammalian cells, the SMC1-dependent S-phase checkpoint activation does not affect the ability of SMC1 to bind to chromatin (55), and both phosphorylated and unphosphorylated SMC1 appear to be competent for sister chromatid cohesion (55). Third, loss of the Mec1 checkpoint signal does not rescue the *srs2*Δ *mrc1*Δ growth defect. In this situation, the cells can grow for one to two additional generations but still cannot sustain further growth. Fourth, specific loss of the Mrc1 checkpoint signaling function does not lead to lethality in an *srs2*Δ mutant.

Thus, we propose that sister chromatid cohesion is set up at a site of DNA damage when the cell engages in mitotic homologous recombination in the context of replication stalling. We do not know whether Mrc1 interacts with homologous recombination components or the Ctf18-RFC-like complex, nor do we know when homologous recombination occurs, in S phase or G_2, when gap repair is inhibited. Nonetheless, our results highlight the importance of the postreplication repair pathway in maintaining cell survival and repair of spontaneous lesions by the correct pathway to avoid genome instability and lethal homologous recombination events.

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