The severe slow growth of Δ*srs2* Δ*rqh1* in *Schizosaccharomyces pombe* is suppressed by loss of recombination and checkpoint genes

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ABSTRACT

Our interest in the *Schizosaccharomyces pombe* RecQ helicase, *rqh1*+, led us to investigate the function of a related putative DNA helicase, *srs2*+. We identified the *srs2*+ homolog in *S.pombe*, and found that *srs2*+ is not essential for cell viability. A Δ*srs2* Δ*rqh1* double mutant grows extremely slowly with aberrant shaped cells and low viability. This slow growth does not appear to be related to stalled replication, as Δ*srs2* Δ*rqh1* cells showed higher survival rates, compared with Δ*rqh1*, when stalled forks were increased by UV irradiation or hydroxy urea treatment. Consistent with this result, we found that Δ*srs2* Δ*rqh1* cells progress through S-phase with a slight delay, but undergo a checkpoint-dependent arrest presumably at G$_2$/M. Further, we found that Δ*srs2* Δ*rqh1* slow growth is related to recombination, as loss of either the *rhp51*+ or *rhp57*+ recombination genes improves cell growth in the double mutant. Δ*srs2* is also synthetic lethal with Δ*rhp54*, another homologous recombination gene. This lethality is suppressed in a Δ*rhp51* background. Together, these results demonstrate a clear genetic interaction between *rqh1*+, *srs2*+ and the genes of the homologous recombination pathway.

INTRODUCTION

The faithful replication of DNA is essential for maintaining the integrity of the cell's genetic information. Many challenges exist for the replication machinery to perform its task. One problem arises when the growing replication fork is halted. Replication fork arrest occurs when the replication complex encounters a site of DNA damage, some secondary DNA structures or nucleotide depletion, as in the case of treatment with hydroxy urea (HU) (1,2). Stalled replication forks have been shown to create sites for recombination, as demonstrated in both bacterial and eukaryotic systems (2–5).

The loss of the *recQ* homolog in various systems leads to hyper-recombination when the cells undergo replication arrest induced by HU or ultraviolet (UV) DNA damage (6–13). These data suggest that the RecQ helicases act to suppress recombination at arrested replication forks, although the RecQ helicases have also been shown to promote certain types of recombination (10,14–16). Different models have been proposed to explain how RecQ helicases might suppress recombination. In *Escherichia coli*, arrested replication forks are believed to be stabilized by RecQ through the formation of a triple helix (10,17). Studies on the human and *Schizosaccharomyces pombe* RecQ helicases (Blm and Rqh1, respectively) suggest that they act to remove Holliday junctions formed at stalled replication forks, by reverse branch migration (18,19). In one of these studies, expression of the bacterial Holliday junction resolvase was shown to significantly suppress UV and HU sensitivity in *S.pombe* Δ*rqh1* cells (18). These results suggest that cells lacking Rqh1 accumulate Holliday junctions at stalled replication forks.

Recent findings in *Saccharomyces cerevisiae* demonstrating a synthetic interaction between *srs2* and the *recQ* homolog *sgs1* suggest the possibility that Srs2 might also function at stalled replication forks (14,20–22). The *S.cerevisiae* Srs2 DNA helicase, which is structurally related to the bacterial helicase II (*UvrD*) (23), was first discovered in a screen for suppressors of trimethoprim sensitivity of *rad6* and *rad18* mutants (24,25). It was independently characterized as a suppressor of homologous recombination (named *HPR5*), as mutants showed a hyper-recombination phenotype (24,26,27). *SRS2* has since been shown to be associated with the *RAD6* post replication repair (PRR) pathway. Specifically, Srs2 functions with the *RAD5*-dependent error-free branch of PRR, where it has been shown to suppress the UV sensitivities of *rad5*, *ubc13* and *mms2* mutants (28). This suppression is lost in cells lacking a functional recombination system, consistent with a role of Srs2 blocking damage from being chanelled into the *RAD52*-dependent homologous recombination pathway (28,29). The synthetic interaction between *srs2* and *sgs1* was shown to be suppressed by mutations in the homologous recombination genes *rad51*, *rad55* and *rad57* (14). It has also been demonstrated that *S.cerevisiae srs2* shows either a lethal

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Table 1. Strains used in this study

Strain	Genotype	Reference
SZ132	*h⁺ ade6-210 leul-32 ura 4-D18*	Laboratory stock
SZ242	*h⁺ srs2::kanᴿ ade6-210 leul-32 ura 4-D18*	This study
SZ243	*h⁺ rqh1:: kanᴿ ade6-210 ura-D18*	This study
SZ215	*h⁺ rhp51:: ura4⁺ ade6-704 leul ura4-D18*	(48)
SZ231	*h⁺ rhp54::ura4⁺ ade6-704 leul-32 ura4-D18*	(49)
SZ258	*h⁺ rhp57::ura4⁺ ade6-216 leul-32 his7-366 ura 4-D18*	(50)
SZ76	*h⁻ rad9:: ura4⁺ ade6-704 leul-32 ura4-D18*	(51)
SZ247	*h⁻ srs2::kanᴿ rqh1:: kanᴿ*	This study
SZ271	*h⁺ srs2::kanᴿ rqh1:: kanᴿ rhp51:: ura4⁺ ade6-210 leul-32 ura 4-D18*	This study
SZ277	*h⁺ srs2::kanᴿ rqh1:: kanᴿ rhp57::ura4⁺ leul-32 ura4-D18*	This study
SZ279	*h⁺ srs2::kanᴿ rhp54:: ura4⁺ rhp51:: ura4⁺ ade6-210 leul-32 ura4-D18*	This study
SZ280	*h⁺ srs2::kanᴿ rqh1:: kanᴿ rad9:: ura4⁺ leul-32 ura4-D18*	This study
SZ256	*h⁺ rqh1:: kanᴿ rhp54:: ura4⁺ ade6-210 leul-32 ura 4-D18*	This study
SZ281	*h⁺ rhp54:: ura4⁺ rhp51:: ura4⁺ ade6-704 leul-32 ura4-D18*	This study
SZ613	*h⁺ cds1::ura4⁺ srs2::kanᴿ rqh1::kanᴿ leul-32 ura4-D18*	This study
SZ614	*h⁺ chk1::ura4⁺ srs2::kanᴿ rqh1::kanᴿ leul-32 ura4-D18*	This study

phenotype or poor growth when combined with a number of other mutants (*rdh54*, *rad50*, *mre11*, *xrs2*, *top3* and *rad54*) (21,30,31). The synthetic interactions of these double mutants are furthermore suppressed by mutating the recombination genes *rad51*, *rad52*, *rad55* and *rad57* (14,30–32). In addition, it was shown that *srs2 rad54* and *srs2 rdh54* lethality can be suppressed by loss of checkpoint function (31). These data are complemented by evidence of genetic interaction between *SRS2* and *RAD52*, based on suppression of *rad52* mutations by deletion of *SRS2* (33,34). *srs2* mutants have also been shown to suppress hyporecombination of some *rad51* and *rad52* alleles (24,32). Together, these results demonstrate that Srs2 function in *S.cerevisiae* is linked to homologous recombination.

Both Srs2 and the RecQ helicases are associated with DNA damage response and/or genomic instability. In *S.cerevisiae*, Sgs1 and Srs2 have been shown to participate in activation of the intra-S checkpoint following exposure to MMS (35,36). In *S.pombe*, *rqh1* mutants show sensitivity to UV and gamma radiation and treatment with HU (7,8,37). *rqh1⁺* was originally identified as *rad12⁺* in a screen for UV sensitive mutants (38). One phenotype associated with *rqh1* mutants is that although cells undergo a normal G₁–S arrest following DNA damage, they recover poorly. Fluorescence microscopy has revealed that the problem is in mitosis as cells divide with torn chromosomes and uneven distribution of nuclear material (7,8). It has been proposed in *S.cerevisiae* that Srs2 acts in recombination repair of double strand breaks to stabilize the invading single strand with its homolog, thereby acting against reverse branch migration (39). Srs2 is phosphorylated in response to intra-S DNA damage, and this phosphorylation is dependent on both checkpoint activation and Cdk1 (cyclin-dependent kinase) (36). Following DNA damage in S-phase, *srs2* mutants do not fully arrest and Rad53 phosphorylation is reduced, suggesting Srs2 functions in an intra-S checkpoint. In addition, *srs2* mutants suppress the lethality of a *rad17* checkpoint mutant following intra-S DNA damage (36).

Our interest in *rqh1⁺* has led us to study other genes related to its activity such as *top3⁺* (40). In this context we have sought to identify other potentially interacting proteins. Therefore, we identified a homolog of *SRS2* by searching

the *S.pombe* genomic database and created a null mutant, Δ*srs2*. As reported previously (41), we found that Srs2 is not essential in *S.pombe* and that the single mutant does not exhibit significant sensitivity to either UV damage or treatment with HU. Furthermore, the Δ*srs2* Δ*rqh1* double mutant shows a severe slow growth defect compared with the single mutants. Our studies found that the Δ*srs2* Δ*rqh1* double mutant progresses through S-phase with a slight delay but arrests in G₂/M with many of these cells ultimately undergoing an aberrant mitosis. The G₂/M arrest is likely due to a checkpoint dependent DNA damage response, as deletion of the *rad9⁺* checkpoint gene suppresses the accumulation of Δ*srs2* Δ*rqh1* cells in G₂/M. This arrest appears to be related to recombination, as deletion of recombination genes also suppresses the slow growth of Δ*srs2* Δ*rqh1* and improves their viability. This result is in contrast to a recently published paper where it was reported that loss of recombination actually worsened the slow growth of Δ*srs2* Δ*rqh1* (41). We will point out why we believe we find these differences. Interestingly, we found that when replication fork stalling is increased by treatment with UV or HU, Δ*srs2* Δ*rqh1* cells show improved survival compared with that of a Δ*rqh1* single mutant. These data imply that the synthetic phenotype of Δ*srs2* Δ*rqh1* is not a direct result of the double mutant inability to cope with stalled replication. Lastly, in studying the effect of loss of recombination on Δ*srs2* Δ*rqh1* we found that Δ*srs2* is synthetically lethal with Δ*rhp54*, and this lethality is suppressed in a Δ*rhp51* mutant background.

MATERIALS AND METHODS

Yeast strains, molecular techniques and media

The strains with their genotypes used in this study are listed in Table 1. All strains were maintained on YEA media (YEA is 5 g/l yeast extract, 30 g/l of dextrose and 75 mg/l of adenine). Edinburgh minimum media (EMM) is supplied by Bio101 Inc. Double and triple mutants were made by either tetrad analysis or, when appropriate markers were available, by random spore analysis. All newly constructed strains were tested for having the correct genotypes by analytical PCR.

Table 2. Oligonucleotide sequences used in these studies

Name	Sequence
SRS2.kanA	ATGGAAACGAAATCATCATACTTGAAGTTTCTGAATGAAGAACAAAGGAT- TAGTGTTCAGAGTCCTCATAAGTATACTCACAGCTGAAGCTTCGTACG
SRS2.kanB	CTATAACATTCGTGAAACTCGTAGCCTTACACCCAATCGTTTTCTCGAACC- AGCAGGACGGGCACTCGCGACCTTAGTAAGGCCACTAGTGGATCTG

Constructing the *rqh1* and *srs2* deletion strains

Both the Δ*rqh1* and Δ*srs2* strains were made by deleting the entire gene and replacing it with the kanamycin resistance gene (42,43). The KanMX4 gene was amplified using 95mer primers, Srs2kanA and Srs2kanB (sequences in Table 2), which contain 15 nt complementary to the KanMX4 gene. This disruption cassette was used to directly transform our wild-type haploid strain (SZ132), with cells subsequently plated onto YEA plates containing G418 (200 mg/l).

Growth kinetics

Analysis of the relative growth rates of strains was carried out by one of two approaches. In the first case, a single colony was picked from a freshly streaked plate and the cells streaked to single colonies on YEA plates. The plates were incubated at 30°C for 3–4 days and photographed. To get a more quantitative value of growth, overnight liquid cultures were started from single colonies and grown to mid log (2×10^5 to 1×10^6 cells/ml) at 30°C overnight. The cells were counted beginning at time 0 and then at every hour for 9 h. The data were plotted as fold increase against time in hours.

UV and HU sensitivity

Overnight liquid cultures were started from single colonies and grown to mid log (1×10^6 to 5×10^6 cells/ml) at 30°C overnight. The cells were counted and an appropriate number of cells for each dose was plated onto YEA plates. Plates were irradiated with 100 J/m^2 of 254 nm UV light, incubated for 3–5 days and counted. For HU sensitivity studies, the appropriate dilution of cells was spotted onto YEA plates containing the indicated concentration of HU. Plates were incubated for 4–10 days, counted and photographed. Alternatively, 12 mM HU was added to mid log cultures of cells that were then incubated with shaking at 30°C. Cells were plated onto YEA plates at time 0 and every 3 h after for 9 h. The plates were incubated at 30°C for 3–5 days and counted.

Synchronization and release of cells in G_1

To synchronize cells in G_1, we used a combination of nitrogen starvation and size fractionation on lactose gradients. Cells were grown to saturation in YEA and then switched to EMM for 24 h. These cells were washed twice with water and then resuspended in 100 ml of EMM without nitrogen and cultured at 30°C for 20 h. The cells were counted and ~5×10^8 cells in 700 µl were layered onto 13 ml 7–30% lactose gradients made in EMM without nitrogen. The gradients were centrifuged for 4 min at ~300 *g*. The tubes were punctured from the bottom and 0.5 ml fractions collected. Fractions were viewed microscopically and fractions containing the smallest cells pooled. These cells were released from the G_1 block by switching them to YEA media. At 30 min intervals 1.5 ml aliquots were taken, collected by centrifugation, fixed in 70% ethanol and stored at 4°C.

FACS analysis

Fixed cells were collected by centrifugation and washed five times in 50 mM sodium citrate. These cells were resuspended in 0.5 ml of 50 mM sodium citrate, 100 µg/ml RNase and incubated at 37°C for at least 2 h. The cells were then stained by adding 0.5 ml 50 mM sodium citrate, 1 µM Sytox Green (Molecular Probes) and analyzed within 2 h. The cells were assayed using a Becton Dickson Facs Calibur with Cell Quest 4.0 software.

Cell viability

Fresh plates were struck for each strain, and day cultures started from single colonies, grown in YEA, with shaking at 30°C for 11 h. The cultures were counted and the cells plated on YEA plates. The plates were incubated at 30°C for 4 days and colonies counted.

Fluorescence microscopy

Fluorescence microscopy was carried out as previously described (41). Cells were fixed in 70% ethanol. Following fixation, cells were collected by centrifugation, washed in 50 mM sodium citrate and stained with DAPI.

RESULTS

Loss of *srs2*$^+$ does not confer UV and HU sensitivity

The *srs2*$^+$ gene of *S.pombe* was identified by screening the *S.pombe* database using the *S.cerevisiae SRS2/HPR5* gene sequence as the query sequence with a BLAST search program. A haploid strain of *S.pombe*, sp30, was used to create Δ*srs2* by replacing *srs2*$^+$ with the KanMX4 disruption cassette which codes for G418/kanamycin resistance (42). Several G418 resistant clones were identified and deletion of *srs2*$^+$ confirmed by PCR. Thus, we concluded that *srs2*$^+$ is not essential for viability in *S.pombe*. The *srs2::kan*^{R} (Δ*srs2*) cells were then examined initially for growth properties and morphology. As described previously (41) these clones were found to have normal morphologies and near normal growth rates.

To investigate the potential role of Srs2 in DNA damage repair, we measured sensitivity to UV radiation and HU treatment in our *S.pombe* Δ*srs2* mutant. We conclude that the Srs2 protein plays no significant role in survival following UV irradiation (Fig. 1A and B) or treatment with HU (Fig. 1A and C).
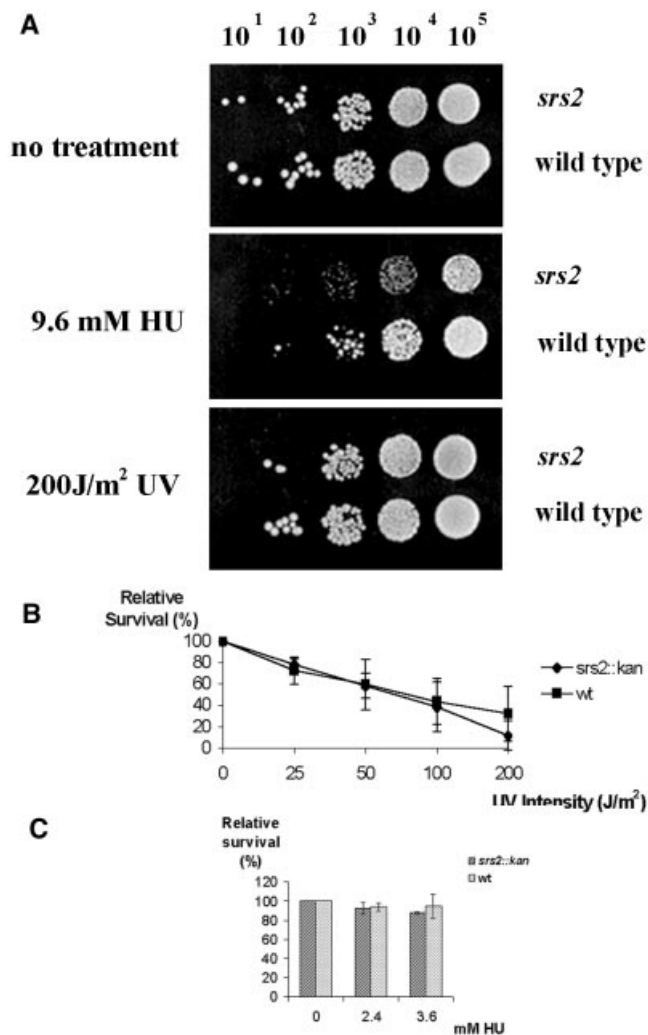
Figure 1. Characterization of the Δ*srs2* mutant. (**A**) Serial dilutions of Δ*srs2* (SZ242) and wild-type (SZ132) cells were plated onto YEA plates (top and bottom panel) or on a YEA plate containing 9.6 mM HU (middle panel). One YEA plate was irradiated with 200 J/m^2 of 254 nm UV light. The other YEA plate was left untreated. Following 4 days of incubation at 30°C these plates were photographed. The plate containing HU was incubated for 6 days and photographed. (**B**) Mid log cultures of Δ*srs2* and wild-type cells were plated onto YEA plates and irradiated with the indicated dose of UV light. Plates were incubated at 30°C for 4 days and colonies counted. This represents the average of three experiments. (**C**) Δ*srs2* and wild-type cells were plated onto YEA plates containing the indicated concentrations of HU, grown for 5 days and colonies counted. This is an average of three experiments.

Schizosaccharomyces pombe Δ*srs2* shows a synthetic slow growth phenotype with Δ*rqh1*

We crossed Δ*srs2* with Δ*rqh1* to create a Δ*srs2* Δ*rqh1* double mutant by random sporulation. Colonies arising from this cross were either normal sized or small. A large screen of over 100 colonies revealed that all normal sized colonies were either single mutants or wild type, while all small colonies were double mutant Δ*srs2* Δ*rqh1*. This demonstrates that the Δ*srs2* Δ*rqh1* double mutant is viable, unlike in *S.cerevisiae* where the corresponding double mutant is synthetic lethal (21). However, the double mutant displays a severe slow
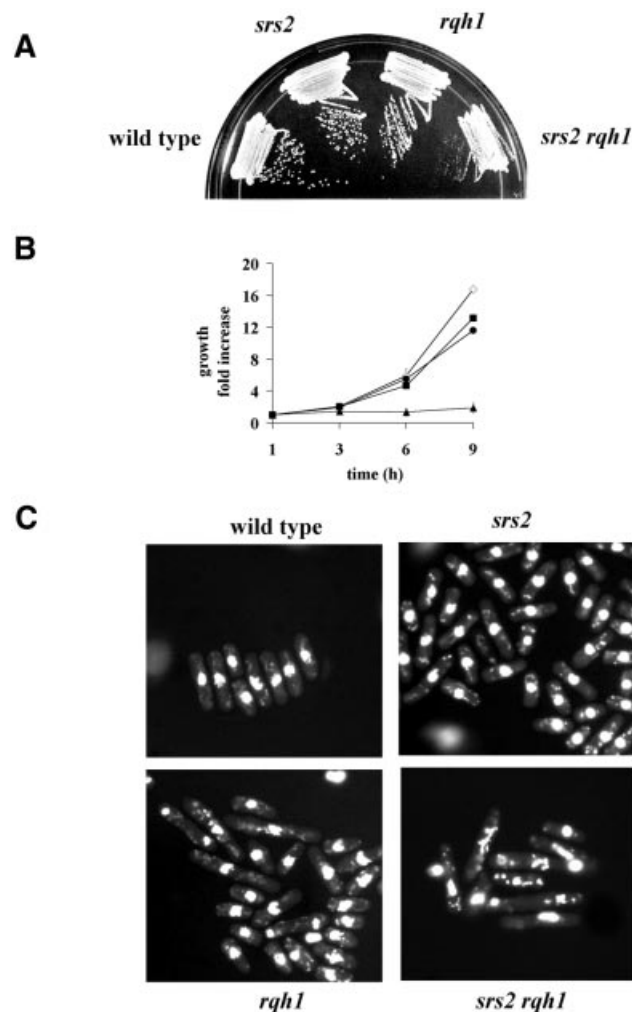

Figure 2. Δ*srs2* shows synthetic slow growth with Δ*rqh1*. The Δ*srs2* Δ*rqh1* double mutant was created from a cross of the single mutants. (**A**) Wild-type (SZ132), Δ*srs2* (SZ242), Δ*rqh1* (SZ243) single mutants and the Δ*srs2* Δ*rqh1* (SZ247) double mutant were struck onto YEA plates. The plates were incubated for 4 days at 30°C and then photographed. (**B**) Growth kinetics were carried out on wild-type (diamonds), Δ*srs2* (squares) Δ*rqh1* (circles) and Δ*srs2* Δ*rqh1* (triangles) cells in mid log (1–5 × 10^5 cells/ml). Cells were collected every hour over a 5 h period and counted in a Coulter Counter. (**C**) Wild-type, Δ*srs2*, Δ*rqh1* and Δ*srs2* Δ*rqh1* cells were isolated from a log growing culture, fixed and then stained with DAPI. A field of cells typical of each strain was photographed.

growth defect (Fig. 2A). Growth kinetic studies demonstrated that the doubling time of the Δ*srs2* Δ*rqh1* mutant is ~5–6 h (Fig. 2B). Wild-type, Δ*srs2*, Δ*rqh1* and Δ*srs2* Δ*rqh1* cells were stained with DAPI and examined microscopically. Figure 2C shows that the double mutant has a phenotype characteristic of fission yeast strains having a growth defect; a mixed population of normal and abnormal appearing cells. The abnormal cells are typically long, often with aberrant cellular morphologies and distorted or fragmented nuclei. The double mutant also shows a very poor plating efficiency (16.7%; see Table 3). We have previously shown that Δ*rqh1* cells not only grow some 30% slower than wild-type cells but the population also contains a number of elongated and abnormally shaped cells (40). The fact that the Δ*srs2* single mutant has normal

Table 3. Plating efficiency of mutant strains

Strain	Wild type	srs2::kan^R	rqh1:: kan^R	rhp51::ura4$^+$	rad9::ura4$^+$	srs2::kan^R rqh1::kan^R	srs2:: kan^R rqh1:: kan^R rhp51::ura4$^+$	srs2:: kan^R rqh1:: kan^R rad9::ura4$^+$
Plating efficiency (%)	95.3 ± 16.7	100.2 ± 34.3	64.1 ± 8.7	63.75 ± 7.1	47.0 ± 14.2	16.7 ± 0.91	38.5 ± 4.2	7.5 ± 2.2

growth characteristics and nearly normal resistance to both UV and HU further suggests that Srs2 alone plays a relatively minor role in cell viability.

Δ*srs2* Δ*rqh1* arrests in G$_2$/M and show a mitotic defect

We initially suspected that the slow growth seen in Δ*srs2* Δ*rqh1* cells might be due to a delay in S-phase based on the proposed role of both of these proteins functioning during DNA replication. To investigate this, we carried out FACS analysis on synchronized cells. Overnight cultures of wild type, Δ*srs2*, Δ*rqh1* and Δ*srs2* Δ*rqh1* were synchronized in G$_1$ by growth in media lacking nitrogen. Cells were released from the block and samples collected for analysis at time intervals over several hours. In our initial studies, FACS profiles of the Δ*srs2* Δ*rqh1* strain were difficult to interpret, presumably due to the abundance of abnormally large cells present in the population (data not shown). In subsequent experiments, following nitrogen starvation, large cells were eliminated by size selection using 7–30% lactose gradients. These size-selected cells were released from the block by switching to YEA media. FACS analysis of these cells gave much improved results. The results in Figure 3 show that Δ*srs2* Δ*rqh1* cells do exhibit a slightly elongated S-phase. The Δ*srs2* Δ*rqh1* cells exit the G$_1$ block and progress through S-phase somewhat slower than wild-type cells or cells containing either single mutation. This is seen in Δ*srs2* Δ*rqh1* as a greater number of cells in S-phase between 1 and 2 h after release from G$_1$. This slight delay in S-phase would not seem to account for all of the slow growth seen in the double mutant.

Since Δ*srs2* Δ*rqh1* cells did not show a significant S-phase delay, we went on to determine if there was a more substantial delay in another part of the cell cycle. To address this, we used cells from the same synchronization experiments described above. Cells, fixed at various times after G$_1$ release, were stained with DAPI and examined microscopically. At each time point, random fields of cells were scored as being either small/normal, elongated, having two nuclei or having torn or abnormal nuclei. On average about 250 cells were counted for each time point. Elongated cells were cells judged to be longer than a typical dividing cell. Figure 4 summarizes the results of wild-type and Δ*srs2* Δ*rqh1* cells. Pictures of DAPI stained cells representative of each classification of cells is shown at the top of each column. The percentage of each cell type seen at each time point are listed below each picture. At 0 h, essentially all wild-type and mutant cells were small, consistent with a good G$_1$ arrest. By 2 h we still saw mainly small or normal sized cells in both strains. By 3.5 h post release we began to see cells from both wild-type and Δ*srs2* Δ*rqh1* that contained two nuclei, consistent with cells having completed mitosis. At 4 h, 28% of wild-type cells and 13% of Δ*srs2* Δ*rqh1* cells were found to contain two nuclei. These
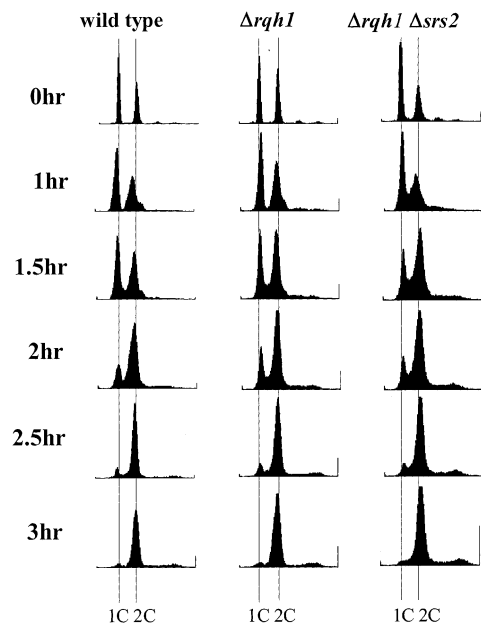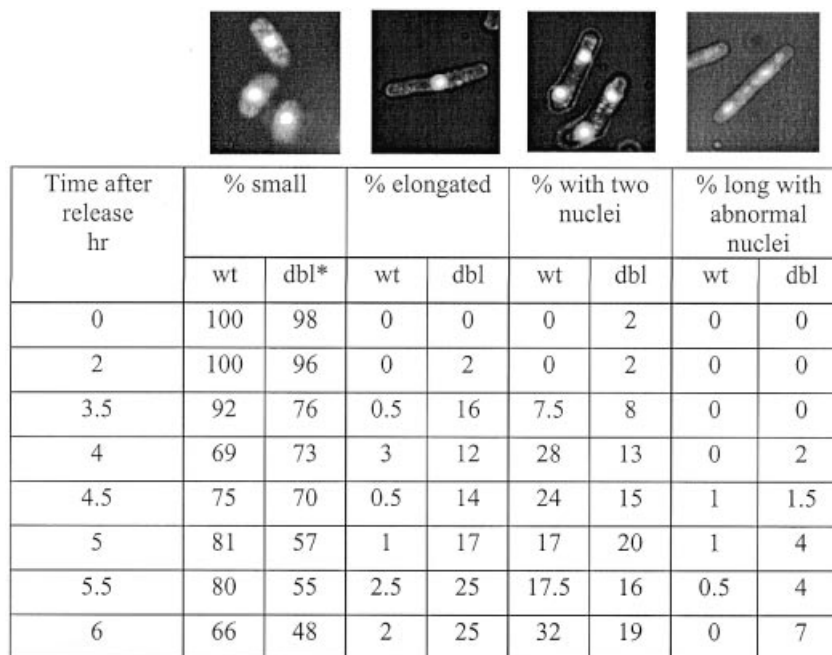

Figure 3. Δ*srs2* Δ*rqh1* cells show a slight delay in S-phase. Wild-type (SZ132), Δ*rqh1* (SZ243) and Δ*srs2* Δ*rqh1* (SZ247) cells were blocked in G$_1$ by nitrogen starvation and size selected on lactose gradients. The cells were released from the G$_1$ block by shifting to YEA media. Cells were isolated every 30 min after release for 3 h and fixed. Fixed cells were stained with Sytox Green and analyzed by FACS. The profiles are shown here.

results demonstrate that a sub-population of Δ*srs2* Δ*rqh1* cells cycle normally. At 4 h many Δ*srs2* Δ*rqh1* were not cycling normally, as indicated by the presence of elongated cells with single nuclei (12% of Δ*srs2* Δ*rqh1* compared with 3% of wild-type cells). This phenotype is indicative of cells delayed in G$_2$/M, suggesting a checkpoint-dependent arrest. These elongated cells appear to accumulate, as their numbers increased to 25% by 5.5 h after release. Only a few percent of elongated cells were ever seen in wild-type cells.

In addition to the elongated cells, at the later time points (5–6 h), there was also an accumulation of cells with torn and abnormally shaped nuclei, indicative of cells that have undergone mitotic catastrophe. This morphology is reminiscent of the phenotype seen in Δ*rqh1* cells following DNA damage or HU arrest. We also examined synchronized Δ*srs2* and Δ*rqh1* single mutants through this same block and release time course. The Δ*srs2* cells were indistinguishable from wild-type cells, while a very small number (<5%) of Δ*rqh1* cells did elongate with a single nucleus or had torn chromosomes (<2%, data not shown). From these results we conclude that the events that lead to cell growth arrest in Δ*srs2* Δ*rqh1* are stochastic, as some cells fail to progress through the cell cycle

Time after release hr	% small		% elongated		% with two nuclei		% long with abnormal nuclei	
	wt	dbl*	wt	dbl	wt	dbl	wt	dbl
0	100	98	0	0	0	2	0	0
2	100	96	0	2	0	2	0	0
3.5	92	76	0.5	16	7.5	8	0	0
4	69	73	3	12	28	13	0	2
4.5	75	70	0.5	14	24	15	1	1.5
5	81	57	1	17	17	20	1	4
5.5	80	55	2.5	25	17.5	16	0.5	4
6	66	48	2	25	32	19	0	7

* dbl refers to Δ*rqh1* Δ*srs2*

Figure 4. Δ*srs2* Δ*rqh1* cells appear to arrest at G$_2$/M with many undergoing mitotic catastrophe. Wild-type (SZ132) and Δ*srs2* Δ*rqh1* (SZ247) cells were blocked in G$_1$ by nitrogen starvation and size selected on lactose gradients. Cells were released from the block by growth in YEA. Samples were collected at 30 min intervals, fixed and stained with DAPI. Random fields of cells were then examined microscopically and each cell scored for being in one of the four morphological types; small/normal, elongated (cells without septi which were as long or longer than dividing cells), cells with two nuclei (these are post mitotic cells) and cells with elongated and abnormal nuclei (these are cells that have undergone mitotic catastrophe). Approximately 300 cells were counted for each time point. An example of cells characteristic for each morphological type are shown at the top of each column.

while others proceed at what appears to be a normal rate of growth. Also, when cells do arrest it appears to be at G$_2$/M although our FACS data do not rule out the possibility that the arrest is in very late S-phase.

Loss of Srs2 and Rqh1 leads to induction of a checkpoint response

One explanation for the G$_2$/M arrest of Δ*srs2* Δ*rqh1* cells is that their DNA is altered or damaged in such a way that a checkpoint response is elicited. To test this, we created a triple mutant strain in which the *rad9*+ checkpoint gene was deleted in a Δ*srs2* Δ*rqh1* background. Figure 5A shows that loss of the *rad9*+ gene suppresses the slow growth of the Δ*srs2* Δ*rqh1* mutation. Furthermore, when these cells (Δ*srs2* Δ*rqh1* Δ*rad9*) were stained with DAPI and visualized by fluorescence microscopy, fewer elongated cells were detected (Fig. 5B). This result suggests that by deleting *rad9*+, the Δ*srs2* Δ*rqh1* cells escape a checkpoint-dependent arrest, reducing their elongated cell cycle time and, therefore, partially suppressing their slow growth. We also reasoned that an apparent suppression of slow growth would occur if deletion of *rad9*+ improved cell survival in Δ*srs2* Δ*rqh1* cells. We suggest this in light of our finding that the Δ*srs2* Δ*rqh1* double mutant shows a high rate of spontaneous cell death, contributing to an appearance of slow growth. Therefore, we measured the plating efficiency of Δ*srs2* Δ*rqh1* and compared it with that of the Δ*srs2* Δ*rqh1* Δ*rad9* triple mutant. The data shown in Table 3 reveal that the plating efficiency of the triple mutant

was actually worse than that of the double mutant, 7.5% for Δ*srs2* Δ*rqh1* Δ*rad9* versus 17.6% for Δ*srs2* Δ*rqh1*. Therefore, the increased growth rate in the triple mutant cannot be explained by improved cell viability. These data support the conclusion that loss of *rad9*+ in Δ*srs2* Δ*rqh1* improves the growth rate because these cells can no longer induce a checkpoint-dependent arrest.

We next wanted to ask whether the arrest of Δ*srs2* Δ*rqh1* cells was specific to S-phase or G$_2$. To answer this question we made Δ*cds1* Δ*srs2* Δ*rqh1* and Δ*chk1* Δ*srs2* Δ*rqh1* triple mutants and examined the growth and morphology of these cells. Cds1 controls S-phase checkpoint arrest while Chk1 controls G$_2$ checkpoint arrest. While the loss of *cds1*+ had no effect on cell growth, loss of *chk1*+ did improve cell growth (data not shown). Consistent with this finding, loss of *chk1*+ suppressed the appearance of elongated cells normally seen in the Δ*srs2* Δ*rqh1* mutant (Fig. 5C), while loss of *cds1*+ had no apparent effect on the morphology of Δ*srs2* Δ*rqh1* cells. Δ*cds1* Δ*srs2* Δ*rqh1* and Δ*chk1* Δ*srs2* Δ*rqh1* triple mutants had plating efficiencies similar to Δ*srs2* Δ*rqh1*, 22 and 14%, respectively. These results are consistent with the Δ*srs2* Δ*rqh1* mutant arresting in G$_2$.

The slow growth of Δ*srs2* Δ*rqh1* double mutants is suppressed by loss of the recombination pathway

Loss of *rqh1*+, as well as other members of the *recQ* family, leads to hyper-recombination, as does loss of *srs2*+ in *S.pombe* (8,41,44,45). Thus, the phenotype associated with
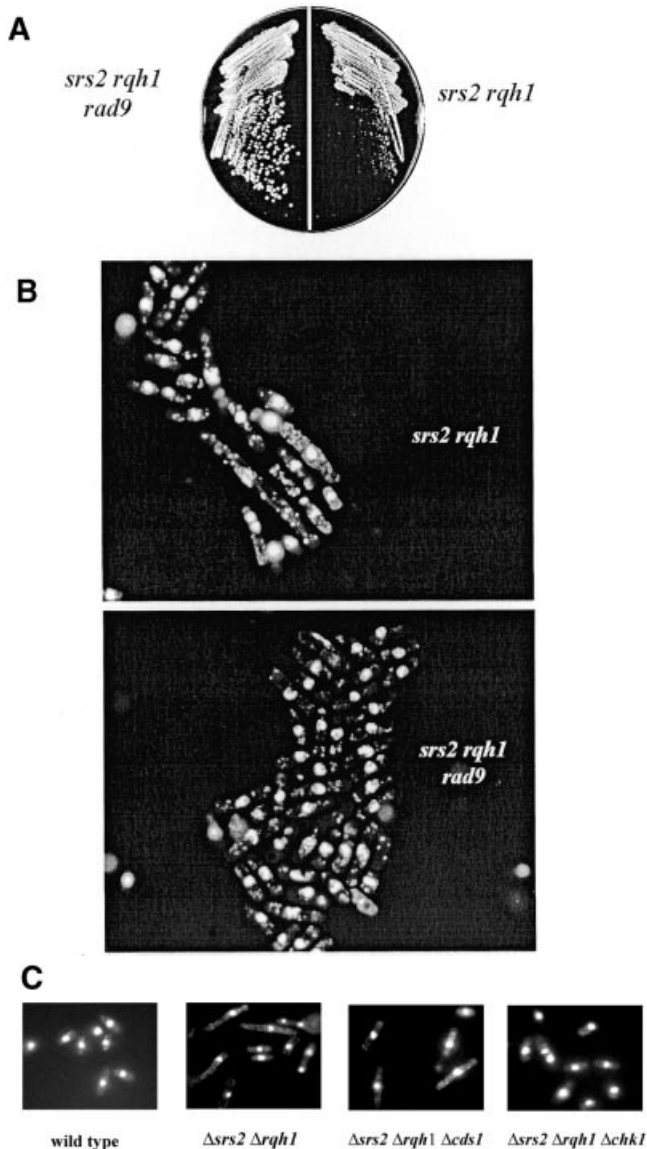
Figure 5. Loss of the checkpoint genes suppresses the slow growth and abnormal morphology of Δ*srs2* Δ*rqh1*. A Δ*rad9* Δ*srs2* Δ*rqh1* (SZ280) triple mutant was created to study the loss of the checkpoint response on cell growth and morphology. (**A**) Strains were struck onto YEA plates and grown at 30°C for 4 days and photographed. The Δ*rad9* Δ*srs2* Δ*rqh1* triple mutant (SZ280) is seen to have better growth than the Δ*srs2* Δ*rqh1* (SZ247) double mutant. (**B**) Fluorescence microscopy of DAPI stained cells reveals that the morphology of the triple mutant is much more like wild-type cells than the Δ*srs2* Δ*rqh1* double mutant. See Figure 2C for wild-type cells. (**C**) Δ*cds1* Δ*srs2* Δ*rqh1* (SZ613) and Δ*chk1* Δ*srs2* Δ*rqh1* (SZ614) triple mutants were constructed and analyzed. Fluorescence microscopy of DAPI stained cells shows that while loss of *chk1*+ suppresses the abnormal morphology of Δ*srs2* Δ*rqh1* cells, loss of *cds1*+ does not.

the simultaneous loss of both *srs2*+ and *rqh1*+ might be explained by increased rates of recombination. It follows that eliminating recombination genes might suppress the slow growth seen in this double mutant. To study this, we created two different recombination deficient strains, Δ*srs2* Δ*rqh1* Δ*rhp51* and Δ*srs2* Δ*rqh1* Δ*rhp57*. One complicating factor with these studies is that in *S.pombe* recombination mutants themselves are slow growing, although not nearly as severe as Δ*srs2* Δ*rqh1*. Despite this, we were able to see clear
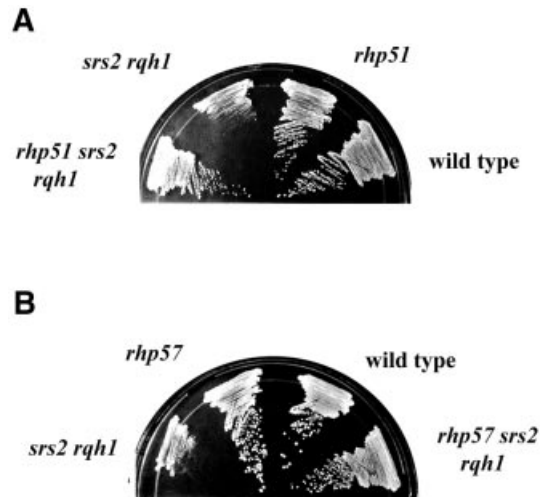

Figure 6. The slow growth of Δ*srs2* Δ*rqh1* is also suppressed in cells deficient in homologous recombination. (**A**) Mid log cultures of wild-type (SZ132), Δ*rhp51* (SZ215), Δ*srs2* Δ*rqh1* (SZ247) and Δ*srs2* Δ*rqh1* Δ*rhp51* (SZ271) cells were struck onto YEA plates and grown at 30°C for 4 days and photographed. The Δ*srs2* Δ*rqh1* Δ*rhp51* strain has growth properties similar to the Δ*rhp51* single mutant. (**B**) Loss of *rhp57*+ (SZ258) suppresses as well the slow growth of Δ*srs2* Δ*rqh1* (SZ247) mutant.

suppression of the slow growth in Δ*srs2* Δ*rqh1* when either recombination gene was deleted (Fig. 6A and B). We created the triple mutant by random spores and characterized over 100 colonies for each strain. Our Δ*rhp51* and Δ*rhp57* strains were disrupted with *ura4*+ while both Δ*srs2* and Δ*rqh1* were disrupted with *kanMX*. This allowed us to rapidly screen our colonies. We found that every slow growing colony that we examined was Δ*srs2* Δ*rqh1* while faster growing colonies were always either Δ*rhp51* or Δ*rhp57*. The figure shows that loss of *rhp51*+ partially suppresses the slow growth of Δ*srs2* Δ*rqh1* double mutants, to a level similar to that of the Δ*rhp51* single mutant (Fig. 6A). Δ*rhp57* mutant was also found to suppress the slow growth of Δ*srs2* Δ*rqh1* (Fig. 6B).

Clearly, loss of recombination suppresses the slow growth of Δ*srs2* Δ*rqh1*, but does it improve cell survival? To test this we measured the plating efficiency of Δ*srs2* Δ*rqh1* Δ*rhp51* and compared it with the double mutant as well as the single mutants. In Table 3, we see that the triple mutant showed a plating efficiency of 40% as compared with Δ*srs2* Δ*rqh1*, which was only 17.4%. The efficiency was not as good as the single Δ*rhp51* mutant, which was 65%.

In light of the discrepancy between our data and that of Wang *et al.* (41) concerning suppression of Δ*srs2* Δ*rqh1* slow growth by loss of recombination genes, we carried out one other experiment to eliminate the possibility that our suppression was the result of some spontaneous suppressor. To determine if a spontaneous suppressor did arise in our strains we crossed Δ*srs2* Δ*rqh1* to Δ*srs2* Δ*rqh1* Δ*rhp51*. The progeny were analyzed by random sporulation. Spores were found to be either large or small at about 50:50. A screen of over 100 colonies from the cross found that all of the large colonies that were tested contained Δ*rhp51* (data not shown). This demonstrates that the suppression is due to the loss of recombination and not due to a spontaneous suppressor. From these results we show conclusively that the synthetic slow growth of Δ*srs2* Δ*rqh1* is suppressed in a recombination deficient background.

These results are consistent with findings reported in *S.cerevisiae* where it was demonstrated that the synthetic lethality or slow growth of *srs2 sgs1* is suppressed by mutations in genes coding for proteins of the recombination machinery (14,30,32).

Loss of *srs2+* partially suppresses the HU and UV sensitivity of Δ*rqh1*

Our FACS analysis of synchronized Δ*srs2* Δ*rqh1* cells indicates that these cells progress through S-phase slightly slower than wild type, which seems to argue against the slow growth being primarily a function of stalled replication. However, it is possible that the impairment seen in Δ*srs2* Δ*rqh1* cells is related to an inability to properly respond to stalled replication forks, even though the major cell cycle arrest occurs outside of S-phase. If stalled forks are the problem, then increasing the number of stalled replication forks in Δ*srs2* Δ*rqh1* cells by DNA damage with UV radiation or depleting the nucleotide pool with HU should have a profound negative effect on cell survival. In Figure 7A, it is seen that rather than being more sensitive to UV the Δ*srs2* Δ*rqh1* double mutant was actually less sensitive to UV than that of the Δ*rqh1* single mutant. Δ*srs2* Δ*rqh1* double mutants showed an equivalent sensitivity to 12 mM HU in liquid culture when compared with the Δ*rqh1* single mutant (Fig. 7B). To consider the possibility that the lack of increased sensitivity to HU in the double mutant was a function of its slow growth, we examined the HU sensitivity of the double mutant with continuous HU exposure by plating cells directly onto HU-containing plates. Figure 7C shows plates containing 2.4 mM HU after 8 days of incubation. The Δ*srs2* Δ*rqh1* plate shows not only increased survival relative to the Δ*rqh1* single mutant but also the presence of larger colonies. Together, these data support the conclusion that loss of *srs2+* partially suppresses the HU and UV sensitivity of Δ*rqh1*. More importantly, this result argues against the idea that the synthetic phenotype seen in Δ*srs2* Δ*rqh1* is solely dependent on stalled replication.

Δ*srs2* and Δ*rhp54* show synthetic lethality, which can be suppressed by loss of *rhp51+*

In our screen for homologous recombination mutants that would suppress the slow growth of Δ*srs2* Δ*rqh1*, we found a synthetic lethality between Δ*srs2* and Δ*rhp54*. This synthetic lethality also exists in the budding yeast (45). The *RAD54* homolog in *S.pombe* is Rhp54. We crossed *srs2::kan^R* to *rhp54:: ura4+* followed by random sporulation onto plates containing G418 to select for *srs2::kan^R* colonies. Two hundred G418 resistant colonies were replica plated on minimum media. No colonies were observed after 4 days of incubation at 30°C indicating that all the colonies were *ura⁻* and therefore *rhp54+*. From this cross we also plated directly onto YEA media and, following this, we identified 100 *ura+* colonies. When replica plated onto G418, none of these colonies grew. Thus, we conclude that these mutants are synthetic lethal in *S.pombe*.

We next looked for a possible suppressor of this lethality. We constructed two double mutant strains Δ*srs2* Δ*rhp51* and Δ*rhp54* Δ*rhp51*. These strains were crossed and screened for the presence of kanamycin resistance on G418 plates. G418 resistant colonies were isolated and screened for the presence
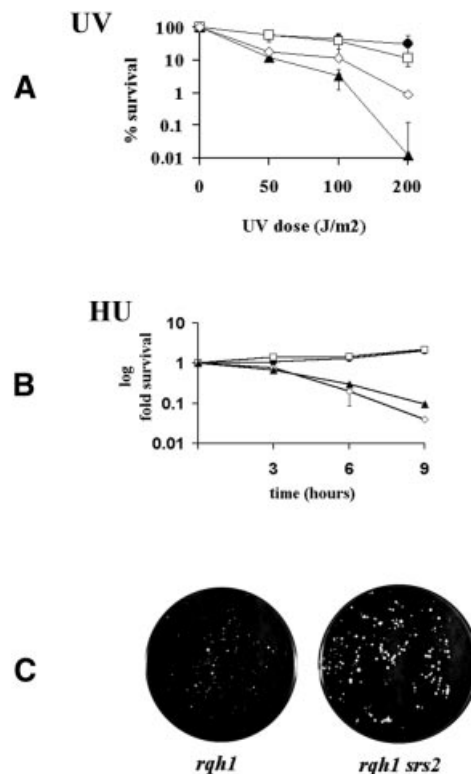

Figure 7. Loss of *srs2+* partially suppresses the UV and HU sensitivities of Δ*rqh1*. (**A**) Mid log cultures of wild-type (SZ132) (circles), Δ*srs2* (SZ242) (squares), Δ*rqh1* (SZ243) (triangles) and Δ*srs2* Δ*rqh1* (SZ247) (diamonds) cells were plated onto YEA plates and irradiated with the indicated doses of UV light. The plates were incubated for 4 days and counted. This is the average of two independent experiments. (**B**) 12 mM HU was added to mid log cultures of wild-type (circles), Δ*srs2* (squares), Δ*rqh1* (triangles) and Δ*srs2* Δ*rqh1* (diamonds) cells and incubated at 30°C. At the indicated time points, aliquots of cells were plated onto YEA plates, which were incubated for 4 days and colonies counted. This is the average of three independent experiments. (**C**) Mid log cultures of Δ*rqh1* and Δ*srs2* Δ*rqh1* cells were plated onto YEA plates containing 2.4 mM HU. Plates were incubated for 8 days and photographed.

of Δ*rhp54* by PCR analysis. In this screen several Δ*srs2* Δ*rhp54* Δ*rhp51* triple mutants were identified. Figure 8 shows one of these strains growing on YEA. Thus, the synthetic lethality of the Δ*srs2* Δ*rhp54* is suppressed by loss of *rhp51+*.

DISCUSSION

A summary of our findings on *srs2+* is as follows: (i) Δ*srs2* single mutants grow normally and have a normal morphology; (ii) Δ*srs2* mutants are not sensitive to UV irradiation and treatment with HU; (iii) simultaneous loss of *srs2+* and *rqh1+* results in a synthetic phenotype in which the cells show extreme slow growth, aberrant cellular and nuclear morphologies and high spontaneous cell death; (iv) a large portion of Δ*srs2* Δ*rqh1* cells undergo a checkpoint-dependent cell cycle arrest, most likely at G_2/M, which is a stochastic event; (v) Δ*srs2* Δ*rqh1* double mutants show high levels of spontaneous mitotic catastrophe; (vi) the slow growth and poor viability of Δ*srs2* Δ*rqh1* are partially suppressed by loss of the *rhp51+* and *rhp57+* homologous recombination genes; (vii) loss of *srs2+* suppresses the UV and HU sensitivity of
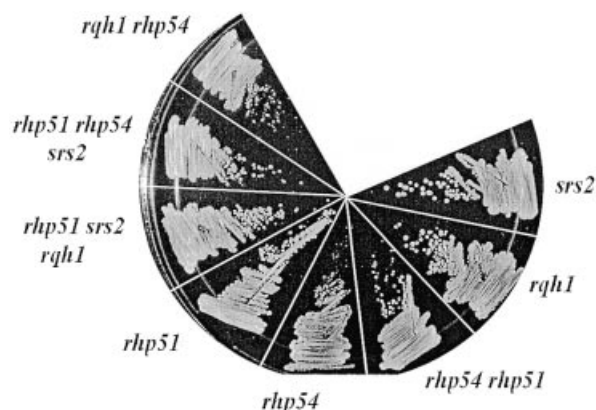
Figure 8. The synthetic lethality of Δ*srs2* (SZ242) and Δ*rhp54* (SZ231) is suppressed by loss of *rhp51⁺*. Mid log cultures of each indicated strain were grown and struck onto YEA plates. The plates were incubated for 4 days and photographed. The triple mutant, Δ*rhp51* Δ*rhp54* Δ*srs2* (SZ279) shows a similar growth rate to the Δ*rhp51* Δ*srs2* Δ*rqh1* (SZ271) triple mutant. Both of these strains grow slower than the Δ*srs2* single mutant. The wild-type strain is not shown, however Δ*srs2* (SZ242) is pictured, which grows essentially like wild-type cells.

Δ*rqh1* mutants; and (viii) Δ*srs2* is synthetic lethal with Δ*rhp54*, and this lethality is suppressed by loss of *rhp51⁺*. Deletion of the *S.pombe srs2⁺* gene shares many properties in common with deletion of *SRS2* in *S.cerevisiae* although the severity of phenotypes sometimes differs. For example, *S.cerevisiae srs2* mutants are more sensitive to UV damage and its synthetic interaction with *sgs1* appears more severe than that of Δ*srs2* and Δ*rqh1*. In addition we have gone on to show that loss of *srs2⁺* partially suppresses the UV and HU sensitivity of Δ*rqh1*. We also find that Δ*srs2* Δ*rqh1* cells do not show a significant arrest in S-phase but rather arrest at what appears to be G₂/M. The spontaneous cell death associated with Δ*srs2* Δ*rqh1* appears to be due to mitotic catastrophe.

Phenotype of Δ*srs2*

In contrast to Rqh1, Srs2 clearly plays a much less prominent role in cell survival following UV damage or HU treatment. This is demonstrated by the level of sensitivity that each single mutant shows when exposed to these agents (Figs 1 and 7). Consistent with our previous studies we find that the Δ*rqh1* single mutant has a mild cell growth defect and lower plating efficiency. By comparison, Δ*srs2* behaves essentially like wild type for both growth and plating efficiency (Fig. 2B and Table 3).

The synthetic interaction between Δ*srs2* and Δ*rqh1*

Schizosaccharomyces pombe Δ*srs2* has a synthetic interaction with Δ*rqh1*, manifested as extreme slow growth, poor plating efficiency, high rates of spontaneous cell death and abnormal cellular and nuclear morphologies. One interpretation of a synthetic interaction is that the two genes function in parallel pathways in an essential or very important process. The role of Rqh1 and its homologs is not known, but most evidence points to it playing some role in suppressing recombination arising by stalled replication. The synthetic phenotype of Δ*srs2* Δ*rqh1* would suggest that Srs2 plays a similar or alternative role at

stalled forks. However, our data do not support this interpretation. First, if the phenotype of the double mutant results from a major defect in the cells' ability to cope with spontaneously arising stalled replication forks, then predictably these cells should arrest or slow in S-phase. Our FACS data from synchronized cells show that Δ*srs2* Δ*rqh1* show only a slight delay in S-phase (Fig. 3). Second, if the phenotype of the double mutant results from a defect in its ability to cope with replication fork stalling, then these cells should be extremely sensitive to any increase in the number of stalled forks. Again we find this not to be the case, as the Δ*srs2* Δ*rqh1* double mutant actually shows increased survival when exposed to UV or treated with HU when compared with Δ*rqh1* (Fig. 7A, B and C). These results argue against the phenotype of the double mutant resulting from stalled replication.

If Δ*srs2* Δ*rqh1* cells are not arresting in S-phase at a significant level, where do they arrest? In an effort to determine this, Δ*srs2* Δ*rqh1*, wild-type and single mutant cells were DAPI stained and examined microscopically at times after release from G₁ synchronization. Cells from each time point were scored as being either small or normal in size, elongated (assumed to be in G₂ arrest), bi-nucleated (cells having completed mitosis) or abnormal (cells with torn or fragmented nuclei). We draw two conclusions from these studies. Our first conclusion, based on the data in Figure 4, is that some double mutant cells appear to cycle normally. Initially both wild-type and Δ*srs2* Δ*rqh1* cells are small with single nuclei. At 3.5 h, which is the earliest time that double nucleated wild-type cells are seen, an equal number of Δ*srs2* Δ*rqh1* are double nucleated. This shows that cells have progressed through mitosis. At later time points we see continued evidence of Δ*srs2* Δ*rqh1* cells cycling normally, as double nucleated cells continue to be present. Our second conclusion is that many Δ*srs2* Δ*rqh1* cells undergo an arrest, most likely at G₂/M. This conclusion is based on our observation that large numbers of Δ*srs2* Δ*rqh1* cells accumulate as single nucleated (pre-mitotic) elongated cells. In combination with our FACS data showing no major accumulation of cells in S-phase, these elongated cells with a single nucleus must be in G₂, unable to enter M. We cannot rule out the possibility that these cells are in very late S-phase. By 6 h there are essentially no elongated cells found in wild-type, yet 25% of Δ*srs2* Δ*rqh1* cells are elongated. In addition to elongated cells, at 6 h 7% of Δ*srs2* Δ*rqh1* cells have aberrant or torn nuclei. This occurs in elongated cells, suggesting that some arrested cells ultimately undergo mitotic catastrophe. While we did not look at time points beyond 6 h, we assume that the number of cells showing mitotic catastrophe will continue to accumulate with time. We speculate that most, if not all, elongated cells will fail to progress through mitosis properly. This cannot be determined by these experiments, as elongated cells will continue to accumulate. However, if this assumption is correct, then the 25% of elongated cells together with the 7% of cells having already undergone mitotic catastrophe represent one-third of the cell population dying during each round of the cell cycle. This number is in good agreement with a doubling time of ~6 h for Δ*srs2* Δ*rqh1*. A recent study in *S.cerevisiae* demonstrated that 15% of *srs2 sgs1* double mutants arrest in mitosis as large budded cells and that this arrest was checkpoint dependent (20). Cells arrested in mitosis were also found in 7% of *srs2* and 2% of *sgs1* single

mutants, demonstrating that this is not strictly a phenotype of the double mutant. Interestingly, when we examined Δsrs2 in a similar experiment, there was no evidence of G₂/M arrested cells or cells undergoing a mitotic catastrophe. We saw evidence for a few elongated Δrqh1 cells suggesting a mild arrest. These data demonstrate that the apparent slow growth of Δsrs2 Δrqh1 cells is not due to a homogeneous slowing of the entire cell population, but that this is a mixed population of normal cycling and arrested cells. This indicates that the cellular events leading to cell cycle arrest are stochastic.

The G₂/M arrest seen in Δsrs2 Δrqh1 cells is likely due to the accumulation of some type of DNA damage or structure that induces a checkpoint response. This is further corroborated by our finding that deletion of the rad9⁺ checkpoint gene suppresses the accumulation of the elongated, G₂/M arrested cells in the Δsrs2 Δrqh1 mutant (Fig. 5B). While we cannot be certain what DNA structure is recognized as damage, it is tempting to speculate that it is recombination intermediates. This is based on the fact that Δsrs2 mutants have been reported to have a hyper-recombination phenotype and our findings that loss of recombination genes suppresses the slow growth of Δsrs2 Δrqh1 cells and improves their viability (Fig. 6A and B, and Table 3). It is known that Cds1 regulates the S-phase arrest while Chk1 regulates the G₂ arrest. Our data, showing that loss of chk1⁺ but not cds1⁺ suppresses the elongated morphology seen in Δsrs2 Δrqh1, further support the conclusion that these cells are arresting in G₂.

Deletion of recombination genes suppress the Δsrs2 Δrqh1 phenotype

Previous studies demonstrated that Δrqh1 mutants have no increase or only slightly elevated recombination rates (8,18). However, loss of Srs2 is associated with a 12-fold increase in spontaneous recombination rates (41,45). The elevated recombination rates in Δsrs2 do not appear to have any obvious deleterious effect on cells, as they grow normally and have normal morphologies. We wondered if the severe phenotype seen in Δsrs2 Δrqh1 was due to the inability of these cells to deal effectively with recombination. This would appear to be the case as we found that deletion of either rhp51⁺ or rhp57⁺ markedly suppresses the slow growth and low viability of Δsrs2 Δrqh1 cells. Loss of recombination also returns Δsrs2 Δrqh1 cells to a more normal cellular phenotype. This normalization suggests that recombination is at least a partial cause of the aberrant phenotype seen in the double mutant. One interpretation of these results is that potential problems caused by hyper-recombination in Δsrs2 cells are suppressed by Rqh1 function, and so the problems are only seen upon simultaneous loss of both genes.

Our findings of slow growth suppression in Δsrs2 Δrqh1 cells by loss of recombination are in contrast to the results of Wang et al. (41). They reported that Δrhp51 failed to suppress the slow growth of Δsrs2 Δrqh1 and, in fact, the triple mutant grew worse. We found clear suppression by not only loss of rhp51⁺, but also by loss of rhp57⁺. Additionally, they reported that their Δsrs2 Δrhp51 double mutant looked like a Δsrs2 Δrqh1 mutant and were more sensitive to UV damage than the Δrhp51 single mutant. Our experiments showed neither a synthetic interaction between Δsrs2 and Δrhp51 nor increased UV sensitivity in the double mutant compared with the Δrhp51 single mutant (data not shown). Other results with Δsrs2 were the same between our laboratories. This suggests that the discrepancies may arise from the use of different Δrhp51 mutants which could contain uncharacterized strain differences. We used the Δrhp51 reported previously (46), while Wang et al. used a Δrhp51 created for their studies. However, we are confident in our conclusion that loss of recombination suppresses the Δsrs2 Δrqh1 phenotype based on the fact that we observed suppression with two different recombination mutants and that comparable results have been established for the budding yeast S.cerevisiae. We also addressed the possibility that our suppression was due to a spontaneous suppressor. Our results argue against the presence of a spontaneous suppressor of the Δsrs2 Δrqh1 phenotype.

Δsrs2 is synthetic lethal with Δrhp54

While searching for recombination mutants that would suppress the slow growth of Δsrs2 Δrqh1, we found synthetic lethality between Δsrs2 and Δrhp54. This lethality was suppressed by deletion of rhp51⁺. Two possible explanations could account for this result. One is that Srs2 functions in recombination in a process in parallel with Rhp54 and that one of these two proteins is necessary to complete recombination initiated by Rhp51. This would explain why deletion of rhp51⁺ suppresses this synthetic lethality. An alternative explanation comes from studies in S.cerevisiae where Srs2 is known to function in the PRR pathway (24,29,47). Loss of srs2 in a rad6 mutant suppresses the UV sensitivity of these strains presumably by allowing repair of DNA damage to occur by homologous recombination. While we do not know if Srs2 plays the same role in PRR in S.pombe, the same synthetic lethality between Δsrs2 and Δrad54 exists in S.cerevisiae (31). We also know that loss of srs2⁺ in S.pombe leads to a hyper-recombination phenotype (41). From this, we can speculate that in the absence of srs2, damaged or incompletely replicated DNA is funneled into the Rhp51-dependent homologous recombination pathway. Presumably the process initiated by Rhp51 in a Δsrs2 background requires Rhp54 to be completed, and the initiation of this process in the absence of Rhp54 leads to lethality. In this scenario, Srs2 plays an indirect role in recombination.

Why does loss of srs2⁺ suppress rqh1 sensitivity to UV and HU?

It is difficult to explain how deletion of srs2⁺ in a Δrqh1 background can have a negative effect on cell growth and viability, yet have a protective effect on the ability of these cells to cope with UV and HU. We have already given a reasonable possible explanation for the synthetic interaction between Δsrs2 and Δrqh1 as it relates to normal growth. It is not necessary to link these two phenotypes since one happens in damaged or replication arrested cells and the other is a spontaneous response. One possibility is as follows: upon replication arrest, caused by DNA damage or HU treatment, recombination takes place at a high rate in the absence of Rqh1 (8,18). It is the process of recombination that leads to the decrease in viability seen in these cells. We base this latter statement on the finding that expression of a recombination resolvase partially suppresses the HU and UV sensitivity of Δrqh1 cells. In addition, unpublished data from our laboratory show that loss of either rhp51⁺ or rhp57⁺ completely or nearly completely suppresses the HU or UV sensitivity of Δrqh1. One

proposed function of *SRS2* in *S.cerevisiae* is to act downstream of *RAD51* to stabilize recombination intermediates (39). By enhancing recombination, Srs2 might increase the HU and UV sensitivity of Δ*rqh1* cells. This proposed function might explain why the loss of Srs2 improves the survival of Δ*rqh1* cells when challenged with UV or HU. Obviously, further investigation will be necessary to answer this question with certainty.

ACKNOWLEDGEMENTS

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