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Srs2 overexpression reveals a helicase-independent role at replication forks that requires diverse cell functions

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Abstract

Srs2 is a 3' to 5' DNA helicase that regulates many aspects of DNA metabolism in *Saccharomyces cerevisiae*. It is best known for its ability to counteract homologous recombination by dismantling Rad51 filaments, but is also involved in checkpoint activation, adaptation and recovery, and in resolution of late recombination intermediates. To further address its biological roles and uncover new genetic interactions, we examined the consequences of overexpressing *SRS2* as well as two helicase-dead mutants, *srs2-K41A* and *srs2-K41R*, in the collection of 4827 yeast haploid deletion mutants. We identified 274 genes affecting a large variety of cellular functions that are required for cell growth when *SRS2* or its mutants are overexpressed. Further analysis of these interactions reveals that Srs2 acts independently of its helicase function at replication forks likely through its recruitment by the sumoylated PCNA replication clamp. This helicase-independent function is responsible for the negative interactions with DNA metabolism genes and for the toxicity of *SRS2* overexpression in many of the diverse cellular pathways revealed in our screens.

Keywords

Replication; Repair; Recombination; Srs2; Overexpression; Genetic interactions

1. Introduction

Maintaining genome integrity is crucial to all living cells. The genome is constantly exposed to different DNA damaging agents that can be endogenous, such as side products of normal metabolism, or exogenous, such as UV irradiation. DNA damage can also arise as a consequence of physiological processes such as DNA replication or transcription, leading to accumulation of single-stranded DNA and eventually to DNA double-strand breaks (DSBs) [1, 2]. Several mechanisms are known to repair different types of lesions. In *Saccharomyces cerevisiae*, homologous recombination (HR) is a major pathway for the repair of DSBs and depends on the *RAD52* epistasis group (for reviews see [3–5]). HR is also important for the

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restart of stalled replication forks (reviewed in [6, 7]), although damage at the fork can also be overcome by post-replicational repair mechanisms (PRR) dependent on the *RAD6* pathway. Repair mechanisms therefore compete at sites of DNA damage, particularly in the context of a damaged replication fork.

The Srs2 helicase likely plays a role in the regulation between PRR and HR at replication forks. The *SRS2* gene encodes a 3' to 5' DNA helicase for which mutants were originally identified as suppressors of the ultraviolet sensitivity of *rad6* and *rad18* mutations, which are defective in PRR [8, 9]. *SRS2* was also independently identified as a hyper-recombination mutant, *hpr5* [10]. As the suppressive effect of *srs2* on *rad6* and *rad18* repair defects requires a functional recombination machinery [11], these studies lead to the notion that Srs2 acts as an "anti-recombinase". Biochemically, Srs2 has been shown to translocate on single-stranded DNA to disrupt Rad51 nucleoprotein filaments, which are required for homologous recombination to occur [12, 13]. More recently, the Srs2 protein was also shown to be recruited to replication forks by the replication clamp PCNA (Proliferating Cell Nuclear Antigen), preferentially when the clamp is SUMOylated [14–16]. These observations suggest the current model in which Srs2 is recruited to replication forks by SUMOylated PCNA where it removes Rad51 filaments thus preventing homologous recombination during replication and favoring PRR [14, 15, 17–19]. However, Srs2 acts as an anti-recombinase also independently of its recruitment to replication forks by PCNA [20, 21], suggesting there are additional roles for the anti-recombinase function outside of DNA replication.

This important regulator of DNA metabolism appears to have multiple roles in different aspects of DNA metabolism (reviewed in [22, 23]) but how these roles are achieved and coordinated is not clearly understood. For example, *SRS2* participates in the checkpoint response upon detection of DNA damage. Cdk1 phosphorylates the Srs2 protein in a checkpoint-dependent manner and in turn likely contributes to the proper activation of the checkpoint [24]. It may further modulate the response to DNA damage together with the Mre11 and Sgs1 proteins [24]. Srs2 has also been proposed to participate in the resolution of late recombination intermediates, promoting synthesis dependent strand annealing (SDSA) rather than crossover formation [25–27]. This SDSA promoting activity was recently linked to its phosphorylation by Cdk1 suggesting functional crosstalk between the checkpoint and repair functions of Srs2 [28]. Thus, Srs2 is at the crossroads of DNA replication and repair processes and the understanding of its complex functions in each remains incomplete. Since the expression of *SRS2* is tightly regulated - both mRNA and protein levels reach a maximum in early S-phase [29, 30] - part of the complexity of its function is likely due to its roles at various stages of the cell cycle in different cellular contexts. In addition, overexpression of Srs2 is known to impair cell growth both in *Saccharomyces cerevisiae* and in *Schizosaccharomyces pombe*, although the basis of this toxicity remains unknown [12, 31, 32]. Therefore, we sought to investigate the regulatory roles of Srs2 by affecting its levels in the cell.

Here, we present three synthetic dosage lethality (SDL) screens designed to gain better insight into the multiple roles of Srs2. In the past, SDL screens have proven extremely powerful in identifying functional relationships between genes involved in the same processes or protein complexes [33–35]. For example, new components of the kinetochore complex were discovered by searching for mutations that are lethal in combination with the overexpression of one kinetochore component [36]. Importantly, SDL interactions do not necessarily overlap with classical synthetic lethal interactions and potentially reveal new genetic and/or physical associations ([37] and present study). SDL thus provides a complementary and global view of the complex genetic interactions in which a gene is

involved. The study of SDL interactions of mutant versions of a gene should also provide additional functional relevance to the genetic interactions with the wild-type gene.

Thus, we have performed screens for genes required for cell growth in conditions of controlled overexpression of either wild-type *SRS2*, or two helicase-dead mutants, *srs2-K41A*, which is unable to bind ATP, and *srs2-K41R*, which can bind ATP but cannot hydrolyze it [38]. Upon screening the haploid yeast disruption library [39], we found 274 SDL interaction with genes involved in very diverse cellular functions. Then, we show that overexpression of *SRS2* specifically perturbs replication progression independently of its helicase function. This effect is not mediated by Rad51, one of the known Srs2-interacting partners. Instead it relies on the interaction of Srs2 with the sumoylated PCNA replication clamp, as overexpression of an *srs2* mutant that cannot interact with the clamp, *srs2-R1*, abolishes the replication defects. Importantly, overexpression of *srs2-R1* also abolishes the sensitivity of many mutants found in the screens that affect diverse cellular functions.

2. Material and methods

2.1. Yeast strains and growth media

Strains deleted for genes in the BY4742 background were obtained from the *Saccharomyces* Genome Deletion Project [39]. The plasmids were transformed in these strains by lithium acetate transformations [40]. The genotypes of other strains used in this study are listed in Table 1. Yeast strains carrying an overexpression plasmid were grown in synthetic complete medium lacking leucine (SC-LEU): 20 μg/ml adenine, 20 μg/ml L-arginine, 20 μg/ml L-histidine, 30 μg/ml L-isoleucine, 30 μg/ml L-lysine, 20 μg/ml L-methionine, 50 μg/ml L-phenylalanine, 20 μg/ml L-tryptophan, 30 μg/ml L-tyrosine, 17 Ug/ml uracil, 150 μg/ml L-valine, 0.17% Yeast Nitrogen Base, 0.5% $(NH_4)_2SO_4$, 2% D-glucose. To allow a proper cell cycle progression after alpha-factor arrest in G1, cells were released in SC-5X medium (5-fold enriched in the above aminoacid, adenine and uracil).

2.2. Plasmids

The *CUP1* promoter was fused to *SRS2*, *srs2-K41A* or *srs2-K41R* by PCR using adaptamers [41]. The resulting PCR products were cloned into a centromeric plasmid pRS415 [42] carrying the selection marker *LEU2*, generating plasmids pWJ1509 (*pCUP1-SRS2*), pWJ1510 (*pCUP1-srs2-K41A*) and pWJ1511 (*pCUP1-srs2-K41R*). The srs2-*R1* C-terminal mutation was amplified by PCR from pRS406-*srs2-R1* (kind gift from Xavier Veaute) and introduced by cloning into pRS415 to generate pAM19 (*pCUP1-srs2-R1*), pAM20 (*pCUP1-srs2-K41A-R1*) and pAM21 (*pCUP1-srs2-K41R-R1*). *ELG1-13 MYC* was amplified by PCR from strain MK11241 (kind gift from Martin Kupiec) and cloned by gap-repair downstream of the *CUP1* promoter of pWJ1512 [43], generating pAM53.

2.3. Synthetic Dosage Lethality screen

The SDL screen was done by selective ploidy ablation as previously reported [43]. Briefly, the universal donor strain W8164-2C was transformed with the *LEU2*-marked overexpression plasmids. This haploid strain carries sixteen *GAL1* promoters that induce transcription across its 16 centromeres upon induction, perturbing correct chromosome segregation. It contains the *K. lactis URA3* gene upstream of each of the *GAL1* promoters, allowing for counter-selection of all of its chromosomes upon plating onto 5-fluoroorotic acid (5-FOA). The plasmid carrying donor strains were crossed to the 4827 MATα strains of the yeast disruption library, previously rearranged in plates containing 384 strains in quadruplicate. The diploids were plated onto galactose to allow missegregation of the chromosomes of the donor, and on 5-FOA plates lacking leucine to counter-select for cells that have kept chromosomes of the donor and select for the overexpression plasmid. Plating

onto medium without or with 100 μM $CuSO_4$ induces overexpression driven by the *CUP1* promoter on the plasmid. The plates obtained were scanned and the images analyzed using *ScreenMill* [44]. We retained as SDL interactors the strains for which the colonies were more than 2-fold smaller with at least one of the overexpression plasmids than with the empty vector in the same growth conditions. To verify individual SDL interactions, the overexpression plasmids were transformed into the library strains. We verified by protein dot-blots that each of the transformants effectively overexpressed *SRS2, srs2-K41A* and *srs2-K41R*. Growth assessment by spot assays were performed upon overnight cultures in SC-LEU and dilution to obtain cultures at 10^7 cells/ml. Five serial dilutions to one tenth were done from this first dilution, and 10 μl of each of the five dilutions was spotted onto SC-LEU plates containing increasing amounts of copper sulfate (0, 50, 100 and 200 μM $CuSO_4$). The plates were incubated for 3 days at 30°C and scanned. Among, the 73 SDL interactions with DNA metabolism-related genes originally found in the screens, eleven could not be tested either because no transformants could be obtained or because no transformants were found to effectively overexpress the *SRS2* plasmids. Among the remaining 62 strains, 51 SDL interactions were definitively validated. Nine additional deletion strains not found in the screen but functionally related to the strains found were tested, 6 of which were found to be SDL with *SRS2* or its mutants (*CTF8, HHF2, RAD50, RAD55, RAD57, RAD59*). Thereafter, we will refer to the SDL interactions of 57 genes related to DNA metabolism.

2.4. Protein analyses

For denaturing protein extractions, overnight cell cultures in SC-LEU were diluted and allowed to grow for 2 hours. Upon addition of 200 μM $CuSO_4$ the cells were incubated for 4 hours to induce *CUP1*-promoter driven gene expression. The cultures were centrifuged, the pellets resuspended in 100 μl of cold 1.85 N NaOH, 7.5% 2-mercaptoethanol and incubated for 10 minutes in ice. Protein was precipitated upon addition of 30 μl of cold 50% Trichloroacetic acid (Sigma, T0699). After vigorous vortexing, the samples were incubated for another 10 minutes in ice, and the precipitates were recovered upon a 5-min centrifugation at 4°C, maximum speed. The precipitates were resuspended in 40 mM Tris pH6.8, 8 M Urea, 5% SDS, 0.1 mM EDTA, 7.5% 2-mercaptoethanol, 10 μg/ml Bromophenol Blue. The supernatants were recovered upon a 5 minute centrifugation at room temperature, maximum speed, frozen in liquid nitrogen and stored at −80°C. For native protein extractions, overnight cell cultures in SC-LEU were diluted and allowed to grow for 2 hours. Upon addition of $CuSO_4$ the cells were incubated for 4 hours to induce *CUP1*-promoter driven gene expression. The extractions were done in ice. The pellets were resuspended in 50 mM Tris pH 8, 1% NP-40, 150 mM NaCl, 1 mM DTT, 30 mM NaF, 1mM PMSF, 0.75 μg/ml Pepstatin A, supplemented with Roche Complete protease inhibitors (Roche, 11836153001). For each phosphatase treatment, three samples were generated. First, an “untreated” sample, corresponding to the native extracts described above immediately denatured upon addition of 6X sample buffer (35 mM Tris pH 6.8, 30% Glycerol, 10% SDS, 0.6 M DTT, 0.012% bromophenol blue). Second and third, a “mock” sample and a “phosphatase-treated” sample. These were incubated for 1 hour at 30°C with 10X phosphatase buffer, 10X $MnCl_2$, 20X Roche Complete phosphatase inhibitors (Roche, 11836153001) and without or with lambda-phosphatase (New England Biolabs, P0753S) respectively. The reactions were stopped by addition of 6X sample buffer. For protein blotting, samples were loaded on SDS-PAGE gels (6% for visualizing Srs2, 8% for visualizing Rad53 and 10% for visualizing alpha-tubulin). Migration was carried out at 150–200V in running buffer (25 mM Tris, 190 mM Glycine, 0.1% SDS), and the proteins were transferred at 25V in transfer buffer (12 mM Tris, 100 mM Glycine, 20% EtOH). The membranes were blocked in 5% milk – PBS Tween for 1 hour at room temperature, incubated with primary antibodies overnight at 4°C in 1% milk – PBS-Tween, and finally

incubated with the secondary antibodies for 1 hour at room temperature. The anti-Srs2 antibody (Santa Cruz Biotechnology, sc-11989) was used as a 1:200 dilution. The anti-Rad53 antibody (Santa Cruz Biotechnology, sc-6749) was used as a 1:750 dilution. The anti-Myc antibody (Santa Cruz Biotechnology, sc-40) was used as a 1:200 dilution. The anti-alpha-Tubulin antibody (AbD Serotec, MCA77G) was used as a 1:2000 dilution. The secondary antibodies used were an HRP-coupled anti-goat antibody (Promega, V8051) as a 1:5,000 dilution, an HRP-coupled anti-rat antibody (Jackson ImmunoResearch Laboratories, 112035062) as a 1:10,000 dilution and an HRP-coupled anti-mouse antibody (Jackson ImmunoResearch Laboratories, 115035146) as a 1:10,000 dilution. Samples from time-courses after alpha-factor arrest were quantified using the RC DC Protein Assay (BioRad, 500-0120) to load the same amount of protein in each well.

2.5. Cell synchronization

*MAT***a** *bar1Δ* cells were grown at 30°C to exponential phase (OD_{600}~0.5) in SC-LEU medium. To induce G1 arrest, cells were incubated for 2 hours with 50 ng/ml alpha-factor (GenWay, 06–271–83056). Thirty minutes prior to release, $CuSO_4$ was added to 200 μM to induce *CUP1* promoter-driven gene expression, and leucine was added to 300 μg/ml for a proper release. To release cells from the alpha-factor arrest, cells were washed twice with one volume of SC-5X medium containing 200 μM $CuSO_4$ and 50 μg/ml pronase (Sigma, P6911). They were finally resuspended in one volume of the latter medium. After release, samples were collected every 15 minutes for FACS analysis and protein extraction.

2.6. Flow cytometry

Cells were prepared for FACS analysis as previously reported [45]. Briefly, cell pellets were fixed on a rotator at 4°C overnight in 70% ethanol. In 50 mM sodium citrate pH 7.0, cells were sonicated for 15 seconds at 30%, incubated with 0.25 mg/ml RNase A for 1 hour at 50°C, washed and finally stained with 16 μg/ml propidium iodide (Sigma, 81845). Samples were then analyzed on a Becton Dickinson FACScalibur, and the resulting data was compiled using WinMDI 2.9.

3. Results

3.1. Genes required for cell viability upon overexpression of SRS2 and/or its helicase mutants srs2-K41A and srs2-K41R

To identify the biological processes dependent on the dosage of the Srs2 protein, we carried out three synthetic dosage lethal screens. Plasmids overexpressing *SRS2*, the helicase mutant *srs2-K41A*, which is unable to bind ATP, and the helicase mutant *srs2-K41R*, which is capable of binding ATP but unable to hydrolyze it [38], were analyzed. To accomplish overexpression, the three genes were cloned into centromeric plasmids under control of the *CUP1* promoter [46] (Fig. 1A). Expression levels of the proteins increase as a function of the amount of $CuSO_4$ in the medium, allowing controlled overexpression (Fig. 1B). Individual plasmids were introduced into the 4827 haploid MATα strains deleted for non-essential open-reading frames [39]. With the exception of the *srs2Δ* strain from this library, all of the other strains contain the endogenous wild-type *SRS2* so cells maintain an unperturbed level of the wild-type protein before overexpression is induced. The three overexpression plasmids and an empty vector control were transferred by selective ploidy ablation (SPA) into an arrayed version of the deletion library in which each strain is quadruplicated ([43] and Materials and methods). The plasmid-carrying strains were then replica-plated onto solid medium containing 100 μM $CuSO_4$ to induce overexpression and were grown for three days. Cell growth was measured using *ScreenMill*, a colony size measurement and analysis tool [44]. We chose to screen for SDL interactions at 100 μM $CuSO_4$ because *SRS2* or the helicase-dead mutants did not affect the growth of wild-type

cells when expressed at this level (Fig. 1C). We identified 274 SDL interactions corresponding to genes required for cell growth in the context of overproduction of at least one of the forms of the Srs2 protein.

Table 2 lists the genes found in the screen sorted by cellular function. As expected for a regulator of homologous recombination, 68 genes involved or related to different aspects of DNA metabolism were found. A large majority was individually confirmed providing a final list of 57 genes (Material and methods). Additionally, genes involved in other diverse cellular functions were found. The major classes are vesicular traffic (40 genes), mitochondrial functions (38 genes), RNA metabolism (26 genes) and ribosome functions (19 genes). To check if these genetic interactions resulted from drastic change in Srs2 overexpression, we examined the level of Srs2 in the DNA metabolism genes further analysed below and in a random subset of 21 strains representing the other Gene Ontology categories. Compared to the wild-type cells, the maximal variation was 1.6-fold overexpression in *bro1Δ* cells (Supplementary Fig. S1). Therefore, the SDL interactions cannot be simply explained by a difference in the Srs2 overexpression levels. Rather, it reveals that, although *SRS2* is mostly known for its role in DNA metabolism, tolerance to changes in its global level requires many cellular functions.

3.2. Cells overexpressing SRS2 or its helicase mutants impact DNA replication-related functions

We first examined the effects of overexpression of *SRS2* on DNA metabolism genes (Table 2). We individually verified each of the SDL interactions related to DNA metabolic processes by transforming the overexpression plasmids into each strain and assessing the growth of the transformants by spot-assays on plates with or without $CuSO_4$ (Materials and methods, Fig. 1C). After validation, 57 strains deleted for genes involved in DNA metabolism show sensitivity to overexpression of wild-type or mutant *SRS2*. These deletion strains behave differently with regard to the three overexpression plasmids. For example, like *elg1Δ*, *mcm22Δ* and *cbf1Δ* (Fig. 1C), a subset of strains is equally sensitive to all three alleles of *SRS2*. In contrast, strains like *mre11Δ* and *rmi1Δ* (Fig. 1C) are very sensitive to overexpression of the helicase mutants *srs2-K41A* and *srs2-K41R*, but are less or not at all sensitive to wild-type *SRS2*. Fig. 1D compiles, in a heat-map, the sensitivity profiles of all the strains that were independently tested. Overall, overexpression of wild-type and helicase-mutant *SRS2* is very toxic when DNA metabolism is compromised.

The sensitivity profiles of the strains shown in Fig. 1D show that functionally-related genes exhibit a similar phenotype. For example, cells deleted for genes involved in DNA replication or in kinetochore structure and chromosome segregation are equally sensitive to wild-type or helicase-dead *SRS2*, whereas cells deficient in DNA repair are more sensitive to the helicase mutants than to wild-type *SRS2*. Moreover, among the mutants deficient in homologous recombination, only a subset (*rad52Δ*, *rad55Δ*, *rad57Δ*, *rad59Δ*, *mre11Δ*, *rad50Δ* and *xrs2Δ*) is sensitive to overexpression of helicase-dead *srs2*, while *rad51Δ* and *rad54Δ* are not.

Surprisingly, no case was found in which sensitivity to wild-type *SRS2* was suppressed by inactivating the helicase function of the gene, suggesting that its helicase function is not responsible for the SDL interactions. Instead, several strains are more sensitive to the helicase mutants than to wild-type *SRS2*. Therefore, the lack of helicase function makes the mutant protein more toxic, further compromising DNA metabolism and requiring an extra set of genes that are involved in DNA repair processes. To decipher the complex effect of overexpression of *SRS2* and its helicase mutants, we more closely examined the molecular and cellular consequences of overproduction of these proteins.

3.3. Overexpression of wild-type and helicase-dead SRS2 requires the DNA replication checkpoint

Deletion of genes involved in DNA replication, including the three checkpoint mediators *MRC1, TOF1* and *CSM3*, causes sensitivity to overexpression of all three versions of *SRS2* (Figs. 1D and 2A). However, deletion of another checkpoint mediator, *RAD9*, does not affect sensitivity to overexpression of any of the alleles of *SRS2* (Fig. 2A). While *MRC1, TOF1* and *CSM3* mediate the replication checkpoint [47–50], *RAD9* mainly mediates the DNA damage checkpoint [48, 49, 51], suggesting that overexpression of wild-type and helicase-dead *SRS2* affects replication but does not directly cause DNA lesions.

Next, we examined the status of the Rad53 checkpoint protein. Slow migrating forms of the Rad53 protein appear upon overexpression of *SRS2* and even more extensively with the helicase-dead mutants (Fig. 2B, left panel). Noticeably, the helicase-dead mutants cause a more extensive shift of Rad53 (Fig. 2B and Fig. 2C), consistent with the observation that these mutants are often more toxic than wild-type *SRS2*. These forms correspond to phosphorylated versions of Rad53, as they are sensitive to a phosphatase treatment (Fig. 2C). These results, together with the requirement of the replication checkpoint mediators *MRC1, TOF1* and *CSM3* argue that overexpression of *SRS2* or the helicase-dead mutants activates the DNA replication checkpoint.

To establish a link between the replication checkpoint mediators and the activation of Rad53, we investigated the phosphorylation state of Rad53 in *mrc1Δ* cells. In the absence of *MRC1*, Rad53 is constitutively phosphorylated in control cells that do not overexpress Srs2 (Fig. 2B, right panel), as previously reported [48], and no additional phosphorylation (upper smear bands) occurs upon overexpression of *SRS2* or the helicase-dead mutants. Finally, we also examined overexpression in *mec1Δ, tel1Δ* and *mec1Δ tel1Δ* strains (where deletion of *MEC1* was combined with deletion of *SML1* to suppress lethality). As illustrated in Fig. 2D, Srs2 overexpression is tolerated in single mutants but is very toxic in the absence of both Mec1 and Tel1. Altogether, these results directly link checkpoint activation, as measured by Rad53 phosphorylation, with the requirement of the Mec1/Tel1 kinases and the Mrc1 mediator for cell growth upon *SRS2* overexpression.

3.4. Overexpression of wild-type and mutant SRS2 delays S-phase progression

Next, we investigated cell cycle progression. Cells overexpressing wild-type or helicase-dead *SRS2* were arrested in G1 using alpha-factor and overexpression was induced by addition of $CuSO_4$ prior to release from the G1 arrest. Progression of the resulting synchronous cultures was assessed by flow cytometry to evaluate DNA content (Fig. 3A). Upon release, control cells containing an empty vector begin entering S-phase by 15 minutes, and complete replication between 45 and 60 minutes. Cells overexpressing *SRS2* show a similar progression through the cell cycle, although a slight delay is detected at 45 minutes post-release. This delay is aggravated in cells overexpressing the helicase mutant *srs2-K41A* as seen by FACS at 30 and 45 minutes post-release. However, the cells manage to fully complete replication between 60 and 75 minutes (Fig. 3A).

Total protein extracts from the same synchronous cultures shown in Fig. 3A reveal the status of Rad53 through the cell cycle (Fig. 3B). In asynchronous populations, before $CuSO_4$ induction, Rad53 is phosphorylated at a low level in cells containing an empty vector, *SRS2* or *srs2-K41A* (as indicated by the slow migrating band in Fig. 3B, lanes labeled “As.”). In the cells containing the empty vector, G1 cells (0 time-point) show no detectable phosphorylation of Rad53. A low level of phosphorylation then appears at 45 minutes, when replication is almost complete. Like the control cells, synchronous cells overexpressing *SRS2* begin to show Rad53 phosphorylation at 45 minutes post-release, with Rad53

becoming more extensively phosphorylated at 60 and 75 minutes. Cells overexpressing *srs2-K41A* show an increased phosphorylation of Rad53 as early as 30 minutes post-release, which correlates with the time at which the S-phase progression defect is detected by flow cytometry. Thus, overexpression of *SRS2* and to a larger extent, overexpression of *srs2-K41A*, causes an S-phase progression delay associated with the induction of Rad53 phosphorylation (Fig. 2E), *i.e*. the activation of the replication checkpoint.

3.5. The toxicity of SRS2 and the helicase-dead mutants on DNA replication is independent of RAD51

Since a well characterized role of Srs2 is its anti-recombinase function and the dismantling of Rad51 presynaptic filaments [12, 13, 21], we hypothesized that the inability to regulate Rad51 filaments could explain at least part of the toxicity induced by overexpression of helicase-dead *SRS2*. This view would account for the fact that *rad51Δ* cells are not sensitive to overexpression of these mutants while other recombination mutants are sensitive and predicts that deleting *RAD51* would abolish the toxic effects of the helicase-dead mutants on DNA integrity and specifically on DNA replication. However, Rad53 is still strongly phosphorylated in a *rad51Δ* strain upon overexpression of wild-type or helicase-dead *SRS2* (Fig. 3C). Furthermore, in a *rad52Δ* strain, deleting *RAD51* does not abolish sensitivity to the helicase mutants (Fig. 3D). These results show that the toxicity of the overexpressed *SRS2* genes is not mediated by the Rad51 protein, but rather the excess Srs2 protein is causing its toxic effects directly. Additionally, Rad51-dependent homologous recombination is not important for overcoming the toxic effects inflicted by the *srs2* helicase mutants or by wild-type *SRS2*, since *rad51Δ* cells are not sensitive to their overexpression.

3.6. The effect on replication depends on the domain of Srs2 responsible for its interaction with SUMOylated PCNA

Srs2 interacts with the PCNA replication clamp when the clamp is SUMOylated during S-phase [14–16]. To test whether this interaction is involved in the effect of overexpressed Srs2 on SDL interactions, DNA replication and Rad53 phosphorylation, we examined the behavior of a mutated version of *SRS2, srs2-R1*, unable to interact with PCNA [20], and also combined *-R1* with the helicase mutations. As illustrated in Fig. 4A, overexpression of *srs2-R1* no longer sensitizes *mrc1Δ* cells and *-R1* suppresses the sensitivity of *mrc1Δ* cells to helicase-dead *srs2*. Globally, *srs2-R1* overexpression suppresses the SDL interactions with several replication mutants (*cnn1Δ, csm3Δ, ctf3Δ, ctf8Δ, ctf18Δ, dcc1Δ, elg1Δ, hhf1Δ, hhf2Δ, nkp1Δ, pol32Δ, tof1Δ)* and partially suppresses the sensitivity of the *mec1Δ tel1Δ* double mutant, in both the presence or absence of helicase function. This is not due to a decrease in protein since the *-R1* mutant protein levels are comparable to Srs2, Srs2-K41A and Srs2-K41R levels at the same $CuSO_4$ concentration (Supplementary Fig. S1C). Surprisingly, in *rad27Δ* cells, *srs2-K41A-R1* or *srs2-K41R-R1* mutations abolish the growth defect associated with *srs2-K41A* or *srs2-K41R* overexpression, but the *-R1* mutation does not abolish the toxicity of *SRS2* overexpression (Fig. 4A). Therefore, our results suggest that the *-R1* mutant proteins have lost the ability to perturb DNA replication. Accordingly, the S-phase progression delay induced by *srs-K41A* is no longer seen in *srs2-K41A-R1* cells (Fig. 4B). Finally, phosphorylation of Rad53 is greatly diminished upon overexpression of *srs2-R1* compared to *SRS2* (Fig. 4C). However, loss of the PCNA interaction did not abolish the Rad53 phosphorylation induced by the *srs2-K41A* mutation, suggesting that part of the checkpoint activation induced by the helicase mutants does not depend on their recruitment to replication forks.

Then we tested whether the *−R1* mutation affects the sensitivity of those mutants that are only sensitive to helicase-dead *srs2-K41A* and *srs2-K41R*. Interestingly, *rad52A* cells are no longer sensitive to *srs2-K41A-R1* or *srs2-K41R-R1* (Fig. 4D). The same suppression is seen

in other repair deficient strains, *ccr4Δ, mre11Δ, rmi1Δ, top3Δ*, suggesting that the helicase defect lead to DNA replication-dependent accumulation of structures that are resolved by recombination and repair.

Moreover, we noticed that *srs2Δ* cells are sensitive to the helicase mutants even in the absence of induction (0 μM $CuSO_4$) and this sensitivity is suppressed by the *−R1* mutation (Fig. 5A). Indeed, in the absence of $CuSO_4$, helicase-mutant protein expression in *srs2Δ* cells is even lower than that of endogenous Srs2 (Fig. 5B).

Finally, unlike overexpression of Srs2, overexpression of Elg1, another protein known to preferentially interact with sumoylated PCNA [52] does not induce a strong Rad53 mobility shift (Fig. 5C). Together, these results demonstrates that even low levels of the helicase mutant proteins can perturb the replication fork, and that their overexpression is not simply clogging the PCNA clamp, nor a general property of PCNA-interacting proteins.

3.7. Srs2-R1 also suppresses the SDL interactions with deletions affecting various cellular functions

As listed in Table 2, overexpression of Srs2 impairs growth not only in mutants deficient in DNA metabolism processes, but also in mutants of RNA metabolism, ribosomal functions, mitochondrial functions, vesicular traffic, etc. To test whether or not the SDL interactions with these various classes of functions depended on the interaction of Srs2 with sumoylated PCNA, we examined the effect on cell growth of overexpressing Srs2-R1 in 18 randomly chosen mutants from these various cellular processes. Remarkably, 17 SDL interactions are suppressed by the *srs2-R1* mutation: *tda1Δ, tda2Δ, tda4Δ, tda6Δ, tda11Δ, bro1Δ, doa4Δ, stp22Δ, vps4Δ, vps24Δ, vps25Δ, vps27Δ, vam3Δ, ynr005cΔ, eap1Δ, nut1Δ, lsm6Δ*. Thus, the sensitivity of these strains to Srs2 overexpression is likely due to replication defects. In contrast, *fet3Δ* cells, deficient in iron ion transport, are very sensitive to both *SRS2* and *srs2-R1* overexpression (Fig. 4E), uncovering a novel type of genetic interaction that will warrant further investigation.

4. Discussion

Srs2 is a 3’-5’ DNA helicase with multiple roles at the crossroads of DNA metabolism processes. To gain better insight into the complexity of these roles, we performed three synthetic dosage lethal screens by overexpression of *SRS2* and two helicase-dead mutants, *srs2-K41A* and *srs2-K41R*, in the 4827 strains of the haploid yeast disruption library. We find that overexpression is toxic in a diverse set of deletion mutant strains showing that many cellular functions are involved in resistance to the activities of *SRS2*. We demonstrate that *SRS2* and the helicase-dead mutants cause toxicity associated with DNA replication defects and depends on the interaction with sumoylated PCNA. In addition, the physiological sensitivity of yeast cells to the overexpression of Srs2 may explain the long-standing observation that it is toxic in some genetic backgrounds [12, 31, 32].

4.1. A variety of cellular functions are required for cell growth upon overexpression of SRS2

From our large-scale SDL screens, we find 274 genes required for cell growth upon overexpression of *SRS2*, *srs2-K41A* and/or *srs2-K41R*. Remarkably, these genes are involved in a variety of cellular processes (Table 2). A large number clearly relate to DNA metabolism functions, as expected for a gene that plays an important role in regulating many aspects of DNA recombination and repair. Consistent with the known functions of Srs2, thirty of these genes show a synthetic growth defect with *RAD51* (Supplementary Table S1). More surprisingly, other well-represented Gene Ontology categories, *e.g.*, vesicular

trafficking, mitochondrial function, RNA metabolism and ribosomal function, are also needed for cell growth upon overexpression of *SRS2* or the helicase mutants. It is important to note that most of these unexpected functions are not systematically uncovered in other SDL screens (([33–37], RJDR, JCD and RR, unpublished observations), indicating that the corresponding processes are not affected by the overexpression of just any protein in the cell. The conclusion that these SDL interactions are specific to a function of Srs2 is reinforced by the observation that cell growth is not impaired in the vast majority of mutants upon overexpression of the *srs2-R1* separation-of-function mutant protein.

Upon literature data mining, we noted that 57 of the 206 genes, which at first approximation are not known to be associated with DNA metabolism, actually have reported phenotypes related to DNA metabolic processes (*e.g*., sensitivity to hydroxyurea, camptothecin, methylmethane sulfonate, ionizing radiation, UV or abnormal telomere length or increased recombination centers) (Supplementary Table S2). Thus, for these 57 genes, the SDL interactions with *SRS2* and its mutants are likely due to an interference with DNA metabolism. Furthermore, although the data from the previously cited screens may contain false positives, these same genes are also SDL with *SRS2* suggesting that they should be classified in DNA metabolism-related gene ontology processes. One of the unexpected classes, vesicular trafficking genes, was also enriched in an SDL screen with a mutant version of *TOP1*, *top1-TA*, which mimics camptothecin-induced damage [43]. The Top1-TA protein nicks double-stranded DNA, but remains covalently attached to it, thus generating DNA damage [53]. It will be interesting to investigate how Top1-TA and Srs2 overexpression, which both affect genome integrity, require genes involved in vesicular trafficking.

4.2. The anti-recombinase function of Srs2 is not responsible for its overexpression effects

Srs2 is an important anti-recombinase that displaces Rad51 presynaptic filaments from single-stranded DNA in a helicase-dependent manner [12, 13, 21, 38]. By overexpressing Srs2, we expected to uncover deletion mutants sensitive to a low cellular level of *RAD51*-dependent recombination. If this view were correct, we would expect the SDL genes from overexpression of *SRS2* to be known synthetic lethal with *RAD51*. However, only 30 of the 274 SDL interacting genes found in our screens are synthetic lethal with *RAD51* (Supplementary Table S1). Moreover, for these 30 genes, most are SDL not only with *SRS2*, but also with the helicase-dead mutants *srs2-K41A* and *srs2-K41R*. Therefore, the SDL interactions do not rely on the helicase function of Srs2 and the lethality is not due to an excessive Srs2 anti-recombinase activity.

4.3. Overproduced Srs2 affects DNA replication through its domain that interacts with sumoylated PCNA

By investigating the cellular consequences of Srs2 overexpression, we find that wild-type or helicase-mutated Srs2 primarily affect progression of DNA replication. First, a number of key non-essential genes involved in DNA replication are required for cell growth when *SRS2*, *srs2-K41A* or *srs2-K41R* are overexpressed (Table 2). Second, *SRS2* and its mutants cause replication checkpoint activation dependent on the Mec1/Tel1 kinases, mediated by Mrc1, Tof1 and Csm3 and resulting in the phosphorylation of Rad53 (Figs. 1 and 2). Third, *SRS2* and to a larger extent the helicase-dead mutants induce an S-phase progression delay, which is temporally associated with the activation of the checkpoint (Fig. 3). Interestingly, most of these defects are abolished by mutating the PCNA interaction domain on *SRS2* or the helicase-dead mutants (*srs2-R1*, *srs2-K41A-R1* or *srs2-K41R-R1*) [20] (Fig. 4). These separation-of-function mutants suggest that Srs2 wild-type or helicase-dead proteins are recruited to replication forks through their interaction with PCNA, possibly generating fork

progression defects that trigger checkpoint activation. Since the *srs2-R1* mutation abolishes the SUMO interacting motif of Srs2 (SIM) [16,20], we assume that it is the interaction with sumoylated PCNA that is important, more than the interaction with the unmodified clamp. Formally, if Srs2 interacts with other sumoylated proteins through the same binding site, other targets cannot be excluded. Alternatively, Srs2 may directly activate the replication checkpoint upon its recruitment by PCNA without affecting or compromising fork stability. However, the growth of *mrc1Δ, tof1Δ* and *csm3Δ* cells would not be affected in this case. Since their viability is compromised upon overexpression of *SRS2* and its mutants, we favor a model in which the excessive presence of Srs2, regardless of the status of its helicase activity, perturbs fork stability, which in turn activates the replication checkpoint controlled by these proteins. Our studies also show that overexpression of the *srs2-K41A* and *srs2-K41R* helicase mutants is more toxic than *SRS2* and requires additional genes involved in DNA repair (*RAD52, MRE11, RAD50, XRS2, NTG2*, etc., Fig. 1D). Interestingly, this toxicity is also abolished with the separation-of-function mutants, *srs2-K41A-R1* or *srs2-K41R-R1*, again suggesting that it is through DNA replication that the helicase-dead mutants generate DNA damage or an aberrant intermediate that requires repair genes for resolution. Thus, as depicted in the model presented in Fig. 6, we propose that excess Srs2 recruited to the PCNA replication clamp affects normal replication progression (Panel 1). Perhaps Srs2 prevents other DNA replication factors from easily accessing the fork. Since Srs2 protein levels are highest at the beginning of S-phase [29, 30], we suggest a helicase-independent role for Srs2 during S-phase, which differs from its anti-recombinase function, to prevent the recruitment of proteins that might compromise replication. Our findings likely reflect this role for the endogenous protein, since even very low levels of Srs2-K41A or Srs2-K41R are toxic to *srs2Δ* and this toxicity is suppressed by mutating the *−R1* domain responsible for the interaction with sumoylated PCNA (Fig. 5 A and 5B).

rad27Δ cells are unique in that Srs2 toxicity is independent of its interaction with the PCNA clamp, but toxicity depends on the helicase function of the protein (Fig. 4A). As *rad27Δ* cells require homologous recombination for survival [54–56], SDL interaction of *SRS2* with *RAD27* may be due to an increased inhibition of recombination by *srs2-R1* that does not occur upon overexpression of the helicase-deficient mutants *srs2-K41A-R1* and *srs2-K41R-R1*. Alternatively, since Rad27 is a flap endonuclease important for Okazaki fragment maturation, this particular SDL may reflect a helicase-dependent role of Srs2 in processing Okazaki fragments.

In contrast to overexpression of the *srs2-R1* mutant, phosphorylation of Rad53 is not abolished or diminished by overexpression of *srs2-K41A-R1* or *srs2-K41R-R1* (Fig. 4C). Therefore, the helicase mutants induce Rad53 phosphorylation independently of their PCNA-dependent effect on DNA replication. It is possible that the overproduced mutant protein itself interacts with checkpoint proteins inducing their activation, but not inducing DNA damage *per se*. This view is supported by the fact that *rad9Δ* cells are not sensitive to overexpression of any of the *SRS2* constructs (Fig. 2B) and that repair deficient strains, *rad52Δ, mre11Δ, rmi1Δ, srs2Δ, top3Δ* and *ccr4Δ*, are not sensitive to the *srs2-K41A-R1* and *srs2-K41R-R1* separation-of-function mutants. Since the helicase-dead mutants do not induce massive Rad53 phosphorylation in *mrc1Δ* (Fig. 2D), it is likely that the artificial checkpoint activation still depends on the Mrc1 mediator.

Together, the observations that deletion of *SRS2* leads to insufficient Rad53 activation [24], while overproduction leads to its hyperactivation highlights the important contribution of Srs2 in the DNA damage response. As illustrated in Fig. 6 (Panel 2), we hypothesize that the overexpressed helicase mutants of *SRS2* trigger checkpoint activation that is not associated with DNA damage or with the interaction of Srs2 with the PCNA replication clamp.

In conclusion, we find that the growth defect that can be induced by Srs2 overexpression [12, 31, 32] is primarily due to its toxicity on DNA replication and depends on its capability to interact with the PCNA replication clamp. In addition, our data suggest a new function for endogenous Srs2 at replication forks that does not depend on its helicase activity. Remarkably, this role is responsible for many of the SDL interactions reported here, suggesting that a wide variety of cellular pathways are affected when the integrity of the replication fork is compromised.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

SDL	Synthetic Dosage Lethality
SPA	Selective Ploidy Ablation

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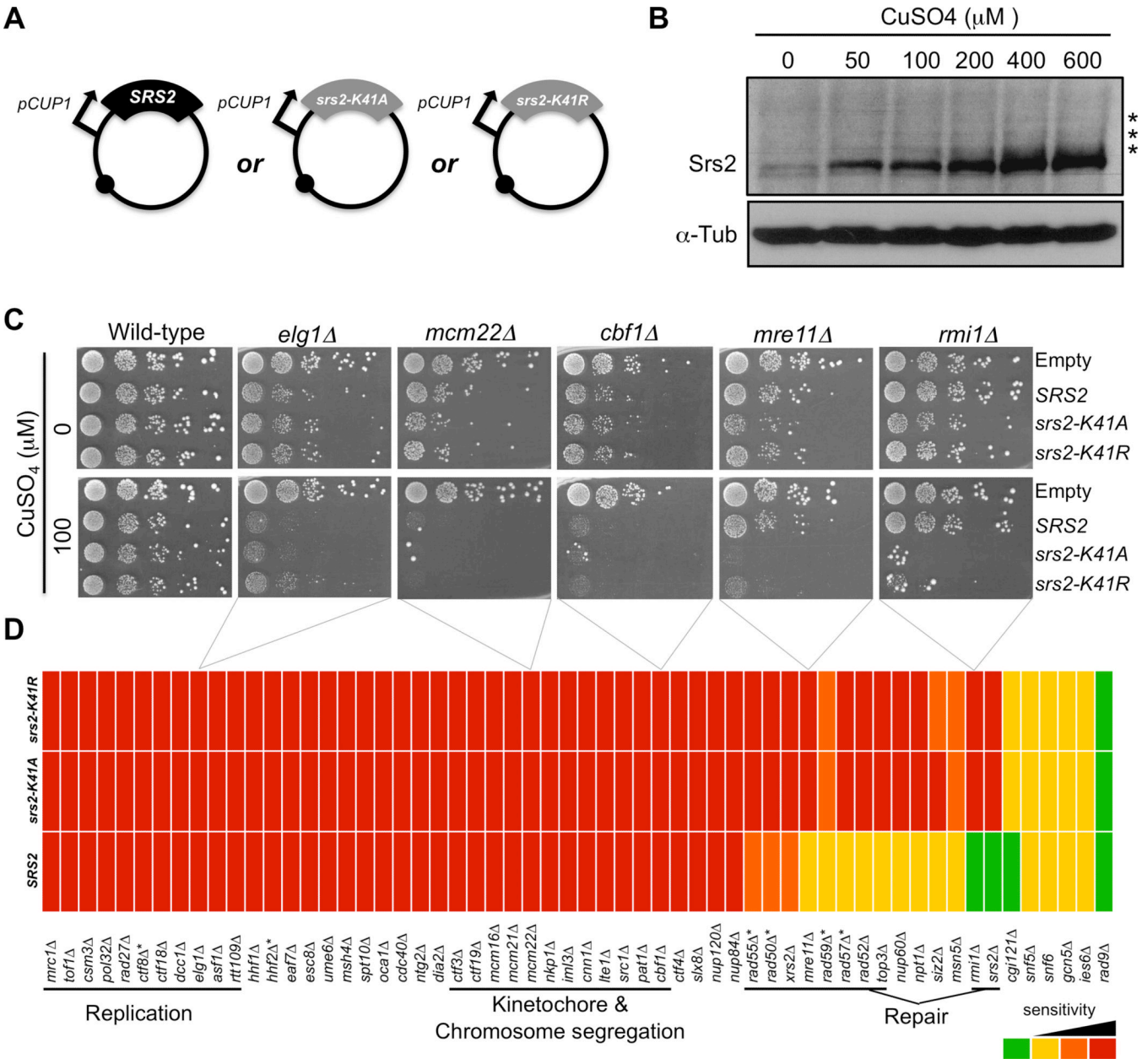

Fig. 1.

Synthetic dosage lethal screen by overexpression of *SRS2*, *srs2-K41A* and *srs2-K41R* in the strains of the yeast disruption library. (A) Centromeric overexpression plasmids containing *SRS2*, *srs2-K41A* or *srs2-K41R* under control of the *CUP1* promoter. (B) Denaturing protein extracts from wild-type cells overexpressing Srs2 upon addition of 0, 50, 100, 200, 400 or 600 μM $CuSO_4$ to the growth medium. The extracts were migrated and revealed with an anti-Srs2 antibody and an anti-alpha-Tubulin antibody for a loading control. Asterisks mark post-translational modifications of the protein. (C) Manual verification of the SDL interactions with DNA metabolism genes. The interactions were found in the high-throughput screen and were verified by transforming the overexpression plasmids and an empty vector control into the deletion strains. The transformants were spotted in 10-fold serial dilutions onto medium without or with 100 μM $CuSO_4$. Overexpression in wild-type cells is presented as a control. (D) Heat-map representing the sensitivity of the DNA

metabolism strains verified manually. For each strain, the three colour boxes correspond to the degree of its sensitivity to each of the three versions of *SRS2:* wild-type *SRS2* or helicase-dead mutants *srs2-K41A* and *srs2-K41R*. Red: severe growth defect. Orange and Yellow: intermediate growth defects. The differences between these two classes are subtle and attributed according to growth of the each strain with the empty control vector. Green: same growth profile as with the empty control vector. * Genes that were not found in the high throughput screen.

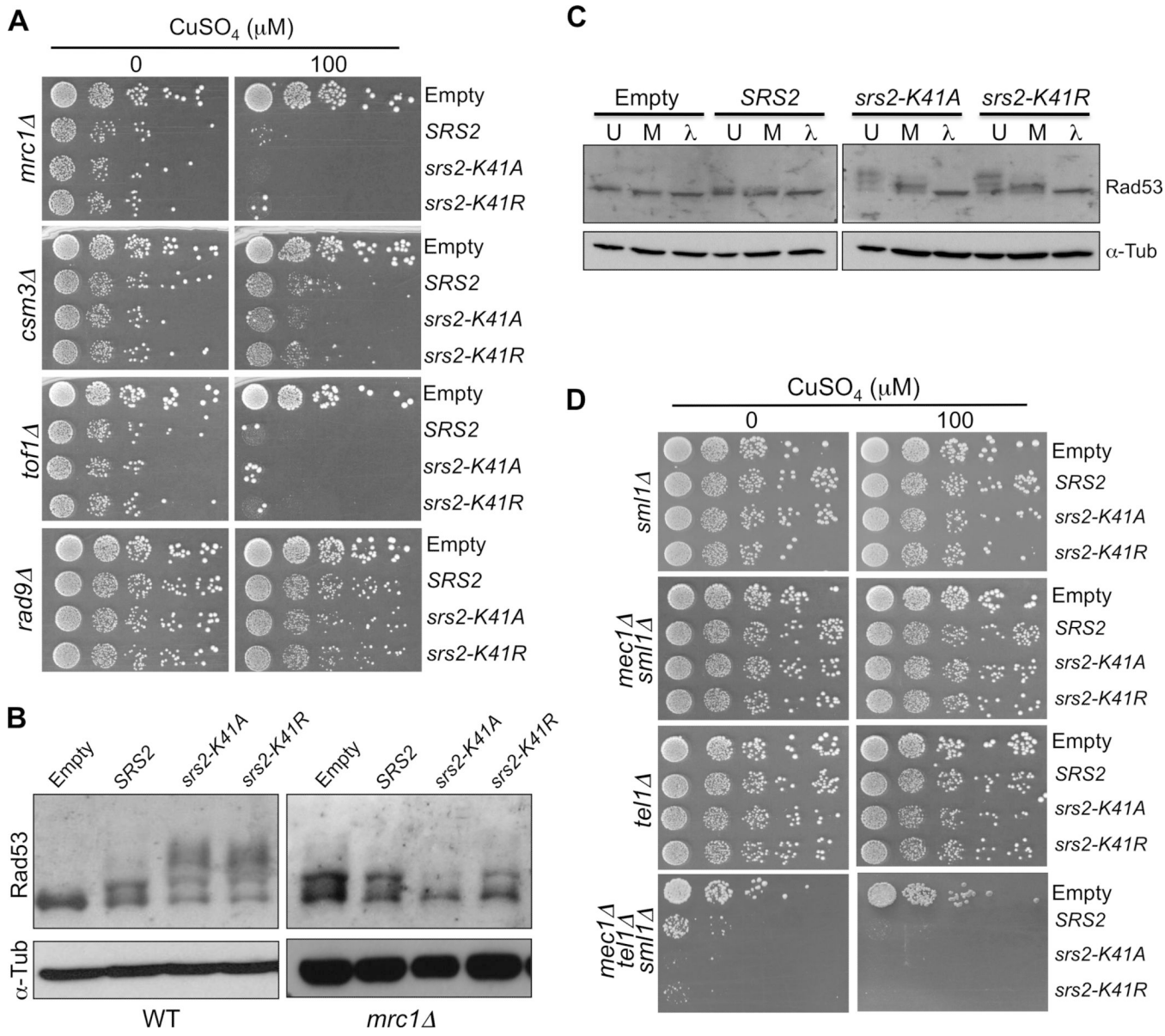

Fig. 2.

Overexpression of wild-type and helicase-dead *SRS2* triggers the activation of the replication checkpoint. (A) *mrc1Δ*, *csm3A*, *tof1Δ* or *rad9Δ* mutants containing the overexpression plasmids were diluted in 10-fold serial dilutions and spotted onto medium without or with copper (100 μM $CuSO_4$). (B) Denaturing protein extracts of wild-type or *mrc1Δ* cells overexpressing wild-type or mutant *SRS2* at 200 μM $CuSO_4$. The proteins were migrated on an acrylamide gel and revealed with antibodies against Rad53 and alpha-Tubulin for a loading control. (C) Rad53 phosphorylation. Native extracts of wild-type cells overexpressing wild-type or mutant *SRS2*. The extracts were either directly denatured after extraction (**U**: untreated), or incubated for at 1 hour at 30°C with phosphatase buffer, $MnCl_2$ and protease inhibitors (**M**: mock), or finally treated with λ-phosphatase for 1 hour at 30°C with phosphatase buffer, $MnCl_2$ and protease inhibitors (**λ**: λ-phosphatase treated). Note that the Rad53 is partially unphosphorylated in "Mock" samples suggesting the presence of phosphatases in the cellular extracts. (D) *sml1Δ*, *mec1Δ sml1Δ*, *tel1Δ* or *mec1Δ tel1Δ sml1Δ*

mutants containing the overexpression plasmids were diluted in 10-fold serial dilutions and spotted onto medium without or with copper (100 μM $CuSO_4$).

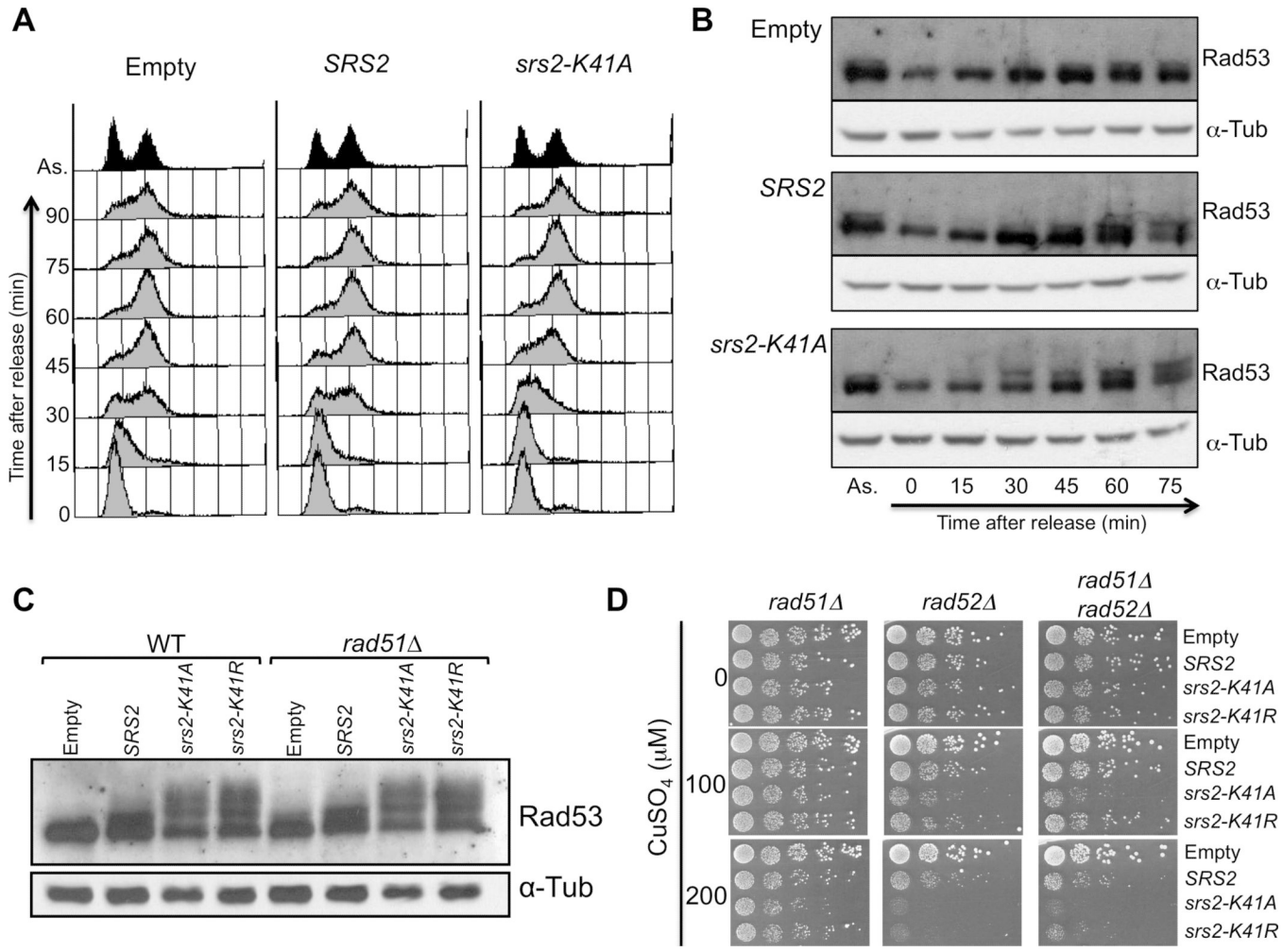

Fig. 3.

Overexpression of wild-type or helicase-mutant *SRS2* delays S-phase progression and acts independently of *RAD51*. (A) *MAT***a** *bar1*Δ cells containing an empty vector control or the plasmids for overexpression of *SRS2* or *srs2-K41A* were synchronized in G1 with alpha factor. To induce overexpression, 200 μM $CuSO_4$ were added 30 minutes prior to release, and the induction was kept upon release. Samples were collected every 15 minutes for FACS analysis. (B) Cells were synchronized as described in (A) and samples were collected for protein extractions. The proteins were migrated on an acrylamide gel and revealed with antibodies againt Rad53 and alpha-Tubulin for a loading control. (C) Denaturing protein extracts of wild-type or *rad51Δ* cells overexpressing wild-type or helicase-dead *SRS2*. The extracts were revealed with antibodies against Rad53 and alpha-tubulin as a loading control. (B) *rad51Δ*, *rad52Δ* or *rad51Δ rad52Δ* mutants containing the overexpression plasmids were diluted in 10-fold serial dilutions and spotted onto medium without or with copper (100 μM and 200 μM $CuSO_4$).

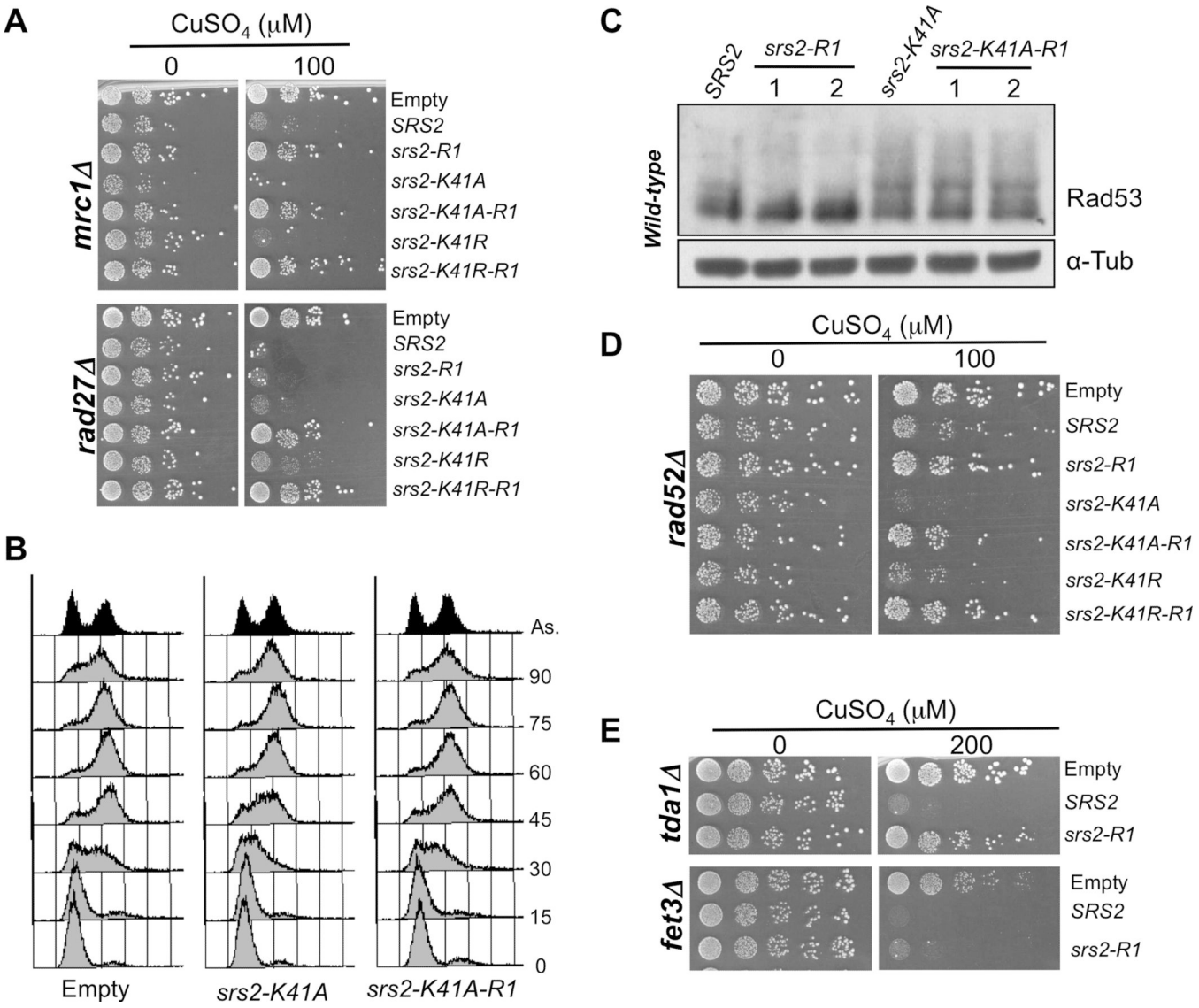

Fig. 4.

The toxicity of Srs2 during replication depends on the domain that interacts with sumoylated PCNA. (A) *mrc1Δ* or *rad27Δ* cells transformed with an empty vector or with overexpression plasmids for *SRS2, srs2-R1, srs2-K41A, srs2-K41A-R1, srs2-K41R* and *srs2-K41R-R1*. 10-fold serial dilutions of the transformants were spotted on medium without or with copper (100 μM $CuSO_4$). (B) *MAT***a** *bar1Δ* cells containing an empty control vector or the plasmids for overexpression of *srs2-K41A* or *srs2-K41A-R1* were synchronized in G1 with alpha factor. To induce overexpression, 200 μM $CuSO_4$ were added 30 minutes prior to release, and the induction was kept upon release. Samples were collected every 15 minutes for FACS analysis. (C) Protein extracts from wild-type cells overexpressing plasmids for *SRS2, srs2-R1, srs2-K41A* or *srs2-K41A-R1*, revealed with antibodies against Rad53 and alpha-Tubulin. 1 and 2 correspond to two independent transformants for *srs2-R1* and *srs2-K41A-R1*. (D) Same as in (A) but with *rad52Δ* cells. (E) *tda1Δ* and *fet3Δ* cells transformed with an empty vector or with overexpression plasmids for *SRS2* and *srs2-R1*. 10-fold serial dilutions of the transformants were spotted on medium without or with copper (200 μM $CuSO_4$).

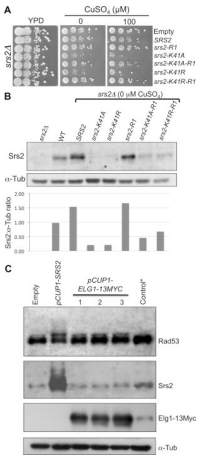

Fig. 5.

Specificity and relevance of Srs2 overexpression. (A) *srs2Δ* cells transformed with an empty vector or with overexpression plasmids for *SRS2, srs2-R1, srs2-K41A, srs2-K41A-R1, srs2-K41R* and *srs2-K41R-R1*. 10-fold serial dilutions of the transformants were spotted on YPD as a spotting control and on medium without or with 100 μM $CuSO_4$. (B) Denaturing protein extracts of *srs2Δ* cells expressing *SRS2, srs2-K41A, srs2-K41R, srs2-R1, srs2-K41A-R1* or *srs2-K41R-R1* at 0 μM $CuSO_4$. Control extracts from *srs2Δ* cells with an empty vector and wild-type cells with an empty vector were used. The proteins were revealed with an anti-Srs2 antibody and anti-alpha-Tubulin antibody as a loading control. Total Srs2 protein was quantified relative to the amount of alpha-tubulin in each extract. (C) Overexpression of Elg1 does not induce a high Rad53 phosphorylation. Denaturing protein extracts of wild-type cells overexpressing *SRS2* or ELG1-13MYC (three different clones) at 200 μM $CuSO_4$. *Control cells with endogenous tagged *ELG1-13MYC*. The proteins were revealed with ntibodies against Rad53, Srs2, Myc and alpha-Tubulin.

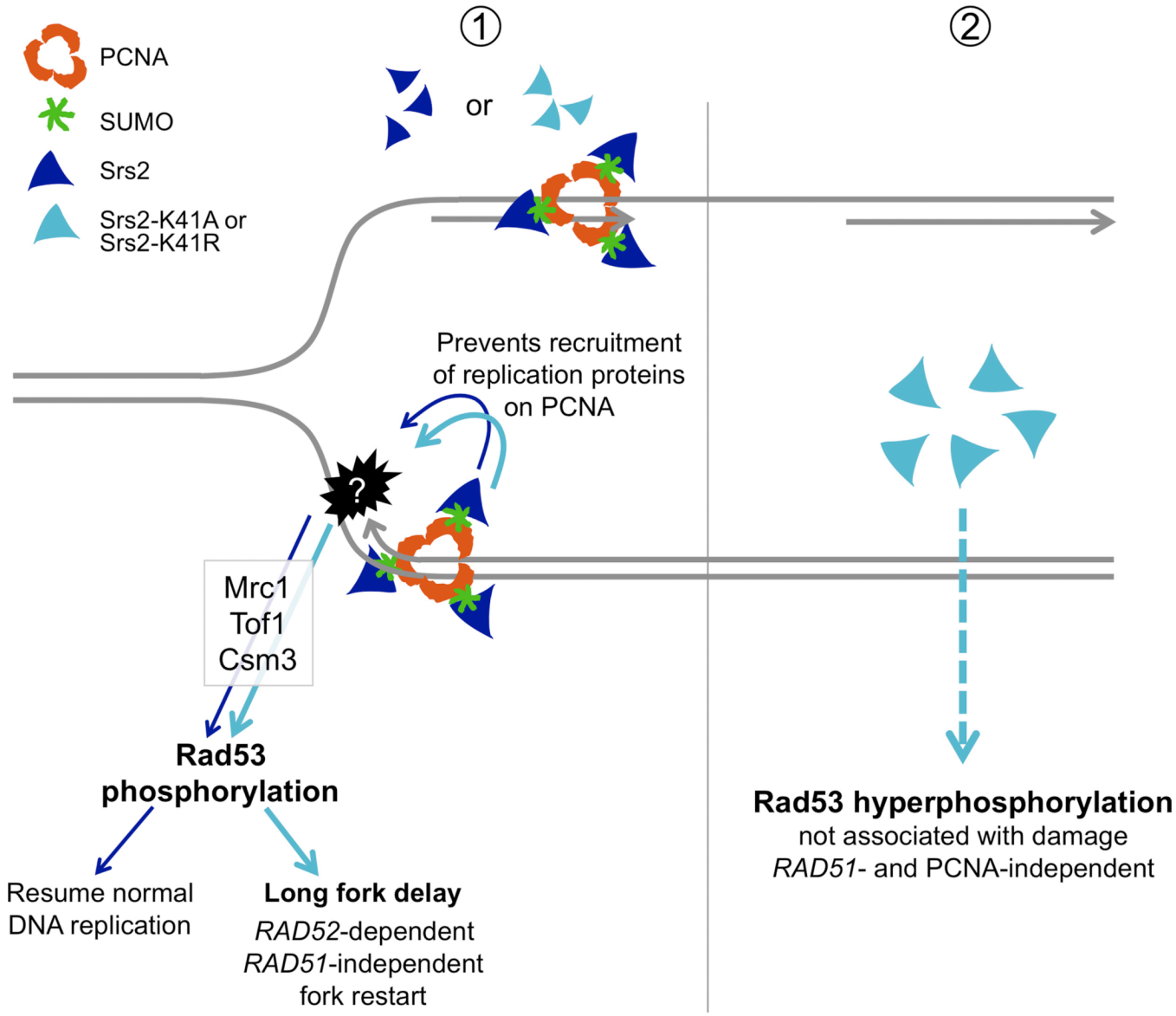

Fig. 6.

Model representing the effects of overexpression of *SRS2* and its helicase-dead mutants on DNA replication. ① Wild-type or helicase-dead *SRS2* are recruited to replication forks by SUMOylated PCNA. This interaction likely prevents the recruitment of other factors required for replication, leading to fork progression delay, which activates the replication checkpoint (Mrc1, Tof1 and Csm3-mediated phosphorylation of Rad53). Cells overexpressing *SRS2* eventually overcome this defect. The helicase-dead mutants *srs2-K41A* or *srs2-K41R* show a greater replication delay that likely leads to the accumulation of aberrant intermediates that require *RAD52*-dependent, *RAD51*-independent recombination to resume replication. ②The helicase-dead mutants induce activation of the DNA integrity checkpoint, resulting in hyperphosphorylation of Rad53. This effect is independent of DNA replication, is not associated with any damage and likely results from the accumulation of an Srs2 protein capable of triggering checkpoint activation.

Table 1

Strains

Strain	Background	Genotype	Reference
BY4742	S288C	*MAT alpha his3Δ0 leu2Δ0 lys2Δ0 ura3Δ0*	[39]
ORD9833-3B	S288C	*MAT alpha tel1::KanMX his3Δ0 leu2Δ0 lys2Δ0 ura3Δ0*	This study
ORD9834-15B	S288C	*MAT alpha mec1::URA3-kanR sml1::KanMX his3Δ0 leu2Δ0 lys2Δ0 ura3Δ0*	This study
ORT7009	S288C	*MAT alpha mec1::URA3-kanR tel1 ::KanMX sml1::KanMX his3Δ0 leu2Δ0 ura3Δ0 met15Δ0*	This study
MK11241	W303	*ELG1 ::13Myc ::KanMX lys2::Ty1Sup ade2-1(o) can1-100(o) ura3-52 leu2 3,112 his3del200 trp1del901 HIS3::lys2::ura3 his4::TRP1::his4*	[52]
W8164-2C	W303	*MATa CEN1-16::Gal-K.lactis-URA3 trp1-1 his3-11,15 leu2-3,112 ura3-1 RAD5+ MET17 ADE2 LYS2*	Reid RJD et al., submitted

Table 2

Genes required for viability upon overexpression of *SRS2*, *srs2-K41A* or *srs2-K41R*.

Function	
DNA metabolism-related genes	
DNA replication	*MRC1*[*] *TOF1*[*] *CSM3*[*] *CDC40*[*] *CTF4*[*] *CTF8*[*] *CTF18*[*] *CTF8 DCC1*[*] *DIA2 ELG1 POL32 RAD27 RNR4*[(a)]
DNA Repair	*MMS22*[(a)] *MRE11 NTG2 RAD50 RMI1 SRS2 TOF1*[*] *TOP3 XRS2 YLR235C*[(a)]
Recombination	*MSH4 RAD52 RAD55 RAD57 RAD59*
Checkpoint	*MRC1*[*] *TOF1*[*] *CSM3*[*]
Chromatin	*HHF1 HHF2*
Chromatin Remodeling	*ASF1 CBF1 EAF7 ESC8 GCN5 IES6 NPT1 RTT109 SNF12*[(a)] *SNF5 SNF6 SPT10 UME6*
Telomere maintenance	*CGI121 BUD32*[*(a)]
Chromosome Segregation	*CSM3*[*] *PAT1*[*] *SRC1*
Kinetochore	*CHL4*[(a)] *CNN1 CTF3 CTF19 IML3 KRE28*[(a)] *LTE1*[*] *MCM16 MCM21 MCM22 NKP1*
Sister Chromatid Cohesion	*CTF4*[*] *CTF8*[*] *CTF18*[*] *DCC1*[*]
Cell Cycle	*CLB2*[(a)] *CLN3*[(a)] *DOC1*[(a)] *LTE1*[*] *OCA1*
SUMO and Ubiquitin	*SIZ2 SLX8*
Nuclear Pore	*MSN5 NUP60 NUP84 NUP120*
Genes unrelated to DNA metabolism	
Budding	*BUD21*[*] *BUD22 BUD31 BUD32*[*]
Cell Wall	*DFG5 SAC1*
Cytoskeleton	*MSB3 SAC6 SAC7 SLA1 SLM6*
Lipid Metabolism	*FAA1*
Mitochondria	*ACO1 ATP14 ATP15 ATP7 FMP33 FZO1 GGC1 GEP5 ISA2 ISM1 MDM12 MRPL11 MRPL15 MRPL22 MRPL23 MRPL27 MRPL28 MRPL37 MRPL38 MRPL7 MRPS5 MRPS9 MSM1 OMA1 POR1 QCR10 QCR2 RRF1 RSM22 RSM24 SAM37*[(b)] *SLS1 SSQ1 TOM5 TSR2 TUF1 YBL100C YKL169C YDR115W*
Peroxisome	*PEX15*
Ribosome	*ARX1*[(b)] *BUD21*[*] *JJJ1 KAP120 RPL16B RPL19A RPL20B RPL24A RPL31B RPL40A RPP2B RPS0B RPS10A RPS19B RPS1B RPS25A RPS9B RSA1 SSF1*
RNA Metabolism	*CDC40*[*(b)] *CKB2 DBP3 DUS4 LSM1 LSM6*[#] *MED1 MSL1 NOP12 NSR1 PAT1*[*(b)] *RNP1 RPA34 RRP6 RRP8*[(b)] *RTC3 SKI2*[(b)] *SKI3 SKI7 SKI8 SPT3 STB5 THP1 TRM10 YGL214W*
Transcription	*CCR4*[(b)] *NUT1*[#] *POP2*[(b)] *SPT2 SPT8 THO2*
Translation	*EAP1*[(b)] *TIF3*
Vacuole	*BSD2 KCS1*[(b)] *PPA1 TFP1 VAM10 VAM3*[#] *VAM6 VMA6*[(b)] *VAM7 VPH2*
Vesicular Traffic	*BRO1*[(b)] *CDC50 COG1 DOA4*[(b)] *EDE1 ERP2 FAB1 IMH1 PEP8 RAV2 RVS167*[(b)] *SNF8 SRN2 STP22*[(b)] *TLG2 VPS1 VPS13 VPS17 VPS20 VPS21 VPS24*[(b)] *VPS25*[(b)] *VPS27*[(b)] *VPS28 VPS29 VPS30 VPS36 VPS38 VPS4*[(b)] *VPS41 VPS45 VPS5 VPS51 VPS60 VPS61*[(b)] *VPS8 VPS9 YPT6 OPI8 YOR331C*
Other	*AIM45 APQ13 CCH1 CIN5 CKA2 CLA4 CPR1 CSG2 CYS4 ELM1 EMC2 FET3*[#] *FTR1 FYV1 FYV5 GRE2 HEK2 HMG2 HSP31 HUR1 KEX1 LCB5 LTV1 MET7 OPI9 PDX3 PMP1 PMP3 PMT2*[(b)] *PRS3 PUF6 RCY1 RRT8 SEC66 SHE4 SRV2 SUR1 TDA1*[(b)] *TDA2*[(b)] *TDA4*[(b)] *TDA6*[(b)] *TDA11*[(b)] *THI20 TNA1*[(b)] *VRP1 YBP1 YVH1*

Function	
DNA metabolism-related genes	
	YAR047C YCR051W YGL242C YGR160W YML094C-A YML122C YNR005C[#] *YNR068C YOR364W*

[(a)] All SDL interactions with DNA metabolism-related genes were individually verified, except for the genes marked with an.

[(b)] Of the SDL interactions with genes unrelated to DNA metabolism, only the ones marked with a were verified.

[#] The SDL interaction with wild-type *SRS2* was verified

[*] Genes involved in more than one function.