The homologous recombination complex of the Rad51 paralogs Rad55-Rad57 avoids translesion DNA polymerase recruitment and counterbalances mutagenesis induced by UV radiation

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Abstract

Bypass of DNA lesions that block replicative polymerases during DNA replication relies on several DNA damage tolerance pathways. The error-prone translesion synthesis (TLS) pathway involves specialized DNA polymerases that incorporate nucleotides in front of base lesions. The template switching and the homologous recombination (HR) pathways are mostly error-free because the bypass is performed by using typically the sister chromatid as a template. This is promoted by the Rad51 recombinase that forms nucleoprotein filaments on single-strand DNA (ssDNA). The balance between error-prone and error-free pathways controls the level of mutagenesis. In yeast, the Rad55-Rad57 complex of Rad51 paralogs is required for Rad51 filament formation and stability, notably by counteracting the Srs2 antirecombinase. Several reports showed that Rad55-Rad57 promotes HR at stalled replication forks more than at DNA double-strand breaks (DSB), suggesting that this complex is more efficient at ssDNA gaps and thus, could control the recruitment of TLS polymerases. To address this point, we studied the interplay between Rad55-Rad57 and the TLS polymerases Polζ and Polη following UV radiation. We confirmed that Rad55-Rad57 protects Rad51 filaments from Srs2 dismantling activity but we found that it is also essential for the promotion of UV-induced HR independently of Srs2. In addition, we observed that cell UV sensitivity, but not DSB sensitivity, is synergistically increased when Rad55 and Polζ deletions are combined. Moreover, we found that mutagenesis and HR frequency were increased in *rad55*Δ mutants and in TLS-deficient cells, respectively. Finally, UV-induced HR was partially restored in Rad55-deficient cells with mutated Polζ or Polη. Overall, our data suggest that the HR and TLS pathways compete for the same ssDNA substrates and that the Rad55-Rad57 complex of Rad51 paralogs prevents the recruitment of TLS polymerases and counterbalances mutagenesis.

Introduction

In all living organisms, genomic DNA undergoes chemical modifications or crosslinking with proteins. These damages greatly compromise DNA replication because they induce replication fork stalling. DNA damage tolerance (DDT) mechanisms have evolved to ensure completion of genome replication [1], and rely on two main mechanisms: translesion synthesis (TLS) DNA polymerases, and the use of a homologous template, typically the newly synthesized sister chromatid. Specialized TLS polymerases efficiently insert nucleotides opposite and beyond lesions on DNA templates that block the replicative DNA polymerases [2]. They possibly extend blocked primer/template junctions at replication forks or at single-stranded DNA (ssDNA) gaps left behind the forks [3,4]. TLS polymerases are intrinsically error-prone and constitute a major source of DNA damage-induced mutagenesis [5,6]. Alternatively, recombination-mediated pathways, such as the template switch and the homologous recombination (HR) pathways, mediate damage bypass through the annealing of the damaged strand to the intact homologous template on the sister chromatid [7–9]. HR, also referred to as the salvage pathway, can use homologous chromosomes as intact donors rather than sister chromatids [10]. Template switch and HR are error-free lesion bypass mechanisms and counterbalance mutagenesis induced by TLS [11,12]. Thus, it is crucial to precisely characterize factors influencing the pathway choice to better understand genetic stability at replication forks.

The template switch pathway involves ubiquitin ligase and the Rad18/Rad6 pathway conjugating activities. In budding yeast, Rad6 and Rad18 induce PCNA mono-ubiquitination at its conserved K164 residue, whereas Rad5 and Mms2-Ubc13-dependent activities lead its poly-ubiquitination at K164 [13,14]. X-shaped intermediates are formed during template switch between sister chromatids when replication is challenged by DNA damages [8].

Interestingly, the formation of these X-shaped intermediates is controlled by PCNA poly-ubiquitination, but relies also on the HR factors Rad51 and Rad52 [7,15].

The HR pathway relies on the recombinase protein Rad51 that oligomerizes on ssDNA to form a right-handed helical nucleoprotein filament covering thousands of bases [16,17]. This nucleoprotein filament performs homology search and catalyzes DNA joint formation between ssDNA and dsDNA homologous partners, thereby leading to strand exchange [18–20]. Eventually, repair DNA synthesis occurs from damaged DNA invading ends on undamaged template homologous DNA sequences [21]. Rad51 loading on RPA-coated ssDNA is a crucial step in HR and is mediated mainly by Rad52 in *Saccharomyces cerevisiae* [22,23]. Rad51 filament assembly also depends on the Rad55-Rad57 heterodimer [24,25]. Rad55 and Rad57 share 20% identity with the RecA/Rad51 ATPase core region [26,27], and thus are referred to as Rad51 paralogs [28]. However, they do not form filaments on ssDNA, and they do not exhibit strand exchange activity [24,29]. Electron microscopy images showed the association of gold-labeled Rad55 with Rad51-ssDNA filaments [29] and two-hybrid experiments revealed the interaction between Rad51 and Rad55 [30,31]. However, recent single-molecule studies suggest that the interaction between Rad55-Rad57 and Rad51 filaments is transient and that Rad55-Rad57 dissociates during filament extension [32]. As similar findings were obtained in nematodes [33], it has been proposed that RAD51 paralogs behave as classical chaperones to temporarily assist Rad51 filament formation.

Rad55-Rad57 role in DNA double strand break (DSB) repair is considered accessory on the basis of the weak sensitivity of *rad55* and *rad57* mutants to ionizing radiation and to site-directed DSBs [34,35]. Interestingly, this sensitivity seems to depend on the Srs2 helicase activity because it is partially suppressed by *SRS2* ablation [29,35]. *In vitro* experiments have shown that the Srs2 translocase activity disrupts Rad51 filaments [36,37] and that Rad55-Rad57 counteracts Srs2 to maintain Rad51 filaments on ssDNA [29,32]. Therefore, it has

been proposed that Rad51 filament assembly and disassembly, which are mediated by Rad55-Rad57 and Srs2 respectively, provide a regulatory mechanism to control HR initiation. However, *SRS2* deletion does not rescue spontaneous HR defects between direct repeats in the *rad55* and *rad57* mutants, indicating that Rad55-Rad57 also acts independently of Srs2 [35].

To explain the different defects observed between spontaneous and DSB-induced HR, it was hypothesized that spontaneous HR between direct repeats is initiated by ssDNA gaps rather than DSBs [35]. Thus, Rad55-Rad57 would play a more prominent role in HR when the initiating lesion is a ssDNA gap. Interestingly, it has been observed that *RAD55* ablation abolishes X-structures formed through the template switch pathway at ssDNA gaps in the rear of replication forks [15]. Therefore, Rad55-Rad57 could be a determining factor in the pathway choice for DDT: template switch and HR at the expense of the TLS pathway.

In the present study, we explored the role of Rad55-Rad57 in Rad51 filament formation on ssDNA gaps and in the balance between HR and TLS in *S. cerevisiae*. For that purpose, we induced interhomolog HR in diploid strains by ionizing radiation (IR) or UV radiation. IR generates DSBs and ssDNA gaps, whereas UV generates mostly ssDNA gaps [38]. We found that Rad55-Rad57 is essential for UV-induced HR and accessory for IR-induced HR. We observed this essential role also in the absence of Srs2, suggesting that it is different from Rad55-Rad57 balancing role against Srs2 activity. Importantly, we observed UV-induced HR in Rad55-Rad57-deficient cells upon inactivation of a TLS polymerase (Polζ or Polη). Therefore, our results strongly suggest that Rad51 filament assembly on ssDNA gaps relies heavily on Rad55-Rad57, thus avoiding the recruitment of TLS polymerases.

Results

1 - The Rad55-Rad57 heterodimer is essential for UV-induced homologous recombination

To investigate Rad55-Rad57 role in HR induced by DSB and ssDNA gaps, we analyzed UV- and IR-induced interhomolog HR in wild type (WT) and *rad55*Δ isogenic diploid strains. As IR generates DSBs much more readily than UV, *rad51* and *rad52* mutant cells are very sensitive to IR, while they display a moderate sensitivity to UV [39,40]. Additionally, genetic evidence suggests that UV-induced ssDNA gaps trigger HR [41]. We measured interhomolog HR using two mutant alleles of *ARG4*: *arg4-RV*, a 2-bp deletion that ablates the *Eco*RV site at position +258, and *arg4-Bg*, a 4-bp insertion by fill-in of a *Bgl*II site at position +1,274 [42] (**Fig. 1A**). Only recombination in heteroallelic *arg4-RV*/*arg4-Bg* cells results in the formation of a WT *ARG4* gene primarily by non-reciprocal transfer covering one mutation [43].

The *rad55*Δ diploid strain was not sensitive to UV radiation (**Fig. 1B**), but remarkably, UV-induced recombinant [Arg^+] frequency was strongly reduced in the *rad55*Δ diploid strain compared with WT cells (10-fold at 120 J/m^2) (**Fig. 1C**). Conversely, *rad55*Δ diploid cells were sensitive to IR (**Fig. 1D**), but γ-ray-induced HR frequencies were identical in *rad55*Δ and WT cells at high doses (400 Gy and 600 Gy) (**Fig. 1E**). Similar results were previously reported for spontaneous HR in *rad57*Δ mutants [34,44], a result we confirmed here in *rad55*Δ cells (**Fig. 1F**). We observed the same phenotypes in the *rad57*Δ mutant (**S1. Fig**). These observations suggest that the Rad55-Rad57 complex plays a specific and essential role in the repair of ssDNA gaps originating from UV radiation, while it seems accessory for DSB repair induced by IR and spontaneous HR.

The apparent absence of Rad55-Rad57 involvement in repair induced by IR and spontaneous HR could be explained by HR promotion by *MAT* heterozygosity [44,45]. Effectively, we observed a synergistic decrease in HR rate in diploids that carried also a *MAT*α allele deletion (**Fig. 1F**), confirming previous results obtained in *rad57*Δ cells [34,44]. Moreover, haploid *rad55*Δ cells were more sensitive to IR than their diploid counterparts that

express both *MAT* alleles (**Fig. 1D**). Thus, a factor controlled by the *MAT***a**/*MAT*α transcription program can compensate for the significant role of Rad55-Rad57 in spontaneous and IR-induced HR. Interestingly, this factor cannot compensate for UV-induced HR in *rad55*Δ and *rad57*Δ mutant cells. Therefore, the Rad55-Rad57 complex plays a specific role in UV-induced heteroallelic HR.

2 – The essential role of Rad55-Rad57 in UV-induced HR is not related to Rad51 filament protection against Srs2

As Rad55-Rad57 limits Rad51 filament destabilization by Srs2 [29,32], we asked whether the defect in UV-induced HR associated with *rad55*Δ or *rad57*Δ was the result of unbalanced Srs2 activity. To address this question, we measured UV-induced HR frequencies in *rad55*Δ *srs2*Δ and *rad57*Δ *srs2*Δ double mutants and found that they were similar to those observed in single *rad55*Δ and *rad57*Δ mutants (**Fig. 2A and B**; **S2A and B Fig**.). *SRS2* deletion did not suppress the strong decrease of UV-induced HR associated with *rad55*Δ and *rad57*Δ. Therefore, the essential role of Rad55-Rad57 in UV-induced HR is not related to Srs2.

We also confirmed that the *srs2*Δ/*srs2*Δ diploid strain was highly sensitive to UV radiation [46]; however, we found that this sensitivity was completely suppressed in *rad55*Δ *srs2*Δ and *rad57*Δ *srs2*Δ mutant cells (**Fig. 2C**; **S2C Fig**.). Early genetic studies have led to the concept of toxic Rad51-dependent recombination intermediates that accumulate in the absence of Srs2 [47–49]. Therefore, on the basis of the complete suppression of *srs2*Δ-associated UV sensitivity by *rad55*Δ and *rad57*Δ, we propose that Rad55-Rad57 participates in the formation of UV-induced Rad51 filaments that are toxic for the cells in the absence of Srs2. In agreement with previous reports [46,50,51], we observed a strong UV-induced hyper-recombination phenotype in Srs2-deficient cells, but not when Rad55 or Rad57 also was

absent (**Fig. 2A**; **S2A Fig**.). This suggests that Rad55-Rad57 can triggers numerous HR events in Srs2-deficient cells.

3 - Channeling UV-induced DNA lesions towards the *REV3* error-prone DNA repair pathway in the *rad55*Δ mutant

Although UV-induced HR was almost abolished in *rad55*Δ cells, these cells were resistant to UV radiation. We hypothesized that UV-induced ssDNA gaps at replication forks could be repaired by TLS instead of HR in *rad55*Δ mutant cells. This implies that *rad55*Δ cells strongly rely on TLS for UV resistance and that UV-induced mutagenesis is increased in *rad55*Δ cells, as previously shown for spontaneous mutagenesis [52].

Polζ is a major TLS polymerase involved in the bypass of UV-induced DNA lesions, as illustrated by the strong UV-sensitivity of cells lacking the Rev3 catalytic subunit of Polζ (**Fig. 3A**) [53]. In addition, Polζ is responsible for almost all UV-induced mutagenesis in yeast cells [53,54]. We confirmed that UV sensitivity is much higher in the *rev3*Δ *rad55*Δ double mutant than in the *rev3*Δ single mutant in haploid cells [52,55,56] (**Fig. 3A**). We also found that the double mutant UV sensitivity was more pronounced in diploid cells (**Fig. 3B**). Therefore, the reduced HR efficiency in *rad55*Δ mutants was compensated by the TLS pathway. This result also suggests that TLS and HR can manage the same lesions.

We then monitored mutagenesis in *rad55*Δ cells with the *CAN1* forward mutation assay. As expected, the frequency of UV-induced canavanine-resistant cells was higher in the *rad55*Δ mutant than in WT cells (**Fig. 3C**). This observation supports the channeling of DNA lesions towards the error-prone Polζ-dependent pathway in *rad55*Δ cells. Noteworthy, the increase of UV-induced mutagenesis in the *rad55*Δ mutant was still present in *rad55*Δ *srs2*Δ cells (**Fig 3C**).

4 - Channeling of UV-induced DNA lesions to the HR pathway in cells deficient for TLS polymerases

The finding that UV-induced DNA lesions can be channeled to the TLS pathway in *rad55*Δ cells suggested that lesions could be managed by the HR pathway in TLS-deficient cells. In that case, TLS-deficient cells should display a hyper-recombinogenic phenotype. To test this hypothesis, we measured the frequencies of UV-induced HR in *rev3*Δ (Polζ mutant) and *rad30*Δ (Polη mutant) cells. As expected, HR frequency was strongly increased in the *rev3*Δ and to a lower extent in the *rad30*Δ mutant (**Fig. 4A and B**). Interestingly, UV sensitivity was similar in diploids harboring the *rev3*Δ or *rad30*Δ mutation (**Fig. 3B; Fig. 4C**), suggesting that fewer UV-induced DNA lesions are bypassed by HR in the *rad30*Δ mutant, probably because they can also be bypassed by Polζ.

5 - Translesion DNA polymerases prevent UV-induced HR in the *rad55*Δ mutant

The strong UV sensitivity of the *rev3*Δ *rad55*Δ double mutant (**Fig. 3A and B**) suggested that in these cells the TLS and HR pathways are strongly impaired. The TLS pathway is highly defective in the *rev3*Δ mutant [53], but HR can be Rad55-independent. Therefore, we measured HR frequencies in the *rev3*Δ *rad55*Δ double mutant, and observed a significant increase in HR frequencies in *rev3*Δ *rad55*Δ cells compared with *rad55*Δ cells (**Fig. 5A**). This increase was even more pronounced in the *srs2*Δ background (**Fig. 5B**). This unexpected observation indicated that HR is somehow functional in *rad55*Δ mutants in the absence of Polζ.

We observed the same increase in UV-induced HR frequency associated with an additive negative effect on survival when a Polη mutation (*rad30*Δ) was combined with *rad55*Δ (**Fig. 5C-E**). Thus, both Polζ and Polη are responsible for the decrease of UV-induced HR in *rad55*Δ mutant cells. From these data, we inferred that Rad55-Rad57 might

negatively regulate TLS polymerases in ssDNA gap repair. In the absence of Rad55-Rad57, TLS polymerases are predominantly recruited and outcompete HR. In *rad55*Δ *rev3*Δ cells, HR functions in a defective manner and cannot provide strong recovery upon UV irradiation, indicating that the Rad55-Rad57 complex is essential for Rad51 filament stability, even in the absence of TLS polymerases.

6- The acute UV sensitivity of the *rad55*Δ *rev3*Δ double mutant is partially suppressed by *MAT* heterozygosity and by *SRS2* deletion

As in *rad55*Δ cells, γ-ray survival and spontaneous HR defects were suppressed by *MAT* heterozygosity (**Fig. 1D and F**), we asked whether the UV-sensitivity of *rad55*Δ *rev3*Δ diploid cells was increased in haploid cells that express only one *MAT* allele. Indeed, survival was 20-fold reduced in haploid cells compared with isogenic diploid cells **(Fig. 6)**. As one of Rad55-Rad57 roles is to counteract Srs2 in displacing Rad51 filaments, Srs2 could be responsible for the HR frequency reduction observed in *rev3*Δ *rad55*Δ cells compared with *rev3*Δ cells. Therefore, Srs2 depletion should suppress the UV sensitivity of *rev3*Δ *rad55*Δ cells. We observed that *rev3*Δ *rad55*Δ *srs2*Δ diploid cells were less sensitive to UV radiation than *rev3*Δ *rad55*Δ cells (**Fig. 6**), suggesting that Rad55-Rad57 prevents also the dismantling by Srs2 of Rad51 filaments nucleated at ssDNA gaps and not only at DSBs [29]. However, the finding that the triple *rev3*Δ *rad55*Δ *srs2*Δ mutant was more sensitive than the single *rev3*Δ mutant (**Fig. 6**) indicates that Rad55 plays another role in UV-induced HR, besides preventing Srs2 activity and competing with TLS polymerases.

7- The translesion DNA polymerases Polζ and Polη are not essential for DSB repair

The large impact of the *rad50*Δ *rev3*Δ double mutant on cell survival after UV exposure suggested that Rad55-Rad57 and Polζ play an important and specific role in UV-induced

DNA repair. Although Rad55-Rad57 is not essential for DSB repair [35], we wanted to determine the role of TLS polymerases in DSB repair in *rad55*Δ cells. We hypothesized that if Polζ and Polη are not required for DSB repair, *REV3* and *RAD30* deletions should not lead to any phenotype upon DSB induction, including in the *rad55*Δ background. To test this hypothesis, we measured the repair of a DSB induced at the *MAT* locus upon expression of the HO endonuclease controlled by a galactose-inducible promoter. The DSB was repaired by HR using an ectopic *MAT***a**-inc sequence inserted in chromosome V as donor [51,57] (**Fig. 7**). After DSB induction, survival was decreased by 3-fold in *rad55*Δ cells compared with WT cells (**Fig. 7B**). Conversely, the *rev3*Δ, *rad30*Δ, and *rev3*Δ *rad30*Δ mutants did not show any UV sensitivity upon DSB induction, and they did not increase the sensitivity of the *rad55*Δ mutant. Therefore, the TLS polymerases are not required for the efficiency of DSB repair.

Discussion

Genetic arguments supporting the hypothesis that ssDNA gaps are common substrates for HR and TLS polymerases

DSB repair is partially impaired in *RAD55* or *RAD57* mutants, as indicated by their weak sensitivity to IR and to an inducible site-specific DSB. The conclusion that Rad55-Rad57 is accessory for DSB repair is strengthened by the observation that *rad55*Δ and *rad57*Δ-associated sensitivity to DSB can be suppressed by *SRS2* deletion and *MAT* heterozygosity [35]. Therefore, functional Rad51 filaments are formed efficiently at DSB sites in the absence of Rad55-Rad57. However, spontaneous HR between direct repeats is deficient in *rad55*Δ and *rad57*Δ mutants and this defect is not suppressed by *SRS2* deletion and *MAT* heterozygosity [34,35]. To provide an explanation for the differential requirement of Rad55-Rad57 in HR, it has been postulated that Rad55-Rad57 plays an accessory role in DSB

repair, and an essential role is ssDNA gap repair [35]. To test this hypothesis, we compared the phenotypes of the *rad55*Δ and *rad57*Δ mutants upon DNA damage of different origins (i.e., UV, IR or site-directed DSB). While IR readily generates DSB, genetic studies provided evidence that ssDNA gaps are the major initiator of spontaneous and UV-induced HR in yeast [41,49]. In addition, electron microscopy and two-dimensional gel electrophoresis showed that UV-irradiated *S. cerevisiae* cells accumulate ssDNA gaps, likely resulting from re-priming events downstream of stalled replication forks at UV-induced DNA lesions [38]. UV-induced ssDNA gaps were also inferred from a physical assay [58] and from the study of RPA foci distribution relatively to sites of ongoing replication [59]. Interestingly, both studies reported that TLS and HR counteract ssDNA gap accumulation. Here, we found that although *rad55*Δ and *rad57*Δ mutants are not sensitive to UV, UV-induced HR between homologs is strongly decreased in these mutants, but not IR-induced HR (**Fig. 1**).

The conclusion that Rad55-Rad57 is essential for ssDNA gap repair by HR is also supported by the synergistic increase of UV sensitivity upon depletion of both Rad55 and Polζ (**Fig. 3**). Remarkably, we did not observe this negative interaction following the induction of a site-directed DSB (**Fig. 7**). We found that UV-induced mutagenesis at the *CAN1* locus was increased in the *rad55*Δ mutant (**Fig. 3C**) and that UV-induced HR was increased in *rev3*Δ and *rad30*Δ mutants **(Fig. 4)**. The channeling of UV-induced lesions to the other pathway when one is inactivated suggests that HR and TLS share a common substrate.

Surprisingly, the increase in UV-induced mutagenesis in the *rad55*Δ mutant was moderate and could be clearly observed only at the highest UV doses (**Fig. 3C**). Indeed, as Rad55-Rad57 heterodimers play a role in the template switching pathway [15] and in UV-induced HR (our present study), we predicted a stronger effect. This discrepancy can be explained by the involvement of Rad52, Rad51, and Rad55-Rad57 in the TLS pathway by facilitating Rad6/Rad18-dependent PCNA ubiquitination [60].

On the other hand, strong phenotypes were associated with the absence of Polζ in *rev3*Δ cells. The diploid homozygous *rev3*Δ displayed a strong hyper-recombinogenic phenotype, similar to the hyper-recombinogenic phenotype of *srs2*Δ cells. *Rad30*Δ cells displayed the same UV sensitivity as *rev3*Δ cells, but their UV-induced HR frequencies were much lower than those of the *rev3*Δ mutant (**Fig. 4**). Therefore, in *rev3*Δ mutants, Polη cannot take over DNA lesions that are normally processed by Polζ and managed by HR. In *rad30*Δ cells, the low HR level suggests that lesions are processed by Polζ.

Rad55-Rad57 plays three prominent roles in UV-induced HR

The UV-induced HR defect observed in *rad55*Δ cells was not suppressed by *SRS2* deletion **(Fig. 2B and C)**, suggesting that Rad51 filament formation on UV-induced DNA lesions requires Rad55-Rad57 independently of its balancing role against the disassembly of Rad51 filaments by Srs2. Moreover, the severe UV sensitivity of the double *rad55*Δ *rev3*Δ mutant was only partially suppressed by *srs2*Δ and *MAT* heterozygosity (**Fig. 6**), showing again that Rad55-Rad57 regulates HR independently of Srs2.

The weak sensitivity to IR or site-directed DSBs displayed by the *rad55* and *rad57* mutants [34,35] depends on the Srs2 helicase activity because it is suppressed by *SRS2* deletion [29,35]. Interestingly, *rad55*Δ-associated UV sensitivity was partially suppressed by *srs2*Δ in *rev3*Δ mutants and this effect could not be seen in *REV3* cells because of the channeling of UV-induced DNA lesions to the TLS pathway (**Fig. 6**). From this observation, we propose that Rad55-Rad57 heterodimers compete with Srs2 at ssDNA gaps as they do at DSB sites [29,32]. Thus, the Rad55-Rad57 complex is required to protect Rad51 filaments, although alternative Esc2- and Elg1-dependent mechanisms regulate negatively Srs2 at sites of stalled replication forks [61,62]. More genetic studies are required to determine the interplay between these regulating factors.

Remarkably, UV-induced HR frequency increased in *rad55*Δ cells upon Polζ or Polη deletion (**Fig. 5**). Therefore, Rad55-Rad57 might also allow HR to outcompete the TLS polymerases. Rad51 filament stabilization by the Srs2-independent role of Rad55-Rad57 on ssDNA at the lesion site could prevent the recruitment of TLS polymerases by PCNA because of structural constraints. In the absence of Rad55-Rad57, unstable Rad51 filaments cannot prevent the loading of the TLS polymerases, inhibiting HR and inducing mutagenesis. Depletion of the TLS polymerases in *rad55*Δ mutants would allow some HR events to occur, but the inherent instability of Rad51 filaments would make them rare.

The Rad55-Rad57 complex is essential for UV-induced Rad51 filament toxicity in Srs2-deficient cells

Early genetic studies have led to the concept of toxic recombination intermediates that accumulate in the absence of Srs2 [47–49]. Notably, the *srs2*Δ allele displays synthetic lethality or slow growth when associated with the deletion of genes involved in DNA replication and HR [63], and most of these negative interactions depends on *RAD51* [47,48,63]. Interestingly, negative interactions between *srs2*Δ and *rad50*Δ, *sgs1*Δ, *top3*Δ or *rrm3*Δ are suppressed also by *rad55*Δ [47,48,64]. These observations suggest that Rad55-Rad57 is required to form toxic recombination intermediates in Srs2-deficient cells. We completed these observations by showing that *rad55*Δ or *rad57*Δ mutants completely suppress *srs2*Δ-associated UV-sensitivity (**Fig. 2**). This indicates that Rad55-Rad57 is involved in the formation of Rad51 filaments, leading to lethal events possibly initiated by ssDNA gaps that could block replication fork restart.

Model for Rad55-Rad57 activity in ssDNA gap repair

Our genetic data suggest three different and essential Rad55-Rad57 functions in UV-induced interhomolog HR initiated at ssDNA gaps (**Fig. 8A**). First, Rad55-Rad57 role in Rad51 filament formation and stabilization is required for efficient strand exchange at ssDNA gaps. The 5'ends of DSBs are resected to generate 3' ssDNA that can invade the homologous donor. Conversely, 3' ssDNA is not directly available at ssDNA gaps. Rad55-Rad57 may be required to form Rad51 filaments that can invade without 3' ssDNA extremities. Alternatively, Rad55-Rad57 can be involved in the denaturation of the 3' end at the lesion site to generate a 3' ended ssDNA by recruitment of a DNA helicase. Second, Rad55-Rad57 within Rad51 filaments counterbalances Srs2 activity. Third, Rad55-Rad57 inhibits TLS polymerases by preventing their loading at the primer/template junction within ssDNA gaps (**Fig. 8A-E**). This competition might be crucial to control mutagenesis resulting from gap-filling by Polζ. Thus, in our model, Rad51 filaments formed with Rad55-Rad57 preserve the genome stability through ssDNA gap repair by HR, but also through direct competition with TLS on common ssDNA gap substrates.

What is the interplay between HR and TLS in mammals?

The interplay between error-prone and error-free lesion-bypass pathways is documented in *E. coli* and yeast (the present study, [13,14]), but it has not been specifically addressed in mammals. In this respect, the base substitution mutational signatures and increased somatic mutational load detected in BRCA1- and BRCA2-deficient breast cancer cells are interesting observations [65–68]. Given the HR defect found in cancer cells with BRCA1/2 mutations, it seems likely that TLS plays a role in this mutation signature and mutation increase. Therefore, it is possible that HR-defective mammalian cells rely much more on TLS for DNA damage bypass, as observed with the acute DNA damage sensitivity displayed by the double *rad55*Δ *rev3*Δ mutant in yeast (**Fig.3**) [70,71]. In this scenario, similarly to the use of inhibitors of

PARP (a protein implicated in DNA repair) to treat HR-deficient cancers [69], combining drug-induced inhibition of TLS [70–72] with HR deficiency could result in specific tumor cell lethality. Moreover, in HR-deficient cancer cells, TLS inactivation could reduce the frequency of secondary somatic mutations that lead to resistance against DNA-damaging chemotherapy [73]. Finally, it will be interesting to study the effect of the ablation or of mutations detected in patients for each of the five RAD51 paralog genes in TLS-deficient cells to determine their effects on cell viability upon replication fork stalling by DNA damage.

Materials and Methods

Yeast strains, plasmids and media

The *S. cerevisiae* strains used in the present study are isogenic derivatives of FF18733 (*his7-2, leu2-3,112, lys1-1, trp1-289, ura3-52*) and are listed in **Table S1**. Gene deletions were performed by PCR-mediated one-step replacement [74,75]. All deletions were confirmed by PCR amplification of genomic DNA. All media were prepared as previously described [76]. Mutants were selected on YPD medium containing 300 mg/L of geneticin (Sigma) or nourseothricin (cloNAT; Werner BioAgents).

Irradiations, cell survival assay and frequencies of DNA damage-induced HR

Cells in stationary phase (UV) or exponentially growing (γ-rays) were plated at the appropriate dilutions on YPD and synthetic plates. UV irradiation was performed using a 264 nm source. γ-irradiation was performed using a 137Cs source. After irradiation (UV or γ-rays), cells were plated at the appropriate dilution on rich medium (YPD) to measure the survival rate, and on synthetic plates without arginine to quantify the number of HR events between heteroalleles of *ARG4*. HR frequencies were determined by dividing the number of recombinant colonies growing on selective medium by the number of unselected colonies subjected to the same dose of irradiation. The values obtained were corrected by subtracting the number of recombinants present on the non-irradiated plates. The mean percentage from at least three independent experiments is presented.

Measurement of spontaneous heteroallelic HR

Rates of spontaneous HR between two heteroalleles of *ARG4* were determined by fluctuation tests using the method of the median [77]. The reported rates are the mean of three

independent experiments, each performed with nine independent 2-ml cultures set up from 2-day-old colonies and incubated at 30°C.

Measurement of UV-induced mutagenesis

UV-induced mutagenesis was assessed with the *CAN1* forward-mutation assay. UV-induced mutagenesis frequencies were obtained by dividing the number of colonies growing on selective medium containing canavanine (*i.e.*, canavanine-resistant, can^R, cells) by the number of cells that survived irradiation. The number of can^R colonies obtained after irradiation was corrected by subtracting the number of can^R colonies present on non-irradiated plates and corresponding to spontaneous mutation events. Medium lacking arginine but containing l-canavanine (Sigma) at 30 mg/l was used for the selective growth of can^R mutants on plates.

Survival following DSB formation

Cells were grown overnight in liquid culture medium containing lactate before plating. Survival following HO-induced DSB was measured as the number of cells growing on galactose-containing medium divided by the number of colonies growing on YPD. The results shown are the mean values of at least 3 independent experiments.

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Figure 1

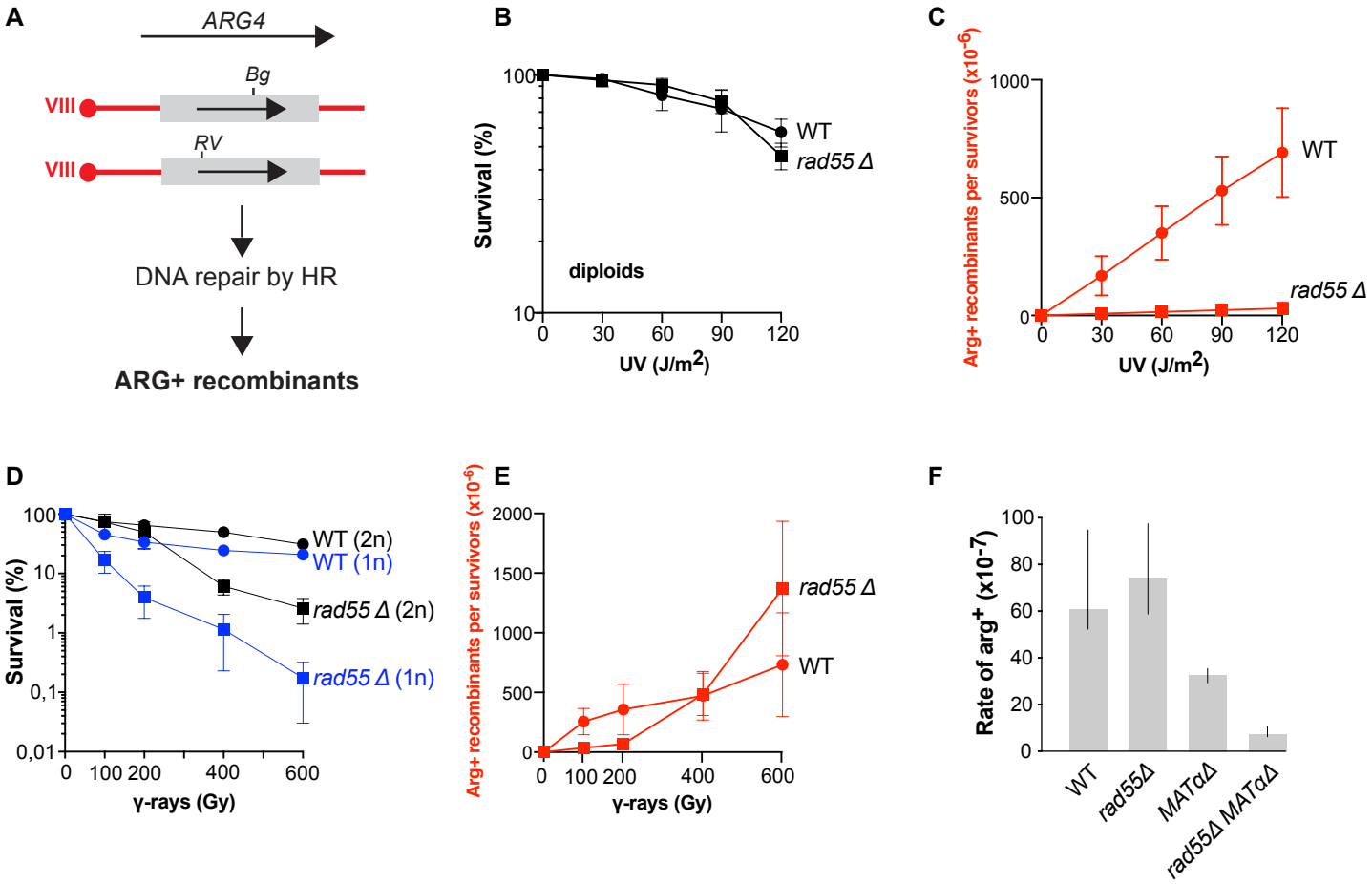

A
ARG4
Bg
VIII
RV
VIII
DNA repair by HR
ARG+ recombinants
B
Survival (%)
100
10
diploids
WT
rad55 Δ
0
30
60
90
120
UV (J/m2)
C
Arg+ recombinants per survivors (x10-6)
1000
500
0
WT
rad55 Δ
0
30
60
90
120
UV (J/m2)
D
Survival (%)
100
10
1
0,1
0,01
WT (2n)
WT (1n)
rad55 Δ (2n)
rad55 Δ (1n)
0
100
200
400
600
γ-rays (Gy)
E
Arg+ recombinants per survivors (x10-6)
2000
1500
1000
500
0
rad55 Δ
WT
0
100
200
400
600
γ-rays (Gy)
F
Rate of arg+ (x10-7)
100
80
60
40
20
0
WT
rad55Δ
MATαΔ
rad55Δ MATαΔ

Fig 1. Rad55 plays a major role in UV-induced HR. (A) Schematic representation of the genetic system used. Frameshift mutations were introduced at the *Eco*RV site (RV, ±2 bp) or the *Bgl*II site (Bg, +4 bp). HR between the two *ARG4* mutant alleles can restore a WT *ARG4* allele associated with the [arg+] phenotype. (B) Survival and (C) [Arg+] recombinant frequencies after UV exposure of WT and *rad55*Δ diploid cells. (D) Survival of haploid (1n; blue curves) and diploid (2n; black curves) WT and *rad55*Δ cells. (E) [Arg+] recombinant frequencies after γ irradiation of WT and *rad55*Δ diploid cells. (F) Mitotic spontaneous [Arg+] recombinant rates ([Arg+] x 10^{-7}/cell/generation) of WT and *rad55*Δ diploid strains, with and without (*MAT*αΔ) *MAT* heterozygosity.

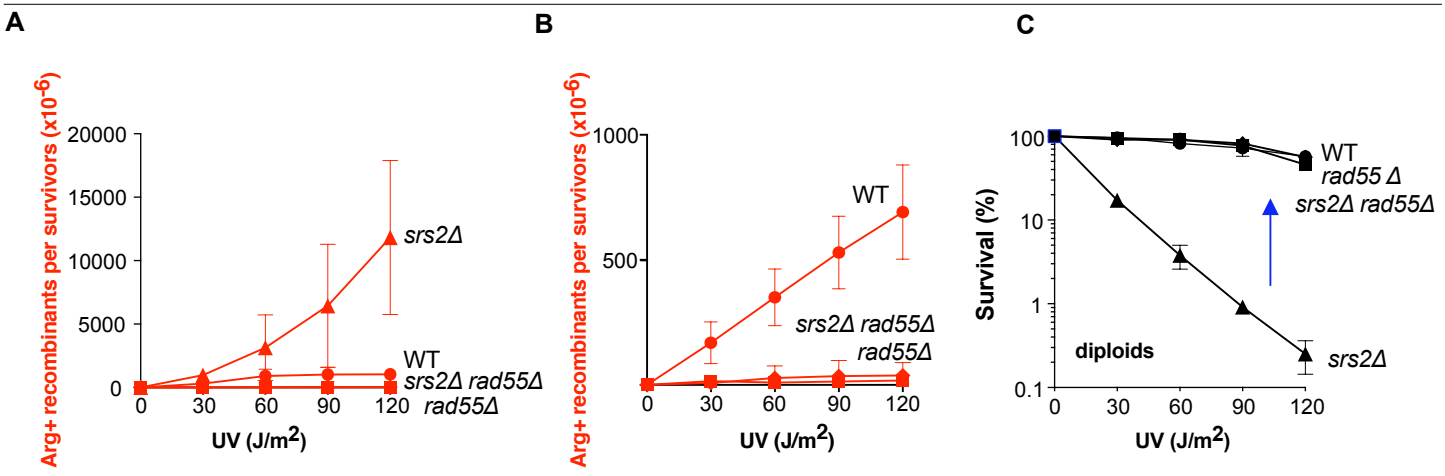

A
B
C
Arg+ recombinants per survivors (x10-6)
20000
15000
10000
5000
0
0
30
60
90
120
UV (J/m2)
srs2Δ
WT
srs2Δ rad55Δ
rad55Δ
1000
500
WT
srs2Δ rad55Δ
rad55Δ
Survival (%)
100
10
1
0.1
diploids
WT
rad55 Δ
srs2Δ rad55Δ
srs2Δ

Figure 2

Fig 2. Deletion of *SRS2* does not rescue the defect in UV-induced HR of the *rad55*Δ mutant. (A) The reduced UV-induced [Arg+] recombinant frequencies of the diploid *rad55*Δ strain are still observed in the *srs2*Δ background at all UV doses applied. Moreover, the high UV-induced [Arg+] recombinant frequencies observed in the diploid *srs2*Δ strain is suppressed by *RAD55* deletion. (B) This graph is a close-up view of (A). (C) Survival curves of diploid cells after exposure to UV light. The acute sensitivity to UV radiation of the diploid *srs2*Δ strain is suppressed by *rad55*Δ (blue arrow).

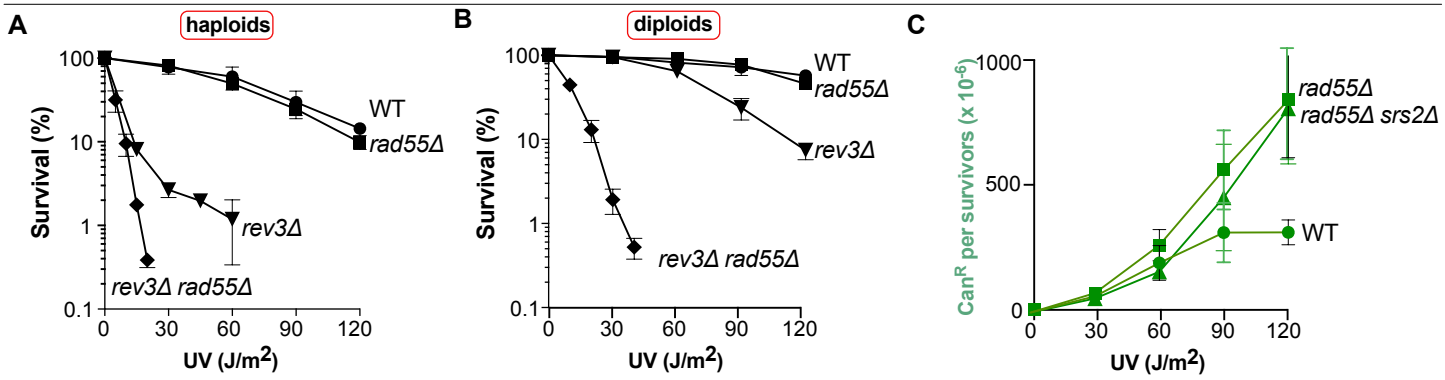

A
haploids
Survival (%)
100
10
1
0.1
0
30
60
90
120
UV (J/m2)
WT
rad55Δ
rev3Δ
rev3Δ rad55Δ
B
diploids
Survival (%)
100
10
1
0.1
0
30
60
90
120
UV (J/m2)
WT
rad55Δ
rev3Δ
rev3Δ rad55Δ
C
CanR per survivors (x 10-6)
1000
500
0
0
30
60
90
120
UV (J/m2)
rad55Δ
rad55Δ srs2Δ
WT

Figure 3

Fig. 3 Channeling UV-induced DNA lesions into the *REV3*-dependent pathway in *rad55*Δ cells. (A) Survival of haploid cells after exposure to UV light. (B) Survival of diploid cells after exposure to UV light. (C) UV-induced mutagenesis in WT and *rad55*Δ cells as observed by quantifying the Can^R mutant frequency for each UV dose applied.

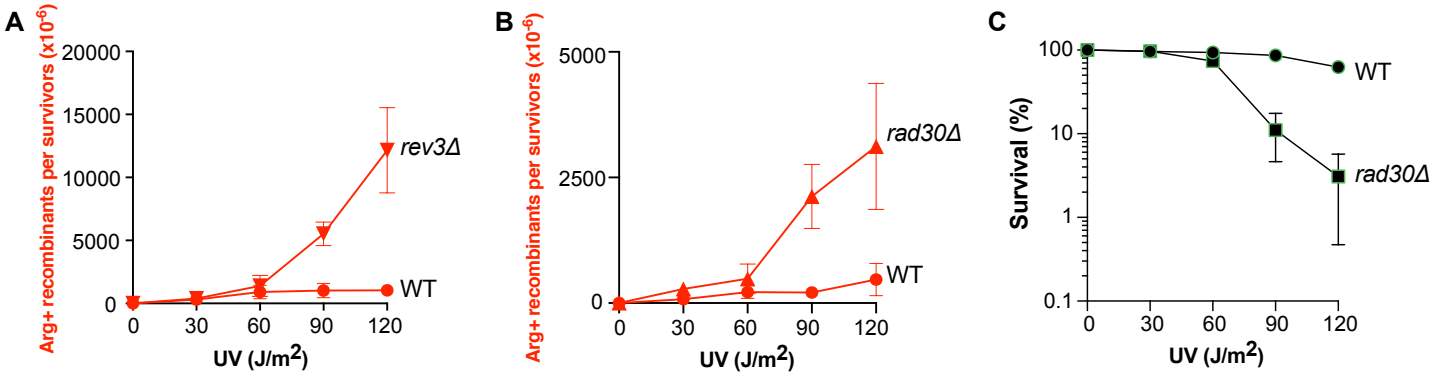

A
Arg+ recombinants per survivors (x10-6)
20000
15000
10000
5000
0
rev3Δ
WT
0
30
60
90
120
UV (J/m2)
B
Arg+ recombinants per survivors (x10-6)
5000
2500
0
rad30Δ
WT
0
30
60
90
120
UV (J/m2)
C
Survival (%)
100
10
1
0.1
WT
rad30Δ
0
30
60
90
120
UV (J/m2)

Figure 4

Fig. 4 Channeling UV-induced DNA lesions into the HR pathway in TLS-deficient cells. (A) UV-induced [Arg+] recombinant frequencies in WT and *rev3*Δ diploid cells. (B) UV-induced [Arg+] recombinant frequencies in WT and *rad30*Δ diploid cells. (C) Survival of diploid *rad30Δ* cells after exposure to UV light.

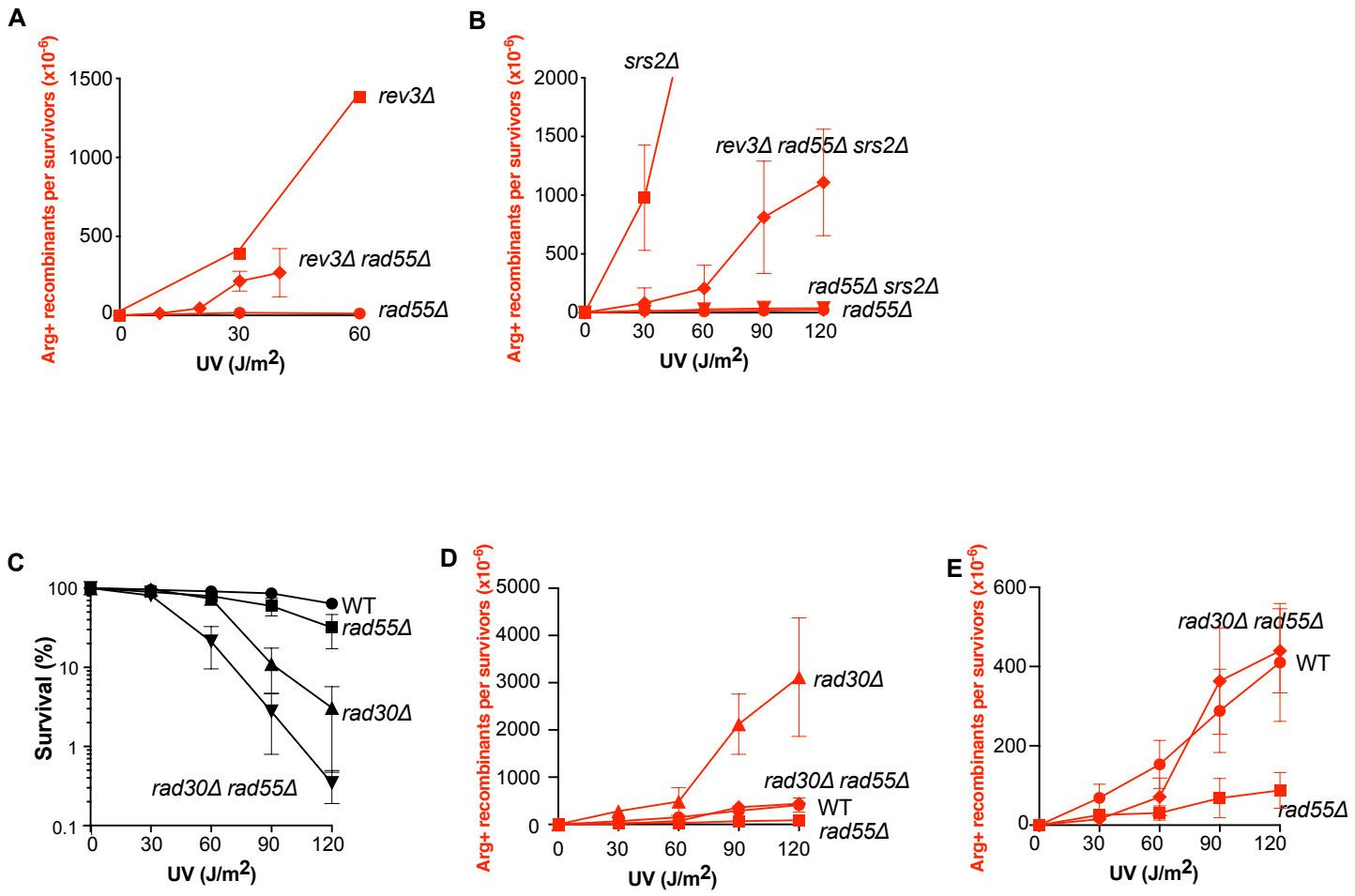

A
Arg+ recombinants per survivors (x10-6)
1500
1000
500
0
0
30
60
UV (J/m2)
rev3Δ
rev3Δ rad55Δ
rad55Δ
B
Arg+ recombinants per survivors (x10-6)
2000
1500
1000
500
0
0
30
60
90
120
UV (J/m2)
srs2Δ
rev3Δ rad55Δ srs2Δ
rad55Δ srs2Δ
rad55Δ
C
Survival (%)
100
10
1
0.1
0
30
60
90
120
UV (J/m2)
WT
rad55Δ
rad30Δ
rad30Δ rad55Δ
D
Arg+ recombinants per survivors (x10-6)
5000
4000
3000
2000
1000
0
0
30
60
90
120
UV (J/m2)
rad30Δ
rad30Δ rad55Δ
WT
rad55Δ
E
Arg+ recombinants per survivors (x10-6)
600
400
200
0
0
30
60
90
120
UV (J/m2)
rad30Δ rad55Δ
WT
rad55Δ

Figure 5

Fig. 5 *RAD55*-independent UV-induced HR in TLS-deficient cells. (A) UV-induced [Arg+] recombinant frequencies in *rad55*Δ *rev3*Δ diploid cells. Note that UV sensitivity displayed by the double mutant precludes the quantification of UV-induced [Arg+] recombinant frequencies at UV doses higher than 40J/m^2. (B) Same as in (A) but in the *srs2*Δ background. (C) Survival of diploid *rad55*Δ *rad30*Δ cells after exposure to UV light. An additive effect on UV sensitivity is observed in the double mutant. (D and E) UV-induced [Arg+] recombinant frequencies in *rad55*Δ and *rad30*Δ diploids cells. Note that (E)) is a close-up view of (D).

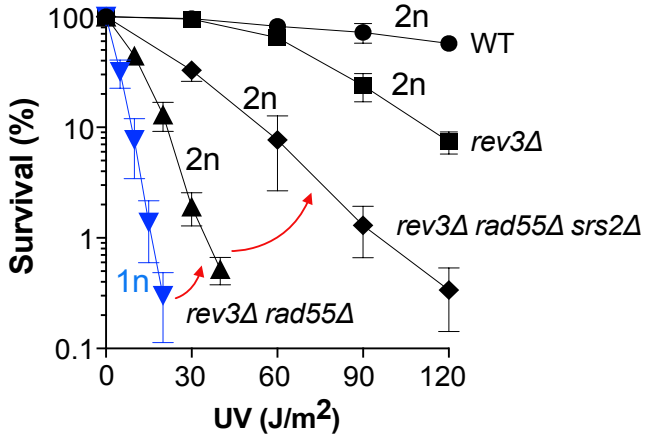

100
10
1
0.1
Survival (%)
0
30
60
90
120
UV (J/m2)
2n
WT
2n
rev3Δ
2n
rev3Δ rad55Δ srs2Δ
2n
rev3Δ rad55Δ
1n

Figure 6

Fig. 6 Partial suppression of *rad55*Δ *rev3*Δ acute UV sensitivity by *MAT* heterozygosity and *SRS2* deletion. The red arrow from haploid cell survival (1n; blue curve) to diploid cell survival (2n; black curves) highlights the better UV resistance of *rev3*Δ *rad55*Δ diploids. The red arrow between the *rev3*Δ *rad55*Δ and *rev3*Δ *rad55*Δ *srs2*Δ survival curves highlights the partial *srs2*Δ suppression. Note that the *rev3*Δ diploid strain is still more resistant than the *rev3*Δ *rad55*Δ *srs2*Δ diploid strain.

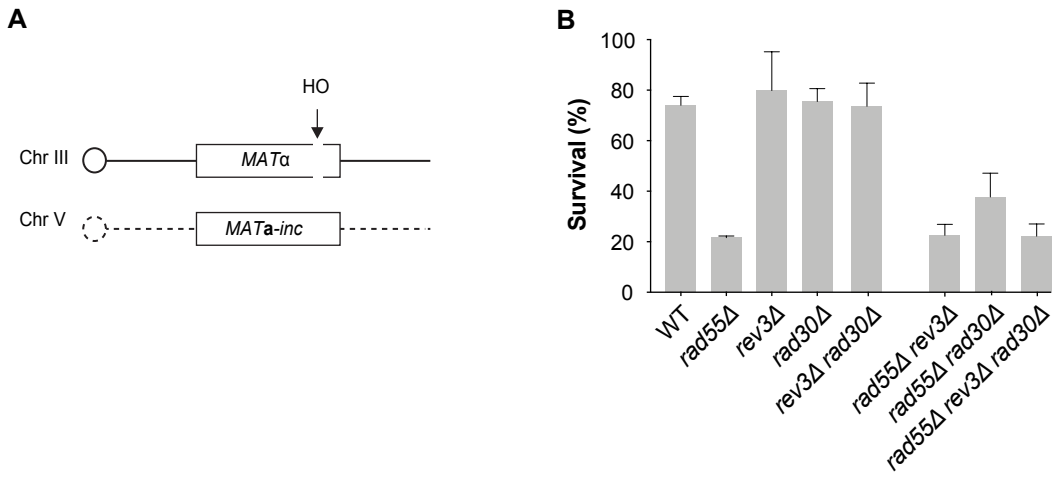

A
HO
Chr III
MATα
Chr V
MATa-inc
B
Survival (%)
100
80
60
40
20
0
WT
rad55Δ
rev3Δ
rad30Δ
rev3Δ rad30Δ
rad55Δ rev3Δ
rad55Δ rad30Δ
rad55Δ rev3Δ rad30Δ

Figure 7

Fig. 7 TLS DNA polymerases are not required for cell survival after a site-specific DSB.

(A) Schematic representation of the HO-induced DSB repair system involving gene conversion between ectopic copies of MAT. (B) Cell viability after DSB formation.

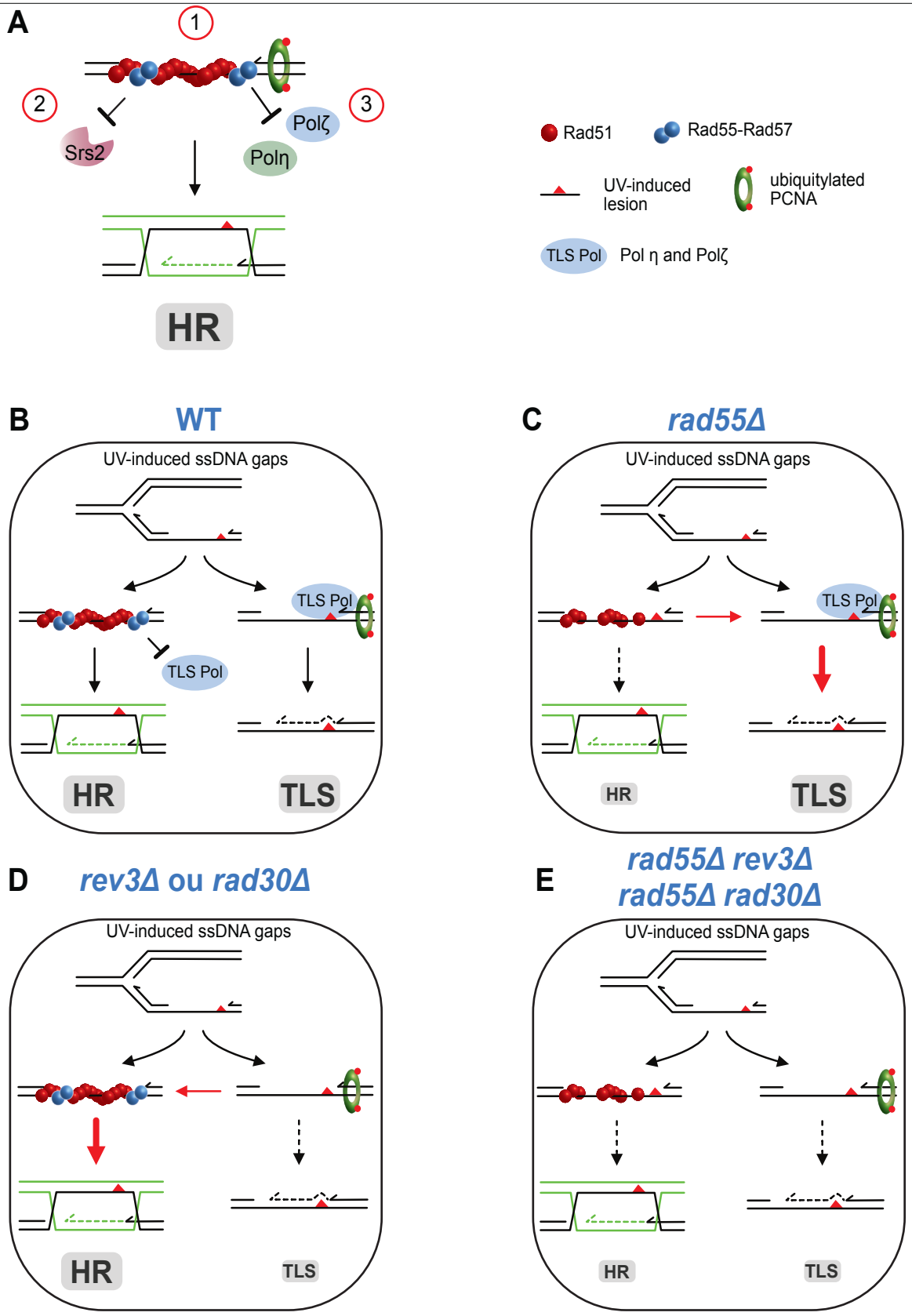
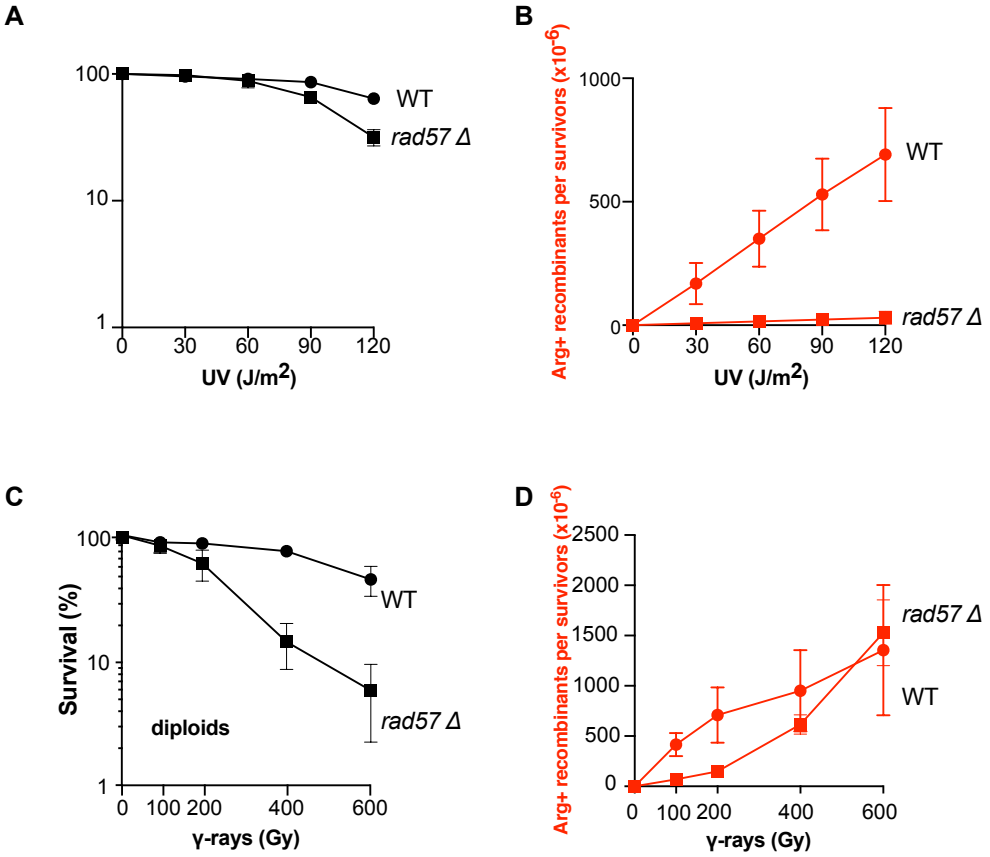

A
1
2
3
Srs2
Polζ
Polη
HR
Rad51
Rad55-Rad57
UV-induced lesion
ubiquitylated PCNA
TLS Pol
Pol η and Polζ
B
WT
UV-induced ssDNA gaps
TLS Pol
TLS Pol
HR
TLS
C
rad55Δ
UV-induced ssDNA gaps
TLS Pol
HR
TLS
D
rev3Δ ou rad30Δ
UV-induced ssDNA gaps
HR
TLS
E
rad55Δ rev3Δ
rad55Δ rad30Δ
UV-induced ssDNA gaps
HR
TLS

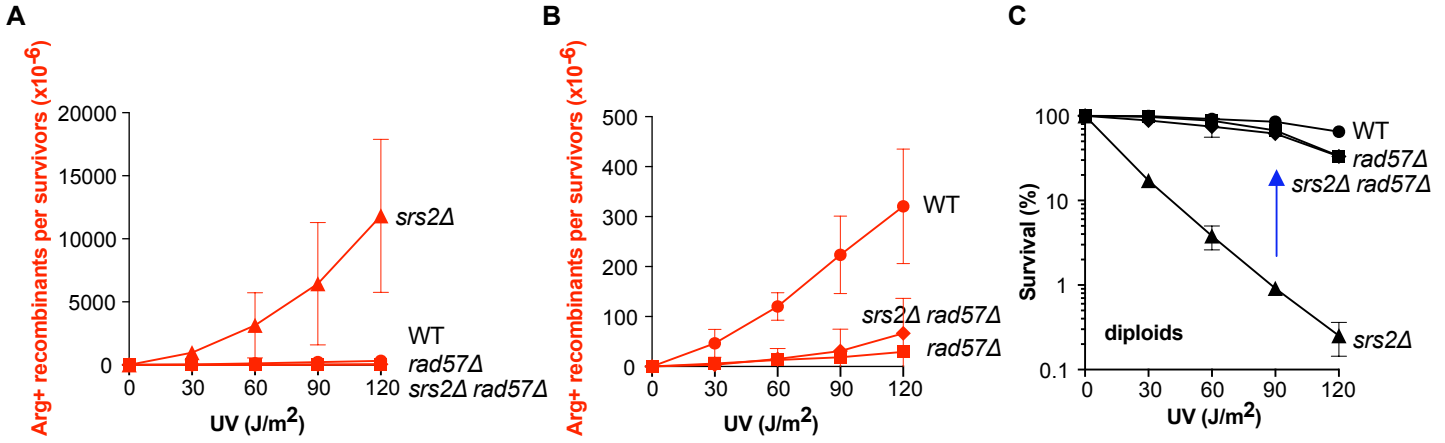
Figure 8

Fig. 8 Rad55-Rad57 roles in ssDNA gap repair by HR behind replication forks. The figure shows an ssDNA gap generated after stalling of a replicative DNA polymerase upon UV exposure. This gap is a common substrate for the HR and TLS pathways. (**A**) The three roles of Rad55-Rad57 in Rad51 filament formation on ssDNA gaps are highlighted: (1) formation of functional Rad51 filaments; (2) protection against Srs2 dismantling activity; (3) prevention of TLS polymerase recruitment at the primer/template junction. The three roles are required to allow WT level of HR. (**B**) In WT yeast cells, Rad55-Rad57 heterodimers (blue spheres) form and stabilize Rad51 filaments (red spheres) on ssDNA leading to HR, and counterbalances TLS. For example, Rad51 filaments can prevent the recruitment of the TLS polymerases Polζ and Polη (collectively named TLS Pol) by covering the 3' extremity blocked at the DNA lesion. (**C**) In the *rad55*Δ mutant, inefficient and/or fewer Rad51 filaments are formed that cannot prevent the recruitment of the TLS polymerases. Therefore, more UV-induced DNA lesions are channeled towards the TLS pathway (red arrows) (**D**) In the *rev3*Δ or in the *rad30*Δ mutant, the absence of Polζ or Polη allows channeling UV-induced DNA lesions towards the HR pathway (red arrows). (**E**) In the double *rad55*Δ *rev3*Δ or *rad55*Δ *rad30*Δ mutants both the HR and the TLS pathways are compromised.

Supplemental Figure S1



S1 Fig. Rad57 plays a major role specifically in UV-induced HR. (A) Survival and (B) [Arg+] recombinant frequencies after UV exposure in WT and *rad57*Δ diploid cells. (C) Survival and (D) [Arg+] recombinant frequencies after γ irradiation in WT and *rad57*Δ diploid cells.



Supplemental Figure S2

S2 Fig. Deletion of *SRS2* does not rescue the defect in UV-induced HR displayed by the *rad57Δ* mutant. (A) The reduced UV-induced [Arg+] recombinant frequencies of the diploid *rad57*Δ strain are still observed in the *srs2*Δ background at all UV doses applied. Moreover, the high UV-induced [Arg+] recombinant frequencies observed in the diploid *srs2*Δ strain are suppressed by *RAD57* deletion. (B) This graph is a close-up view of (A). (C) Survival curves of diploid cells after exposure to UV light. The acute sensitivity to UV radiation of the diploid *srs2*Δ strain is suppressed by *rad57*Δ (blue arrow).