MOLECULAR BIOLOGY

The Mgs1/WRNIP1 ATPase is required to prevent a recombination salvage pathway at damaged replication forks

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DNA damage tolerance (DDT) is crucial for genome integrity maintenance. DDT is mainly carried out by template switch recombination, an error-free mode of overcoming DNA lesions, or translesion DNA synthesis, which is error-prone. Here, we investigated the role of Mgs1/WRNIP1 in modulating DDT. Using budding yeast, we found that elimination of Mgs1 in cells lacking Rad5, an essential protein for DDT, activates an alternative mode of DNA damage bypass, driven by recombination, which allows chromosome replication and cell viability under stress conditions that block DNA replication forks. This salvage pathway is *RAD52* and *RAD59* dependent, requires the DNA polymerase δ and PCNA modification at K164, and is enabled by Esc2 and the PCNA unloader Elg1, being inhibited when Mgs1 is present. We propose that Mgs1 is necessary to prevent a potentially toxic recombination salvage pathway at sites of perturbed replication, which, in turn, favors Rad5-dependent template switching, thus helping to preserve genome stability.

INTRODUCTION

The presence of DNA damage is largely inevitable and a main source of genomic instability (*1*). DNA lesions can cause pathological conditions that may lead to disease or cell death, and in consequence, cells require efficient mechanisms that first detect and then either repair or tolerate DNA insults (*1*, *2*). Cells are especially vulnerable to DNA damage during chromosome replication, as unrepaired lesions at the time of replication may hamper the progression of replication forks. These lesions need to be tolerated, leaving their repair for a later time, to avoid permanent fork stalling or fork breakdown that would result in incomplete genome replication (*2*, *3*).

In eukaryotes, the DNA damage tolerance (DDT) is mainly carried out by the *RAD6/RAD18* pathway (*2*, *3*). When DNA replication forks stall due to DNA lesions or replicative stress, the DNA polymerases and the replicative helicase can partially uncouple, leading to long stretches of single-stranded DNA (ssDNA) that are coated by the replication protein A. This coated ssDNA is the signal for the activation of DDT, triggering the recruitment to chromatin of the E3-ubiquitin ligase Rad18, which, in turn, recruits the E2-conjugating enzyme Rad6 (*4*). Both proteins form a heterodimer that monoubiquitylates the proliferating cell nuclear antigen (PCNA) sliding clamp protein at K164 (*5*). This PCNA modification activates translesion DNA synthesis (TLS) by favoring its interaction with bypass (TLS) polymerases (*6*). TLS polymerases have low fidelity and are able to replicate across the DNA lesions, a mode of DNA damage bypass that is frequently error-prone. The monoubiquitin modification of PCNA can be further extended to K63-linked polyubiquitin chains, a process that is carried out by the E3-ubiquitin ligase Rad5 in budding yeast (*5*) (HLTF and SHPRH in mammals) together with the E2 complex Ubc13-Mms2 (UBC13-UEV1 in mammals). PCNA polyubiquitylation mediates a second mode of DNA damage bypass that requires the DNA-dependent adenosine triphosphatase (ATPase)/helicase activity of Rad5 and is driven by transient template switch recombination (*7*, *8*). In this type of bypass, the blocked DNA nascent strand uses the recently synthesized undamaged strand of the sister chromatid as a template for replication over the lesion, and the process is error-free. Both modes of DDT are interconnected, as Rad5 is also required for the recruitment of TLS polymerases to stressed replication forks and for TLS activity (*9*).

In addition to ubiquitylation, PCNA is also modified during chromosome replication by SUMOylation at K164 and, to a minor extent, at K127 (*5*). PCNA SUMOylation promotes the recruitment of Srs2, an antirecombinogenic helicase that prevents unscheduled recombination at replication forks (*10*, *11*) by dismantling Rad51 filaments (*12*, *13*). In higher eukaryotes, a similar antirecombinogenic role is carried out by PARI (*14*, *15*). Both modifications of PCNA, polyubiquitylation and SUMOylation, cooperate to facilitate template switching (*16*, *17*), and, at least in budding yeast, SUMO-PCNA is the physiological substrate of Rad18 (*17*).

The fact that homologous recombination is inhibited by Srs2 after its recruitment by SUMO-PCNA, while template switch recombination works as an efficient error-free DNA damage bypass mechanism, raised an apparent paradox and an interesting biological problem that was recently deciphered (*18*). This work showed that whereas homologous recombination is inhibited globally during chromosome replication by the Srs2 helicase at ongoing forks, the template switching mode of recombination is allowed locally via the action of the SUMO-like domain protein Esc2, which counteracts Srs2 at damaged or stalled replication forks (*18*). Esc2 binds to sites of stalled replication and promotes Elg1-dependent local unloading of SUMO-PCNA, together with bound Srs2, and Slx5-Slx8–mediated proteasome degradation of the antirecombinase, which notably reduces the levels of Srs2 at the stalled forks. These low Srs2 levels allow local Rad51 filament formation and recombination-mediated damage bypass via template switching at sites of perturbed replication (*18*). A question derived from these findings, however, is how the template switch mode of recombination is favored, whereas, despite the local

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counteraction of Srs2 at damaged forks, a salvage pathway of recombination is still inhibited or postponed for later in the cell cycle. This action is important because, unlike template switching, a salvage pathway is potentially toxic as it can lead to deleterious genomic rearrangements during replication or the accumulation of DNA intermediates that are not properly resolved.

Posttranslational modifications of PCNA are central for DDT, and therefore, the study of the proteins that interact with modified PCNA may provide a better understanding of how the mechanisms of DDT are modulated. Among them, budding yeast maintenance of genome stability 1 [Mgs1; MgsA/RarA in bacteria and WRNIP1 (Werner helicase interacting protein 1) in humans] is an evolutionarily conserved DNA-dependent AAA+ ATPase with ssDNA annealing activity (*19*), whose function is poorly understood. Mgs1 interacts with PCNA in vivo and in vitro (*19*, *20*) and shows preference for the association with polyubiquitylated PCNA via its ubiquitin-binding zinc finger (UBZ) domain, which allows its recruitment to sites of replication stress (*20*). Mgs1 also interacts genetically and physically with the polymerase δ (Pol δ) (*21*–*23*). By interfering with the Pol δ–PCNA interaction, Mgs1 might facilitate the release of this polymerase during different processes (*20*). Mgs1 levels are important for its function because overexpression affects mutation rates and recombination and makes cells sensitive to genotoxic stress (*19*, *21*). However, *MGS1* deletion does not confer sensitivity to DNA-damaging agents, but it causes an increase in the frequency of mitotic recombination (*19*, *21*). Although the function of Mgs1 is unclear, this protein has been linked to DDT due to the interaction with PCNA described above and because Mgs1 becomes essential in the absence of Rad6/Rad18 (*22*, *23*). In addition, consistent with the requirement of Mgs1 and its homologs for genome stability during chromosome replication, it was recently shown that human WRNIP1, with proposed roles in DNA transactions (*24*), is important for the maintenance of the integrity of stalled forks and for replication resumption (*25*). Likewise, it has been proposed that *Bacillus subtilis* RarA assembles at blocked forks and plays a role in preventing pathological replication fork restart (*26*).

In this work, we have investigated the contribution of Mgs1 to the DDT and have found that, in the absence of Rad5, this protein is required to prevent a recombination salvage pathway at damaged and stalled replication forks. We propose that Mgs1 contributes to channeling DDT to error-free template switch recombination by helping to block other potentially detrimental recombination processes at the replication fork, an action that is fundamental for genome stability.

RESULTS

The MMS and HU sensitivity of cells lacking the DDT protein Rad5 is suppressed by deletion of *MGS1* or elimination of Mgs1-ATPase activity

To start investigating the contribution of Mgs1 to DDT, we first studied its functional relevance in the absence of Rad5. We analyzed in *Saccharomyces cerevisiae* the sensitivity of a double mutant *mgs1*Δ*rad5*Δ to methyl methanesulfonate (MMS) or hydroxyurea (HU) (Fig. 1). As described (*22*, *23*), *mgs1*Δ*rad5*Δ cells showed a growth defect with respect to the parental strains when spores germinate after tetrad dissection (fig. S1A), but this defect was not apparent when cells growing exponentially were spotted onto rich-medium plates (Fig. 1A). As previously reported (*19*, *21*), cells lacking *MGS1* did not present differences in the sensitivity to MMS or HU with respect to wild-type cells, whereas *rad5*Δ mutant cells were highly sensitive to the treatment with both compounds (Fig. 1A). Previous reports had indicated a similar sensitivity of *rad5*Δ and *mgs1*Δ*rad5*Δ cells to MMS and a higher sensitivity of the *mgs1*Δ*rad5*Δ double mutant to HU than the single *rad5*Δ (*22*). Unexpectedly, however, and probably as a result of the different experimental approach used, our drop dilution assays showed that *mgs1*Δ*rad5*Δ cells were significantly more resistant to chronic exposure to MMS or HU than *rad5*Δ cells (Fig. 1A), indicating that deletion of *MGS1* causes a significant suppression of the sensitivity of cells lacking Rad5 to those agents. To rule out that this result was due to the genetic background used (W303), we carried out the same kind of experiments using DF5 *S. cerevisiae* cells (fig. S1B). The recovery of the viability of the *rad5*Δ mutant after MMS or HU treatment when *MGS1* was deleted was similar to that obtained with W303 (Fig. 1A and fig. S1B), which eliminated a potential influence of the background on our data.

In the drop dilution assays described above, cells were treated with MMS or HU for several generations. As Rad5 is required for the completion of chromosome replication and the maintenance of viability during S phase in the presence of MMS-damaged DNA (*27*), we also examined the sensitivity to MMS of *mgs1*Δ*rad5*Δ cells and the corresponding individual mutants during a single S phase (Fig. 1B). Cells were first synchronized in G_1 phase with the α factor pheromone and then released into S phase in fresh medium containing different concentrations of MMS. The analysis of the viability along the experiment indicated that wild-type control and *mgs1*Δ cells were not sensitive to the treatment with MMS during S phase, unlike *rad5*Δ cells, which were highly sensitive to all MMS doses used (Fig. 1B). Similar and consistent to the results obtained with the drop dilution assays (Fig. 1A), elimination of *MGS1* allowed cells lacking Rad5 to significantly reduce their sensitivity to MMS at all the concentrations used, even at the highest doses of MMS and at the longest exposure times during S phase (Fig. 1B). Thus, deletion of *MGS1* notably reduces the sensitivity of *rad5*Δ mutant cells to the treatment with MMS, not only after chronic exposure to this DNA-damaging agent but also during a single S phase.

Mgs1 contains an ATPase domain in its central region (*19*) and a zinc finger domain (UBZ) at its C terminus that is necessary for the interaction of this protein with PCNA (*20*). To differentiate whether the effect of *MGS1* deletion on *rad5*Δ cells was due to the absence of the whole Mgs1 protein or can just be explained by the elimination or some of its properties, we examined the consequences of mutating the ATPase or the UBZ domains of Mgs1 on the sensitivity of *rad5*Δ to MMS or HU. We constructed strains combining previously characterized mutants of these domains [*mgs1-K183A* for ATPase (*19*) and *mgs1-D31A* for UBZ (*20*)] with *rad5* deletion and analyzed their sensitivity to MMS and HU after treatment for several generations (Fig. 1C). Drop dilution assays (Fig. 1C) showed that cells lacking Rad5 were highly sensitive to MMS or HU and that this sensitivity was significantly suppressed after *MGS1* deletion, in agreement with the data in Fig. 1A. The mutation of the UBZ domain of Mgs1 did not have any effect on the sensitivity to MMS or HU of *rad5*Δ cells (*mgs1-D31A rad5*Δ strain; Fig. 1C), but the mutation of the ATPase activity of Mgs1 suppressed, to a large extent, the sensitivity of the *rad5*Δ mutant to the same drugs (*mgs1-K183A rad5*Δ strain; Fig. 1C). Likewise, the inactivation of the ATPase activity of Mgs1 allowed a significant suppression of the sensitivity of *rad5*Δ cells to MMS during a single S phase (Fig. 1D), at all MMS concentrations

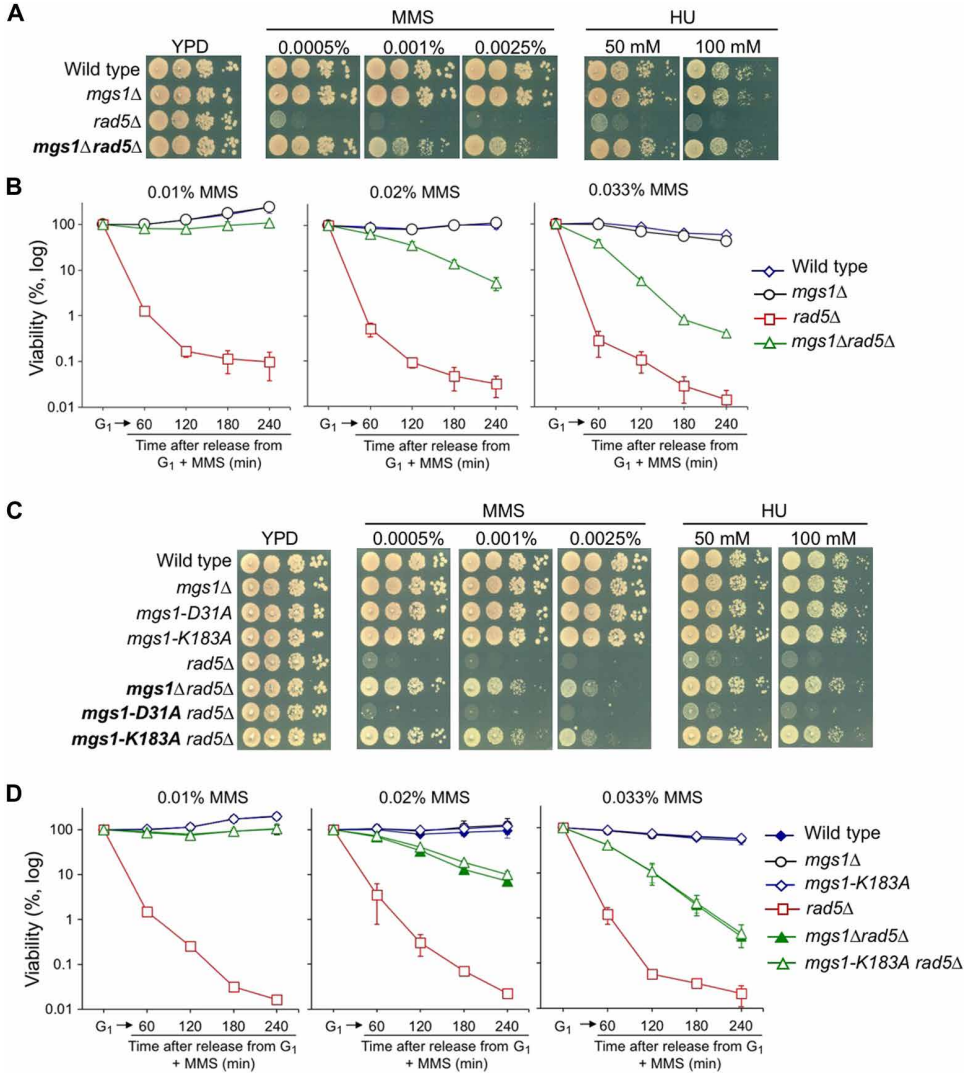

Fig. 1. *MGS1* deletion or elimination of Mgs1-ATPase activity reduce the sensitivity of *rad5*Δ cells to MMS or HU. (**A**) Drop dilution assays. Sensitivity of the strains to chronic treatment with MMS or HU. Serial dilutions (10-fold) of normalized exponentially growing cultures were spotted onto YPD plates containing different amounts of MMS or HU, as indicated, and incubated for 48 hours at 30°C. Strains: Wild type (SY2080), *mgs1*Δ (YSG15), *rad5*Δ (SY2214), and *mgs1*Δ*rad5*Δ (YAJ48) (W303 background). (**B**) Sensitivity to MMS during S phase. Cells were synchronized in G_1 phase with α factor and then released into S phase in medium containing different MMS concentrations. Strains are as in (A). The plots represent the means ± SD from three independent experiments. (**C**) Drop dilution assays, as in (A). Strains: Wild type (YAJ111), *mgs1*Δ (YAJ110), *mgs1-D31A* (YAJ112), *mgs1-K183A* (YAJ113), *rad5*Δ (YAJ115), *mgs1*Δ*rad5*Δ (YAJ114), *mgs1-D31A rad5*Δ (YAJ116), and *mgs1-K183A rad5*Δ (YAJ117). (**D**) Sensitivity to MMS during S phase, as in (B). Strains are as in (C). The plots represent the means ± SD from three independent experiments.

used, to a similar extent as the deletion of *MGS1* (Fig. 1, B and D). Thus, elimination of the ATPase activity of Mgs1 is sufficient to significantly suppress the high sensitivity to treatment with MMS or HU of cells lacking the DDT protein Rad5.

Mgs1 elimination in *rad5*Δ cells facilitates a pol δ– and PCNA-K164 modification–dependent tolerance pathway

Rad5 is required for the completion of DNA replication in the presence of DNA-damaging agents such as MMS or adozelesin (*27*–*29*), making possible the progression of replication forks through damaged DNA and thus contributing to the maintenance of cell viability (*27*). As chromosome replication in the presence of MMS-induced DNA damage is halted in *rad5*Δ cells (*27*), we investigated whether the absence of Mgs1 could revert that situation, which would help to explain the *mgs1*Δ*rad5*Δ results in Fig. 1. With this purpose, we analyzed the dynamics of chromosomal replication by pulsed-field gel electrophoresis (PFGE) after treating cells with MMS (Fig. 2A). *mgs1*Δ*rad5*Δ and wild-type, *mgs1*Δ, and *rad5*Δ control cells were synchronized in G_1 with α factor and then released into S phase in fresh medium containing MMS. After 60 min, the drug was removed, and the cells were allowed to progress through S phase. To avoid entry into a new cell cycle, cells were blocked in G_2-M by adding nocodazole to the medium (Fig. 2A, left top). PFGE resolves linear chromosomes from agarose-embedded cells, while the DNA containing replication bubbles stays in the loading wells. In the four strains, intact chromosomal DNA from G_1-blocked cells was separated as discrete bands. In all cases, after 60-min treatment with MMS during S phase, no bands were detected, indicating ongoing replication [Fig. 2A,

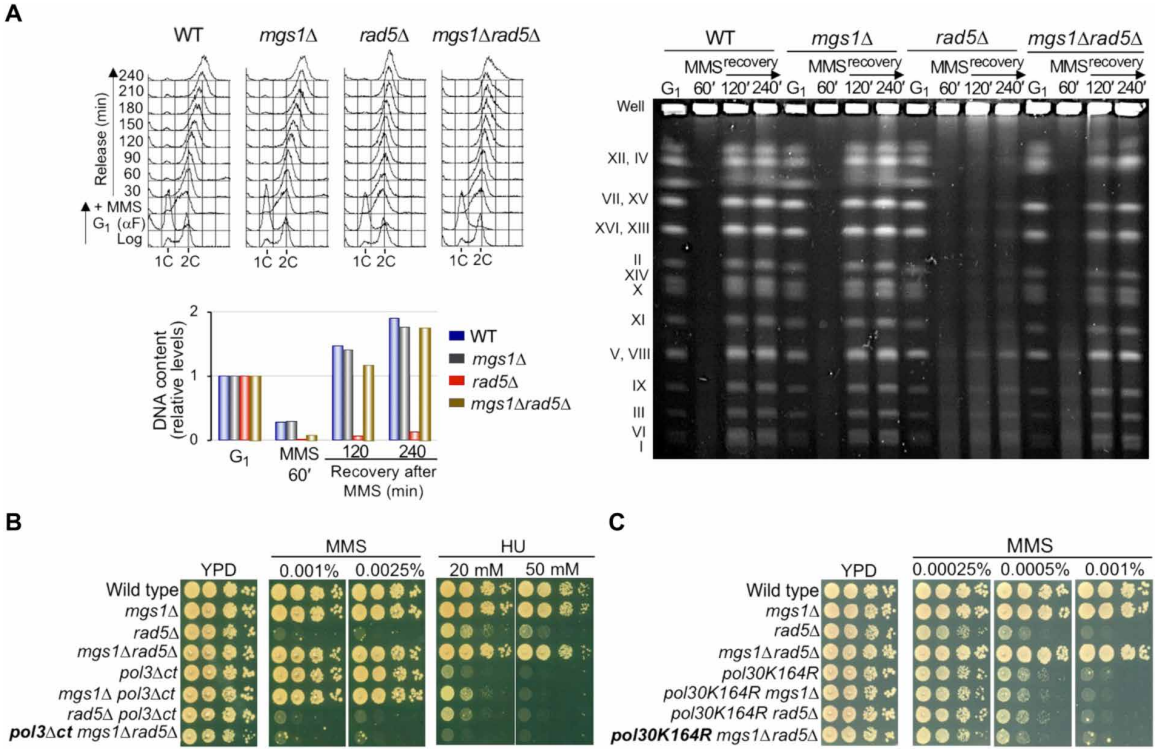

Fig. 2. *MGS1* deletion in cells lacking Rad5 allows the completion of DNA replication under genotoxic stress conditions. (**A**) PFGE analysis. Cells were synchronized in G_1 and released into S phase in medium containing 0.02% MMS and nocodazole for 60 min. The MMS was then removed, and cells were allowed to progress through S phase in medium with nocodazole. Cell cycle progression was monitored by flow cytometry (left top). An ethidium bromide–stained pulse-field gel is shown (right). Chromosomes are labeled with Roman numerals. The quantification of the relative DNA levels of each sample with respect to those in G_1 is indicated (left bottom). Strains: Wild type (WT; SY2080), *mgs1*Δ (YSG15), *rad5*Δ (SY2214), and *mgs1*Δ*rad5*Δ (YAJ48). (**B**) Suppression of the sensitivity to MMS or HU of *rad5*Δ cells after Mgs1 elimination is Pol δ–dependent. Drop dilution assays. Serial dilutions (10-fold) of normalized exponentially growing cultures were spotted onto YPD plates containing different amounts of MMS or HU and incubated for 48 hours at 30°C. Strains: Wild type (SY2080), *mgs1*Δ (YSG15), *rad5*Δ (SY2214), *mgs1*Δ*rad5*Δ (YAJ48), *pol3*Δ*ct* (YSG18), *pol3*Δ*ct mgs1*Δ (YSG21), *pol3*Δ*ct rad5*Δ (YAJ133), and *pol3*Δ*ct mgs1*Δ*rad5*Δ (YAJ135). (**C**) Reduction of the sensitivity to MMS or HU of *rad5*Δ cells after Mgs1 elimination requires modification of the K164 residue of PCNA. Drop dilution assays as in (B). Strains: Wild-type *MGS1*$^{+}$*RAD5*$^{+}$ (SY2080), *mgs1*Δ (YSG15), *rad5*Δ (SY2214), *mgs1*Δ*rad5*Δ (YAJ48), *pol30K164R* (TH291), *pol30K164R mgs1*Δ (YAJ104), *pol30K164R rad5*Δ (YAJ106), and *pol30K164R mgs1*Δ*rad5*Δ (YAJ130).

right (PFGE) and left bottom (quantification)], consistent with flow cytometry (Fig. 2A, left top). In wild-type control and *mgs1*Δ cells, full-length chromosomes reentered the gel after 120-min recovery in medium without MMS, as shown by a clear signal from discrete bands that increased after 240 min to nearly 2× with respect to that in G_1 [Fig. 2A, right and left (bottom)]. These data indicated that, in most cells, the chromosomes recovered from the DNA lesions induced by MMS and completed replication. In contrast, in *rad5*Δ cells, and in agreement with the requirement of Rad5 for replication of damaged DNA (*27*–*29*), most of the DNA was retained in the wells even 240 min after recovery from MMS treatment, indicating that chromosomes were not replicated [Fig. 2A, right and left (bottom)]. However, the elimination of Mgs1 suppressed to a high extent the replication problems of cells lacking Rad5. Thus, unlike *rad5*Δ cells and similar to wild-type and *mgs1*Δ controls, in *mgs1*Δ*rad5*Δ cells, there were discrete bands corresponding to intact chromosomes 120 min after recovery from MMS exposure, with band signals increasing to similar levels to those of wild type and *mgs1*Δ after 240 min [Fig. 2A, right and left (bottom)], indicating that, in most cells, chromosomal replication had been completed. Therefore, the absence of Mgs1 in *rad5*Δ cells allows DNA damage bypass and chromosome replication, which may explain why cells lacking Rad5 reduce their sensitivity to agents causing DNA damage or replication stress when *MGS1* is deleted.

To understand how DNA damage bypass takes place in *mgs1*Δ*rad5*Δ cells, we first asked whether the replicative Pol δ, whose interaction with PCNA is modulated by Mgs1 (*20*), is necessary for the suppression described so far. Although the gene encoding the catalytic subunit of Pol δ, Pol3, is essential, the removal of the last four amino acids of Pol3 (*pol3*Δct mutant) allows cell survival while conferring sensitivity to certain agents (*30*). We made a triple mutant *pol3*Δct *mgs1*Δ*rad5*Δ, which was viable, and carried out sensitivity assays to MMS or HU, as before (Fig. 2B). Drop dilution assays showed that the double mutants *mgs1*Δ*pol3*Δct and *rad5*Δ*pol3*Δct behaved as their corresponding individual parental mutants, whereas *mgs1*Δ*rad5*Δ cells were more resistant to MMS or HU than the *rad5*Δ mutant. However, the suppression of the sensitivity to MMS or HU of *rad5*Δ by *MGS1* deletion was not possible when the last amino acids of Pol3 were deleted (*pol3*Δct *mgs1*Δ*rad5*Δ strain). This result indicates that Pol δ is necessary for the process that allows DNA replication and viability of *mgs1*Δ*rad5*Δ cells after MMS or HU treatment.

The absence of the E3-ubiquitin ligase Rad5 impedes PCNA polyubiquitylation, but this protein can be still modified by monoubiquitylation at K164 and by SUMOylation at the same residue and,

to a minor extent, at K127 (*5*, *6*). To analyze whether PCNA modification was relevant for the replication through damaged DNA in *mgs1*Δ*rad5*Δ cells, we used a PCNA mutant not modifiable at K164, *pol30K164R*, and performed drug sensitivity assays as before (Fig. 2C). Drop dilution assays showed that *pol30K164R* cells were as sensitive to MMS as *rad5*Δ cells. Moreover, deletion of *MGS1* in a *pol30K164R rad5*Δ mutant did not suppress the sensitivity of these cells to MMS treatment (Fig. 2C). Therefore, together with the requirement for Pol δ, PCNA modification at K164 residue is necessary for DNA replication and cell viability under stress conditions in *rad5*Δ cells when Mgs1 is eliminated.

Translesion synthesis polymerases make only a minor contribution to replication stress tolerance in *mgs1*Δ*rad5*Δ cells

As template switching is not present in the *mgs1*Δ*rad5*Δ mutant due to the lack of Rad5, it could be expected that DNA damage bypass could rely solely on translesion DNA synthesis. In *MGS1*$^{+}$*rad5*Δ cells, TLS activity is not enough to allow the completion of chromosome replication under conditions of MMS-damaged DNA (*27*), but spontaneous mutagenesis is increased in *mgs1*Δ*rad5*Δ cells (*22*), which could be the result of a higher activity of TLS polymerases in this mutant. Taking these data and the results above into account, we analyzed whether TLS activity is responsible for allowing DNA damage bypass and thus for the completion of chromosome replication and cell viability in the *mgs1*Δ*rad5*Δ mutant, at least in response to MMS treatment. According to this hypothesis, TLS polymerases would bypass the DNA lesions in *mgs1*Δ*rad5*Δ cells for which the modification of PCNA at K164 is necessary, and then Pol δ would continue DNA synthesis.

*rev1*Δ*mgs1*Δ*rad5*Δ and *rev3*Δ*mgs1*Δ*rad5*Δ mutants exhibit severe growth defects [fig. S2 and (*23*)], but the use of the *mgs1-K183A* allele, which in combination with *rad5*Δ behaves similarly to *mgs1*Δ (Fig. 1, C and D), allowed the construction of viable strains (*mgs1-K183A rev1*Δ*rad5*Δ, *mgs1-K183A rev3*Δ*rad5*Δ, and *mgs1-K183A rev1*Δ*rev3*Δ*rad30*Δ*rad5*Δ—the last one is referred to as *mgs1-K183A tls*Δ*rad5*Δ; Fig. 3, A and B) that were useful to test the mentioned hypothesis. Drop dilution assays (Fig. 3B) indicated that cells lacking Rad5 were highly sensitive to MMS or HU and that elimination of *MGS1* or its ATPase activity (*mgs1-K183A rad5*Δ strain) significantly suppressed this sensitivity, in agreement with the data in Fig. 1. However, in the absence of any of the TLS polymerases or even of all of them (Fig. 3B, last four lanes), there was only a small reduction in the suppression of the sensitivity of *rad5*Δ cells to MMS when Mgs1-ATPase activity was eliminated, and there was no effect on the sensitivity to HU. This result indicates that TLS polymerases have only a minor role in the process that allows DNA replication and cell viability in the absence of Rad5 when *MGS1* is deleted or Mgs1-ATPase activity is eliminated, and therefore, other mechanisms that explain the *mgs1*Δ*rad5*Δ phenotype must be involved.

Elimination of Mgs1 in cells lacking Rad5 promotes a Rad52- and Rad59-dependent recombination-driven tolerance pathway

Previous studies had shown that the elimination of the Srs2 helicase, which binds SUMO-PCNA causing inhibition of homologous recombination (*10*, *11*), suppresses the sensitivity of *rad5*Δ cells to ultraviolet (UV) light or γ-irradiation (*31*, *32*). Considering these data, we sought to analyze whether homologous recombination was

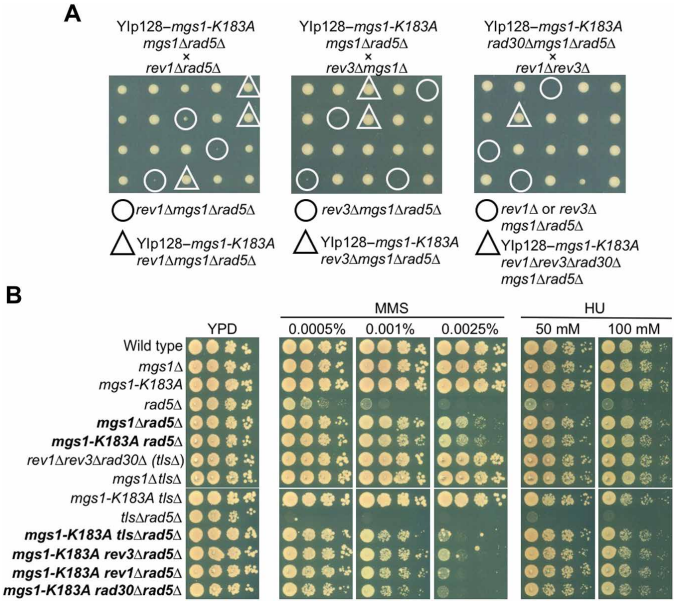

Fig. 3. TLS polymerases have a minor role in the suppression of the sensitivity of *rad5*Δ cells to MMS or HU when the Mgs1-ATPase activity is eliminated. (**A**) The elimination of the ATPase activity of Mgs1 is compatible with the deletion of *RAD5* and the genes encoding TLS polymerases. Examples of tetrad dissection after combining *RAD5* deletion in the absence of Mgs1-ATPase activity (*mgs1-K183A*) with deletions of *REV1* (YAJ117 × YAJ96 strains; left), *REV3* (YAJ117 × YAJ76; center), or all TLS (*REV1*, *REV3*, and *RAD30*; YAJ183 × YAJ206; right). Spores were grown at 30°C for 48 hours. (**B**) Drop dilution assays. Serial dilutions (10-fold) of normalized exponentially growing cultures were spotted onto YPD plates containing different amounts of MMS or HU and incubated for 48 hours at 30°C. Strains: Wild-type (YAJ111), *mgs1*Δ (YAJ110), *mgs1-K183A* (YAJ113), *rad5*Δ (YAJ115), *mgs1*Δ*rad5*Δ (YAJ114), *mgs1-K183A rad5*Δ (YAJ117), *rev1*Δ*rev3*Δ*rad30*Δ (*tls*Δ) (YAJ231), *mgs1*Δ*tls*Δ (YAJ233), *mgs1-K183A tls*Δ (YAJ235), *tls*Δ*rad5*Δ (YAJ237), *mgs1-K183A tls*Δ*rad5*Δ (YAJ230), *mgs1-K183A rev3*Δ*rad5*Δ (YAJ165), *mgs1-K183A rev1*Δ*rad5*Δ (YAJ196), and *mgs1-K183A rad30*Δ*rad5*Δ (YAJ205).

involved in the mechanism that allows chromosome replication in *mgs1*Δ*rad5*Δ in the presence of DNA damage or replicative stress, which, as shown above, cannot be explained (only) by the action of TLS polymerases. We first tested in our genetic background the consequences of eliminating Srs2 in combination with *mgs1*Δ or *rad5*Δ and found that, similar to what happens in *mgs1*Δ*rad5*Δ cells and consistent with the aforementioned reports (*31*, *32*), *SRS2* deletion reduced the sensitivity of *rad5*Δ cells to MMS or HU (fig. S3). Moreover, in the absence of Srs2, Mgs1 elimination did not have apparent consequences on *rad5*Δ cells and vice versa.

The similarity of the phenotype of the *mgs1*Δ*rad5*Δ mutant to that of the strains harboring *SRS2* deletion (fig. S3) could support the hypothesis that, as in *srs2*Δ cells, homologous recombination was facilitated in *rad5*Δ cells after *MGS1* deletion. To directly test this idea, we analyzed recombination molecularly, studying the formation/disappearance of X-shaped structures in the proximity of DNA replication forks by two-dimensional (2D) gel electrophoresis, in cells treated with MMS (Fig. 4A and fig. S4). The presence of these types of structures following DNA damage indicates recombination events, which are largely due to Rad5-dependent template switching mechanisms (*16*, *33*). Cells lacking Rad5 show a reduction of these X-DNA intermediates under DNA-damaging conditions (*29*). We synchronized cells in G_1 phase with α factor and then released them into

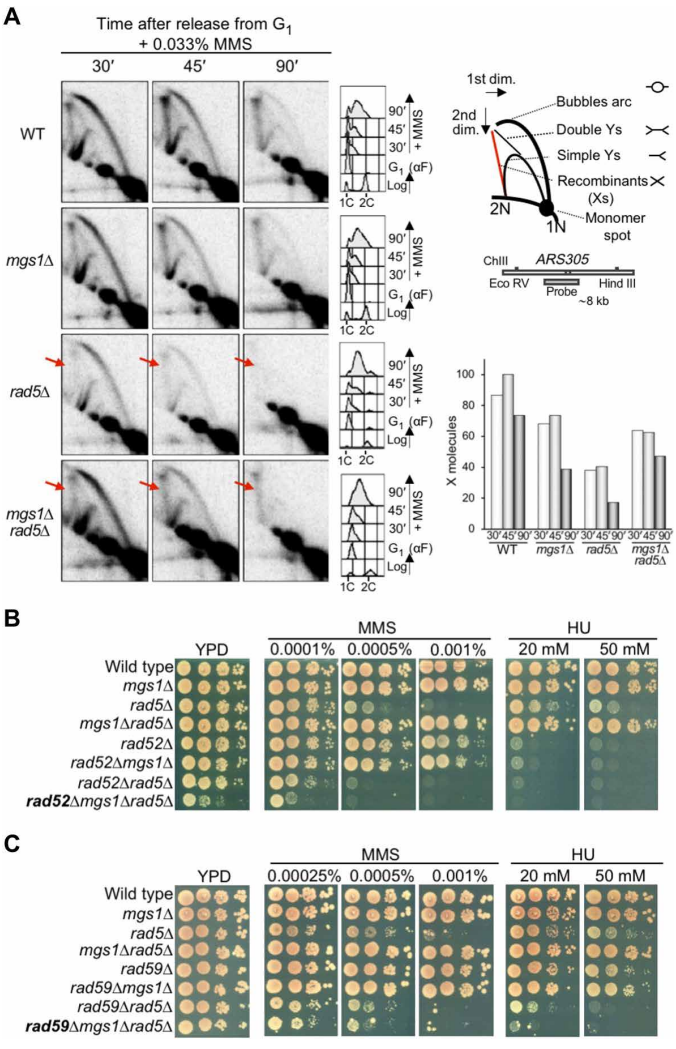

Fig. 4. Replication and viability of *mgs1Δrad5Δ* cells in the presence of genotoxic stress depend on recombination. (**A**) Mgs1 elimination allows the formation of X structures in *rad5Δ* cells under DNA-damaging conditions. 2D gel electrophoresis analysis. Samples were taken at the indicated time points after release from G_1 arrest in the presence of 0.033% MMS. The genomic DNA was digested with Eco RV–Hind III and analyzed by 2D gel with a probe recognizing the *ARS305* early replication origin. Representative autoradiograms of the 2D gels (left) and flow cytometry to monitor cell cycle progression are shown (right). A schematic representation of the main 2D gel signals, the location of the *ARS305* probe, and X-molecule quantification are shown. The highest X signal was set as 100%. The red arrows indicate X molecules (recombinants) to compare differences between *rad5Δ* and *mgs1Δrad5Δ* cells. Strains: Wild type (HY1976), *mgs1Δ* (YAJ319), *rad5Δ* (YAJ321), and *mgs1Δrad5Δ* (YAJ323). (**B**) Suppression of the sensitivity to MMS or HU of *rad5Δ* cells by Mgs1 elimination requires Rad52. Drop dilution assays. Serial dilutions (10-fold) of normalized exponentially growing cultures were spotted onto YPD plates containing different amounts of MMS or HU and incubated for 52 hours at 30°C. Strains: Wild type (SY2080), *mgs1Δ* (YSG15), *rad5Δ* (SY2214), *mgs1Δrad5Δ* (YAJ48), *rad52Δ* (YAJ11), *rad52Δmgs1Δ* (YAJ51), *rad52Δrad5Δ* (YAJ138), and *rad52Δmgs1Δrad5Δ* (YAJ140). (**C**) Suppression of MMS and HU sensitivity of *rad5Δ* cells when Mgs1 is eliminated requires Rad59. Drop dilution assays as in (B). Strains: Wild type (SY2080), *mgs1Δ* (YSG15), *rad5Δ* (SY2214), *mgs1Δrad5Δ* (YAJ48), *rad59Δ* (YCL29), *rad59Δmgs1Δ* (YCL30), *rad59Δrad5Δ* (YCL31), and *rad59Δmgs1Δrad5Δ* (YCL32).

S phase in medium containing MMS. The pattern of replication intermediates at the *ARS305* early origin of replication was analyzed at different times during chromosome replication (Fig. 4A and fig. S4). In agreement with previous studies (*29*), *rad5Δ* cells showed an important reduction in the percentage of X molecules with respect to wild-type and *mgs1Δ* cells. Notably, this situation was significantly reverted when *MGS1* was eliminated in the *rad5Δ* mutant. Thus, in *mgs1Δrad5Δ* cells, there was a notable increase in the percentage of X-shaped intermediates with respect to *rad5Δ* at 45 and 90 min after release from the G_1 block when, according to flow cytometry, cells were in S phase (Fig. 4A). This result indicates that, in cells lacking Rad5 and under conditions of MMS-induced DNA damage, the absence of Mgs1 allows the formation of recombination structures at damaged replication forks that very likely facilitate replication. These structures are not originated by template switching, as this mechanism is absent in this strain due to the lack of Rad5.

To genetically support the 2D gel data in Fig. 4A, we next studied the involvement of the recombination proteins Rad51 and Rad52 in the phenotype of *mgs1Δrad5Δ* cells. The triple mutant *rad51Δmgs1Δrad5Δ* was inviable, and unlike the case of the strategy used to study the role of TLS polymerases (Fig. 3), the combination of *rad51Δ* with the *mgs1*-ATPase mutant and *rad5Δ* (*mgs1-K183A rad51Δrad5Δ*) did not yield a viable strain either (fig. S5). On the contrary, although they exhibited a growth defect, *rad52Δmgs1Δrad5Δ* cells were viable (Fig. 4B). Drop dilution assays showed that *RAD52* deletion increased the sensitivity of cells lacking Rad5 to MMS or HU. Moreover, even considering the growth defect of the triple mutant, these drop dilution assays indicated that, in the absence of Rad52, elimination of Mgs1 could not rescue the viability of *rad5Δ* cells after treatment with the drugs (Fig. 4B). Together, and in agreement with the data obtained by 2D gel electrophoresis (Fig. 4A), the results suggest that homologous recombination is crucial to allow replication and viability of *mgs1Δrad5Δ* cells when treated with MMS or HU.

To further understand the requirement of recombination for replication and viability in the *mgs1Δrad5Δ* mutant after MMS or HU treatment, we analyzed the potential implication of Rad59 in the studied process. *RAD59* is a paralog of *RAD52*, and although the Rad59 protein contributes to a subset of homologous recombination events, it is not required for template switching (*34*). We combined the deletion of *RAD59* with those of *MGS1* and *RAD5* and analyzed the sensitivity of this strain and the corresponding controls to MMS or HU by drop dilution assays (Fig. 4C). The data obtained indicated that Rad59 is necessary for the mechanism that allows viability of *mgs1Δrad5Δ* cells after exposure to MMS or HU, thus reinforcing the importance of homologous recombination for this process and showing that the mode of recombination in these cells is genetically different from template switching.

Survival of *mgs1Δrad5Δ* cells under genotoxic stress conditions depends on the SUMO-like domain protein Esc2 and the PCNA unloader Elg1

The requirement of homologous recombination for the replication and survival of *mgs1Δrad5Δ* cells following genotoxic replicative stress raises the question of how this mechanism is allowed at damaged or stalled forks, as SUMOylation of PCNA recruits the antirecombinase Srs2 helicase and prevents potentially toxic recombination (*10*, *11*). As explained previously, recombination is globally inhibited at forks during chromosome replication in "wild-type" (*MGS1*$^+$*RAD5*$^+$) cells,

but the SUMO-like domain protein Esc2 counteracts Srs2 locally at perturbed forks, facilitating recombination-mediated DNA damage bypass by error-free template switching (*18*). This is achieved by Elg1-dependent local unloading of SUMOylated PCNA and increased turnover of Srs2, which reduces Srs2 levels at sites of perturbed replication (*18*). The question is how a recombination mode alternative to template switching is allowed in a *mgs1*Δ*rad5*Δ mutant, but not in *MGS1*$^{+}$*rad5*Δ cells, in which forks stall in the presence of DNA damage causing cell death (*27*). We thought that a possibility was that this mode of recombination, which could be considered a potentially mutagenic "salvage pathway," was facilitated in the *mgs1*Δ*rad5*Δ mutant by the same factors that promote template switching at stalled forks in wild-type cells but inhibited when Mgs1 is present.

To analyze whether similar requirements to those that facilitate template switching in *MGS1*$^{+}$*RAD5*$^{+}$ cells make possible a recombination salvage pathway in the *mgs1*Δ*rad5*Δ mutant, we examined the potential involvement of Esc2 (Fig. 5A) and Elg1 (Fig. 5B) in the phenotype of these latter cells. Drop dilution assays (Fig. 5A) showed that, like wild-type and *mgs1*Δ cells, *esc2*Δ and *esc2*Δ*mgs1*Δ cells had little sensitivity to MMS or HU treatment similar to *mgs1*Δ*rad5*Δ and that *esc2*Δ*rad5*Δ cells were as sensitive to these drugs as the *rad5*Δ mutant. Notably, deletion of *ESC2* in cells lacking Rad5 impeded the suppression of their sensitivity to MMS or HU when Mgs1 is eliminated (*esc2*Δ*mgs1*Δ*rad5*Δ strain). Drop dilution assays (Fig. 5B) also showed that *elg1*Δ and *elg1*Δ*mgs1*Δ cells had similar sensitivity to MMS or HU as wild-type or *mgs1*Δ cells. In agreement with previous data (*35*), *ELG1* deletion allowed a modest recovery of the viability of *rad5*Δ cells after MMS treatment. However, *ELG1* elimination prevented the suppression of the sensitivity of the *rad5*Δ mutant to MMS or HU when *MGS1* was deleted (*elg1*Δ*mgs1*Δ*rad5*Δ strain) (Fig. 5B). Moreover, both interacting peptide and SUMO-interacting motifs of Elg1 (*35*) were necessary for the suppression of the sensitivity of *rad5*Δ cells by *MGS1* deletion (fig. S6), consistent with the requirement of PCNA modification at K164 for this process (Fig. 2C). These results indicate that both Esc2 and Elg1 are required to allow recombination-driven replication and cell survival in the presence of genotoxic replication stress in *mgs1*Δ*rad5*Δ cells, which strongly suggests that the mechanism allowing for this process is similar to that facilitating template switching at stalled forks in wild-type cells (*18*). Notably, although Esc2 and Elg1 are present in cells lacking Rad5, this mechanism does not work if they have Mgs1.

To further study how Mgs1 influences this alternative recombination mechanism providing tolerance in *mgs1*Δ*rad5*Δ cells, we asked whether this protein affects recruitment of Elg1 to damaged replication forks. To test this possibility, we used chromatin immunoprecipitation (ChIP)–on-chip to analyze the binding of Elg1 along the entire genome

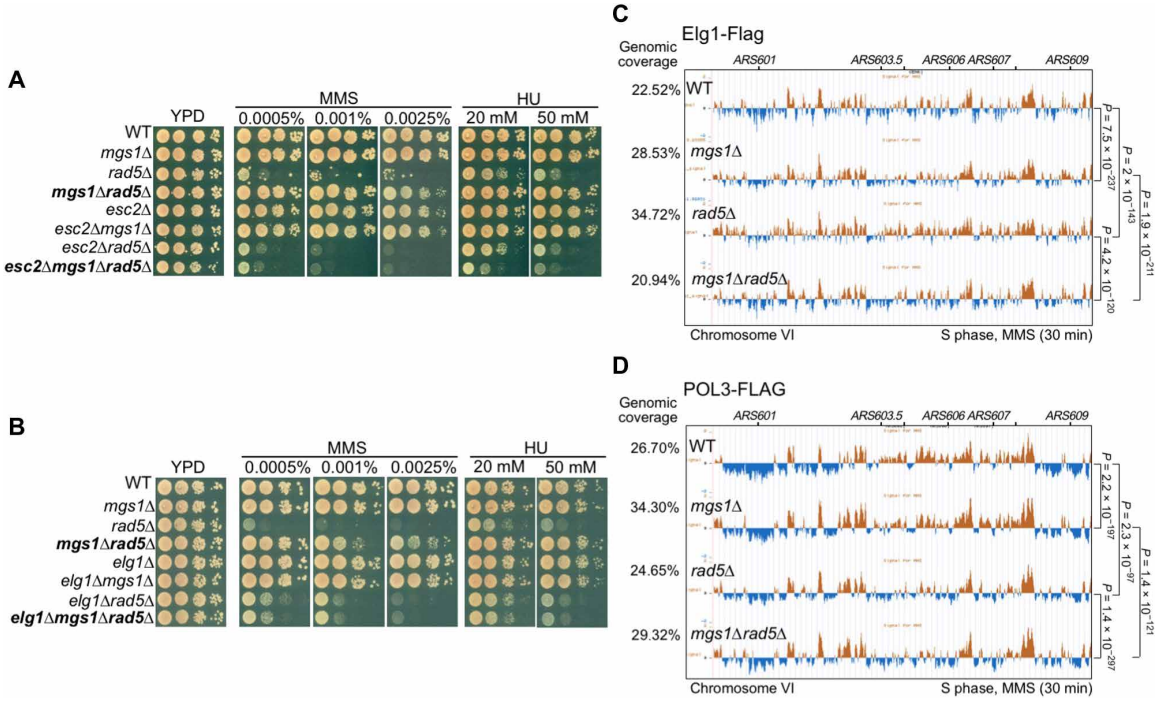

Fig. 5. Esc2 and Elg1 are required for the survival of *mgs1*Δ*rad5*Δ cells in the presence of MMS or HU. (**A**) Reduction of the sensitivity to MMS or HU of *rad5*Δ cells after Mgs1 elimination requires Esc2. Drop dilution assays. Serial dilutions (10-fold) of normalized exponentially growing cultures were spotted onto YPD plates containing different amounts of MMS or HU and incubated for 48 hours at 30°C. Strains: Wild type (SY2080), *mgs1*Δ (YSG15), *rad5*Δ (SY2214), *mgs1*Δ*rad5*Δ (YAJ48), *esc2*Δ (YSG436), *esc2*Δ*mgs1*Δ (YSG437), *esc2*Δ*rad5*Δ (YSG438), and *esc2*Δ*mgs1*Δ*rad5*Δ (YSG442). (**B**) Suppression of the MMS and HU sensitivity of *rad5*Δ cells after Mgs1 elimination is Elg1-dependent. Drop dilution assays, as in (A). Strains: Wild type (SY2080), *mgs1*Δ (YSG15), *rad5*Δ (SY2214), *mgs1*Δ*rad5*Δ (YAJ48), *elg1*Δ (YAJ71), *elg1*Δ*mgs1*Δ (YAJ72), *elg1*Δ*rad5*Δ (YAJ73), and *elg1*Δ*mgs1*Δ*rad5*Δ (YAJ74). (**C**) Genome-wide binding pattern of Elg1 by ChIP-on-chip after release of cells from G_1 block in medium containing 0.02% MMS for 30 min. The histogram peaks on the *y* axis depict the genome browser view of Elg1-Flag binding represented as the average signal ratio in $\log_2$ scale of loci enriched in the immunoprecipitated fraction along the indicated regions. The *x* axis shows chromosomal coordinates. The *P* values relate to the genome-wide overlap among Elg1 clusters in the different strains. Chromosome VI is shown as an example. The location of some replication origins is indicated. Strains: *ELG1-10FLAG* (wild type, HY1976), *ELG1-10FLAG mgs1*Δ (YAJ319), *ELG1-10FLAG rad5*Δ (YAJ321), and *ELG1-10FLAG mgs1*Δ*rad5*Δ (YAJ323). (**D**) Genome-wide binding pattern of Pol3 by ChIP-on-chip. Experimental conditions and picture details are as in (C). Strains: *POL3-3FLAG* (wild type, YAJ347), *POL3-3FLAG mgs1*Δ (YAJ348), *POL3-3FLAG rad5*Δ (YAJ349), and *POL3-3FLAG mgs1*Δ*rad5*Δ (YAJ350).

in *rad5*Δ, *mgs1*Δ*rad5*Δ cells, and the corresponding wild-type and *mgs1*Δ controls (Fig. 5C). Cells were first synchronized in G_1 with α factor and then released into S phase in fresh medium containing MMS. The genome-wide clusters of Elg1 were examined after 30-min treatment with this drug. ChIP-on-chip analysis showed that the overall genomic coverage of Elg1 was significantly higher in *rad5*Δ cells than in the rest of the strains. The genomic coverage of this protein in cells lacking Rad5 was reduced to levels close to those of wild-type cells when *MGS1* was eliminated (Fig. 5C). We also studied the genomic coverage of the Pol δ catalytic subunit Pol3 in the same strains (Fig. 5D). The ChIP-on-chip analysis showed that the main peaks that indicate Pol3 binding significantly coincide in their location with those of Elg1 under the same experimental conditions (Fig. 5, C and D). This similar ChIP-on-chip pattern shown by both proteins indicates that Elg1 is enriched at regions containing replication forks, which are marked by the polymerase. Concerning the binding of Pol3, the fact that the genomic coverage of this protein is higher in *mgs1* cells than in *MGS1*$^+$ cells is consistent with the proposed role for Mgs1 in the modulation of Pol δ interaction with PCNA (*20*). These data indicate that in the absence of Rad5 and under DNA-damaging conditions, there is a direct correlation between the presence of Mgs1 and the accumulation of Elg1 at damaged stalled forks. In this situation, a recombination-driven tolerance pathway that allows replication does not take place, all of which is reverted when Mgs1 is eliminated.

Together, our results strongly suggest that Mgs1 is required to prevent unscheduled and potentially mutagenic recombination at damaged forks in the absence of Rad5. Likewise, from these data, it is possible to deduce that in wild-type cells, in which recombination by template switching, but not other modes of recombination, is facilitated locally at damaged forks (*18*), Mgs1 contributes to avoid a Rad52/Rad59-dependent recombination salvage pathway, thus helping to channel DNA damage bypass to the error-free mode of DDT.

DISCUSSION

The evolutionarily conserved AAA+ ATPase Mgs1 is involved in the maintenance of genome stability (*19*–*23*), but its precise function has remained enigmatic in part due to the absence of a clear phenotype of Mgs1-deficient cells. In this work, we addressed the relevance of this ATPase in cells lacking Rad5, a protein that is central for DDT by template switching and TLS (*9*). This approach revealed a role for Mgs1 in preventing a recombination salvage pathway at damaged or stalled replication forks.

We found that the elimination of Mgs1 or its ATPase activity significantly suppresses the sensitivity of *rad5*Δ cells to agents that cause DNA damage, such as MMS, or replicative stress, such as HU. This suppression can be explained by the activation of an alternative pathway in *mgs1*Δ*rad5*Δ cells, but not in *rad5*Δ cells, where forks stall and cells die under genotoxic stress conditions (*27*–*29*). We showed that the mechanism that allows cell viability by overcoming DNA obstacles during replication in *mgs1*Δ*rad5*Δ cells is dependent on Pol δ and requires modification of PCNA at K164. Notably, translesion synthesis polymerases have only a minor role in this process, despite being the main known DDT branch in the absence of template switching, perhaps because TLS is not fully functional in the absence of Rad5 (*9*). Instead, we uncovered that replication in *mgs1*Δ*rad5*Δ cells in the presence of DNA damage is mainly driven by homologous recombination. This conclusion is based on (i) physical evidence provided by the increase in the percentage of X molecules at forks under DNA damage conditions in *mgs1*Δ*rad5*Δ cells with respect to *rad5*Δ and (ii) genetic data such as the dependency of *mgs1*Δ*rad5*Δ resistance to MMS and HU on *RAD52* and *RAD59*, the latter, in turn, indicates that this type of recombination is genetically distinguishable from template switching (*34*). Notably, Rad59 is not required for the alternative recombination pathway that is facilitated in *rad18siz1* cells treated with UV light (*10*, *11*), suggesting distinct mechanisms for the bypass of different DNA insults. As recombination-mediated template switching is absent in *mgs1*Δ*rad5*Δ cells, and the observed mode of recombination is independent of PCNA polyubiquitylation, it is possible to conclude that elimination of Mgs1 or its ATPase activity in cells lacking Rad5 allows a recombination salvage pathway that facilitates chromosome replication in the presence of genotoxic stress. The extrapolation of these results to wild-type (*RAD5*$^+$) cells might help to understand why the *mgs1*Δ mutant shows an increase in the frequency of mitotic recombination (*19*, *21*).

The salvage pathway of recombination described before is normally restricted to late S phase or G_2-M (*7*, *36*) and inhibited in principle by the antirecombinase Srs2 (*10*, *11*). However, this or a related salvage pathway is permitted at perturbed replication forks in *mgs1*Δ*rad5*Δ cells. Urulangodi *et al.* (*18*) revealed that although recombination is globally inhibited during replication by Srs2, the SUMO-like domain protein Esc2, together with Elg1, can counteract Srs2 locally, allowing recombination-mediated DNA damage bypass by template switching at damaged stalled forks (Fig. 6A). We found that Esc2 and Elg1 are also required for replication stress tolerance in *mgs1*Δ*rad5*Δ cells (Fig. 6B). These dependencies strongly suggest that template switching at damaged forks in wild-type cells and the salvage recombination pathway operating in *mgs1*Δ*rad5*Δ cells are driven in a similar fashion by a mechanism facilitated by Esc2 and Elg1. Thus, in cells lacking both Rad5 and Mgs1, as in wild-type cells (*18*), Esc2 might bind to sites of stalled replication promoting Elg1 association to damaged replication forks, which, in turn, would lead to unloading of Srs2 bound to SUMO-PCNA followed by its degradation. Reduced local levels of Srs2 would allow binding of Rad51 and subsequently a recombination-driven replication pathway that is Rad52 and Rad59 dependent and requires DNA synthesis by Pol δ (Fig. 6B). Our data showed that this recombination-driven replication process is inhibited when Mgs1 is present (Fig. 6C) and that Mgs1 manifests its inhibitory function in a manner driven by its ATPase activity. In *MGS1*$^+$*rad5*Δ cells, we observed an accumulation of Elg1 at forks that is reverted by Mgs1 elimination, which indicates a correlation between Elg1 accumulation and the presence of Mgs1. Elg1 accumulation may lead to PCNA stabilization or, conversely, may be a consequence of it. Either way, the result could be the retention of Srs2 at damaged stalled forks, which would prevent recombination in a Mgs1-dependent way, impeding the completion of chromosome replication in *MGS1*$^+$*rad5*Δ cells (Fig. 6C) (*27*–*29*).

Our results suggest an important role for Mgs1 in preventing unscheduled recombination at damaged replication forks. They offer a rationale to explain why in wild-type cells a recombination-mediated error-free template switching, which depends on Rad5, is facilitated at damaged stalled forks, whereas a potentially toxic recombination salvage pathway that could lead to genomic rearrangements or faulty replication is inhibited. The discrimination between these two modes of recombination exists, although both might be in principle enabled locally at sites of stalled replication by the same Esc2- and Elg1-dependent mechanism that results in low

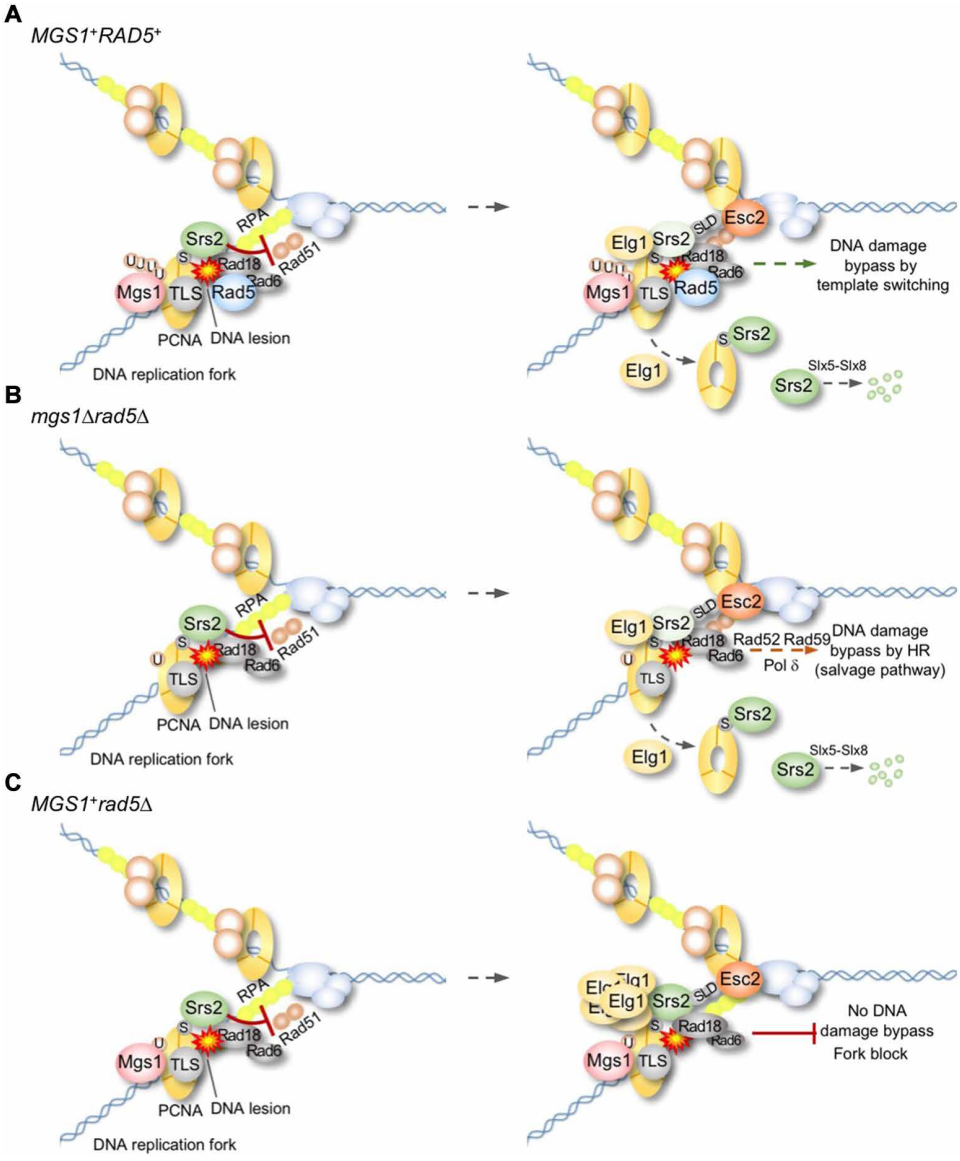

Fig. 6. Model for DDT during chromosomal replication, in the presence or absence of Rad5 or Mgs1. (**A**) *MGS1*$^{+}$*RAD5*$^{+}$ cells. DNA damage is tolerated predominantly by error-free Rad5-dependent template switching during chromosome replication (*27*). Recombination is globally inhibited during replication, at ongoing forks, by recruitment of the Srs2 antirecombinase helicase to SUMO-PCNA (*10*, *11*), but recombination-mediated damage bypass by template switching is locally allowed, at damaged stalled forks, by the SUMO-like domain protein Esc2, which counteracts Srs2 (*18*). Esc2 binds at sites of stalled replication and promotes Elg1 binding at forks, which, in turn, induces regulated unloading of Srs2 bound to SUMO-PCNA by Elg1 and degradation of the helicase by Slx5-Slx8–mediated proteasome-dependent degradation, thus reducing Srs2 levels locally (*18*). RPA, replication protein A. (**B**) *mgs1Δrad5Δ* cells. In the absence of Rad5, Mgs1 elimination allows DNA damage bypass at sites of perturbed replication. The absence of Mgs1 in cells lacking Rad5 facilitates a recombination-driven replication mechanism that is Rad52, Rad59, and Pol δ dependent. As in the template switching pathway used by *MGS1*$^{+}$*RAD5*$^{+}$ cells (A), this salvage pathway of recombination requires Esc2 and Elg1 and, therefore, is very likely conducted in the same manner. HR, homologous recombination. (**C**) *MGS1*$^{+}$*rad5Δ* cells. The absence of Rad5 impedes DNA damage bypass by template switching, which causes forks block, and cells die because replication cannot be completed (*27*–*29*) as an alternative mechanism to overcome replication perturbations is prevented. This correlates with the presence of Mgs1 and the accumulation of Elg1 at stalled forks, which might reflect SUMO-PCNA stabilization. See details in the main text.

levels of the Srs2 antirecombinase (*18*). We propose that Mgs1 is a key factor required to prevent a salvage pathway of recombination at damaged or stalled forks. This, in turn, would help to channel DNA damage bypass to template switching, thus importantly contributing to the maintenance of genome stability during chromosome replication.

MATERIALS AND METHODS

Strains, media, and cell cycle experiments

The budding yeast strains used in this work are derivatives of W303 or DF5. Their relevant genotypes are indicated in table S1. All the strains were constructed by standard techniques or genetic crosses. The pML (*37*) and pYM (*38*) plasmid series were used as templates

for polymerase chain reaction. Yeasts were routinely grown at 30°C in YP medium (1% yeast extract and 2% Bacto Peptone) containing 2% glucose. Bacto agar (2%) was added for solid medium. Cells were synchronized in G_1 with the α factor pheromone (5 to 10 μg/ml). Nocodazole was used at 5 μg/ml. Samples for flow cytometry were collected and processed as described (*39*) and analyzed using a FACSCalibur flow cytometer (BD Biosciences).

Drug sensitivity assays

Cell viability after MMS treatment during a single S phase was determined by plating cells in triplicate onto YP-glucose plates and counting colony-forming units after 3 days of incubation at 30°C. For drop dilution assays, cells growing exponentially at 30°C were normalized to 1×10^7 cells/ml, and 10-fold serial dilutions were spotted onto YP-glucose plates containing different concentrations of MMS or HU. The plates were incubated at 30°C for 48 to 72 hours.

Pulse-field gel electrophoresis

Genomic DNA was obtained from 10^8 cells and prepared in plugs of low melting agarose, as previously described (*39*). The chromosomes were separated in a 1% agarose–tris-borate EDTA (TBE) gel by PFGE at 14°C using a CHEF-DR II system (Bio-Rad). The electrophoresis were carried out at 200 V (6 V/cm) for 24 hours, with 60- and 90-s pulses for 15 and 9 hours, respectively. The gels were stained with ethidium bromide and scanned after UV exposure. Quantification of the chromosome bands was performed using the ImageJ program (National Institutes of Health).

2D gel analysis

Purification of DNA intermediates, 2D gel analysis, and X-molecules quantification were performed as previously described (*40*).

ChIP-on-chip

The ChIP-on-chip experiments and the analysis of genome-wide clusters were carried out as previously described (*18*).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/15/eaaz3327/DC1

View/request a protocol for this paper from *Bio-protocol*.

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