The SUMO Isopeptidase Ulp2p Is Required to Prevent Recombination-Induced Chromosome Segregation Lethality following DNA Replication Stress

Ming-Ta Lee, Abla A. Bakir, Kristen N. Nguyen, Jeff Bachant*
Department of Cell Biology and Neuroscience, University of California Riverside, Riverside, California, United States of America

Abstract
SUMO conjugation is a key regulator of the cellular response to DNA replication stress, acting in part to control recombination at stalled DNA replication forks. Here we examine recombination-related phenotypes in yeast mutants defective for the SUMO de-conjugating/chain-editing enzyme Ulp2p. We find that spontaneous recombination is elevated in ulp2 strains and that recombination DNA repair is essential for ulp2 survival. In contrast to other SUMO pathway mutants, however, the frequency of spontaneous chromosome rearrangements is markedly reduced in ulp2 strains, and some types of rearrangements arising through recombination can apparently not be tolerated. In investigating the basis for this, we find DNA repair foci do not disassemble in ulp2 cells during recovery from the replication fork-blocking drug methyl methanesulfonate (MMS), corresponding with an accumulation of X-shaped recombination intermediates. ulp2 cells satisfy the DNA damage checkpoint during MMS recovery and commit to chromosome segregation with similar kinetics to wild-type cells. However, sister chromatids fail to disjoin, resulting in abortive chromosome segregation and cell lethality. This chromosome segregation defect can be rescued by overproducing the anti-recombinase Srs2p, indicating that recombination plays an underlying causal role in blocking chromatid separation. Overall, our results are consistent with a role for Ulp2p in preventing the formation of DNA lesions that must be repaired through recombination. At the same time, Ulp2p is also required to either suppress or resolve recombination-induced attachments between sister chromatids. These opposing effects may synergize to greatly increase the toxicity of DNA replication stress.

Introduction
As part of the DNA damage response, homologous recombination (HR), particularly template switch recombination through the post-replication DNA repair pathway (PRR), provides an important mechanism for restarting stalled replication forks and filling in un-replicated gaps in DNA (reviewed in [1,2]). These recombination events must be managed carefully, however, DNA strand exchange during HR, followed by re-initiating replication using the nascent sister chromatid as a template, can result in the formation of DNA linkages between daughter chromosomes. Failure to resolve these linkages, called sister chromatid junctions (SCJs), leads to chromosome breakage or aneuploidy, and may contribute to genome instability in many forms of cancer (reviewed in [3]).

A variety of studies implicate SUMO post-translational modification as an important regulator of HR in response to replication stress. Following activation of the SUMO precursor protein, SUMO modification is catalyzed by the E2 conjugating enzyme Ubc9p, which typically acts through one of several E3 ligases to covalently join SUMO moieties to lysine residues on substrate proteins (reviewed in [4]). One SUMO substrate that plays an especially prominent role in controlling HR at replication forks is Pol30p/PCNA, which is modified to recruit different activities to the replisome. During S phase, Ubc9p works through the E3 ligase Siz1p to sumoylate PCNA on K164 and K127 [5]. SUMO modified PCNA recruits the Srs2p helicase [6,7], which suppresses unscheduled HR by disassembling Rad51p nucleoprotein filaments [8-10]. Following replication fork stalling at MMS-induced DNA lesions, however, PRR proteins catalyze either mono- or poly-ubiquitylation of PCNA K164 [5]. These modifications recruit trans-lesion bypass polymerases or induce template switching HR, respectively, providing alternative mechanisms to bypass the lesion and restart replication [5,11-13]. The existence of additional SUMO substrates that control HR is suggested by the observations that mutations affecting both Ubc9p and the E3 ligase Mms21p, which is not required for PCNA sumoylation, confer sensitivity to the replication impeding drugs hydroxyurea (HU) and methyl methansulfonate (MMS) [5,14-19]. Mms21p mutants exist in a complex with two members of the structural maintenance of chromosomes family of proteins, Smc5p and Smc6p, which are also required for HU and MMS-resistance [15,16,20-22]. Notably, in response to MMS, ubc9, mms21, smc5 and smc6 mutants show an accumulation of X-shaped DNA structures that are thought to represent either repressed forks-a possible intermediate in fork restart-or hemi-catenate SCJs [17,19,23]. In this sense, they resemble mutants defective for the Sgs1p/Top3p/Rmi1p complex, which, through concerted heli-
Author Summary

DNA damage, arising from environmental stress or errors in DNA metabolism, can interfere with DNA replication. Cells respond by using homologous recombination to bypass the damage, resulting in DNA strand linkages between the replicated chromosomes. It is crucial to undo these linkages so chromosomes can segregate properly. Previously, a regulatory mechanism known as SUMO modification was shown to be important in controlling recombination following replication interference by the DNA damaging agent MMS. We show that mutations in a yeast enzyme called Ulp2p, which reverses SUMO modification, increase recombination and impose a requirement for recombination to maintain survival. MMS–treated ulp2 mutants also accumulate recombination intermediates and fail to separate their chromosomes, leading to a permanent block to cell division. Further analysis suggests this block may not simply be due to a failure to resolve recombination intermediates, but may reflect a role for Ulp2p in undoing additional chromosome attachments that accompany recombination. In sum, our data indicate that cells defective for Ulp2p develop a love/hate relationship with recombination, requiring recombination for viability while failing to resolve chromosome attachments induced by recombination repair. Identification of Ulp2p substrates that ensure chromosome separation following recombination will shed light on how SUMO modification maintains genome stability.

Ulpl mutants may be necessary to restart the chromosome segregation machinery once the checkpoint block to mitosis has been relieved [41,49]. But whether Ulp2p, like other components of the SUMO pathway, is also involved in controlling HR during DNA damage or replication stress has not yet been examined. In this study, we find that, following replication fork stalling by MMS, ulp2 mutants accumulate persistent recombination intermediates that are likely to correspond to SCJs. This mis-regulation is accompanied by a severe, recombination-dependent, block to chromosome segregation, revealing a critical role for Ulp2p in allowing sister chromatids to disjoin following HR DNA repair.

Results

Recombination is elevated and essential in ulp2 mutants

We initially set out to determine if ulp2 mutants displayed a similar dependency on recombination as ulp1-I615N strains [47]. A ulp2 deletion mutant (ulp2Δ) was mated to rad52Δ, rad51Δ and rad6Δ strains. Rad51p and Rad52p are required for most forms of HR [2], while Rad6p controls trans-lesion synthesis and template switching PRR [1]. 1ulp2Δ rad52Δ, ulp2Δ rad51Δ and ulp2Δ rad6Δ double mutants were either not obtained or were obtained at lower than expected frequencies from these crosses (Table 1, Table 2, Table 3). For rad52Δ, we examined this apparent synthetic lethality further by isolating ulp2Δ rad52Δ segregants harboring a wild type (WT) copy of RAD52 on a UR3 minichromosome (pRAD52). ulp2Δ rad52Δ/pRAD52 mutants grew weakly, if at all, on media containing 5-FOA, a drug that only allows growth if cells are capable of losing pRAD52 (Figure 1A). Thus, Rad52p is essential for proliferation of ulp2Δ cells.

The essential role of Rad52p prompted us to examine whether HR was elevated in the absence of Ulp2p. Yeast cells exhibit a uniform nuclear distribution of fluorescent Rad52p-GFP in the absence of DNA damage (Figure 1B, [49]), but Rad52p-GFP rapidly assembles into intra-nuclear foci during HR DNA repair [49]. We found that an average of 17% of ulp2Δ cells in mid-logarithmic phase cultures displayed Rad52p-GFP foci, a significant increase (p = 0.0074) compared to less than 1% in WT cells. (Figure 1B). As a second assay, we utilized a reporter in which recombination events between direct repeats on chromosome XV can be selected because they restore an intact LEU2 locus (Figure 1D). Thus, Rad52p is essential for proliferation of ulp2Δ cells.

Ulpl substrates may be necessary to restart the chromosome segregation machinery once the checkpoint block to mitosis has been relieved [41,49]. But whether Ulp2p, like other components of the SUMO pathway, is also involved in controlling HR during DNA damage or replication stress has not yet been examined. In this study, we find that, following replication fork stalling by MMS, ulp2 mutants accumulate persistent recombination intermediates that are likely to correspond to SCJs. This mis-regulation is accompanied by a severe, recombination-dependent, block to chromosome segregation, revealing a critical role for Ulp2p in allowing sister chromatids to disjoin following HR DNA repair.

Recombination is elevated and essential in ulp2 mutants

We initially set out to determine if ulp2 mutants displayed a similar dependency on recombination as ulp1-I615N strains [47]. A ulp2 deletion mutant (ulp2Δ) was mated to rad52Δ, rad51Δ and rad6Δ strains. Rad51p and Rad52p are required for most forms of HR [2], while Rad6p controls trans-lesion synthesis and template switching PRR [1]. 1ulp2Δ rad52Δ, ulp2Δ rad51Δ and ulp2Δ rad6Δ double mutants were either not obtained or were obtained at lower than expected frequencies from these crosses (Table 1, Table 2, Table 3). For rad52Δ, we examined this apparent synthetic lethality further by isolating ulp2Δ rad52Δ segregants harboring a wild type (WT) copy of RAD52 on a UR3 minichromosome (pRAD52). ulp2Δ rad52Δ/pRAD52 mutants grew weakly, if at all, on media containing 5-FOA, a drug that only allows growth if cells are capable of losing pRAD52 (Figure 1A). Thus, Rad52p is essential for proliferation of ulp2Δ cells.

The essential role of Rad52p prompted us to examine whether HR was elevated in the absence of Ulp2p. Yeast cells exhibit a uniform nuclear distribution of fluorescent Rad52p-GFP in the absence of DNA damage (Figure 1B, [49]), but Rad52p-GFP rapidly assembles into intra-nuclear foci during HR DNA repair [49]. We found that an average of 17% of ulp2Δ cells in mid-logarithmic phase cultures displayed Rad52p-GFP foci, a significant increase (p = 0.0074) compared to less than 1% in WT cells. (Figure 1B). As a second assay, we utilized a reporter in which recombination events between direct repeats on chromosome XV can be selected because they restore an intact LEU2 locus (Figure 1D). Thus, Rad52p is essential for proliferation of ulp2Δ cells.

Ulpl substrates may be necessary to restart the chromosome segregation machinery once the checkpoint block to mitosis has been relieved [41,49]. But whether Ulp2p, like other components of the SUMO pathway, is also involved in controlling HR during DNA damage or replication stress has not yet been examined. In this study, we find that, following replication fork stalling by MMS, ulp2 mutants accumulate persistent recombination intermediates that are likely to correspond to SCJs. This mis-regulation is accompanied by a severe, recombination-dependent, block to chromosome segregation, revealing a critical role for Ulp2p in allowing sister chromatids to disjoin following HR DNA repair.

Recombination is elevated and essential in ulp2 mutants

We initially set out to determine if ulp2 mutants displayed a similar dependency on recombination as ulp1-I615N strains [47]. A ulp2 deletion mutant (ulp2Δ) was mated to rad52Δ, rad51Δ and rad6Δ strains. Rad51p and Rad52p are required for most forms of HR [2], while Rad6p controls trans-lesion synthesis and template switching PRR [1]. 1ulp2Δ rad52Δ, ulp2Δ rad51Δ and ulp2Δ rad6Δ double mutants were either not obtained or were obtained at lower than expected frequencies from these crosses (Table 1, Table 2, Table 3). For rad52Δ, we examined this apparent synthetic lethality further by isolating ulp2Δ rad52Δ segregants harboring a wild type (WT) copy of RAD52 on a UR3 minichromosome (pRAD52). ulp2Δ rad52Δ/pRAD52 mutants grew weakly, if at all, on media containing 5-FOA, a drug that only allows growth if cells are capable of losing pRAD52 (Figure 1A). Thus, Rad52p is essential for proliferation of ulp2Δ cells.

The essential role of Rad52p prompted us to examine whether HR was elevated in the absence of Ulp2p. Yeast cells exhibit a uniform nuclear distribution of fluorescent Rad52p-GFP in the absence of DNA damage (Figure 1B, [49]), but Rad52p-GFP rapidly assembles into intra-nuclear foci during HR DNA repair [49]. We found that an average of 17% of ulp2Δ cells in mid-logarithmic phase cultures displayed Rad52p-GFP foci, a significant increase (p = 0.0074) compared to less than 1% in WT cells. (Figure 1B). As a second assay, we utilized a reporter in which recombination events between direct repeats on chromosome XV can be selected because they restore an intact LEU2 locus (Figure 1D). Thus, Rad52p is essential for proliferation of ulp2Δ cells.

Ulpl substrates may be necessary to restart the chromosome segregation machinery once the checkpoint block to mitosis has been relieved [41,49]. But whether Ulp2p, like other components of the SUMO pathway, is also involved in controlling HR during DNA damage or replication stress has not yet been examined. In this study, we find that, following replication fork stalling by MMS, ulp2 mutants accumulate persistent recombination intermediates that are likely to correspond to SCJs. This mis-regulation is accompanied by a severe, recombination-dependent, block to chromosome segregation, revealing a critical role for Ulp2p in allowing sister chromatids to disjoin following HR DNA repair.
To further monitor chromosome rearrangements we examined two circular dicentric minichromosomes. In one (p2XCE\textsuperscript{direct}), two copies of a CEN sequence were oriented in a direct repeat configuration. In the other (p2XCE\textsuperscript{inverted}), the same CEN duplication was oriented as inverted repeats. Previous studies have shown that both direct and inverted repeat dicentrics can be efficiently transformed into yeast, and are initially retained through a combination of co-orientation of the two CENs on the spindle and non-disjunction following dicentric bridging \cite{54,55}. During outgrowth, however, rearranged minichromosomes that have deleted one of the CENs accumulate. For direct CEN repeats these deletions tend to arise through loop out events, whereas inverted CEN repeats are resolved through more complex re-arrangements. Consistent with this characterization, in WT transformants p2XCE\textsuperscript{direct} and p2XCE\textsuperscript{inverted} exhibited similar mitotic stabilities to p1XCE controls (Figure 2D). Analysis of minichromosomes rescued from these cells revealed precise CEN excision for p2XCE\textsuperscript{direct} and a diversity of plasmid species for p2XCE\textsuperscript{inverted} (not shown). In ulp2\Delta mutants, p1XCE was only retained in \(~30\%\) of the cells; this result is in keeping with previous studies showing reduced minichromosome stability in the absence of Ulp2p \cite{29}. p2XCE\textsuperscript{direct} demonstrated a similar stability to p1XCE (Figure 2D), and underwent the same precise CEN deletions observed in WT (not shown). In contrast, p2XCE\textsuperscript{inverted} proved extremely unstable, with less than 1\% of ulp2\Delta cells maintaining the mini-chromosome. These results suggest that some chromosome re-arrangements either fail to occur or cannot be tolerated in ulp2\Delta mutants.

Table 1. Genetic interactions between ulp2\Delta and rad52\Delta mutants.

| Genotype               | # Expected Spores | # Obtained (% of Expected) |
|------------------------|-------------------|----------------------------|
| WT                     | 26                | 18 (69\%)                  |
| ulp2\Delta             | 26                | 18 (69\%)                  |
| rad52\Delta            | 26                | 32 (123\%)                 |
| ulp2\Delta rad52\Delta | 26                | 2 (8\%)                    |
| Total                  | 104               | 70 (67\%)                  |

doi:10.1371/journal.pgen.1001355.t001

Table 2. Genetic interactions between ulp2\Delta and rad51\Delta mutants.

| Genotype               | # Expected Spores | # Obtained (% of Expected) |
|------------------------|-------------------|----------------------------|
| WT                     | 26                | 16 (69\%)                  |
| ulp2\Delta             | 26                | 22 (85\%)                  |
| rad51\Delta            | 26                | 24 (92\%)                  |
| ulp2\Delta rad51\Delta | 26                | 6 (23\%)                   |
| Total                  | 104               | 68 (65\%)                  |

doi:10.1371/journal.pgen.1001355.t002

Table 3. Genetic interactions between ulp2\Delta and rad6\Delta mutants.

| Genotype               | # Expected Spores | # Obtained (% of Expected) |
|------------------------|-------------------|----------------------------|
| WT                     | 22                | 18 (82\%)                  |
| ulp2\Delta             | 22                | 10 (45\%)                  |
| rad6\Delta             | 22                | 18 (82\%)                  |
| ulp2\Delta rad6\Delta  | 22                | 0 (0\%)                    |
| Total                  | 88                | 46 (52\%)                  |

doi:10.1371/journal.pgen.1001355.t003

MMS-induced HR intermediates accumulate in ulp2\Delta mutants

In order to more directly examine the consequences of HR in ulp2\Delta mutants, we used MMS to induce recombination. As an initial experiment, we examined chromosome integrity following exposure to MMS by pulse-field gel electrophoresis. WT and ulp2\Delta cells were arrested in G1, released into media containing 0.01\% MMS for 2 hr, and then allowed to recover in MMS-free media. Following MMS treatment a lower molecular weight DNA smear was observed in both WT and ulp2\Delta strains (Figure 3A), reflecting MMS-induced chromosome breakage \cite{17}. For both strains, a one hr recovery largely restored the normal chromosome banding pattern. This suggests Ulp2p is not obviously required for healing MMS-induced DNA breaks.

We next examined processing of MMS-induced DNA lesions. In the experiment shown in Figure 3B, WT cells and ulp2\Delta mutants expressing RAD52-GFP were treated with 0.01\% MMS and allowed to recover. After a 2 hr recovery, \(~30\%\) of WT cells accumulated Rad52p-GFP foci (Figure 3B). By 6 hr, however, the percentage of cells with Rad52p-GFP foci had substantially declined and many cells were proceeding with the next round of cell division. In contrast, ulp2\Delta mutants showed a much stronger accumulation of Rad52p-GFP foci, reaching a maximum of \(~60\%\) (Figure 3B), and these foci tended to persist for the duration of the recovery period. We also examined Rad22p-GFP foci in ulp2\Delta cells treated with 200 mM HU. HU does not normally induce Rad52p foci because the integrity of the replisome is maintained by the S phase checkpoint (Figure 3B, \cite{56}). HU treated ulp2\Delta cells, however, exhibited a strong induction of Rad52p-GFP foci.

In response to MMS, proper regulation of HR is required to prevent X-shaped recombination intermediates from accumulating in the vicinity of origins of replication \cite{17,19,23}. On two-dimensional gels these structures migrate as a “X-spike” that is distinct from replication forks and bubbles \cite{57,58}. To determine whether ulp2\Delta mutants accumulated this type of HR intermediate, ulp2\Delta cells, along with WT and sgs1\Delta controls, were released from a G2/M nocodazole block and treated with 0.033\% MMS for 3 hr as previously described \cite{17}. Genomic DNAs were a G2/M nocodazole block and treated with 0.033\% MMS for 3 hr as previously described \cite{17}. Genomic DNAs were

Table 3. Genetic interactions between ulp2\Delta and rad6\Delta mutants.

| Genotype               | # Expected Spores | # Obtained (% of Expected) |
|------------------------|-------------------|----------------------------|
| WT                     | 22                | 18 (82\%)                  |
| ulp2\Delta             | 22                | 10 (45\%)                  |
| rad6\Delta             | 22                | 18 (82\%)                  |
| ulp2\Delta rad6\Delta  | 22                | 0 (0\%)                    |
| Total                  | 88                | 46 (52\%)                  |

MMS-induced HR intermediates accumulate in ulp2\Delta mutants

In order to more directly examine the consequences of HR in ulp2\Delta mutants, we used MMS to induce recombination. As an initial experiment, we examined chromosome integrity following exposure to MMS by pulse-field gel electrophoresis. WT and ulp2\Delta cells were arrested in G1, released into media containing 0.01\% MMS for 2 hr, and then allowed to recover in MMS-free media. Following MMS treatment a lower molecular weight DNA smear was observed in both WT and ulp2\Delta strains (Figure 3A), reflecting MMS-induced chromosome breakage \cite{17}. For both strains, a one hr recovery largely restored the normal chromosome banding pattern. This suggests Ulp2p is not obviously required for healing MMS-induced DNA breaks.

We next examined processing of MMS-induced DNA lesions. In the experiment shown in Figure 3B, WT cells and ulp2\Delta mutants expressing RAD52-GFP were treated with 0.01\% MMS and allowed to recover. After a 2 hr recovery, \(~30\%\) of WT cells accumulated Rad52p-GFP foci (Figure 3B). By 6 hr, however, the percentage of cells with Rad52p-GFP foci had substantially declined and many cells were proceeding with the next round of cell division. In contrast, ulp2\Delta mutants showed a much stronger accumulation of Rad52p-GFP foci, reaching a maximum of \(~60\%\) (Figure 3B), and these foci tended to persist for the duration of the recovery period. We also examined Rad22p-GFP foci in ulp2\Delta cells treated with 200 mM HU. HU does not normally induce Rad52p foci because the integrity of the replisome is maintained by the S phase checkpoint (Figure 3B, \cite{56}). HU treated ulp2\Delta cells, however, exhibited a strong induction of Rad52p-GFP foci.

In response to MMS, proper regulation of HR is required to prevent X-shaped recombination intermediates from accumulating in the vicinity of origins of replication \cite{17,19,23}. On two-dimensional gels these structures migrate as a “X-spike” that is distinct from replication forks and bubbles \cite{57,58}. To determine whether ulp2\Delta mutants accumulated this type of HR intermediate, ulp2\Delta cells, along with WT and sgs1\Delta controls, were released from a G2/M nocodazole block and treated with 0.033\% MMS for 3 hr as previously described \cite{17}. Genomic DNAs were a G2/M nocodazole block and treated with 0.033\% MMS for 3 hr as previously described \cite{17}. Genomic DNAs were
Ulp2p Counteracts Recombination DNA Linkages

Figure 1. Spontaneous HR in ulp2Δ mutants. (A) rad52Δ and ulp2Δ rad52Δ segregrants harboring pRAD52-URA3 were derived from a cross between rad52Δ/pRAD52-URA3 (MLY031; pRAD52 is pVL191) and ulp2Δ(JBY238) haploid strains, cultured in the absence of selection for pRAD52-URA3, and a 10-fold dilution series was stamped onto Ura-/SC (growth requires pRAD52-URA3) and 5-FOA media (growth requires loss of pRAD52-URA3). Plates were cultured at 30°C and photographed after 3 days. (B) Aliquots of mid-log phase cultures of WT (MLY061) and ulp2Δ (MLY060) RAD52-GFP strains were fixed and scored to determine the percentage of unbudded and budded cells exhibiting distinct Rad52p-GFP foci. Micrographs (superimposed bright field and fluorescent images) depict dispersed nuclear Rad52p-GFP signal in WT cells and Rad52p-GFP foci in ulp2Δ mutants. Scale bar, 4 μm. Graph displays the average percentage of budded and un-budded cells with and without Rad52p foci determined from three separate experiments. Error bars, ± one standard deviation. A total of 1,018 WT and 523 ulp2Δ cells were scored; 4 WT cells displayed distinct foci (0.4%). The p value (Student’s t-test) for comparing total number of cells with foci from the WT and ulp2Δ datasets is 0.0074. (C) WT (MLY066; 8 cultures) and ulp2Δ (MLY067; 10 cultures) strains were constructed in which a plasmid insertion at the HIS3 locus is flanked by 414 bp direct repeats. Recombination events between the repeats can be selected because they restore HIS3 function. Box plot graphs display the median number of His+ clones per 10^6 viable cells, 25th and 75th percentiles, and range of data (p = 0.044; Student’s t-test).

doi:10.1371/journal.pgen.1001355.g001

study has shown that a single prominent SUMO species of Sgs1p accumulates after MMS exposure, and K621 has been identified as the acceptor lysine that is responsible for this modification [59]. We were able to confirm that treatment with 0.3% MMS resulted in a substantial fraction of Sgs1p-myc shifting into a reduced mobility species (Figure 4A and Figure S1), and that a decreased amount of this form was observed following treatment with a lower MMS concentration (0.033%; Figure 4B, 4C). The appearance of this form was abolished in ulp2Δ strains (Figure 4B) and a sgs1-K621R mutant (Figure S1), indicating it is likely to correspond to the previously reported K621 conjugate. In ulp2Δ strains, however, a marked increase in this putative Sgs1p SUMO species was observed (Figure 4B, 4C), which persisted for at least 3 hr after removal of MMS (Figure 4C). In sum, these results suggest that sumoylation of Sgs1p is likely to be regulated by Ulp2p.

If failure to properly control Sgs1p sumoylation was responsible for ulp2Δ HR defects, SUMO-resistant Sgs1p might ameliorate these phenotypes. We therefore examined whether a plasmid-born copy of the sgs1-K621R allele could prevent Rad52p foci accumulation. Following a two hr treatment with 0.001% MMS, however, no significant reduction in ulp2Δ sgs1-K621R cells displaying Rad52p-GFP foci was observed (Figure 4D). Previous studies have shown that a form of Smt3 (smt3-3KR) that cannot form polymeric SUMO chains can rescue the HU and MMS sensitivity of ulp2 mutants [43], leading us to test whether smt3-3KR could prevent Rad52p foci accumulation. This proved to be the case, as smt3-3KR ulp2Δ double mutants did in fact show a substantial reduction in the accumulation of both spontaneous and MMS-induced Rad52p foci (Figure 4F). Thus, proper SUMO chain editing through Ulp2p is likely to be important in controlling HR.

ulp2Δ mutants fail in chromosome segregation after exposure to MMS

In our experiments, it was apparent that ulp2Δ cells frequently remained blocked in the cell cycle during recovery from MMS, similar to previous results examining ulp2 recovery following HU treatment and in response to an irreparable DNA double strand break [41]. We took four experimental approaches to investigate the basis for the apparent MMS recovery defect of ulp2Δ cells. First, phospho-activation of the Rad53p checkpoint kinase during the DNA damage response results in a series of slower migrating gel mobility variants [60], and collapse of these forms provides a means to assess silencing of the checkpoint. In WT cells, Rad53p phospho-variants almost completely disappeared during a 2–4 hr recovery after treatment with 0.01% MMS (Figure 5A). A similar pattern was observed in ulp2Δ strains, although the accumulation and disappearance of shifted Rad53p appeared to be slightly delayed.

Second, we examined degradation of Pds1p/securin. Pds1p is a downstream target of the DNA damage checkpoint that is stabilized to block cohesin proteolysis and anaphase entry [61,62]. The kinetics of Pds1p degradation therefore provides a read-out of commitment to anaphase. In these experiments, we used the cdc14-1 allele to block Pds1p re-synthesis once cells recovered from the checkpoint. cdc14-1 PDS1-myc and cdc14-1 ulp2Δ PDS1-myc cells were treated with 0.001%, 0.005% and 0.01% MMS for 2 hr, allowed to recover at a cdc14-1 non-
permissive temperature, and Pds1p-myct abundance was monitored over a 24 hr period. In cdc14-1 cells, Pds1p-myct degradation proceeded in a dose-dependent manner until 10 hr post-treatment (Figure 5B, 5C). At this point, Pds1p started to increase in the 0.001% and 0.005% MMS cultures, probably reflecting leakage through the cdc14-1 arrest. These degradation kinetics were virtually indistinguishable in cdc14-1 ulp2D cells, although resynthesis of Pds1p was not observed (Figure 5B, 5C). These results suggest that MMS treated ulp2D cells can terminate checkpoint signaling and commit to anaphase.

Third, we used micro-colony analysis to determine whether getting rid of the checkpoint relieved the restraint on cell division. Cells from MMS treated and untreated cultures were positioned on agar plates, and the appearance of cell bodies was examined over time. A budded yeast cell arrested at the DNA damage checkpoint consists of two cell bodies. If this cell completes mitosis and a long right arm containing 332 kbp of human DNA (adapted from [53]). GCRs deleting ADE2/URA3 at the terminus of right arm can be selected and distinguished from YAC loss through segregation errors. (B) WT (MLY068; 30 cultures), ulp2D (MLY069; 30 cultures), smt3-331 (MLY070; 10 cultures), ulp1-333 (MLY071; 10 cultures) and ubc9-1 (MLY072; 10 cultures) strains harboring the YAC were plated onto 5-FOA media to select for loss of URA3 and further genotyped to identify GCRs. Box plot graphs display median GCR events per 10^7 viable cells. p values (Student’s t-test) were obtained from pair-wise comparisons between indicated mutants and the WT control. (C) Data as in B, but with lowered y-axis scale to show the reduction of GCRs in the ulp2D strain. It is possible to calculate that an average of 97.8 ± 1.7% of WT cells and 93.6 ± 4.4% of ulp2D cells retained the YAC at the time of plating to select for GCRs. (D) WT (CRY1) and ulp2D (JBY242) strains were transformed with three circular URA3 minichromosomes: monocentric p1Xcen, dicentric p2Xcen^invi, and dicentric p2Xcen^inv. Nine transformants for each strain/plasmid combination were cultured in parallel YPD (no selection for minichromosome) or Ura^-SC (to maintain selection) media, and equivalent volumes were plated onto YPD and Ura^-SC media. Graphs display the average percentage of cells retaining the minichromosome (mitotic stability), ± one standard deviation. doi:10.1371/journal.pgen.1001355.g002

Figure 2. Chromosome re-arrangements in ulp2D mutants. (A) The YAC used for GCR analysis consists of an origin of replication (ARS1), a CEN, and a long right arm containing 332 kbp of human DNA (adapted from [53]). GCRs deleting ADE2/URA3 at the terminus of right arm can be selected and distinguished from YAC loss through segregation errors. (B) WT (MLY068; 30 cultures), ulp2D (MLY069; 30 cultures), smt3-331 (MLY070; 10 cultures), ulp1-333 (MLY071; 10 cultures) and ubc9-1 (MLY072; 10 cultures) strains harboring the YAC were plated onto 5-FOA media to select for loss of URA3 and further genotyped to identify GCRs. Box plot graphs display median GCR events per 10^7 viable cells. p values (Student’s t-test) were obtained from pair-wise comparisons between indicated mutants and the WT control. (C) Data as in B, but with lowered y-axis scale to show the reduction of GCRs in the ulp2D strain. It is possible to calculate that an average of 97.8 ± 1.7% of WT cells and 93.6 ± 4.4% of ulp2D cells retained the YAC at the time of plating to select for GCRs. (D) WT (CRY1) and ulp2D (JBY242) strains were transformed with three circular URA3 minichromosomes: monocentric p1Xcen, dicentric p2Xcen^invi, and dicentric p2Xcen^inv. Nine transformants for each strain/plasmid combination were cultured in parallel YPD (no selection for minichromosome) or Ura^-SC (to maintain selection) media, and equivalent volumes were plated onto YPD and Ura^-SC media. Graphs display the average percentage of cells retaining the minichromosome (mitotic stability), ± one standard deviation. doi:10.1371/journal.pgen.1001355.g002
two-dimensional gels, transferred to nitrocellulose, and hybridized to a DNA fragment encompassing ARS305 (an early-firing origin of replication) to
synchronized in G2/M using nocodazole and released into media containing 0.033% MMS. After 3 hr, genomic DNA samples were fractionated on

forced for the appearance of distinct Rad52p foci; at least 100 cells were scored per time point. On graph, WT in HU and MMS
(triangles); ulp2Δ in HU and MMS (circles). Micrographs display fluorescent images of MMS-treated cells following either a 2 hr or 6 hr recovery period. Scale bar, 4 µm. (C) Two-dimension gel analysis of HR intermediates. WT (MLY080), ulp2Δ (MLY085), and sgs1Δ (MLY082) cells were synchronized in G2/M using nocodazole and released into media containing 0.033% MMS. After 3 hr, genomic DNA samples were fractionated on two-dimensional gels, transferred to nitrocellulose, and hybridized to a DNA fragment encompassing ARS305 (an early-firing origin of replication) to detect HR or DNA replication structures. Schematic depicts the relative migration of replication bubbles, forks and X-spike HR intermediates.

Blocking HR restores chromosome segregation in MMS–
treated ulp2Δ mutants

If defective HR in MMS treated ulp2Δ cells is causally linked to the chromosome separation defect that we observed in our experiments, blocking recombination should restore chromosome segregation. Given that HR is essential in ulp2Δ mutants (Figure 1) our approach to test this was to overproduce (OP) the Srs2p helicase. In addition to antagonizing nucleoprotein filament assembly [8-10], Srs2p also appears to exert anti-recobinogenic activity by unwinding D-loop intermediates [63,64]. Srs2p OP should therefore be an effective way to short circuit early stages of HR. cdc14-1, cdc14-1 rad9Δ, cdc14-1 ulp2Δ and cdc14-1 rad9Δ ulp2Δ strains were transformed with a vector control or a high copy endogenous promoter (pSRS2). The transformants were then treated with 0.01% MMS for 2 hr and allowed to recover at a cdc14-1 non-permissive temperature. Compared to vector controls, cdc14-1/pSRS2 cells remained blocked in a pre-anaphase configuration for the duration of the recovery period (Figure 9A). This delay was abolished in cdc14-1 rad9Δ/pSRS2 transformants, suggesting Srs2p OP was able to prolong DNA damage checkpoint arrest. In the absence of Ulp2p, however, inactivating the checkpoint in the cdc14-1 rad9Δ ulp2Δ/vector strain was insufficient to allow cells to proceed with chromosome segregation (Figure 9A, 9B). Significantly, Srs2p OP demonstrated a remarkable ability to allow ulp2Δ strains to escape this mitotic

Since sgs1Δ and ulp2Δ mutants both accumulate HR intermediates that might be expected to link sister chromatids (Figure 5C), we additionally examined chromosome segregation during MMS recovery in cdc14-1 sgs1Δ cells. Compared to the ulp2Δ defect, the fraction of MMS treated cdc14-1 sgs1Δ cells that could segregate their chromosomes to an extent necessary to form two distinct nuclear masses was only slightly reduced compared to cdc14-1 controls (Figure 8A; see Figure S4 for a more complete description). Taken as a whole, these results allow us to conclude that, although they commit to anaphase, ulp2Δ mutants are unable to separate their chromosomes efficiently following MMS treatment. Furthermore, this non-disjunction defect appears more severe than that observed in a sgs1Δ strain.
block, with ~50% of cdc14-1 rad9Δ ulp2Δ/pSRS2 cells now segregating their chromosomes in a seemingly normal anaphase (Figure 9A, 9B). Thus, Srs2p OP substantially relieves the block to chromosome separation in MMS treated ulp2Δ cells.

Discussion

HR and genome stability in ulp2Δ mutants

One principal finding of this study is that, even in the absence of exogenous DNA replication stress, spontaneous recombination is increased in ulp2Δ cells. This conclusion is based on two observations. First, by genetic criteria, spontaneous recombination at a genomic location on chromosome XV is elevated in ulp2Δ strains. Second, ulp2Δ mutants also display an increase in the frequency of spontaneous Rad52p DNA repair foci. A similar increase in Rad52p foci has been observed in a number of other SUMO pathway mutants, and has been shown to be largely attributable to a requirement for SUMOylation in preventing inappropriate recombination events involving the 2 μm circle, an endogenous plasmid found in most S. cerevisiae strains [65]. Since we have not directly examined the effect of the 2 μm circle on recombination in ulp2 mutants, destabilization of this extrachromosomal element may well contribute to the ulp2Δ increase in Rad52p foci. However, as the 2 μm circle is not required for S. cerevisiae growth, our finding that HR DNA repair becomes essential in ulp2Δ strains strongly suggests that Ulp2p acts to suppress the formation of genomic DNA lesions that must be repaired through recombination. Previous analyses of the SUMO pathway support this possibility. For example, SUMO conjugation-defective ubc9-1 mutants exhibit synthetic growth defects in the absence of HR and, at the non-permissive temperature, accumulate DNA structures that activate Rad53p [17]. Furthermore, as described in the Introduction, ulp1-I615N mutants also show increased HR and require HR for viability; in this case, the requirement for HR was shown to correspond with single-stranded DNA gaps arising during S phase [47]. It is striking that perturbations to Ulp1p and Ulp2p, which appear to target largely distinct sets of SUMO substrates [29], impose such seemingly similar dependencies on HR. Another observation that lends credence to the idea that Ulp2p suppresses recombinogenic DNA lesions is that ulp2Δ mutants greatly induce the formation of Rad52p foci following HU treatment. Such foci are not observed...
mosomes are consistent with the idea that repair events leading to propagated efficiently. Our observations with dicentric minichromosomes required for cells that would give rise to GCRs to recover and certain types of GCRs [66]. Alternatively, Ulp2p could be PRR, Siz1p-mediated sumoylation of PCNA was required to form as a previous study found that, in the absence of template switch stimulating error prone DNA repair. There is precedence for this, spontaneous GCRs are increased, ulp2

In contrast to HR foci in pol30-K164R rad18 [11], ulp2-D mutants terminate checkpoint signaling and commit to anaphase after MMS treatment. In analyzing genome stability in ulp2-D mutants, we observed two interesting differences compared to other SUMO pathway mutants. First, whereas our data indicate that Rad6p-dependent PRR is essential in ulp2-D mutants, mis-regulation of SUMO conjugation in ulp1-I165N rad18 [17], ubc9-1 rad18 [19], siz1 rad18 [11], pol30-K164R rad18 and pol30-K164R rad6 [5] mutants can actually compensate for defective PRR. One scenario that might account for this difference is if poly-sumoylation of a Ulp2p substrate(s) caused a distinct perturbation to DNA replication that was repaired through PRR-mediated HR. In keeping with this interpretation, we find that blocking poly-SUMO chain formation reduces the accumulation of both spontaneous and MMS-induced HR foci in ulp2-D mutants.

A second apparent difference concerns the formation of GCRs. In contrast to small3-331, ubc9-1 and ulp1-D strains, where spontaneous GCRs are increased, ulp2-D mutants show reduced GCRs. Formally, Ulp2p could promote GCR formation by stimulating error prone DNA repair. There is precedence for this, as a previous study found that, in the absence of template switch PRR, Siz1p-mediated sumoylation of PCNA was required to form certain types of GCRs [66]. Alternatively, Ulp2p could be required for cells that would give rise to GCRs to recover and propagate efficiently. Our observations with dicentric minichromosomes are consistent with the idea that repair events leading to some GCRs may not be tolerated in ulp2-D strains. We were able to recover re-arranged dicentrics from ulp2-D mutants when duplicated CEV sequences were present in a direct repeat configuration. Such deletions can occur through single-strand annealing, an intra-chromosomal form of recombination [67]. In contrast, CEV deletion GCRs were not recovered when the two CEVs were oriented as inverted repeats. Recent studies have shown that faulty template switch PRR is frequently involved in initiating deletions between inverted repeats [68,69]. As discussed below, one possibility is that such recombination events are accompanied by formation of SCJs or other types of chromatid attachments that fail to be resolved in ulp2 cells.

Ulp2p prevents accumulation of HR intermediates

Our results led us to suspect that HR DNA repair, while required for viability, might at the same time be toxic to ulp2 cells, prompting us to examine processing of MMS-induced recombination events. From this analysis, one conclusion is that, similar to Ube9p, Mms21p, Smc3p/Smc6p, and Sgs1p/Top3p [17,19], Ulp2p is required to prevent X-shaped DNA structures from accumulating at sites of replication fork stalling/collapse. We also find that, whereas Rad52p foci disappear after cells recovered from MMS. Protein extracts were prepared at the indicated times, protein concentrations were quantified, and equal amounts of protein were fractionated on SDS-PAGE gels. Pds1p-myc abundance was examined by 7-myc immunoblotting. Ponceau staining was used to confirm equivalency of protein load (not shown). A protein sample from a mid-logarithmic phase culture of a WT strain (CRY1) was used as a no-tag control. (C) The gel analysis tools of NIH Image J were used to quantify the Pds1p bands shown in (B). Values were normalized to the 0 time point and expressed as a percent. WT (squares); ulp2-D (circles).

doi:10.1371/journal.pgen.1001355.g005

Figure 5. ulp2-D mutants terminate checkpoint signaling and commit to anaphase after MMS treatment. (A) Rad53p activation/deactivation. WT (CRY1) and ulp2-D (BY240) mutants were synchronized in G1 using mating pheromone, then released into media containing 0.01% MMS (~2 hr time point). After a 2 hr MMS treatment (0 hr time point) cells were allowed to recover in MMS-free media, and mating pheromone was restored to re-arrest cells in the next G1. Protein samples were analyzed by α-Rad53p immunoblotting to monitor phospho-electrophoretic mobility variants of Rad53p. (B) Pds1p degradation. cdc14-1 (MLY181) and cdc14-1 ulp2-D (MLY183) PDS1-myc strains were grown to logarithmic phase at 23°C and then washed into media containing either 0.001%, 0.005%, or 0.01% MMS. After a 2 hr MMS treatment, cells were washed into MMS-free media (0 time point) and shifted to 35°C to inactivate cdc14-1, thereby blocking mitotic exit after cells recovered from MMS. Protein extracts were prepared at the indicated times, protein concentrations were quantified, and equal amounts of protein were fractionated on SDS-PAGE gels. Pds1p-myc abundance was examined by α-myc immunoblotting. Ponceau staining was used to confirm equivalency of protein load (not shown). A protein sample from a mid-logarithmic phase culture of a WT strain (CRY1) was used as a no-tag control. (C) The gel analysis tools of NIH Image J were used to quantify the Pds1p bands shown in (B). Values were normalized to the 0 time point and expressed as a percent. WT (squares); ulp2-D (circles).

doi:10.1371/journal.pgen.1001355.g005

in HU treated WT cells [56], consistent with an underlying replication problem in ulp2-D mutants that is exacerbated by slowed fork progression.

In analyzing genome stability in ulp2-D strains, we observed two interesting differences compared to other SUMO pathway mutants. First, whereas our data indicate that Rad6p-dependent PRR is essential in ulp2-D mutants, mis-regulation of SUMO conjugation in ulp1-I165N rad18 [47], ubc9-1 rad18 [19], siz1 rad18 [11], pol30-K164R rad18 and pol30-K164R rad6 [5] mutants can actually compensate for defective PRR. One scenario that might account for this difference is if poly-sumoylation of a Ulp2p substrate(s) caused a distinct perturbation to DNA replication that was repaired through PRR-mediated HR. In keeping with this interpretation, we find that blocking poly-SUMO chain formation reduces the accumulation of both spontaneous and MMS-induced HR foci in ulp2-D mutants.

A second apparent difference concerns the formation of GCRs. In contrast to small3-331, ubc9-1 and ulp1-D strains, where spontaneous GCRs are increased, ulp2-D mutants show reduced GCRs. Formally, Ulp2p could promote GCR formation by stimulating error prone DNA repair. There is precedence for this, as a previous study found that, in the absence of template switch PRR, Siz1p-mediated sumoylation of PCNA was required to form certain types of GCRs [66]. Alternatively, Ulp2p could be required for cells that would give rise to GCRs to recover and propagate efficiently. Our observations with dicentric minichromosomes are consistent with the idea that repair events leading to some GCRs may not be tolerated in ulp2-D strains. We were able to recover re-arranged dicentrics from ulp2-D mutants when duplicated CEV sequences were present in a direct repeat configuration. Such deletions can occur through single-strand annealing, an intra-chromosomal form of recombination [67]. In contrast, CEV deletion GCRs were not recovered when the two CEVs were oriented as inverted repeats. Recent studies have shown that faulty template switch PRR is frequently involved in initiating deletions between inverted repeats [68,69]. As discussed below, one possibility is that such recombination events are accompanied by formation of SCJs or other types of chromatid attachments that fail to be resolved in ulp2 cells.
Ulp2p Counteracts Recombination DNA Linkages

- MMS

- MMS

WT

ulp2Δ

rad9Δ

ulp2Δ rad9Δ

mec1Δ

ulp2Δ mec1Δ

mad2Δ

ulp2Δ rad9Δ mad2Δ

Number of Cell Bodies

Day 1
Day 2
Day 3

Number of Cell Bodies

15 +/- 1%
64 +/- 15%
13% +/- 14%
47 +/- 4%
73 +/- 21%
38 +/- 20%
77 +/- 11%
4 +/- 3%
90% +/- 6%
Figure 6. Microcolony analysis of MMS–treated ulp2Δ mutants. Cultures of WT (MLY061), ulp2Δ (MLY060), rad9Δ (MLY112), ulp2Δ rad9Δ (MLY064), mec1Δ GAP-RNR3 (JBY321; MEC1) essential function provided by overproduction of Rnr3p; (86)), ulp2Δ mec1Δ GAP-RNR3 (MLY108), mad2Δ (JBY554), and ulp2Δ rad9Δ mad2Δ (MLY160) strains were split, and half the cultures were treated with 0.01% MMS for 2 hr. For both treated (+MMS) and untreated (−MMS) cultures, 50 large-budded cells (equivalent to 2 cell bodies) were micro-manipulated onto a grid pattern on a YPD plate, and the number of cell bodies in the resulting micro-colonies were monitored over the next 3 days. The graphs display the average of three separate experiments for each strain, ± one standard deviation. Numbers on the +MMS graphs indicate the average percent of cells that remain blocked with 2–3 cell bodies. For the WT and ulp2Δ strains, the average percentage of cells capable of recovering to form microcolonies of ≥ 16 cell bodies is also indicated.

doi:10.1371/journal.pgen.1001355.g006

function is related to or separate from Ulp2p’s role in Rad52p foci disassembly, are important future questions.

Based on current information, Ulp2p could be connected to HR through a number of different SUMO substrates. First, Mms21p-mediated sumoylation of unknown substrates, probably in conjunction with Smc3p/Smc6p [22,70], has been proposed to prevent excessive template switch recombination through PRR [19]. Alternatively, more recent evidence suggests Smc3p/Smc6p may instead act downstream of PRR to facilitate the dissolution of HR intermediates [71]. Second, Sgs1p is sumoylated under conditions when it is active in SCJ dissolution [17,59], although apparently through an Mms21-independent pathway [17]. Third, Ubc9p/Siz1p-controlled sumoylation of PCNA and recruitment of Srs2p may suppress PRR-independent recombination at replication forks [6,7,19]. Fourth, Srs2p has also been shown to be sumoylated, with poly-sumoylation being proposed to trigger Srs2p degradation through the Skx3p/Shx0p pathway [72]. Fifth, a fraction of Rad52p [73-75], and other HR proteins [76], are sumoylated in response to MMS, which may be involved in fine-tuning processing of broken DNA. Finally, a growing number of protein-protein interactions within HR foci have been found to be controlled by sumoylation [reviewed in [77]]. As part of completion of repair, Ulp2p may catalyze the disassembly of these networks.

As a first step in placing Ulp2p in these pathways, we tested whether mis-regulation of Sgs1p sumoylation was connected to ulp2Δ HR defects. Overproduction of Ulp2p was recently shown to block Sgs1p sumoylation on K621 following MMS treatment [59], and, as we report here, MMS-induced sumoylation of Sgs1p is elevated in the absence of Ulp2p. It is therefore likely that Ulp2p acts as the SUMO deconjugating enzyme for Sgs1p. Despite this, short-circuiting Sgs1p sumoylation using the sgs1Δ-K621R mutation
Pds1p on schedule, suggesting they were competent to silence the mutants. (A) Bulk chromosome separation. cdc14-1 (MLY181, JBY531; circles), cdc14-1 ulp2Δ (MLY183, triangles in left graph), and cdc14-1 sgs1Δ (JBY1809, triangles in right graph) strains were cultured at 23°C, treated with 0.01% MMS for 2 hr, and allowed to recover (0 time point) at 35°C to inactivate cdc14-1. At the indicated times, samples were fixed, stained with DAPI, and scored for binucleate cells indicative of successful nuclear division. ≥ 100 cells were scored per time point. (B) Chromosome separation and segregation at TRP1-GFP. cdc14-1 (JBY642, circles) and cdc14-1 ulp2Δ (JBY643, triangles) TRP1-GFP strains were treated with MMS and allowed to recover as in (A). At the indicated times, cells were fixed and scored for TRP1-GFP disjunction (two distinct, separated TRP1-GFP foci) and segregation (separated TRP1-GFP foci that have been partitioned between mother and daughter cells). ≥ 50 cells have been scored for each time point.

doi:10.1371/journal.pgen.1001355.g008

Figure 8. Chromosome disjunction in MMS–treated ulp2Δ mutants. (A) Bulk chromosome separation. cdc14-1 (MLY181, JBY531; circles), cdc14-1 ulp2Δ (MLY183, triangles in left graph), and cdc14-1 sgs1Δ (JBY1809, triangles in right graph) strains were cultured at 23°C, treated with 0.01% MMS for 2 hr, and allowed to recover (0 time point) at 35°C to inactivate cdc14-1. At the indicated times, samples were fixed, stained with DAPI, and scored for binucleate cells indicative of successful nuclear division. ≥ 100 cells were scored per time point. (B) Chromosome separation and segregation at TRP1-GFP. cdc14-1 (JBY642, circles) and cdc14-1 ulp2Δ (JBY643, triangles) TRP1-GFP strains were treated with MMS and allowed to recover as in (A). At the indicated times, cells were fixed and scored for TRP1-GFP disjunction (two distinct, separated TRP1-GFP foci) and segregation (separated TRP1-GFP foci that have been partitioned between mother and daughter cells). ≥ 50 cells have been scored for each time point.

doi:10.1371/journal.pgen.1001355.g008

HR and the ulp2 recovery defect

The failure of ulp2 mutants to resume cell division following DNA damage is one of the most intriguing aspects of the ulp2 phenotype. The first study to document this phenomenon showed that, following adaptation to a persistent DNA break, only a fraction of ulp2 cells were able to proceed with nuclear division, frequently accompanied by abnormally extended or broken mitotic spindles [41]. Inactivating the DNA damage checkpoint rescued this defect, suggesting a critical role for Ulp2p in re-initiating chromosome segregation following completion of the checkpoint response [41,48].

While our results are largely in accord with this study, we observed a potentially informative difference in the role of the checkpoint in manifesting the ulp2Δ recovery defect. During MMS recovery, ulp2Δ cells dephosphorylated Rad33p and degraded Pds1p on schedule, suggesting they were competent to silence the checkpoint and initiate anaphase. Despite this, sister chromatids failed to disjoin, resulting in a dramatic failure in chromosome segregation. OP of Srs2p, which antagonizes HR [8-10,63,64], was able to largely restore chromosome segregation. In addition to modulating nucleo-protein filament assembly, Srs2p has also been shown to be required for full activation of the DNA damage checkpoint and for recovery from DNA damage checkpoint arrest [78,79]. In our experiments, we observed that Srs2p OP greatly extended DNA damage checkpoint arrest in MMS treated WT cells. Based on the above considerations, this extended arrest would presumably reflect either mis-regulation of the checkpoint pathway, or, by interfering with HR DNA repair, elevated Srs2p could simply prolong normal checkpoint signaling. While the effects of Srs2 OP on checkpoint signaling and HR may be multifaceted, the key point we wish to emphasize here is that abolishing the DNA damage checkpoint (or the SAC) did not allow ulp2Δ cells to divide more times during recovery from MMS treatment.

Furthermore, preventing DNA damage checkpoint arrest in MMS treated ulp2Δ rad9Δ cells was insufficient to relieve the block to chromosome separation; OP of Srs2 was also necessary. In sum, these findings strongly suggest that, following replication fork stalling by MMS, downstream events initiated through HR, rather than checkpoint arrest per se, appear to play a causal role in interfering with chromosome segregation.

A key question concerns how HR could have this effect. Perhaps the simplest idea is that unresolved SCJs block chromatid disjunction. Whether this is a sufficient explanation, however, is unclear. First, in the experiments examining ulp2 adaptation to a persistent, endonuclease-targeted DNA break, both chromatids would be expected to be cut, preventing HR strand exchange [41]. Thus, the only way in which DNA linkages could form between chromosomes in these cells would be if extensive resection during prolonged checkpoint arrest triggered illegitimate recombination events. Second, we show that MMS treated sgs1Δ mutants, which are clearly defective in the dissolution of SCJs [17,25,27], do not show as severe a block to chromosome separation as Ulp2p-deficient cells. This is consistent with a recent study that showed, from among a collection of helicase-, nuclease-, and topoisomerase-deficient mutants, only smc5, smc6 and mms21 strains showed chromosome segregation defects after a pulse of MMS delivered in G1 [71]. This suggests a role for Mms21p-mediated sumoylation and the Smc5p/Smc6p complex in resolving SCJs or other types of chromatid linkages outside the Sgs1p/Top3p pathway [71].

Along these lines, it is notable that Ulp2p has been implicated in multiple facets of chromatid separation, including controlling sumoylation of cohesin regulatory proteins [37,42], condensin [35,38], and DNA topoisomerase II [36,40]. Speculatively, following induction of HR, there may be an increased requirement for Ulp2p in the vicinity of DNA lesions, not only to prevent accumulation of HR intermediates, but also to complete replication, to disentangle DNA or to release protein-based forms of cohesion. Given the dramatic way in which the absence of Ulp2p potentiates the ability of replication toxins to block cell proliferation, a further understanding of the ulp2 recovery defect could lead to insights that are relevant to cancer treatment.

**Materials and Methods**

**Yeast strains and culture**

All *S. cerevisiae* strains used in this study were derived from the W303-related CRY1 strain and are listed in Table S1. A description of how different genetic elements were introduced into the CRY1 background can be found in Text S1. For all experiments, cells were cultured in standard formulations of yeast...
extract/peptone/dextrose (YPD) and synthetic complete minimal (SC) media. For G1 synchronization, alpha factor (Bio-Synthesis Corp.) was used at 10 μg/ml. For arresting cells in G2/M, nocodazole (Sigma-Aldrich) was used at 15 μg/ml in YPD. MMS and HU were purchased from Sigma-Aldrich. 5-FOA was purchased from Biovectra/Fisher and used at 1 mg/ml. G418 was purchased from Mediatech/Fisher and used at 200 μg/ml in YPD.

Recombination frequency

pLAY202 ([50]; provided by A. Bailis, City of Hope National Medical Center, Duarte, CA) was linearized with BstXI and targeted to the HIS3 locus, placing a URA3 marker between partially duplicated HIS3 sequences. pLAY202 integrants were propagated in Ura2/SC media, and, following overnight incubation, cell density was quantified using a hemacytometer. Viable cell counts were determined by plating a defined number of cells onto YPD and counting the resulting colonies. Recombination events were selected by plating a larger number of cells onto His2/SC media, and replica plating colonies that arose onto 5-FOA. Colonies that reverted to a His+, Ura2 phenotype were scored as recombinants.

GCR frequency

YAC yWss1572-1 ([53]; provided by D. Koshland, Univ. of California at Berkeley, Berkeley, CA) was modified so that the TRP1 marker on the left arm of the YAC was replaced with kanMX. This was performed by PCR amplifying a trp1Δ:kanMX disruption cassette using the following primers:

5'-GCATATAAAAATAGTTCAGGCACTCCGAAATACT-TGGTTGGCGTGTTTC
GTCAGCTGAAGCTTCGTACGC (CO354)
5'-TCTGGCGTCAGTCCACCAGCTAACATAAAATGTA-AGCTTTCGGGGCAT
AGGCCACTAGTGGATCTG (CO355)
AGGCCACTAGTGGATCTG (CO355) and pFA6a/kanMX2 [80] as template. G418Res, Trp− transformants were analyzed by PCR to verify correct targeting. The resulting YAC, named yWss1572Δtrp1Δ, was subsequently transferred between strains using cytotransduction [81] or standard genetic crosses. To isolate GCRs, strains containing yWss1572Δtrp1Δ were grown in Ura−/SC media at 30°C for WT, ulp2Δ, ube9-1 and smt3-331 strains, and 34°C for ulp1-333 mutants; these represent semi-permissive temperatures for the ube9-1, smt3-331 and ulp1-333 alleles. Cell densities were quantified using a hemacytometer, and dilutions of the cultures were plated onto YPD to monitor plating efficiency. Aliquots of 105, 106, 107 and

Figure 9. Effect of Srs2p OP and inactivating the DNA damage checkpoint on chromosome segregation during MMS recovery. cdc14-1 (MLY181), cdc14-1 rad9Δ (MLY186), cdc14-1 ulp2Δ (MLY183), and cdc14-1 ulp2Δ rad9Δ (MLY188) strains were transformed with a vector control (pRS426; [84]) or a high copy plasmid expressing SRS2 under control of its endogenous promoter (pSRS2; YEpLac195-SRS2; [85]). Transformants were cultured under selection for the plasmid at 23°C, treated with 0.01% MMS for 2 hrs, and allowed to recover (0 time point) at 35°C to inactivate cdc14-1. (A) Cell aliquots were removed at the indicated times, stained with DAPI, and scored for nuclear segregation. Graphs display results from two separate experiments; vector transformants (circles); Srs2 OP transformants (triangles). For one experiment, cdc14-1 and cdc14-1 rad9Δ strains were only scored to 4 hr. (B) Representative images of cdc14-1 ulp2Δ rad9Δ/vector cells with undivided nuclei and examples of cdc14-1 ulp2Δ rad9Δ/pSRS2 cells that would be scored as having completed chromosome segregation. All images taken 4 hr post-treatment. Scale bar, 4 μm.

doi:10.1371/journal.pgen.1001355.g009
10^6 cells were plated on 5-FOA to select for loss of the \textit{URA3} marker on the YAC. Colonies arising on 5-FOA were replica plated to YPD/G418 and Ade^-/SC media. Clones growing on 5-FOA and YPD/G418, but not on Ade^-/SC (G418^{res}, 5-FOA^{sens}, Ade^-) were considered to arise from GCRs deleting the right arm of the YAC. In contrast, clones that were able to grow on 5-FOA, but could not grow on YPD/G418 or Ade^-/SC (G418^{sens}, 5-FOA^{sens}, Ade^-) were considered to arise through YAC mis-segregation events. For each culture, the total number of GCR clones arising on all the assay plates was used to calculate GCR frequency.

Minichromosome loss
To monitor the mitotic stability of dicentric minichromosomes, p2XCEX^{direct} (pJBN152; a YRp14-derived minichromosome containing two copies of a 1.7 kb GEVI DNA fragment in a direct repeat configuration, see Text S1) and p2XCEX^{inverted} (pJBN151; similar to pJBN152 but with the GEVI duplication oriented as an inverted repeat) were transformed into WT and \textit{ulp2} strains and compared to p1XCEV (YRp14/GEV) controls. Transformants were inoculated into parallel YPD and Ura^-/SC cultures and incubated at 30°C. After ~15 hr of outgrowth, appropriate dilutions were plated onto YPD and Ura^-/SC media. Mitotic stability was calculated by dividing the number of Ura^+ colonies by the total number of colonies obtained on YPD.

Microscopy and flow cytometry
Cultures for microscopy were supplemented with 50 µg/ml adenine to quench auto-fluorescence. To visualize Rad52p-GFP and TRP1-GFP, cells were fixed in 1% formaldehyde for 1.5 min and washed into PBS. DAPI staining was performed using VectaShield (Vector Laboratories) containing 10 µg/ml DAPI. TUB1-GFP and HHF2-FYP strains were visualized as live mounts. HHF2-FYP is typically propagated as a heteroygous diploid (HHF2-FYP-HIS3^+/+) to minimize selective pressure for rearranged variants that lose the fluorescent marker. However, in order to compare the response of HHF2-FYP strains to MMS concentrations similar to those used in our other recovery experiments, we chose to examine HHF2-FYP haploid segregants that were generated on an experiment-by-experiment basis. This proved to allow propagation of haploid strains with robust Hsf2-FYP fluorescence. In all cases, cells were visualized on Nikon E-800 or Nikon Eclipse 80i microscopes equipped with fluorescence optics and 100X (1.4 NA) or 60X (1.4 NA) objectives. Rad52p-GFP foci were typically scored using a number 4 neutral density filter to minimize photobleaching. A Zeiss Axioskop 40 microscope equipped with a 25 µm diameter optical fiber dispersion needle was used to micromanipulate yeast cells for microcolony analysis. FACS analysis was performed by staining ethanol fixed yeast cells with propidium iodide as previously described [82].

Pulse field gel electrophoresis
10 ml aliquots of OD_{600} 0.8 cultures were harvested by centrifugation and concentrated into 400 µl cell suspension buffer (10 mM Tris, 20 mM NaCl, 50 mM EDTA, pH 7.2). The cell suspension was warmed to 55°C and mixed with 400 µl 2% low melting temperature agarose (SeaKem) dissolved in TBE gel electrophoresis buffer (kept molten at 55°C) containing lyticase (Sigma L4025; final concentration 1 mg/ml). The cell suspension was transferred into molds and allowed to solidify to form plugs (4°C, 15 min). Plugs were pushed out into 50 ml conical tubes and incubated with 5 ml 1 mg/ml lyticase dissolved in 10 mM Tris, 50 mM EDTA, pH 7.2 for one hr at 37°C, followed by treatment 1 mg/ml Proteinase K (Sigma) dissolved in 100 mM EDTA, 0.2% Na Deoxycholate, 1% Na lauryl sarcosine, pH 8.0 at 50°C overnight. Plugs were washed (20 mM Tris, 50 mM EDTA, pH 8.0) 4 times one hour each and stored in wash buffer. Prior to electrophoresis, plugs were placed on a glass plate and trimmed to fit electrophoresis wells. Samples were then fractionated on 1% agarose gels in TBE using a Bio-Rad CHEF-DR II pulsed field electrophoresis system at 6V/cm for 22 hrs with a switch ramp time ramped from 50 to 90 sec at 14°C. Gels were stained with ethidium bromide (0.5 µg/ml, 15 min) prior to photography.

Two-dimensional gel analysis
Genomic DNA preparations and two-dimensional gel electrophoresis were performed according to detailed online methods available from the Brewer-Raghuraman laboratory:

[http://fangman-brewer.genetics.washington.edu/DNA_prep.html](http://fangman-brewer.genetics.washington.edu/DNA_prep.html)

In brief, cells were grown in 500 ml YPD until the cultures reached an OD_{600} of 0.6. The cultures were synchronized in nocodazole for 2 hr, washed, and released into fresh YPD containing 0.03% MMS. After a 3 hr treatment, cells were harvested by centrifugation and stored in 5 ml of NIB buffer (17% propidium iodide as previously described [82]. For first dimension separation, ~30 µg of digested DNA was loaded onto 0.35% agarose gels and fractionated at 22 volts for 42-48 hr at room temperature. Gel slices containing DNA in the 3–10 kb range were excised and positioned onto a 0.95% agarose gel. Electrophoresis in the second dimension was performed at 4°C at 80 volts for 17 hr at room temperature and 130 volts for another 1.5 hr. Following transfer to nylon membranes (Hybond-XL, GE Healthcare), samples were hybridized with a 280 bp ARS305 DNA fragment PCR amplified from genomic DNA using the following primers:

9'-CTCCGTTTTTAGCCCCCGTG/GATTGAGGCCACAGCAAGACCG-
9'-GATTGAGGCCACAGCAAGACCG-

The PCR product was radio-labeled (Megaprime DNA labeling system, GE Healthcare) and hybridized using Southern blot procedures as previously described [83].

Protein techniques
Protein extracts were prepared by mechanical beakage of cells in 20% TCA as previously described [36]. 6% SDS-PAGE gels were used to fractionate samples for analysis of Sgs1p-myc and Pds1p-myc, while 12% SDS-PAGE gels (acylamide: bis = 30:0.39) were used to analyze phosphorylated species of Rad53p. α-myc (9E10, 1:1000, Covance), α-Rad53p (SC-6749, 1:2000, Santa Cruz), and HRP conjugated secondary (1:25,000; Jackson ImmunoResearch) antibodies were used for immunoblotting.

Supporting Information

**Figure S1** Analysis of Sgs1p SUMO conjugation. A sgs1A mutant strain (MLY200) was transformed with plasmids encoding untagged SGS1 (no tag; YCplac33/SGS1), SGS1-10Xmyc (pJBN276), or the sgs1-K621R-18Xmyc mutant allele (pJBN277). Duplicate cultures of these transformants were treated with 0.03% MMS for 2 hrs or were maintained in MMS-free media, and protein samples were analyzed by α-myc immunoblotting. As observed previously [11], a shifted form of Sgs1p is observed in...
MMS-treated Sgs1Δ transformants but not in cells transformed with the sgs1-K62R allele.

Figure S2 Microcolony analysis of MMS recovery in u2Δ mad2Δ mutants. In conjunction with the experiments shown in Figure 6, mid-logarithmic phase cultures of a u2Δ mad2Δ strain (JBY733) were split. One half was treated with 0.01% MMS for 2 hr while the other half was cultured for an equivalent period in MMS-free media. For both treated (+MMS) and untreated (−MMS) cultures, 50 large-budded cells (2 cell bodies) were micro-manipulated onto a grid pattern on a YPD plate, and the number of cell bodies in the resulting micro-colonies were monitored over 3 days. The graphs display the average from three separate experiments, ± one standard deviation. The average percent of MMS treated cells that remained blocked with 2–3 cell bodies is indicated.

Figure S3 FACS analysis of MMS treated u2Δ cells. WT (MLY061) and u2Δ (MLY060) strains were treated with 0.03% MMS for 2 hrs (2 to 0 timepoints) and allowed to recover in MMS-free media. At the indicated times, samples were withdrawn and DNA content was examined by flow cytometry. Although u2Δ cells are blocked for chromosome segregation, they show an apparent increase in DNA content, suggesting that following this mitotic catastrophe they may attempt an addition round of DNA replication. 

Figure S4 Chromosome segregation in MMS treated cdc14-1 sgs1Δ cells. cdc14-1 sgs1Δ RAD52-GFP cells (JBY1808) were cultured at 23°C, treated with 0.01% MMS for 2 hr, and allowed to recover at 35°C to inactivate cdc14-1. After a 5 hr recovery, aliquots were fixed, stained with DAPI and visualized DNA and Rad52p-GFP foci. Micrographs depict cells in which chromosome separation and nuclear segregation are largely complete, but with faint DAPI-staining fibers that appear to connect the nuclei. Persistent Rad52p-GFP foci are often observed in the nuclei. In some cases, the Rad52p-GFP foci appear to colocalize on the DAPI-staining fibers, while in others the foci appear to have partitioned with the bulk of chromosomal DNA. BLM-deficient human cells (BLM is the human homologue of Sgs1) exposed to replication stress have been shown to accumulate ultra-fine sister chromatid bridges that are thought to arise from unresolved DNA linkages [12]. Speculatively, MMS treated sgs1Δ cells may form similar types of structures. 

Table S1 Yeast strains used in this study. 

Text S1 Additional description of methods. 

Acknowledgments The authors wish to thank L. Xu and C. Nugent (University of California Riverside) for construction of the yWss1572ΔΔyp1 YAC described in this study. Other colleagues who generously provided strains, plasmids, or other reagents are described in Text S1. 

Author Contributions Conceived and designed the experiments: MTL JB. Performed the experiments: MTL AAB KNN JB. Analyzed the data: MTL AAB KNN JB. Wrote the paper: MTL AAB KNN JB.

References 

1. Broomfield S, Hryciw T, Xiao W (2001) DNA postreplication repair and mutagenesis in Saccharomyces cerevisiae. Mutat Res 486: 167–184. 
2. San Filippo J, Sung P, Klein H (2002) Mechanism of eukaryotic homologous recombination. Annu Rev Biochem 77: 229–257. 
3. Braun D, Foiani M (2010) Maintaining genome stability at the replication fork. Nat Rev Mol Cell Biol 11: 208–219. 
4. Johnson ES (2004) Protein modification by SUMO. Annu Rev Biochem 73: 355–382. 
5. Hoege C, Pflander B, Moldovan GL, Pyrowolakis G, Jentsch S (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419: 135–141. 
6. Pflander B, Moldovan GL, Sacher M, Hoege C, Jentsch S (2005) SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. Nature 436: 426–433. 
7. Papouli E, Chen S, Davies AA, Hartner D, Kreji L, et al. (2005) Crossover between SUMO and ubiquitin on PCNA is mediated by the helicase Srs2p. Mol Cell 19: 123–133. 
8. Kreji L, Van Komen S, Li Y, Vignal JM, Reddy MS, et al. (2003) DNA helicase Srs2p disrupts the Rad51 presynaptic filament. Nature 425: 305–309. 
9. Vearue X, Jeste J, Soustelle C, Kowalczykowski SC, Le Cam E, et al. (2003) The Srs2p helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature 423: 309–312. 
10. Antony E, Tomko EJ, Xiao Q, Kreji L, Lohman TM, et al. (2009) Srs2 disassembles Rad51 filaments by a protein-protein interaction triggering ATP turnover and dissociation of Rad51 from DNA. Mol Cell 35: 105–115. 
11. Stelter P, Ulrich HD (2003) Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature 425: 188–191. 
12. Haracska L, Torres-Ramos CA, Johnson RE, Prakash S, Prakash L (2004) Opposing effects of ubiquitin conjugation and SUMO modification of PCNA on replicational bypass of DNA lesions in Saccharomyces cerevisiae. Mol Cell Biol 24: 4267–4274. 
13. Kanneuche PL, Wing J, Lehmann AR (2008) Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. Mol Cell 14: 491–500. 
14. Maeda D, Seki M, Onoda F, Brajezi D, Kawaih Y, et al. (2004) Ubc9 is required for damage-tolerance and damage-induced interchromosomal homologous recombination in S. cerevisiae. DNA Repair (Amst) 3: 335–341. 
15. Andrews EA, Palecek J, Sergeant J, Taylor E, Lehmann AR, et al. (2005) Nse2, a component of the Smc3-6 complex, is a SUMO ligase required for the response to DNA damage. Mol Cell Biol 25: 185–196. 
16. Zhao X, Blobel G (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc Natl Acad Sci U S A 102: 4777–4782. 
17. Brajezi D, Sollor J, Liberi G, Zhao X, Maeda D, et al. (2006) Ubc9- and mms21-mediated sumoylation counteracts recombinogetic events at damaged replication forks. Cell 127: 509–522. 
18. van Waardenburg RC, Duda DM, Lancaster CS, Schulman BA, Bjornmi MA (2003) Distinct functional domains of Ubc8 dictate cell survival and resistance to genotoxic stress. Mol Cell Biol 23: 4958–4969. 
19. Brajezi D, Vanioli F, Foiani M (2008) SUMOylation regulates Rad18-mediated template switch. Nature 456: 913–920. 
20. Onoda F, Takeda M, Seki M, Maeda D, Tajima J, et al. (2004) SMC6 is required for MMS-induced interchromosomal and sister chromatid recombination in Saccharomyces cerevisiae. DNA Repair (Amst) 3: 429–439. 
21. Torres-Rosell J, Machin F, Farmer S, Jarmynz A, Eydmann T, et al. (2005) SMC5 and SMC6 genes are required for the segregation of repetitive chromosome regions. Nat Cell Biol 7: 412–419. 
22. Ampatoudios E, Irmisch A, O’Connell MJ, Murray JM (2006) Smc5/6 is required for repair at collapsed replication forks. Mol Cell Biol 26: 9387–9401. 
23. Sollor J, Driscoll R, Casteluccic F, Foiani M, Jackson SP, et al. (2009) The Saccharomyces cerevisiae Ecb1 and Smc3-6 proteins promote sister chromatid junction-mediated intra-S repair. Mol Cell Biol 20: 1671–1682. 
24. Karow JK, Constantinoiu A, Li JL, West SC, Hickson ID (2000) The Bloom’s syndrome gene product promotes branch migration of Holliday junctions. Proc Natl Acad Sci U S A 97: 6509–6518. 
25. Liberi G, Maisioli G, Lauda C, Ciardo I, Baranikova A, et al. (2005) Rad51-dependent DNA structures accumulate at damaged replication forks in sgs1 mutants defective in the yeast ortholog of BLM RecQ helicase. Genes Dev 19: 339–350. 
26. Plank JL, Wu J, Hsieh TS (2006) Topoisomerase IIIalpha and Bloom’s helicase can resolve a mobile double Holliday junction substrate through convergent branch migration. Proc Natl Acad Sci U S A 103: 11118–11123. 
27. Mankouri HW, Ngo HP, Hickson ID (2007) Shu proteins promote the formation of homologous recombination intermediates that are processed by Sgs1-Rmi1-Top3. Mol Cell Biol 27: 4062–4073.
55. Koshland D, Rutledge L, Fitzgerald-Hayes M, Hartwell LH (1987) A genetic
52. Lemoine FJ, Degtyareva NP, Kokoska RJ, Petes TD (2008) Reduced levels of
50. Maines S, Negritto MC, Wu X, Manthey GM, Bailis AM (1998) Novel
46. Mullen JR, Brill SJ (2008) Activation of the Slx5-Slx8 ubiquitin ligase by poly-
43. Bylebyl GR, Belichenko I, Johnson ES (2003) The SUMO isopeptidase Ulp2
41. Schwartz DC, Felberbaum R, Hochstrasser M (2007) The Ulp2 SUMO
40. Takahashi Y, Yong-Gonzalez V, Kikuchi Y, Strunnikov A (2006) SIZ1/SIZ2
35. Strunnikov AV, Aravind L, Koonin EV (2001) Saccharomyces cerevisiae SMT4
34. Meluh PB, Koshland D (1995) Evidence that the MIF2 gene of Saccharomyces
33. Kroetz MB, Su D, Hochstrasser M (2009) Essential role of nuclear localization
32. Panse VG, Kuster B, Gerstberger T, Hurt E (2003) Unconventional tethering of
30. Schwienhorst I, Johnson ES, Dohmen RJ (2000) SUMO conjugation and
29. Li SJ, Hochstrasser M (2000) The yeast ULP2 (SMT4) gene encodes a novel
28. Li SJ, Hochstrasser M (1999) A new protease required for cell-cycle progression
25. Rana SS, Hoppe LF, Haber JE (1992) Deficient DNA replication and requires Srs2 and homologous
24. Schwartz DC, Kellogg JS, Hochstrasser M (2002) The Rnf4 SUMO ligase specifically
23. Toonstra SP, Hoppe LF, Haber JE (1992) Two alternative pathways of
22. Mullen JR, Kalbisch G, Puchades C, Smith AJ, Cusick ME, et al. (2004) The Smc5-Smc6 complex affects sumoylation of DNA repair proteins and negatively regulates recombinational repair. PLoS Genet 6: e1000858. doi:10.1371/journal.pgen.1000858
21. Sanchez Y, Desany BA, Jones WJ, Liu Q, Wang B, et al. (1996) Regulation of RAD53 by the ATM-like kinases MEC1 and TEL1 in yeast cell cycle checkpoint pathways. Science 275: 357–360.
20. Li SJ, Hochstrasser M (1999) A new protease required for cell-cycle progression in yeast. Nature 398: 246–251.
19. Li SJ, Hochstrasser M (2000) The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. Mol Cell Biol 20: 2021–2028.
18. Schwienhorst I, Johnson ES, Dohmen RJ (2000) SUMO conjugation and deconjugation. Mol Gen Genet 263: 771–786.
17. Li SJ, Hochstrasser M (2003) The Upl1 SUMO isopeptidase: distinct domains required for viability, nuclear envelope localization, and substrate specificity. J Cell Biol 160: 1069–1071.
16. Pane VG, Kuster B, Grosbergter T, Hurt E (2003) Unconventional tethering of Upl1 to the transport channel of the nuclear pore complex by karyopherins. Nat Cell Biol 5: 21–27.
15. Kroetz MB, Su D, Hochstrasser M (2009) Essential role of nuclear localization for yeast Ulp2 SUMO protease function. Mol Cell Biol 20: 2196–2206.
14. Meluh PB, Koshland D (1995) Evidence that the MIF2 gene of Saccharomyces cerevisiae encodes a centromere protein with homology to the mammalian centromere protein CENPC. Mol Cell Biol 6: 793–807.
13. Strunnikov AV, Aravind L, Koonin EV (2001) Saccharomyces cerevisiae SMT4 encodes an evolutionarily conserved protease with a role in chromosome condensation regulation. Genetics 158: 95–107.
12. Bachant J, Alcasabas A, Blat Y, Kleckner N, Elledge SJ (2002) The SUMO-1 isopeptidase Smt1 is linked to centromeric cohesion through SUMO-1 modification of DNA topoisomerase II. Mol Cell 9: 1169–1182.
11. Scott J, Aguilar C, Hartman T, Drenel M, Meluh P, et al. (2003) Pdc1p regulates the maintenance of sister chromatid cohesion and is sumoylated to promote the dissolution of cohesion. J Cell Biol 163: 729–741.
10. D’Amours D, Stegemeyer F, Amon A (2004) Cdk4 and condensin control the dissolution of cohesion-independent chromosomal linkages at repeated DNA. Cell 117: 455–469.
9. Bachant J, Jessen SR, Kavanaugh SE, Fielding CS (2005) The yeast S phase checkpoint enables replicating chromosomes to bi-orient and restrain spindle elongation during phase distortion. J Cell Biol 166: 999–1011.
8. Takahashi Y, Yong-Gonzalez V, Kikuchi Y, Strunnikov A (2006) SIZ1/SIZ2 control of chromosome transmission fidelity is mediated by the sumoylation of topoisomerase II. Genetics 172: 783–794.
7. Schwartz DC, Felberbaum R, Hochstrasser M (2007) The Ulp2 SUMO protease is required for cell division following termination of the DNA damage checkpoint. Mol Cell Biol 27: 6948–6961.
6. Baldwin ML, Julius TA, Tang X, Wang Y, Bachant J (2009) The yeast SUMO isopeptidase Smt1/Ulp2 and the polo kinase Cdc53 act in an opposing fashion to regulate sumoylation in mitosis and cohesion at centromeres. Cell Cycle 8: 3404–3419.
5. Byfeldt GR, Belchenko I, Johnson ES (2003) The SUMO isopeptidase Ulp2 prevents accumulation of SUMO chains in yeast. J Biol Chem 278: 44115–44120.
4. Uozumo K, Gotsche K, Mitve M, Weishaar SR, Glänemann C, et al. (2007) Ubiquitin-dependent proteolytic control of SUMO conjugates. J Biol Chem 282: 34167–34173.
3. Tatham MH, Geoffroy MC, Shen L, Plechanova A, Hatternsey N, et al. (2008) RNF3 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. Nat Cell Biol 10: 538–546.
2. Mann JR, Bell JA (2008) Activation of the Smc3-Skls8 ubiquitin ligase by poly-small ubiquitin-like modifier conjugates. J Biol Chem 283: 19912–19921.
1. Soustelle C, Verma S, Freon K, Reynaud-Angelin A, Chanet R, et al. (2004) A new Saccharomyces cerevisiae strain with a mutant Smt3-deconjugating Upl1 protease provides insight into DNA replication and requires Srs2 and homologous recombination for its viability. Mol Cell Biol 24: 5130–5143.
84. Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P (1992) Multifunctional yeast high-copy-number shuttle vectors. Gene 110: 119–122.
85. Mankouri HW, Craig TJ, Morgan A (2002) SGS1 is a multicopy suppressor of srs2: functional overlap between DNA helicases. Nucleic Acids Res 30: 1103–1113.
86. Desany BA, Alcasabas AA, Bachant JB, Elledge SJ (1998) Recovery from DNA replicational stress is the essential function of the S-phase checkpoint pathway. Genes Dev 12: 2956–2970.