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A Tale of Tails: Insights into the Coordination of 3' End Processing during Homologous Recombination

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Summary

Eukaryotic genomes harbor a large number of homologous repeat sequences that are capable of recombining at high frequency. Their potential to disrupt genome stability highlights the need to understand how homologous recombination processes are coordinated. The *S. cerevisiae* Rad1-Rad10 endonuclease performs an essential role in recombination between repeated sequences by processing 3' single-stranded intermediates formed during single-strand annealing and gene conversion events. Several recent studies have focused on factors involved in Rad1-Rad10-dependent removal of 3' nonhomologous tails during homologous recombination, including Msh2-Msh3, Slx4, and the newly identified Saw1 protein (1–4). In addition, these studies suggest mechanisms for how DNA repair is coordinated by the DNA damage checkpoint machinery (1). This review aims to integrate these new findings with previous work to create a comprehensive model for how DNA repair and checkpoint factors act in concert to process 3' nonhomologous tail intermediates that arise during homologous recombination.

Keywords

Rad1-Rad10; Saw1; Slx4; Msh2-Msh3; nonhomologous tail

Homologous stretches of DNA sequences scattered throughout the genome are a threat to genome stability because of their potential to recombine. It is estimated that nearly half of the human genome consists of repetitive DNA (5,6), and genome rearrangements caused by recombination between repeats are known to contribute to a variety of human diseases, including many cancers (7–9). Repetitive sequences such as *Alu* elements are particularly susceptible to non-conservative homologous recombination via single-strand annealing (SSA), which results in the deletion of sequences located between the repeats (10–12). The abundance of such sequences in the human genome underscores the need for a comprehensive understanding of the homologous recombination processes that act on them.

SSA is a major recombination pathway for repairing spontaneous and induced double-strand breaks (DSBs) that arise between repeated sequences (10,13,14). During SSA in *S. cerevisiae*, DSBs are resected 5' to 3' to reveal single-stranded homologous sequences (Figure 1). Following Rad52- and Rad59-dependent annealing of the homologous sequences, the 3' single-stranded DNA ends are nonhomologous to the new flanking regions, and must be cleaved in order to complete repair of the broken strands. The Rad1-Rad10 endonuclease and Msh2-Msh3 mismatch recognition complex are required for cleaving 3' single-stranded nonhomologous tails on either side of the annealed region (15–19). Once both 3' tails have

been removed, SSA is completed by DNA synthesis initiated off of the newly cleaved 3' ends followed by ligation (reviewed in 10).

Rad1-Rad10 is a structure-specific endonuclease that functions in both nucleotide excision repair (NER) and homologous recombination (10,11,19,20). The significance of the role of Rad1-Rad10 in recombination is demonstrated by the severe developmental abnormalities in mice lacking the mammalian *RAD10* homolog *ERCC1* which, unlike NER mutant phenotypes, include severe runting, reduced liver function, and death before weaning (21,22). A recently described human patient with *ERCC1* deficiency also exhibited severe fetal development defects that are clearly distinct from NER-related phenotypes (23). In yeast, the absence of Rad1-Rad10 leads to cell death or plasmid loss (depending on the assay) during recombination by SSA due to lack of repair, since 3' nonhomologous tail removal is an essential step in SSA (15,17). Several recent papers have highlighted factors involved in Rad1-Rad10-dependent 3' nonhomologous tail removal during homologous recombination in *S. cerevisiae* (1–4), and it is these non-NER functions of Rad1-Rad10 that are reviewed here. The role of Rad1-Rad10 in nucleotide excision repair has been reviewed elsewhere (19,20).

Homologous recombination by gene conversion also involves the removal of 3' nonhomologous tails. Most mitotic gene conversion events are thought to occur by a synthesis-dependent strand annealing mechanism (10,11,24). During such gene conversion events, the DSB is resected 5' to 3', and one of the 3' ends undergoes Rad51-mediated strand invasion into a duplex region of DNA containing a homologous sequence (Figure 2A). DNA synthesis initiating from the 3' invading strand allows for copying of DNA sequence from the donor template, and unwinding of the invading strand from the donor template allows it to anneal back to its native locus. The non-invading strand is then able to be repaired using the invading strand as a template (reviewed in 10,11).

Rad1-Rad10-dependent nonhomologous tail removal during gene conversion can occur during the strand invasion step as well as after annealing, depending on whether one or both 3' ends contain nonhomology with respect to the donor locus. If both sides of a DSB are nonhomologous to the donor (Figure 2B), the invading strand contains a 3' nonhomologous tail that must be removed in order to prime repair synthesis off of the donor. When nonhomologous sequence resides on only one side of a DSB (Figure 2A), the 3' end of the break that shares homology with the donor sequence performs the strand invasion step, and there is thus no barrier to initiate new DNA synthesis on the invading strand.

Differential requirements for Rad1-Rad10-dependent 3' nonhomologous tail removal during gene conversion

The requirement for Rad1-Rad10 during gene conversion depends on both the number and length of nonhomologous tails. Rad1-Rad10 is critical for gene conversion when both ends of a DSB contain 30 or more nucleotides of nonhomologous sequence (3,17,25,26), but DSB repair is more subtly reduced in *rad1Δ* mutants when only one nonhomologous tail is present (3,25,27). The differential requirement for Rad1-Rad10 during gene conversion when one or two ends of a DSB contain nonhomology has been ascribed to the structural nature of the DNA junctions. The initial invasion of 3' single-stranded DNA into a homologous duplex is proposed to create an unstable paranemic joint, which might be a better substrate for Rad1-Rad10 (17). In contrast, when nonhomologous sequence is only on one side of a DSB, the homologous 3' end can perform strand invasion, leaving the nonhomologous tail on the second, non-invading end (Figure 2A). The sequence adjacent to this nonhomologous 3' end would likely form a stable plectonemic joint, since the rest of the strand can fully base pair. It is possible that Rad1-Rad10 is only minimally required when only one nonhomologous tail is present because

plectonemic joints are not ideal substrates for Rad1-Rad10, and/or Rad1-Rad10 is only one of a host of other factors that process these types of structures.

When 3' nonhomologous tails are only 10 nucleotides in length, gene conversion remains efficient in the absence of *RAD1*, *MSH2*, or *MSH3*, and the short 3' tails are removed by the proofreading 3' to 5' exonuclease activity of Polymerase δ (26). A second Rad1-Rad10- and Msh2-Msh3-independent pathway of 3' nonhomologous tail removal is proposed to exist (3, 16,26,27), though the inefficiency of this proposed pathway suggests that Rad1-Rad10-dependent end processing is preferred. There is no evidence that known factors play a role in this backup pathway of nonhomologous tail removal, as neither Mus81-Mms4 nor the proofreading activities of Pol δ and Pol ϵ appear to contribute (3,26). Replication of partially repaired recombination intermediates might also bypass the requirement for 3' nonhomologous tail removal (3,28).

Msh2-Msh3 facilitates Rad1-Rad10-dependent 3' nonhomologous tail removal

The Msh2-Msh3 DNA mismatch recognition complex functions in Rad1-Rad10-dependent 3' end processing during homologous recombination (Figures 1, 2B; 17, 25, 26, 29, 30). Msh2-Msh3 specifically recognizes insertion/deletion loops of up to 17 base pairs in DNA mismatch repair (31–33). During homologous recombination, Msh2-Msh3 is proposed to act in the recognition and stabilization of 3' tails at the junction of double-stranded and single-stranded DNA to aid in either the recruitment or cleavage activity of Rad1-Rad10 (17, 34–36). A similar mechanism involving Msh2-Msh3 and Rad1-Rad10 also functions to remove large loops during meiosis (32, 37, 38). Consistent with its role in 3' tail removal, an *in vitro* DNA binding study showed that purified Msh2-Msh3 binds specifically to branched DNA substrates containing 3' single-stranded ends, with an affinity comparable to that of its binding to +8 mismatch loops (4). Msh2-Msh3 appears to bind asymmetrically around double-strand/single-strand junctions, and binding opens the conformation of the junction slightly, potentially to facilitate Rad1-Rad10-dependent cleavage of 3' tails (4).

Msh2 physically interacts with both Rad1 and Rad10 independently of other mismatch repair factors (35), and no other mismatch repair factors are required for Rad1-Rad10-dependent 3' end processing besides Msh3 (17,29,36). The role of Msh2-Msh3 in nonhomologous tail removal during recombination can be distinguished from its role in DNA mismatch repair, since mutations have been isolated in *MSH2* that disrupt mismatch repair but are functional for recombination (34). Msh2 localizes rapidly to DSBs flanked by nonhomologous sequence on chromosomal and plasmid substrates (3,30,39), and Msh2 and Msh3 have been reported to physically interact with subunits of the single-strand binding protein RPA (40–42). Together these data support a very early role for the Msh2-Msh3 complex in 3' nonhomologous tail removal that might aid in 3' tail recognition.

Rad1-Rad10-dependent 3' end processing does not always require Msh2-Msh3. During SSA, the requirement for Msh2-Msh3 depends upon the length of the annealed region. Strains lacking *MSH2* or *MSH3* are defective in SSA when the annealed region is only 205 bp, but show only a small reduction in repair relative to wild-type when the annealed region is more than 1 kb (17). Decreased dependence on Msh2-Msh3 is also observed with larger loop sizes during Rad1-Rad10-dependent meiotic loop repair (38). Despite a predicted role and clear localization of Msh2 to DSBs, Msh2-Msh3 is dispensable for gene conversion during mating type switching, where Rad1-Rad10 plays a significant role (3). Only when nonhomologous sequence is inserted on the invading strand does the role of Msh2-Msh3 in Rad1-Rad10-dependent 3' nonhomologous tail removal become apparent (3). Altogether, these results

support the idea that Msh2-Msh3 plays a role in stabilizing 3' tail intermediates in preparation for Rad1-Rad10-dependent cleavage.

Slx4 is a critical component of the Rad1-Rad10 3' tail removal pathway

Recent work by Flott *et al.* (1) identified Slx4 as a essential component of the Rad1-Rad10 3' nonhomologous tail removal pathway. Slx4 was initially characterized as a subunit of the Slx1-Slx4 endonuclease, deletion of which is synthetically lethal with *sgs1Δ* (43). Slx1-Slx4 is a 5' flap endonuclease, of which Slx1 is thought to be the catalytic subunit (44). In addition to its function as a heterodimer with Slx1, Slx4 appears to have at least two other separate functions, one involving Rad1-Rad10, and another that is independent of both Slx1 and Rad1-Rad10 and promotes cellular resistance to MMS (1,2,45,46). A screen for mutants defective in SSA recently found that *slx4Δ* mutants are blocked at the 3' tail removal step of SSA (2), and Slx4 was found to play an important role during mating type switching in the same pathway as the Rad1-Rad10 complex (3). Additionally, the Flott *et al.* study found that at least three residues on Slx4 are directly phosphorylated by the Mec1 and Tel1 checkpoint kinases, and that this phosphorylation is essential for the SSA functions of Slx4 but not for resistance to MMS or viability in *sgs1Δ* mutants (1,47).

These new findings support a model in which Slx4 is acted upon directly by the checkpoint machinery in response to DSBs to recruit or activate Rad1-Rad10 and promote 3' nonhomologous tail removal. The DNA damage response is thought to be activated during SSA because of the relatively slow kinetics of repair and the extensive resection required. Since phosphorylation of Slx4 is absolutely essential for 3' nonhomologous tail removal during SSA, but is dispensable for its other functions (1), this phosphorylation is likely to provide the specificity to channel Slx4 to its recombination function.

While gene conversion involving a single 3' nonhomologous tail does not require checkpoint activation, recent work has shown that *rad1Δ* and *slx4Δ* mutants exhibit *RAD9*- and *MAD2*-dependent checkpoint activation during mating type switching, a single-nonhomology gene conversion event (3). It is possible that the recruitment or activity of Rad1-Rad10-Slx4 is required to turn off the DNA damage checkpoint by signaling that repair is proceeding normally.

Slx4 is not required for checkpoint activation, but provides a crucial link between DNA damage sensing and activation of DNA repair. Slx4, but not its endonuclease partner Slx1, is required for the repair of alkylation damage (47). In addition to SSA, Slx4 is phosphorylated in a Mec1/Tel1-dependent manner in response to a variety of DNA-damaging agents, including MMS, camptothecin, hydroxyurea, ionizing radiation, and the UV mimetic 4-NQO, and is required for efficient DNA repair throughout the cell cycle (47). Thus it appears that Slx4 plays a critical role in the response to many types of DNA damage, and the strict requirement for its checkpoint-dependent phosphorylation in 3' nonhomologous tail removal provides a beautiful example of how DNA damage sensed by the checkpoint machinery directly promotes DNA repair.

Saw1 is a component of the Rad1-Rad10 end-processing machinery

The *SAW1* (*YAL027W*) gene was recently identified in a microarray-based screen for mutants defective in SSA (2). Saw1, for single-strand annealing weakened 1, physically interacts with Rad1-Rad10, Msh2-Msh3, and Rad52 (2,40,42), all of which function in SSA, and like *slx4Δ* mutants, *saw1Δ* mutants are defective specifically in 3' nonhomologous tail removal (2). Li *et al.* (2) found that the Rad1 protein fails to localize to SSA intermediates in *saw1Δ* mutants, and *saw1Δ* 18–24 mutants, whose mutant Saw1 protein fails interact with Rad1 but still interacts with Msh2 and Rad52, are completely defective in SSA. These new findings

provide strong evidence that Saw1 recruits Rad1-Rad10 to recombination intermediates containing Rad52.

Rad14 is thought to target Rad1-Rad10 to its substrates during NER (20,48,49), but it has been unclear how Rad1-Rad10 is targeted to 3' tails during recombination, especially since Msh2-Msh3 is dispensable for some gene conversion and SSA events. In human cell-free extracts, Rad52 and the Rad1-Rad10 homolog XPF-ERCC1 stably associate through interaction of XPF with the N-terminus of Rad52 (50). This physical interaction stimulates the endonuclease activity of XPF-ERCC1 and attenuates the strand annealing activity of Rad52, both of which promote the removal of 3' tails from recombination intermediates (50). Though an equivalent interaction between Rad1-Rad10 and Rad52 has not been observed, it appears that Saw1 provides this physical interaction in *S. cerevisiae* (2). These new findings reveal how Saw1, and potentially Slx4, may recruit and regulate Rad1-Rad10 and 3' nonhomologous tail removal during homologous recombination.

A comprehensive model for Rad1-Rad10-dependent 3' nonhomologous tail removal during SSA

The new findings reviewed here provide a more comprehensive understanding of how Rad1-Rad10-dependent 3' end processing is coordinated during homologous recombination. As shown in Figure 3, resection of DSBs during SSA creates 3' single-stranded tails that are coated by the RPA single-stranded DNA binding protein. The extensive single-stranded DNA activates the DNA damage response, which promotes phosphorylation of Slx4 by the Mec1 and Tel1 checkpoint kinases. Once resection has revealed regions of homology, the Rad52 strand annealing protein anneals the homologous sequences, creating 3' nonhomologous tails on either side of the intermediate. When the length of homology is limited and creates an unstable paranemic intermediate, Msh2-Msh3 stabilizes the junction and slightly opens it to create a more suitable substrate for Rad1-Rad10 cleavage. Saw1 recruits Rad1-Rad10 to Rad52-containing annealed sequences at double-strand/single-strand junctions bound by Msh2-Msh3. Phosphorylated Slx4 binds to Rad1-Rad10, but does not appear to interact with Rad52, Saw1, or Msh2-Msh3. The result of these physical interactions is the positioning of Rad1-Rad10 at the double-strand/single-strand junction between Saw1 and Slx4. Rad1-Rad10 cleaves the strand near the junction, removing the 3' nonhomologous tail and providing a free 3' OH for extension by DNA polymerases.

It is not clear whether these physically interacting factors arrive at recombination intermediates in a sequential fashion, or whether a stable complex of Saw1-Msh2-Msh3-Rad1-Rad10-Slx4 exists *in vivo* and is simply recruited to Rad52-containing DNA, perhaps in response to Slx4 phosphorylation by Mec1/Tel1. It is striking that *saw1Δ* and *slx4Δ* mutants exhibit opposing phenotypes with regard to rDNA stability (2), so additional work is needed to understand the distinct functions of Slx4 and Saw1 in recombination and how they relate to coordination of Rad1-Rad10 3' tail cleavage. It will be interesting to identify the critical function of Slx4 in this process, whether it is primarily to transmit a repair signal from the DNA damage checkpoint or whether there are additional roles in recruiting, positioning, or activating the endonuclease activity of Rad1-Rad10. While the findings reviewed here provide a clearer picture of how Rad1-Rad10 identifies and cleaves its substrates during recombination, many questions still remain. What is the physical activity of Saw1? How does Slx4 arrive at junctions containing 3' tails? What is the function of phosphorylated Slx4? Why is Msh2-Msh3 essential in some cases and dispensable for others? Addressing these questions will edge us closer to understanding the link between DNA damage recognition, checkpoint signaling, and efficient DNA repair.

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Abbreviations

DSB	double-strand break
NER	nucleotide excision repair
SSA	single-strand annealing

References

1. Flott S, Alabert C, Toh GW, Toth R, Sugawara N, et al. Phosphorylation of Slx4 by Mec1 and Tel1 Regulates the Single-Strand Annealing Mode of DNA Repair in Budding Yeast. *Mol Cell Biol* 2007;27:6433–6445. [PubMed: 17636031]
2. Li F, Dong J, Pan X, Oum J-H, Boeke JD, et al. Microarray-Based Genetic Screen Defines *Saw1*, a Gene Required for Rad1/Rad10-Dependent Processing of Recombination Intermediates. *Mol Cell Biol* 2008;30:325–335. [PubMed: 18471978]
3. Lyndaker AM, Goldfarb T, Alani E. Mutants Defective in Rad1-Rad10-Slx4 Exhibit a Unique Pattern of Viability during Mating-Type Switching in *Saccharomyces cerevisiae*. *Genetics* 2008;179:1807–1821. [PubMed: 18579504]
4. Surtees JA, Alani E. Mismatch Repair Factor Msh2-Msh3 Binds and Alters the Conformation of Branched DNA Structures Predicted to Form During Genetic Recombination. *J Mol Biol* 2006;360:523–526. [PubMed: 16781730]
5. Li W-H, Gu Z, Wang H, Nekrutenko A. Evolutionary Analyses of the Human Genome. *Nature* 2001;409:847–849. [PubMed: 11237007]
6. Lander E, Linton L, Birren B, Nusbaum C, Zody M, et al. Initial Sequencing and Analysis of the Human Genome. *Nature* 2001;409:860–921. [PubMed: 11237011]
7. Stenger JE, Lobachev KS, Gordenin D, Darden TA, Jurka J, et al. Biased Distribution of Inverted and Direct Alus in the Human Genome: Implications for Insertion, Exclusion, and Genome Stability. *Genome Res* 2001;11:12–27. [PubMed: 11156612]
8. Kolomietz E, Meyn SM, Pandita A, Squire JA. The Role of *Alu* Repeat Clusters as Mediators of Recurrent Chromosomal Aberrations in Tumors. *Genes, Chrom Canc* 2002;35:97–112.
9. Deininger PL, Batzer MA. *Alu* Repeats and Human Disease. *Mol Gen Metab* 1999;67:183–193.
10. Pâques F, Haber JE. Multiple Pathways of Recombination Induced by Double-Strand Breaks in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 1999;63:349–404. [PubMed: 10357855]
11. Symington LS. Role of *RAD52* Epistasis Group Genes of Homologous Recombination and Double-Strand Break Repair. *Microbiol Mol Biol Rev* 2002;66:630–670. [PubMed: 12456786]
12. Hedges DJ, Deininger PL. Inviting Instability: Transposable Elements, Double-Strand Breaks, and the Maintenance of Genome Integrity. *Mut Res* 2007;616:46–59. [PubMed: 17157332]
13. Liang F, Han M, Romanienko PJ, Jasin M. Homology-Directed Repair Is a Major Double-Strand Break Repair Pathway in Mammalian Cells. *Proc Natl Acad Sci USA* 1998;95:5172–5177. [PubMed: 9560248]
14. Elliott B, Richardson C, Jasin M. Chromosomal Translocation Mechanisms at Intronic *Alu* Elements in Mammalian Cells. *Mol Cell* 2005;17:885–894. [PubMed: 15780943]
15. Fishman-Lobell J, Haber J. Removal of Nonhomologous DNA Ends in Double-Strand Break Recombination: The Role of the Yeast Ultraviolet Repair Gene *RAD1*. *Science* 1992;258:480–484. [PubMed: 1411547]
16. Ivanov E, Haber J. *RAD1* and *RAD10* but Not Other Excision Repair Genes, Are Required for Double-Strand Break-Induced Recombination in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1995;15:2245–2251. [PubMed: 7891718]

17. Sugawara N, Pâques F, Colaiacovo M, Haber JE. Role of *Saccharomyces cerevisiae* Msh2 and Msh3 Repair Proteins in Double-Strand Break-Induced Recombination. *Proc Natl Acad Sci USA* 1997;94:9214–9219. [PubMed: 9256462]
18. Bardwell A, Bardwell L, Tomkinson A, Friedberg E. Specific Cleavage of Model Recombination and Repair Intermediates by the Yeast Rad1-Rad10 DNA Endonuclease. *Science* 1994;265:2082–2085. [PubMed: 8091230]
19. Ciccio A, McDonald N, West SC. Structural and Functional Relationships of the Xpf/Mus81 Family of Proteins. *Annu Rev Biochem* 2008;77:259–287. [PubMed: 18518821]
20. Prakash S, Prakash L. Nucleotide Excision Repair in Yeast. *Mut Res* 2000;451:13–24. [PubMed: 10915862]
21. McWhir J, Selfridge J, Harrison DJ, Squires S, Melton DW. Mice with DNA Repair Gene (*ERCC-1*) Deficiency Have Elevated Levels of p53, Liver Nuclear Abnormalities and Die before Weaning. *Nat Genet* 1993;5:217–224. [PubMed: 8275084]
22. Weeda G, Donker I, de Wit J, Morreau H, Janssens R, et al. Disruption of Mouse *ERCC1* Results in a Novel Repair Syndrome with Growth Failure, Nuclear Abnormalities and Senescence. *Curr Biol* 1997;7:427–439. [PubMed: 9197240]
23. Jaspers NGJ, Raams A, Silengo MC, Wijgers N, Niedernhofer LJ, et al. First Reported Patient with Human *Ercc1* Deficiency Has Cerebro-Oculo-Facio-Skeletal Syndrome with a Mild Defect in Nucleotide Excision Repair and Severe Developmental Failure. *Am J Hum Gen* 2007;80:457–466.
24. Ira G, Satory D, Haber JE. Conservative Inheritance of Newly Synthesized DNA in Double-Strand Break-Induced Gene Conversion. *Mol Cell Biol* 2006;26:9424–9429. [PubMed: 17030630]
25. Colaiacovo MP, Pâques F, Haber JE. Removal of One Nonhomologous DNA End During Gene Conversion by a *RAD1*- and *MSH2*-Independent Pathway. *Genetics* 1999;151:1409–1423. [PubMed: 10101166]
26. Pâques F, Haber JE. Two Pathways for Removal of Nonhomologous DNA Ends During Double-Strand Break Repair in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1997;17:6765–6771. [PubMed: 9343441]
27. Holmes AM, Haber JE. Double-Strand Break Repair in Yeast Requires Both Leading and Lagging Strand DNA Polymerases. *Cell* 1999;96:415–424. [PubMed: 10025407]
28. Kang LE, Symington LS. Aberrant Double-Strand Break Repair in *rad51* Mutants of *Saccharomyces cerevisiae*. *Mol Cell Biol* 2000;20:9162–9172. [PubMed: 11094068]
29. Saparbaev M, Prakash L, Prakash S. Requirement of Mismatch Repair Genes *MSH2* and *MSH3* in the *Rad1-Rad10* Pathway of Mitotic Recombination in *Saccharomyces Cerevisiae*. *Genetics* 1996;142:727–736. [PubMed: 8849883]
30. Evans E, Sugawara N, Haber JE, Alani E. The *Saccharomyces cerevisiae* Msh2 Mismatch Repair Protein Localizes to Recombination Intermediates *in Vivo*. *Mol Cell* 2000;5:189–199. [PubMed: 10678181]
31. Habraken Y, Sung P, Prakash L, Prakash S. Binding of Insertion/Deletion DNA Mismatches by the Heterodimer of Yeast Mismatch Repair Proteins Msh2 and Msh3. *Curr Biol* 1996;6:1185–1187. [PubMed: 8805366]
32. Jensen LE, Jauert PA, Kirkpatrick DT. The Large Loop Repair and Mismatch Repair Pathways of *Saccharomyces cerevisiae* Act on Distinct Substrates During Meiosis. *Genetics* 2005;170:1033–1043. [PubMed: 15879514]
33. Sia EA, Kokoska RJ, Dominska M, Greenwell P, Petes TD. Microsatellite Instability in Yeast: Dependence on Repeat Unit Size and DNA Mismatch Repair Genes. *Mol Cell Biol* 1997;17:2851–2858. [PubMed: 9111357]
34. Studamire B, Price G, Sugawara N, Haber JE, Alani E. Separation-of-Function Mutations in *Saccharomyces cerevisiae* *MSH2* That Confer Mismatch Repair Defects but Do Not Affect Nonhomologous-Tail Removal during Recombination. *Mol Cell Biol* 1999;19:7558–7567. [PubMed: 10523644]
35. Bertrand P, Tishkoff DX, Filosi N, Dasgupta R, Kolodner RD. Physical Interaction between Components of DNA Mismatch Repair and Nucleotide Excision Repair. *Proc Natl Acad Sci USA* 1998;95:14278–14283. [PubMed: 9826691]

36. Langston LD, Symington LS. Opposing Roles for DNA Structure-Specific Proteins Rad1, Msh2, Msh3, Sgs1 in Yeast Gene Targeting. *EMBO J* 2005;24:2214–2223. [PubMed: 15920474]
37. Kirkpatrick DT, Petes TD. Repair of DNA Loops Involves DNA-Mismatch and Nucleotide-Excision Repair Proteins. *Nature* 1997;387:929–931. [PubMed: 9202128]
38. Kearney HM, Kirkpatrick DT, Gerton JL, Petes TD. Meiotic Recombination Involving Heterozygous Large Insertions in *Saccharomyces cerevisiae*: Formation and Repair of Large, Unpaired DNA Loops. *Genetics* 2001;158:1457–1476. [PubMed: 11514439]
39. Goldfarb, T.; Alani, E. Chromatin Immunoprecipitation to Investigate Protein-DNA Interactions during Genetic Recombination. In: Waldman, AS., editor. *Genetic Recombination: Reviews and Protocols*. Humana Press Inc; Totowa, NJ: 2004. p. 223-237.
40. Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, et al. Global Landscape of Protein Complexes in the Yeast *Saccharomyces cerevisiae*. *Nature* 2006;440:637–643. [PubMed: 16554755]
41. Gavin A-C, Aloy P, Grandi P, Krause R, Boesche M, et al. Proteome Survey Reveals Modularity of the Yeast Cell Machinery. *Nature* 2006;440:631–636. [PubMed: 16429126]
42. Gavin A-C, Bosche M, Krause R, Grandi P, Marzioch M, et al. Functional Organization of the Yeast Proteome by Systematic Analysis of Protein Complexes. *Nature* 2002;415:141–147. [PubMed: 11805826]
43. Mullen JR, Kaliraman V, Ibrahim SS, Brill SJ. Requirement for Three Novel Protein Complexes in the Absence of the Sgs1 DNA Helicase in *Saccharomyces cerevisiae*. *Genetics* 2001;157:103–118. [PubMed: 11139495]
44. Fricke WM, Brill SJ. Slx1-Slx4 Is a Second Structure-Specific Endonuclease Functionally Redundant with Sgs1-Top3. *Genes Dev* 2003;17:1768–1778. [PubMed: 12832395]
45. Roberts TM, Kobor MS, Bastin-Shanower SA, Li M, Horte SA, et al. Slx4 Regulates DNA Damage Checkpoint-Dependent Phosphorylation of the Brct Domain Protein Rtt107/Esc4. *Mol Biol Cell* 2006;17:539–548. [PubMed: 16267268]
46. Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, et al. A Comprehensive Two-Hybrid Analysis to Explore the Yeast Protein Interactome. *Proc Natl Acad Sci USA* 2001;98:4569–4574. [PubMed: 11283351]
47. Flott S, Rouse J. Slx4 Becomes Phosphorylated after DNA Damage in a Mec1/Tel1-Dependent Manner and Is Required for Repair of DNA Alkylation Damage. *Biochem J* 2005;391:325–333. [PubMed: 15975089]
48. Guzder SN, Sommers CH, Prakash L, Prakash S. Complex Formation with Damage Recognition Protein Rad14 Is Essential for *Saccharomyces cerevisiae* Rad1-Rad10 Nuclease to Perform Its Function in Nucleotide Excision Repair in vivo. *Mol Cell Biol* 2006;26:1135–1141. [PubMed: 16428464]
49. Guzder SN, Sung P, Prakash L, Prakash S. Nucleotide Excision Repair in Yeast Is Mediated by Sequential Assembly of Repair Factors and Not by a Pre-Assembled Repairosome. *J Biol Chem* 1996;271:8903–8910. [PubMed: 8621533]
50. Motycka TA, Bessho T, Post SM, Sung P, Tomkinson AE. Physical and Functional Interaction between the Xpf/Ercc1 Endonuclease and hRad52. *J Biol Chem* 2004;279:13634–13639. [PubMed: 14734547]

Figure 1. Single-strand annealing mechanism of double-strand break (DSB) repair between repeated sequences

DSBs that arise between repeated sequences are resected by 5' to 3' exonuclease activity, and the single-stranded DNA is annealed at regions of homology. Pairing of these sequences reveals 3' nonhomologous tails on either side of the intermediate that are cleaved off in a Rad1-Rad10- and Msh2-Msh3-dependent manner. Removal of the 3' nonhomologous ends allows initiation of repair synthesis to produce final recombinant products.

Figure 2A

Figure 2B

Figure 2. Synthesis-dependent strand annealing mechanism of gene conversion involving the removal of either one (A) or two (B) 3' nonhomologous tails

A. After DSB formation and 5' to 3' resection, one 3' end invades a donor locus containing a homologous sequence. DNA synthesis is primed from this invading 3' end and copies the donor sequence, and unwinding of this strand allows it to reanneal to its native locus. When the newly repaired strand differs in sequence from the original sequence, a 3' nonhomologous tail remains at the non-invading strand. Removal of this 3' tail involves Rad1-Rad10 and Slx4, but not Msh2-Msh3. 3' nonhomologous tail removal allows for completion of repair by gene conversion. B. If nonhomologous sequence flanks both sides of a DSB, the 3' invading strand must also be processed in order to have productive strand invasion. 3' nonhomologous tail

removal on the invading strand requires both the Rad1-Rad10 and Msh2-Msh3 complexes. After 3' tail removal, gene conversion proceeds via synthesis-dependent strand annealing as described in A.

Figure 3. Model for coordination 3' nonhomologous tail removal factors during single-strand annealing

Following DSB formation, 5' to 3' resection creates single-stranded DNA that is coated by RPA. This process also signals the Mec1 and Tel1 checkpoint kinases that, once activated, phosphorylate Slx4. The Rad52 strand annealing protein facilitates pairing of the homologous sequences, displacing RPA from the annealed regions. The Msh2-Msh3 complex recognizes the junction of double-stranded and 3' single-stranded DNA, potentially interacting with RPA, and opens the DNA junction slightly to create a better substrate for Rad1-Rad10 cleavage. The Saw1 protein recruits Rad1-Rad10 endonuclease to the junction of Rad52- and Msh2-Msh3-containing DNA, and association of Rad1-Rad10 with phosphorylated Slx4 allows it to cleave the DNA near the double-strand/single-strand junction, freeing the 3' nonhomologous tail.