Mechanisms and Regulation of Mitotic Recombination in *Saccharomyces cerevisiae*

Lorraine S. Symington,* Rodney Rothstein,† and Michael Lisby‡

*Department of Microbiology and Immunology, and †Department of Genetics and Development, Columbia University Medical Center, New York, New York 10032, and ‡Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark

ABSTRACT Homology-dependent exchange of genetic information between DNA molecules has a profound impact on the maintenance of genome integrity by facilitating error-free DNA repair, replication, and chromosome segregation during cell division as well as programmed cell developmental events. This chapter will focus on homologous mitotic recombination in budding yeast *Saccharomyces cerevisiae*. However, there is an important link between mitotic and meiotic recombination (covered in the forthcoming chapter by Hunter *et al.* 2015) and many of the functions are evolutionarily conserved. Here we will discuss several models that have been proposed to explain the mechanism of mitotic recombination, the genes and proteins involved in various pathways, the genetic and physical assays used to discover and study these genes, and the roles of many of these proteins inside the cell.

TABLE OF CONTENTS

Abstract 795

I. Introduction 796

II. Mechanisms of Recombination 798

A. Models for DSB-initiated homologous recombination 798

DSB repair and synthesis-dependent strand annealing models 798

Break-induced replication 798

Single-strand annealing and microhomology-mediated end joining 799

B. Proteins involved in homologous recombination 800

DNA end resection 800

Homologous pairing and strand invasion 802

Rad51 mediators 803

Single-strand annealing 803

DNA translocases 804

DNA synthesis during HR 805

Resolution of recombination intermediates 805

III. Genetic Assays of Mitotic Recombination 806

A. Allelic recombination in diploids 806

Continued

Copyright © 2014 by the Genetics Society of America

doi: 10.1534/genetics.114.166140

Manuscript received May 12, 2014; accepted for publication August 15, 2014

Available freely online through the author-supported open access option.

[1]Corresponding authors: Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY 10032. E-mail: lss5@columbia.edu; Department of Genetics and Development, Columbia University Medical Center, New York, NY 10032. E-mail: rothstein@columbia.edu; and Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark. E-mail: mlisby@bio.ku.dk

CONTENTS, *continued*

B. Recombination between dispersed repeats 808
Direct-repeat recombination 809
Inverted-repeat recombination 809
C. Plasmid gap repair 811
D. Assays for BIR 811
IV. The Nature of the Recombinogenic DNA Lesion 812
A. Induction of recombination by DSBs and nicks 812
B. Replication-coupled recombination 813
C. Fragile sites and noncanonical structures 813
D. Transcription-stimulated recombination 814
V. Cell Biology of Recombination 814
A. Recombination foci 814
B. Choreography of focus formation 814
C. DSB dynamics and recombination centers 816
D. Nuclear compartments 817
VI. Regulation of Homologous Recombination 817
A. Transcriptional regulation of homologous recombination 817
B. Regulation of homologous recombination by post-translational modifications 817
Regulation of DSB repair by PTMs 818
Regulation of recombinational restart of replication by PTMs 819
C. Role of chromatin in controlling mitotic recombination 820
D. Role of cohesin in regulating mitotic recombination 821
E. Ploidy/aneuploidy 821
VII. Postscript 821

IN the course of this review, we will touch on many of the genes and processes conserved between mitosis and meiosis. Indeed, early studies in yeast and other fungi showed that mitotic recombination exhibited many of the same properties of meiotic recombination. For example, gene conversion, the nonreciprocal transfer of genetic information (see below), is sometimes associated with exchange (*i.e.*, crossover). Heteroduplex DNA, which is detected by the failure to repair mismatches between genetically distinct DNA molecules, is indicative of strand exchange and is often found at or near sites of crossovers. Importantly, the unrepaired mismatched sequences segregate after the next round of DNA replication and can be seen as sectored colonies, similar to postmeiotic segregation observed by tetrad analysis.

For simplicity, homologous recombination (HR) is minimally defined as the repair of DNA lesions using homologous sequences. During S phase and G2, in both haploid and diploid cells, repair of the damage uses the unbroken sister chromosome as the homologous sequence (Figure 1A). Such repair is the main role of mitotic recombination and it can lead to genetic consequences. When sister chromatid repair is accompanied by a crossover, it results in sister-chromatid exchange (SCE). If the repair event occurs between misaligned repetitive sequences in a tandem array, it results in an unequal SCE (USCE) (Figure 1B). In diploids, repair can also be templated from the unbroken homologous chromosome and if associated with a crossover, the exchange can lead to loss of heterozygosity (LOH) (Figure 1C). Mitotic gene conversion results when there is a nonreciprocal transfer of genetic information from one chromosome to the other during the repair event (Figure 1D). DNA repair from homologous sequences at nonallelic positions, called ectopic recombination, can lead to deletions, inversions, translocations, and acentric or dicentric chromosomes if repair is associated with a crossover (Figure 1, E and F).

Most of our attention focuses on the repair of double-strand breaks (DSBs); however, the exact nature of the initiating spontaneous lesion is unknown. Indeed nicks can be processed into single-stranded DNA (ssDNA) gaps or DSBs as the result of ligation failure from the previous round of DNA replication or during the repair of damaged or misincorporated nucleotides via processes such as nucleotide excision repair (NER), mismatch repair (MMR), base excision repair (BER), or transcription-coupled repair (TCR). Nicks can also be formed after the failed catalysis of Top1 covalently attached to DNA. Upon subsequent replication, these protein-bound nicks can also become DSBs. Reactive oxygen

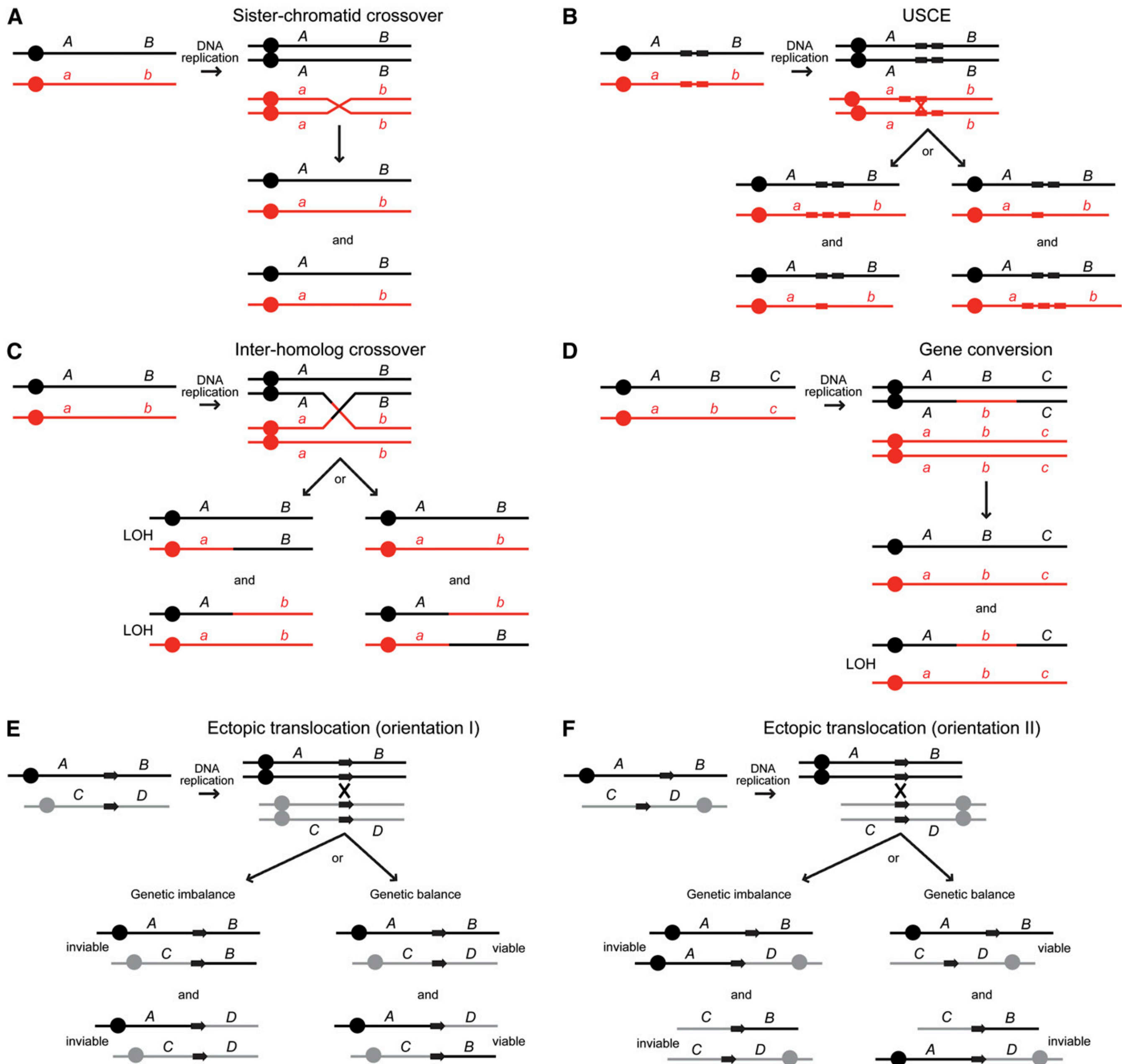

Figure 1 Genetic outcomes of homologous recombination. The letters A/a and B/b indicate heteroalleles. Circles indicate centromeres. Colors red and black indicate homologous chromosomes in diploid cells. (A) Sister-chromatid crossover. A crossover between sister chromatids results in two genetically identical cells. (B) Unequal sister-chromatid exchange (USCE). Within repetitive sequence elements (boxes), a crossover between misaligned repeats results in repeat copy number expansion and contraction. (C) Interhomolog crossover. A crossover between homologs leads to loss of heterozygosity (LOH), if the recombinant molecules segregate to different cells in the ensuing cell division. (D) Gene conversion. A nonreciprocal genetic exchange between homologs leads to LOH in one of the resulting cells. (E) Productive ectopic translocation. A crossover between homologous sequences (boxes with arrows) with the same orientation relative to the centromere (circles) on different chromosomes in gray and black results in a productive ectopic translocation. Cosegregation of the recombinant molecules results in genetically balanced cells, shown on the right. Segregation of the recombinant molecules to different cells, shown on the left, leads to lethality if the regions represented by B and D are essential. (F) Nonproductive ectopic translocation. If the recombining sequences have opposite orientation with respect to the centromere, the reciprocal translocation results in inviable dicentric and acentric chromosomes.

species (ROS), cellular metabolism, and exogenous damage from ultraviolet light or gamma-irradiation can also produce nicks. Similarly, collapsed and stalled replication forks can lead to structures that can be processed into DSBs. Finally, DNA ends are produced when gamma-irradiation breaks both strands or when telomeres are uncapped due to problems in assembling the shelterin complex. In some cases, simple ligation of the ends, so-called nonhomologous end joining (NHEJ), results in repair that may or may not be error-free. For example, in a G1 cell, NHEJ is likely the preferred repair choice. However, given the many different sources of DNA lesions, it is clear that one of the central questions in the

regulation of recombination is how the cell "determines" how to repair a DSB—NHEJ or HR. Among some of the issues the cell must confront are the necessity to interpret whether it is haploid or diploid (controlled by the *MAT* locus), where it is in the cell cycle (controlled by CDK), what kind of processing the DNA ends need [controlled in part by the Mre11–Rad50–Xrs2 (MRX) complex] and the chromatin environment of the DNA lesion. All of this information must be integrated for the appropriate repair decision to be made. Understanding this integration will clarify how all of the pathways that impact recombination are intertwined to lead to repair of a DNA lesion and a viable cell.

Many of the genes described in this chapter that affect genetic recombination were originally identified by their requirement to repair radiation-induced DNA damage. For example, most of the genes of the *RAD52* epistasis group are ionizing radiation (IR) sensitive, while *RAD1* and *RAD10* are ultraviolet light sensitive (Game and Cox 1971; Game and Mortimer 1974). In addition, genes have been identified that are sensitive to other types of DNA damaging agents, such as methyl methanesulfonate (MMS), camptothecin (CPT), 4-nitroquinoline 1-oxide (4-NQO), etc. Over the years, genes involved in many pathways have been shown to affect genetic recombination. Some of these genes were discovered in mutation analyses that looked for effects on recombination and repair assays, while others were identified when the yeast gene disruption library was systematically tested for gross chromosomal rearrangements (Huang and Koshland 2003; Smith *et al.* 2004), mitotic crossing over (Andersen *et al.* 2008), sensitivity of polyploidy (Storchova *et al.* 2006), Rad52 foci (Alvaro *et al.* 2007), doxorubicin sensitivity (Westmoreland *et al.* 2009), sensitivity to R-loops (Gomez-Gonzalez *et al.* 2011), fragility of triplex structure-forming GAA/TTC (Zhang *et al.* 2012), stability of quasipalindromes (Zhang *et al.* 2013), and many more. Bioinformatic analyses have also revealed many genes involved in genome stability (Putnam *et al.* 2012). Furthermore, screens for hyperrecombination (hyper-rec) uncovered genes involved in a multitude of pathways (Aguilera and Klein 1988; Keil and McWilliams 1993; Scholes *et al.* 2001).

Finally, it is worth mentioning that most assays that have been constructed to identify genes that affect recombination were applied without knowing the precise recombination pathway being assayed. By isolating mutations that affect that pathway, we can start to understand the mechanism of the assay. At the same time, the precise function of the gene is refined by understanding its role in the assay. This "yin–yang" situation makes the study of homologous recombination so challenging.

II. Mechanisms of Recombination

A. Models for DSB-initiated homologous recombination

DSB repair and synthesis-dependent strand annealing models: The DSB repair (DSBR) model was first proposed to explain the mechanism of plasmid gap repair and is currently the most accepted model to rationalize the association of crossing over with gene conversion during homologous recombination (Orr-Weaver *et al.* 1981; Szostak *et al.* 1983). In this model, the 5′ ends at the DSB are degraded to yield 3′ ssDNA tails, one of which invades a homologous double-stranded DNA (dsDNA) to form a displacement loop (D-loop) and is used to prime DNA synthesis, templated by the donor duplex (Figure 2). The 3′-terminated strand at the other side of the break anneals to the displaced strand from the donor duplex and primes a second round of leading strand synthesis. After ligation of the newly synthesized DNA to the resected 5′ strands, a double Holliday junction intermediate (dHJ) is generated. To segregate the recombinant duplexes, the HJs must be removed, which can occur by the activity of a helicase and topoisomerase to produce only noncrossover (NCO) products (dissolution), or by endonucleolytic cleavage (resolution). Cutting the inner strands of both HJs yields NCO products, whereas cleavage of the inner strands of one HJ and the outer strands of the other generates crossovers (COs). Heteroduplex DNA (hDNA), which is formed by pairing one strand from one duplex with a complementary strand from the other duplex, is a hallmark of homologous recombination and can be formed during the initial strand invasion and/or by second end capture. Repair of mismatches present in hDNA can give rise to gene conversion or restoration to the original sequence (Boiteux and Jinks-Robertson 2013).

While the DSBR model explains many properties of meiotic recombination, mitotic recombination generally shows a lower association of crossing over with gene conversion than observed during meiotic recombination. This observation led to two variations of the DSBR model, the synthesis-dependent strand annealing (SDSA) and migrating D-loop models, to explain the lower incidence of associated COs during mitotic DSB repair (Nassif *et al.* 1994; Ferguson and Holloman 1996; Paques *et al.* 1998). The SDSA model proposes that both 3′ ssDNA tails invade the homologous duplex(es) and after limited DNA synthesis are displaced by DNA helicases; the nascent complementary strands anneal and after fill-in synthesis and ligation generate exclusively NCO products (Nassif *et al.* 1994). The migrating D-loop model proposes that only one of the two 3′ ssDNA tails invades the homologous DNA duplex and after limited DNA synthesis is dissociated and anneals to the 3′ ssDNA tail at the other side of the DSB (Ferguson and Holloman 1996). Gap filling and ligation yields only NCO products (Figure 2). SDSA is the acronym generally used to refer to both models.

Break-induced replication: Break-induced replication (BIR) is a recombination-dependent replication process that results in the nonreciprocal transfer of DNA from the donor to recipient chromosome. For repair by BIR, a single end of a DSB invades a homologous duplex DNA and initiates replication to the chromosome end (Kraus *et al.* 2001; Llorente *et al.* 2008) (Figure 2). As BIR from one of the two ends of a DSB would result in extensive loss of heterozygosity (LOH), it suggests BIR is suppressed when DSBs have two homologous ends in order for repair to occur by a more conservative HR mechanism.

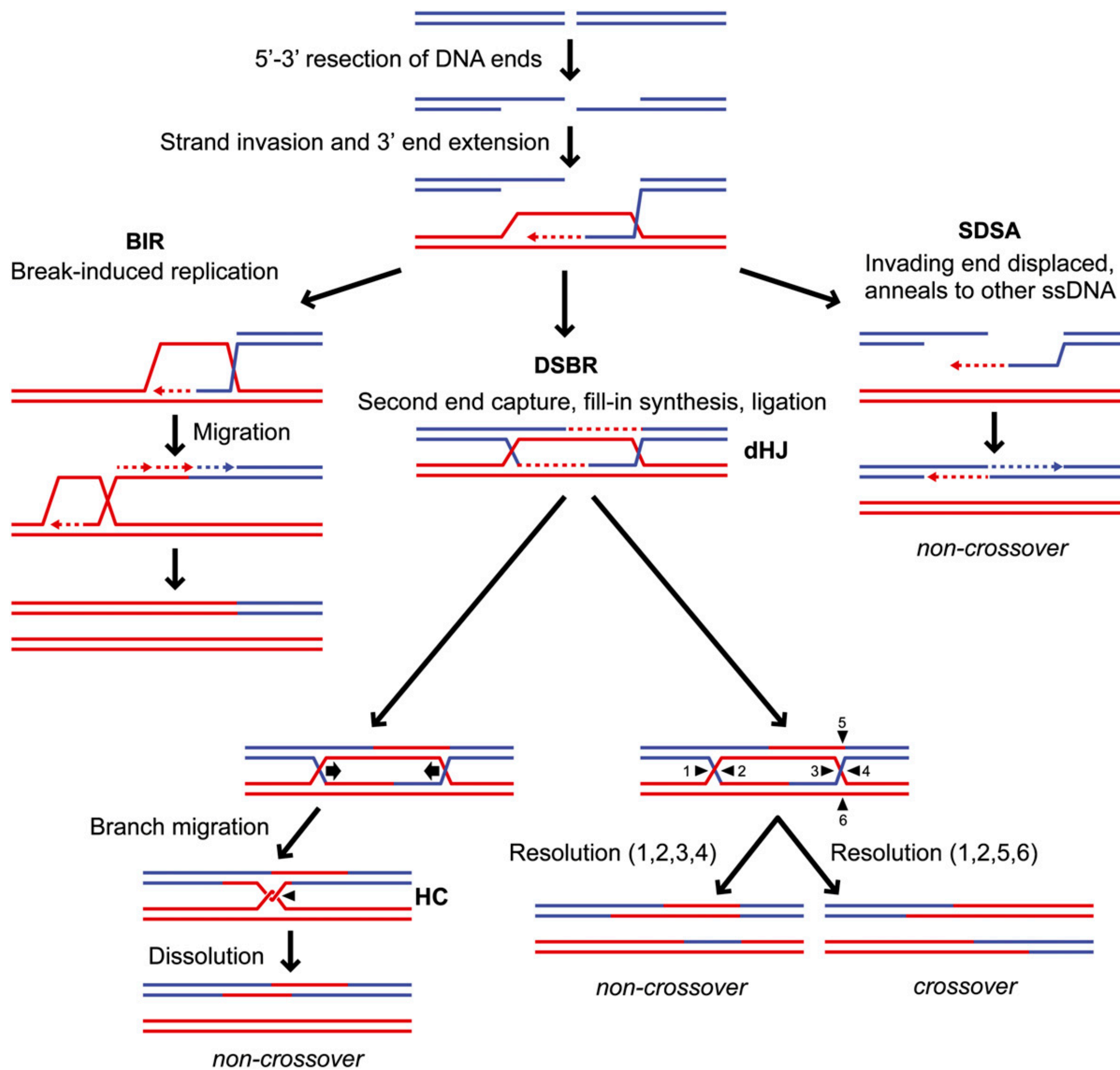

Figure 2 Models for homology-dependent DSB repair. Recombinational repair of a DSB is initiated by 5′ to 3′ resection of the DNA end(s). The resulting 3′ single-stranded end(s) invades an intact homologous duplex (in red) to prime leading strand DNA synthesis. For one-ended breaks, a migrating D-loop is established to facilitate break-induced replication (BIR) to the end of the chromosome and the complementary strand is synthesized by conservative replication. For two-ended breaks, the classical double-strand break repair (DSBR) model predicts that the displaced strand from the donor duplex pairs with the 3′ ssDNA tail at the other side of the break and primes a second round of leading strand synthesis. After ligation of the newly synthesized DNA to the resected 5′ strands, a double Holliday junction intermediate (dHJ) is generated. The dHJ can be either dissolved by branch migration into a hemicatenane (HC) leading to noncrossover (NCO) products or resolved by endonucleolytic cleavage to produce NCO (positions 1, 2, 3, and 4) or CO (positions 1, 2, 5, and 6) products. In mitotic cells, the invading strand is often displaced after limited synthesis and the nascent complementary strand anneals with the 3′ single-stranded tail of the other end of the DSB and after fill-in synthesis and ligation generate exclusively NCO products (synthesis-dependent strand annealing, SDSA).

Indeed, the frequency of BIR at an endonuclease-induced DSB is <1%, but can be substantially higher if gene conversion is prevented by limiting homology to one side of the break (Malkova *et al.* 1996, 2005; Bosco and Haber 1998). The initial steps of BIR appear to be similar to DSBR and SDSA in requiring end resection, homologous pairing, and strand invasion (Davis and Symington 2004). Recent studies identified a migrating D-loop intermediate during BIR and demonstrated that synthesis is conservative (Donnianni and Symington 2013; Saini *et al.* 2013a), suggesting the "lagging" strand initiates on the nascent strand extruded from the trailing end of the D-loop. BIR can occur by several rounds of strand invasion, DNA synthesis, and dissociation, resulting in chromosome rearrangements when dissociation and reinvasion occur within dispersed repeated sequences (Smith *et al.* 2007; Ruiz *et al.* 2009). Thus, the highly mutagenic nature of BIR could contribute to genome evolution and disease development in humans.

Single-strand annealing and microhomology-mediated end joining: The single-strand annealing (SSA) mechanism has been most extensively studied in the context of repair of an induced DSB formed between direct repeats (Paques and

Haber 1999). SSA might also be responsible for spontaneous deletions between direct repeats, but other mechanisms could operate. SSA efficiently repairs DSBs formed between repeats of >200 bp, but the frequency drops significantly for repeats of <50 bp (Sugawara *et al.* 2000). After resection of the DSB ends, the 3′ ssDNA tails can anneal when resection is sufficient to reveal complementary single-stranded regions corresponding to the repeats (Figure 3). Following annealing of the complementary ssDNA, heterologous flaps are formed if the repeats are separated from the break site by unique sequence. The flaps are removed by nucleases prior to gap filling and ligation (Fishman-Lobell *et al.* 1992; Ivanov and Haber 1995). Microhomology-mediated end joining (MMEJ) applies to an end joining mechanism that, like SSA, involves end resection and annealing between short (5–25 nt) direct repeats flanking a DSB (Ma *et al.* 2003; Decottignies 2007; Villarreal *et al.* 2012; Deng *et al.* 2014). These mechanisms are always mutagenic because they result in deletions, and MMEJ may be responsible for gross chromosome rearrangements (GCRs) that exhibit microhomologies at the junctions (Putnam *et al.* 2005).

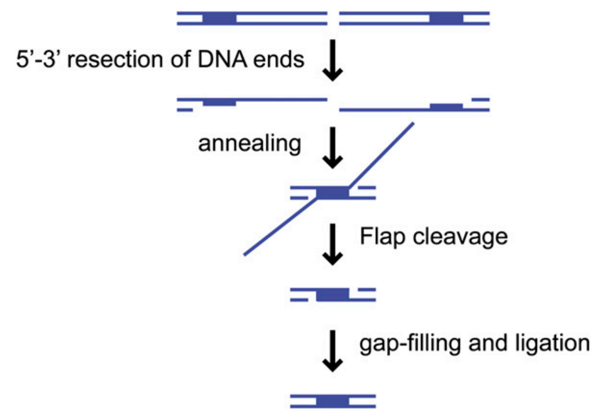

Figure 3 Single-strand annealing. Repair of a DSB flanked by direct repeat sequences (boxes) can occur by single-strand annealing (SSA), if 5′ to 3′ resection is allowed to progress past the repeats. The complementary single-stranded repeats can anneal, leaving heterologous flaps to be removed by the Rad1–Rad10 endonuclease, before gap-filling and ligation completes the repair thereby deleting one of the repeats and the intervening sequence.

B. Proteins involved in homologous recombination

Here we summarize the activities of proteins known to function at discrete steps of homology-dependent repair, as determined by biochemical analysis using defined DNA substrates and from the phenotypes of mutants in genetic and physical assays (for a list of human homologs and associated human diseases see Table 1). Physical assays, such as the *MAT* switching system, have been particularly useful to identify DNA intermediates formed during DSB repair and the genetic control of discrete steps in HR (Haber 2012). *MAT* switching is initiated by HO endonuclease cleavage of a specific site at the *MAT* locus and repair occurs by gene conversion using one of the transcriptionally silent donor cassettes, *HML* or *HMR*. The system has been adapted for studies of DSB repair by placing the *HO* gene under the control of the *GAL1* promoter to synchronously induce HO in a population of cells by addition of galactose to the growth medium (Jensen and Herskowitz 1984). The HO recognition site can be inserted at other genomic sites unrelated to *MAT* to induce recombination (Nickoloff *et al.* 1986). I-SceI, a site-specific endonuclease responsible for intron mobility in yeast mitochondria, has also been used to initiate DSB-induced recombination in the yeast nuclear genome by expressing an engineered version from the *GAL1* promoter (Plessis *et al.* 1992). The advantage of both systems is that 50–100% of the target sequences are cut within 1 hr following induction of the nuclease, and, by taking DNA samples at different times after DSB formation, intermediates in the process, for example, resected DNA ends and strand invasion intermediates, can be identified by Southern blot hybridization and/or PCR methods.

DNA end resection: Homology-dependent DSB repair initiates by nucleolytic degradation of the 5′ strands to yield 3′ ssDNA tails, a process referred to as 5′–3′ resection (reviewed by Mimitou and Symington 2009) (Figure 4). The *RAD50* and *XRS2* genes were initially implicated in controlling end resection because the mutants show a marked delay in the initiation of resection at endonuclease-induced DSBs; however, recombination products are still produced, but with delayed kinetics and lower yield than wild-type cells (Ivanov *et al.* 1994). Since recombination is not completely defective, it is puzzling why *mre11*, *rad50*, and *xrs2* mutants exhibit extreme sensitivity to DNA damaging agents and could be due to other functions of the proteins, such as in telomere maintenance, processing of ends with adducts, and nonhomologous end joining repair. The Mre11, Rad50, and Xrs2 proteins form a stable heterotrimeric complex (MRX) that plays structural and catalytic roles in the initiation of end resection (Mimitou and Symington 2009). Mre11 contains conserved phosphoesterase motifs that are essential for 3′–5′ dsDNA exonuclease and ssDNA endonuclease activities *in vitro* (Furuse *et al.* 1998; Usui *et al.* 1998; Moreau *et al.* 1999; Trujillo and Sung 2001). Rad50 has a similar domain organization to the structural maintenance of chromosomes (SMC) family of proteins (Alani *et al.* 1989; Lammens *et al.* 2011; Lim *et al.* 2011; Williams *et al.* 2011). The integrity of the long coiled-coil domains, as well as the conserved Cys-X-X-Cys dimerization motif (Rad50 hook) at the apex of the coiled-coil domains, is important for all functions of the MRX complex, suggesting tethering of DNA ends or sister chromatids via MR binding and dimerization is critical for repair (De Jager *et al.* 2001; Hopfner *et al.* 2002; Wiltzius *et al.* 2005; Hohl *et al.* 2011). Mre11 binds to the base of the Rad50 coiled-coils forming a "head" region composed of the Mre11 nuclease and Rad50 ATPase domain that together provides DNA binding and end processing activities that are regulated by ATP binding and hydrolysis (Lammens *et al.* 2011; Lim *et al.* 2011; Williams *et al.* 2011). Interaction with Xrs2 is required for translocation of Mre11 to the nucleus,

Table 1 Evolutionary conservation of homologous recombination proteins and examples of related human diseases

Functional class	*S. cerevisiae*	*H. sapiens*	Associated disease(s)	References
End resection	Mre11-Rad50-Xrs2	MRE11-RAD50-NBS1	Nijmegen breakage syndrome; AT-like disorder	(Varon et al., 1998)
	Sae2	CtIP		
	Exo1	EXO1	Colorectal cancer	(Yang et al., 2014)
	Dna2-Sgs1-Top3-Rmi1	DNA2-BLM-TOP3-RMI1-RMI2	Bloom syndrome	(Kaneko and Kondo, 2004)
Adaptors	Rad9	53BP1, MDC1	Breast cancer	(Bartkova et al., 2007; Rapakko et al., 2007)
	-	BRCA1	Breast cancer	(Petrucelli et al., 2010)
Checkpoint signaling	Tel1	ATM	Ataxia-telangiectasia	(Gatti et al., 2001)
	Mec1-Ddc2	ATR-ATRIP	Seckel syndrome	(O'Driscoll et al., 2003)
	Rad53	CHK2		
	Rad24-RFC	RAD17-RFC		
	Ddc1-Mec3-Rad17	RAD9-HUS1-RAD1		
	Dpb11	TOPBP1	Breast cancer	(Karppinen et al., 2006)
Single-stranded DNA binding	Rfa1-Rfa2-Rfa3	RPA1-RPA2-RPA3		
Single-strand annealing	Rad52	RAD52		
	Rad59	-		
Mediators	Rad52	-		
	-	BRCA2-PALB2	Breast cancer	(Petrucelli et al., 2010)
Strand exchange	Rad51	RAD51	Breast cancer	(Nissar et al., 2014; Sun et al., 2011)
	Rad54	RAD54A, RAD54B		
	Rdh54	-		
Rad51 paralogs	Rad55-Rad57	RAD51B-RAD51C-RAD51D-XRCC2-XRCC3	Breast cancer	(Silva et al., 2010; Vuorela et al., 2011)
	Psy3-Csm2-Shu1-Shu2	RAD51D-XRCC2-SWS1		
Anti-recombinases	Srs2	FBH1, PARI		
	Mph1	FANCM	Fanconi Anemia	(Meetei et al., 2005)
	-	RTEL1	Hoyeraal-Hreidarsson syndrome	(Ballew et al., 2013)
Resolvases and nucleases	Mus81-Mms4	MUS81-EME1		
	Slx1-Slx4	SLX1-SLX4	Fanconi Anemia	(Crossan *et al*., 2011; Kim *et al*., 2011; Stoepker *et al*., 2011)
	Yen1	GEN1		
	Rad1-Rad10	XPF-ERCC1	Xeroderma pigmentosum	(Gregg et al., 2011)

See also http://www.malacards.org.

and Xrs2 is also thought to regulate the MR complex and to mediate DNA damage signaling via interactions with Tel1 and Sae2 (Nakada *et al.* 2003; Tsukamoto *et al.* 2005; Lloyd *et al.* 2009; Schiller *et al.* 2012).

MRX can act as an endonuclease with Sae2 to cleave oligonucleotides from the 5′ strands resulting in short (∼100 nucleotides) 3′ ssDNA tails, or it can promote resection indirectly by recruitment of the Exo1 and/or Dna2 nucleases (Mimitou and Symington 2008; Zhu *et al.* 2008; Shim *et al.* 2010). The Mre11 nuclease activity and Sae2 are essential to remove covalent adducts, such as Spo11 (which forms a covalent attachment to 5′ ends to initiate meiotic recombination) or hairpin caps, from DNA ends, but not for resection of endonuclease-induced DSBs (Mimitou and Symington 2009). There is a delay of ∼30 min before resection of an HO-induced DSB initiates in the *sae2* mutant and this results in an increased frequency of NHEJ repair (Lee *et al.* 2008; Mimitou and Symington 2008; Deng *et al.* 2014). Sae2 exhibits its endonuclease activity *in vitro* that is stimulated by MRX, but unlike Mre11 has no obvious nuclease motifs (Lengsfeld *et al.* 2007). Which of these two nucleases, or if both, contributes to the initiation of resection is currently unknown. A class of separation-of-function *rad50* alleles, *rad50S*, confers similar phenotypes to the *sae2* and *mre11* nuclease-defective mutants (Alani *et al.* 1990). One attractive model for resection that has emerged from studies of meiotic recombination is for MRX and Sae2 to incise the 5′ strand at a distance from the end, followed by bidirectional resection from the nick using the Mre11 3′–5′ exonuclease and Exo1 5′–3′ exonuclease (Zakharyevich *et al.* 2010; Garcia *et al.* 2011). This model rationalizes how the Mre11 3′–5′ exonuclease participates in end resection and how Exo1 can overcome the block imposed by Ku binding at DNA ends.

Extensive resection is catalyzed by the 5′–3′ dsDNA exonuclease, Exo1, or by the combined action of the Sgs1 helicase and Dna2 endonuclease (Gravel *et al.* 2008; Mimitou and Symington 2008; Zhu *et al.* 2008) (Figure 4). The Sgs1 interacting partners Top3 and Rmi1 are also required for end resection,

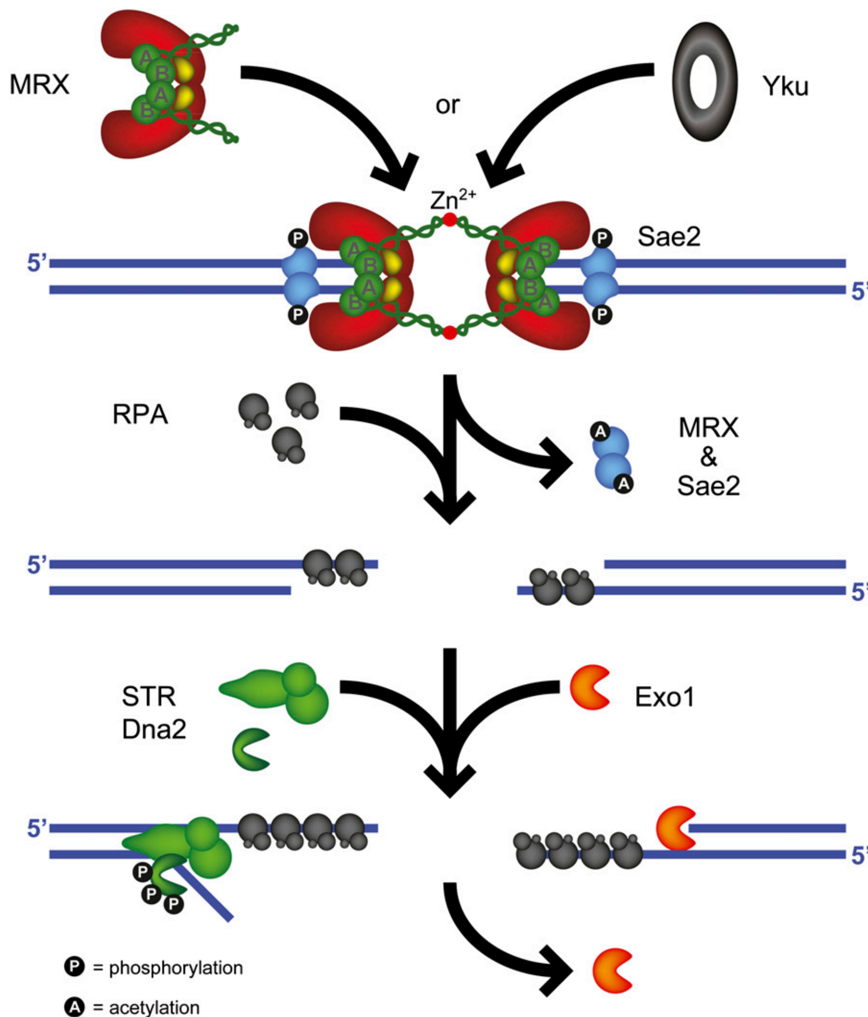

Figure 4 Resection of DSB ends. Resection of DSB ends progress 5′ to 3′ and in two steps. First, MRX and Sae2 catalyze short-range resection of ~100 nt. The initial resection by MRX and Sae2 is particularly important for cleaning up "dirty" ends harboring chemical adducts, secondary structures, or covalently attached proteins. The Yku complex inhibits initial end resection by competing with MRX for binding to ends. Second, extensive resection for up to 50 kb is catalyzed by Exo1 and/or STR–Dna2. Phosphorylation of Sae2 at serine 267 is required for resection. Sae2 is degraded upon acetylation. Dna2 nuclear localization and recruitment to DSBs require its phosphorylation at threonine 4 and serines 17 and 237 (see text for details).

but their roles appear to be structural rather than catalytic (Zhu *et al.* 2008; Niu *et al.* 2010). *In vitro* reconstitution of the Sgs1-Dna2 reaction revealed an essential role for the heterotrimeric replication protein A (RPA, Rfa1–Rfa2–Rfa3) to stimulate Sgs1 unwinding and Dna2 cleavage of the 5′ strand (Cejka *et al.* 2010a; Niu *et al.* 2010). Depletion of RPA from cells at the time of HO cleavage results in a block to extensive resection and failure to recruit Dna2 to DSBs. RPA is also required to prevent formation of secondary structures within the 3′ ssDNA tails that can generate hairpin capped ends or can be degraded by MRX and Sae2 (Chen *et al.* 2013). Although Exo1 or Sgs1–Dna2 can directly process free DNA ends with no covalent adducts, they are unable to remove end-blocking lesions, such as Spo11. One possible reason for the greatly delayed initiation of resection observed for the *mre11*, *rad50*, or *xrs2* mutants is the poor recruitment of Exo1 and Sgs1–Dna2 in the absence of the MRX complex. The other possible reason is the presence of Ku, a heterodimeric dsDNA end binding protein essential for NHEJ, which inhibits Exo1-mediated resection and is greatly enriched at DSBs in the absence of the MRX complex (Ivanov *et al.* 1994; Clerici *et al.* 2008; Mimitou and Symington 2010; Nicolette *et al.* 2010; Shim *et al.* 2010). Indeed, the resection defect of the *mre11* mutant is suppressed by elimination of Ku or overexpression of *EXO1* (Bressan *et al.* 1999; Tsubouchi and Ogawa 2000; Moreau *et al.* 2001; Lewis *et al.* 2002). Similarly, the resection defect of the *sae2* mutant is suppressed by *yku70* in an Exo1-dependent manner (Limbo *et al.* 2007; Mimitou and Symington 2010).

The rate of extensive end resection is ~4 kb/hr and can remove up to 50 kb of DNA if there is no donor sequence to template repair of the DSB (Zhu *et al.* 2008). However, extensive resection is unlikely to be necessary for HR, and even the short 3′ ssDNA tails resulting from MRX–Sae2-dependent cleavage in the absence of Exo1 and Sgs1–Dna2 are sufficient for Rad51-dependent recombination (Mimitou and Symington 2008; Zhu *et al.* 2008). Although extensive resection is not required for HR, resection of >10 kb is needed to activate the DNA damage checkpoint (Gravel *et al.* 2008; Zhu *et al.* 2008; Roberts *et al.* 2012). Elimination of MRX, the Mre11 nuclease or Sae2 in the *exo1 sgs1* background results in a complete block to end resection and lethality (Mimitou and Symington 2008).

Homologous pairing and strand invasion: The critical step of HR is pairing between the ssDNA formed by end resection and one strand of the donor duplex to form hDNA, a reaction

catalyzed by the Rad51/RecA family of recombinases (Shinohara *et al.* 1992; Symington 2002). Although Rad51 can catalyze DNA strand exchange under certain conditions *in vitro*, efficient exchange requires the activity of several other proteins, including RPA, Rad52, Rad54, Rad55, and Rad57 *in vivo*. Rad51 binds to ssDNA and dsDNA in an ATP-dependent fashion to form right-handed helical nucleoprotein filaments that can span thousands of nucleotides (Ogawa *et al.* 1993; Sung 1994; Sehorn *et al.* 2004; Sheridan *et al.* 2008; Ferrari *et al.* 2009). The contour length of DNA within the nucleoprotein filaments is extended by 50% relative to B-form DNA. Although Rad51 binds dsDNA *in vitro*, only the Rad51–ssDNA filament is active for homologous pairing and strand exchange. Rad51 has two DNA binding sites, one of which is required for high-affinity DNA binding and filament formation, and the second is required for homologous pairing (Cloud *et al.* 2012). Alone, Rad51 exhibits weak strand exchange activity that is stimulated by RPA, but this stimulation only occurs if Rad51 is allowed to nucleate on single-stranded DNA prior to the addition of RPA (Sung 1994, 1997a; Sugiyama *et al.* 1997). RPA is thought to stimulate Rad51 binding to ssDNA by removing secondary structures to allow assembly of a contiguous Rad51 nucleoprotein filament. If RPA and Rad51 are added simultaneously to ssDNA then RPA inhibits the reaction. This inhibition can be overcome by the addition of mediator proteins, such as Rad52 (Sung 1997a; New *et al.* 1998; Shinohara and Ogawa 1998). RPA also stimulates Rad51-mediated strand transfer by sequestering the displaced ssDNA that can inhibit the pairing reaction (Eggler *et al.* 2002).

Rad51 mediators: Rad51 mediators include proteins that enable loading of Rad51 on RPA-coated ssDNA, stabilize Rad51 nucleoprotein filaments and/or promote strand exchange by Rad51 (Sung *et al.* 2003). Rad52 interacts directly with both RPA and Rad51 and is thought to mediate Rad51 filament assembly by delivering Rad51 to RPA-bound ssDNA where it binds cooperatively to DNA, displacing RPA (Sung 1997a; New *et al.* 1998; Krejci *et al.* 2002; Sugiyama and Kowalczykowski 2002) (Figure 5). These *in vitro* studies are consistent with Rad52 being required for Rad51 recruitment to an HO-induced DSB *in vivo* as measured by chromatin IP and fluorescence microscopy (Sugawara *et al.* 2003; Lisby *et al.* 2004). Rad52 interaction with Rad51 occurs through a domain in the nonconserved C-terminal region of the protein (Milne and Weaver 1993; Krejci *et al.* 2002). Mutants expressing Rad52 C-terminal truncations that remove the Rad51 interacting domain exhibit intermediate IR sensitivity as compared to wild type and *rad52* mutants, and the IR sensitivity is suppressed by overexpression of *RAD51* or by deleting *SRS2*, which encodes a helicase that disrupts Rad51–ssDNA complexes *in vitro* (Boundy-Mills and Livingston 1993; Milne and Weaver 1993; Kaytor *et al.* 1995). An acidic region of Rad52, encompassing residues 308–311 is required for interaction with RPA and for DNA damage resistance (Plate *et al.* 2008).

Rad55 and Rad57 are referred to as Rad51 paralogs because they share ~20% identity to the Rad51/RecA core region (Kans and Mortimer 1991; Lovett 1994). Rad55 and Rad57 interact to form a stable heterodimer and Rad55 also interacts with Rad51 (Hays *et al.* 1995; Johnson and Symington 1995; Sung 1997b). The Rad55-Rad57 complex is implicated as a mediator of Rad51 filament assembly because it alleviates the RPA inhibition to Rad51-catalyzed strand exchange *in vitro* (Sung 1997b). Rad51 recruitment to an HO-induced DSB occurs more slowly in the absence of Rad55, consistent with a role for Rad55–Rad57 in Rad51 filament formation or stabilization (Sugawara *et al.* 2003). The IR sensitivity of *rad55* and *rad57* mutants is partially bypassed by overexpression of *RAD51*, or by *RAD51* gain-of-function alleles, such as *rad51-I345T*, that encode proteins with higher affinity for DNA than wild-type Rad51 (Hays *et al.* 1995; Johnson and Symington 1995; Fortin and Symington 2002). Deletion of *SRS2* also suppresses the IR sensitivity of *rad55* and *rad57* mutants (Fung *et al.* 2009). *In vitro* studies show the Rad55–Rad57 complex stabilizes Rad51 nucleoprotein filaments and counteracts Srs2-mediated displacement of Rad51 from ssDNA (Liu *et al.* 2011).

The Psy3, Csm2, Shu1, and Shu2 proteins (collectively referred to as the Shu proteins) are thought to function in early HR because mutation of any of the *SHU* genes suppresses the *top3* slow growth defect and *sgs1* HU sensitivity, similar to *rad51*, *rad55*, and *rad57* mutations (Shor *et al.* 2002, 2005). The *shu* mutants are not sensitive to IR, but do exhibit sensitivity to MMS, an alkylating agent that stalls replication, and show increased mutation rates dependent on the translesion synthesis (TLS) DNA polymerase, Polζ (Huang *et al.* 2003; Shor *et al.* 2005), which is consistent with a role in error-free postreplicative repair (Ball *et al.* 2009). The *shu* mutations partially suppress the accumulation of sister-chromatid joint molecules in the *sgs1* mutant, suggesting they function specifically in early HR events to fill gaps during replication (Mankouri *et al.* 2007). The Shu proteins appear to antagonize the activity of the Srs2 antirecombinase, possibly through Rad51 filament stabilization (Bernstein *et al.* 2011). The Shu proteins interact to form a complex and structural studies of the Psy3–Csm2 subcomplex indicate structural similarity to a Rad51 dimer, raising the possibility that the Shu complex is incorporated into the Rad51 filament, as suggested for Rad55–Rad57 (Liu *et al.* 2011; Tao *et al.* 2012; Sasanuma *et al.* 2013). The Psy3–Csm2 complex exhibits DNA binding *in vitro*, with a preference for forked and 3′ overhang DNA substrates, and is able to stabilize Rad51 binding to ssDNA (Godin *et al.* 2013; Sasanuma *et al.* 2013).

Single-strand annealing: Rad52 also promotes annealing of ssDNA *in vitro* (Mortensen *et al.* 1996; Shinohara *et al.* 1998) and this activity is likely important for capture of the second end in DSBR and SDSA and for the SSA mechanism of recombination (Sugawara and Haber 1992; Sugiyama *et al.* 2006; Lao *et al.* 2008). However, Rad52 is not required for MMEJ unless the microhomologies are >14 bp in length (Villarreal *et al.* 2012). This Rad51-independent function of Rad52 may explain the greater defect in most recombination

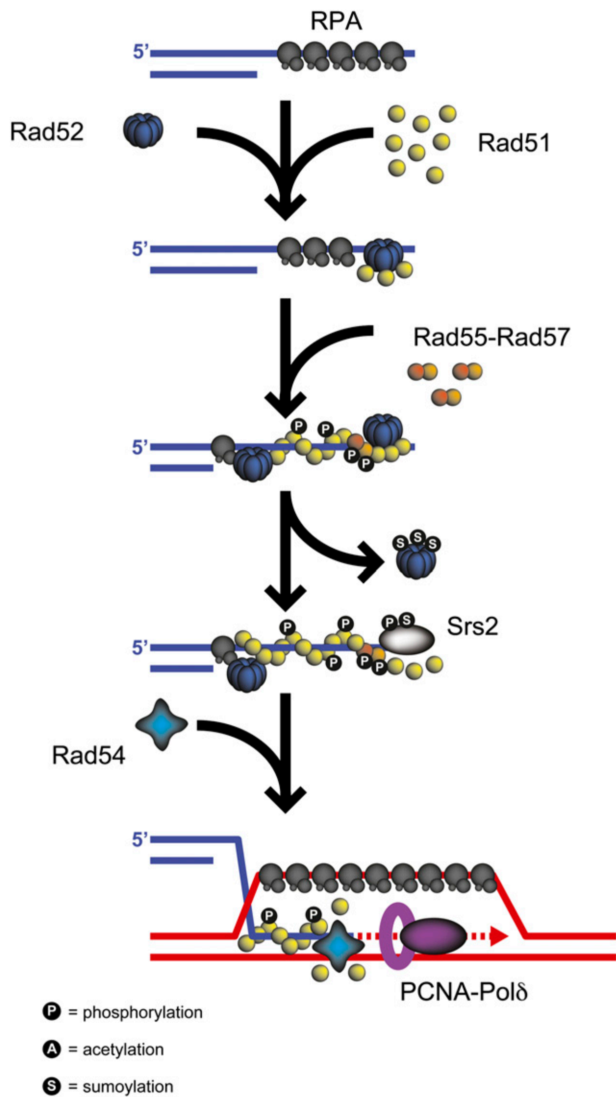

Figure 5 Rad51 filament dynamics. During Rad51-catalyzed strand invasion, Rad52 mediates the loading of Rad51 onto RPA-coated ssDNA to facilitate formation of a Rad51 nucleoprotein filament. The Rad51 filament is further stabilized by Rad55–Rad57. In contrast, the Srs2 helicase counteracts the Rad51 mediators by displacing Rad51 from ssDNA to disrupt toxic recombination intermediates. Similarly, Rad54 can displace Rad51 from dsDNA to allow loading of PCNA–Polδ at the 3′ end of the invading strand. Phosphorylation of Rad51 at serine 192 is required for ATP hydrolysis and DNA binding. Rad55 is phosphorylated at serines 2, 8, and 14. Sumoylation of Rad52 at lysines 43, 44, and 253 mediates its dissociation from ssDNA. Phosphorylation and sumoylation of Srs2 have pro- and antirecombination functions, respectively (see text for details).

assays reported for *rad52* mutants compared to *rad51*, *rad54*, *rad55*, and *rad57* mutants (Symington 2002). RPA is inhibitory to ssDNA annealing *in vitro*; however, Rad52 is able to overcome this inhibition (Sugiyama *et al.* 1998). *In vivo*, RPA prevents annealing between microhomologies that are too short to be annealed by Rad52 and prevents SSA in the absence of Rad52 (Smith and Rothstein 1999; Villarreal *et al.* 2012; Deng *et al.* 2014). Rad59, a protein with homology to the N-terminal DNA binding domain of Rad52 (Bai and Symington 1996), interacts with Rad52 and augments the strand-annealing activity (Petukhova *et al.* 1999; Sugawara *et al.* 2000; Davis and Symington 2001; Wu *et al.* 2006). The N-terminal domain of Rad52 is required for strand annealing and multimerization of Rad52 and is essential for Rad52 function *in vivo* (Kagawa *et al.* 2002; Mortensen *et al.* 2002; Singleton *et al.* 2002).

DNA translocases: Rad54 and Rdh54/Tid1, members of the Swi2/Snf2 family of chromatin remodeling proteins, stimulate homologous pairing by Rad51 *in vitro* (Petukhova *et al.* 1999, 2000). Rad54 stimulates D-loop formation between ssDNA and a homologous supercoiled plasmid, but also promotes dissociation of D-loop structures. The dissociation function is decreased when long ssDNA substrates are used or when duplex regions flank the invading ssDNA (Wright and Heyer 2014). Rad54 mediates chromatin remodeling and is able to promote Rad51-dependent D-loop formation on chromatinized templates (Alexiadis and Kadonaga 2002; Alexeev *et al.* 2003; Jaskelioff *et al.* 2003). Rad54 and Rdh54 exhibit dsDNA-specific ATPase activity and translocate on dsDNA to generate unconstrained negative and positive supercoils (Mazin *et al.* 2000; Van Komen *et al.* 2000). Strand separation at unwound regions is expected to facilitate the search for homology between dsDNA and the incoming Rad51 nucleoprotein filament. Interestingly, Rad54 enables Rad51-dependent D-loop formation between ssDNA and linear duplex DNA, a reaction not observed for the *Escherichia coli* RecA protein (Wright and Heyer 2014). Since homologous pairing between recipient and donor sequences is still observed *in vivo* in *rad54* mutants, it has been suggested that the primary function of Rad54 is postsynaptic rather than during the search for homology (Sugawara *et al.* 2003). Indeed, Rad54 is important to remove Rad51 from the end of strand invasion intermediates to permit access to DNA polymerases to extend the invading end (Li and Heyer 2009) (Figure 5). Rad54 and Rdh54 displace Rad51 from dsDNA and this could be important to remove unproductive association of Rad51 with dsDNA during presynapsis, thus increasing the pool of Rad51 available for HR, for turnover of Rad51 upon completion of recombination, or to uncover the 3′ end of paired intermediates to allow initiation of DNA synthesis (Solinger *et al.* 2002; Holzen *et al.* 2006; Shah *et al.* 2010). Overexpression of *RAD51* in the *rdh54* mutant results in the formation of toxic Rad51 foci on undamaged chromatin, suggesting Rdh54 is the major translocase to remove Rad51 in undamaged cells, whereas Rad54 acts on damaged chromatin (Shah *et al.* 2010).

Srs2 is a DNA helicase that plays both positive and negative roles in recombination. Srs2 mutants show elevated levels of spontaneous recombination, but in some DSB-induced recombination assays, the recovery of recombinants is reduced (Rong *et al.* 1991; Vaze *et al.* 2002). *In vitro*, Srs2 translocates on ssDNA, displacing Rad51 (Krejci *et al.* 2003; Veaute *et al.* 2003). The current view is that Srs2 prevents

initiation of recombination events presynaptically by disrupting the Rad51 nucleoprotein filament, and this activity could be important to prevent unwanted recombination at replication forks, where Srs2 colocalizes with PCNA (Pfander *et al.* 2005; Burgess *et al.* 2009). A direct interaction of Srs2 with PCNA may also regulate DNA synthesis during HR to suppress formation of crossover products (Burkovics *et al.* 2013). Although Srs2 is able to disrupt D-loop intermediates *in vitro*, it is less effective than Mph1, and the pattern of hDNA products recovered from plasmid gap repair in the *srs2* mutant is not consistent with a simple D-loop dissociation mechanism (Sebesta *et al.* 2011; Mitchel *et al.* 2013).

DNA synthesis during HR: DNA synthesis is essential to extend the 3′ end within the D-loop and is likely to be required after strand displacement to fill gaps adjacent to the annealed sequences, replacing the nucleotides lost by end resection. Genetic studies suggest these two phases of DNA synthesis may use different polymerases. The reversion frequency of a marker located 300 bp from an HO cut site was higher during DSB repair than during normal growth, and the mutagenesis was largely DNA Polζ dependent (Holbeck and Strathern 1997; Rattray *et al.* 2002). Polζ-dependent mutagenesis of nearby genes has also been reported for other recombinogenic initiating lesions, such as inverted repeats, GAA repeats, and interstitial telomere repeats (Shah *et al.* 2012; Aksenova *et al.* 2013; Saini *et al.* 2013b; Tang *et al.* 2013). In contrast, another study demonstrated that the mutagenic DNA synthesis associated with gap repair is independent of the TLS polymerases, Polζ or Polη (Hicks *et al.* 2010). The DNA synthesized in the context of the D-loop appears to be carried out by DNA Polδ operating at much lower fidelity and processivity than during S-phase synthesis (Maloisel *et al.* 2008; Hicks *et al.* 2010). Consistent with these findings, the Polδ complex is able to extend D-loop intermediates generated *in vitro* by Rad51 and Rad54 (Li and Heyer 2009). It is possible that long tracts of ssDNA formed by end resection are subject to base modification and gap-filling synthesis by DNA Polζ causing mutations in sequences close to the DSB, but not in the context of D-loop synthesis.

Conditional alleles of essential replication genes have been used to determine the role of replication proteins during DSBR by physical monitoring of *MAT* switching. Gene conversion of the *MAT* locus is independent of ORC, the Cdc7–Dbf4 kinase, the MCM complex, Cdc45, DNA Polα, and Okazaki fragment processing proteins, but requires PCNA, Dpb11, and either Polδ or Polε (Wang *et al.* 2004; Germann *et al.* 2011; Hicks *et al.* 2011). In contrast to gene conversion repair of a two-ended DSB, BIR requires lagging strand as well as leading strand synthesis (Lydeard *et al.* 2007). The extensive DNA synthesis associated with BIR needs the nonessential subunit of the Polδ complex, Pol32, and is also compromised by the *pol3-ct* mutation, which affects stability of the Polδ complex (Lydeard *et al.* 2007; Deem *et al.* 2008; Payen *et al.* 2008; Smith *et al.* 2009; Brocas *et al.* 2010). The Pif1 helicase is required for BIR and *in vitro* studies show Pif1 functions with the Polδ complex to extend the 3′ end of a Rad51-generated D-loop (Saini *et al.* 2013a; Wilson *et al.* 2013). Pif1 facilitates extensive DNA synthesis by liberating the newly synthesized ssDNA to establish a migrating D-loop, in agreement with the current model for BIR synthesis (Figure 2). All three replicative DNA polymerases are required for BIR, but the need for Polε occurs later than for Polα and Polδ (Lydeard *et al.* 2007).

Resolution of recombination intermediates: Resolution of D-loop intermediates by displacement of the extended invading strand is the primary mode of DSB repair in mitotic cells, at least for events initiated by endonucleases (Mitchel *et al.* 2010) (Figure 2). The Mph1 helicase dissociates Rad51-generated D-loop intermediates *in vitro* and mutants show increased levels of COs during DSB-induced recombination, in agreement with a role in promoting SDSA repair (Sun *et al.* 2008; Prakash *et al.* 2009; Tay *et al.* 2010; Sebesta *et al.* 2011; Lorenz *et al.* 2012; Mazon and Symington 2013; Mitchel *et al.* 2013). The increased COs observed in the *mph1* mutant are dependent on *MUS81* (Mazon and Symington 2013). Joint molecules, detected by two-dimensional agarose gel electrophoresis, accumulate transiently in the absence of *MPH1*, and persist at high levels in the *mph1 mus81* double mutant (Mazon and Symington 2013). Dissociation of D-loop intermediates is expected to have a negative impact on BIR and indeed overexpression of *MPH1* reduces the frequency of BIR, while the *mph1* mutant exhibits an increased BIR frequency (Luke-Glaser and Luke 2012; Stafa *et al.* 2014). The *srs2* mutant also exhibits increased levels of COs associated with DSB-induced recombination, but, as noted above, the pattern of hDNA products observed is not consistent with D-loop dissociation.

The DSBR model predicts the formation of a dHJ intermediate, which must be resolved for segregation of the recombinant duplexes. The Sgs1–Top3–Rmi1 complex can resolve dHJ intermediates *in vitro* by a process called dissolution (Wu and Hickson 2003; Cejka *et al.* 2010b). The helicase activity of Sgs1 branch migrates the constrained HJs and the topoisomerase activity of Top3 is thought to remove the supercoils between the two HJs eventually leading to NCO products (Figure 2). Consistent with the *in vitro* studies, *sgs1* and *top3* mutants show increased levels of COs associated with spontaneous recombination and DSB-induced gene conversion (Wallis *et al.* 1989; Watt *et al.* 1996; Ira *et al.* 2003). Furthermore, joint molecules (JMs) containing a dHJ intermediate are detected at higher levels during DSB-induced interhomolog recombination in *sgs1* diploid cells than in wild type (Bzymek *et al.* 2010).

X-shaped JMs are detected in the highly repetitive ribosomal DNA (rDNA) locus during S-phase and their abundance increases when DNA Polα/primase is limiting (Zou and Rothstein 1997). Replication-dependent X-structures at unique sequences that are independent of Rad51 and Rad52 have been found in unperturbed cells. These structures

are thought to be hemicatenanes due to their physical properties and resistance to cleavage by HJ resolvases *in vitro* (Lopes *et al.* 2003). Late forming replication-dependent X-structures accumulate in the absence of the Sgs1 helicase when cells are treated with MMS and in this case require HR functions, as well as the template-switching branch of post-replication repair for their formation, suggesting they are formed at ssDNA gaps as a means to bypass lesions (Figure 6) (Liberi *et al.* 2005; Branzei *et al.* 2008).

The alternative means to remove HJ-containing intermediates is through endonucleolytic cleavage. Several structure-selective nucleases (Mus81–Mms4 heterodimer, Yen1 and Slx1–Slx4 heterodimer) have been shown to cleave branched DNA structures, including HJs, *in vitro* (Boddy *et al.* 2001; Kaliraman *et al.* 2001; Fricke and Brill 2003; Ip *et al.* 2008). Interestingly, *mus81*, *mms4*, *slx1*, and *slx4* mutations were all identified on the basis of synthetic lethality with *sgs1* (Mullen *et al.* 2001). Because Mus81–Mms4 (Eme1 in *Schizosaccharomyces pombe*) exhibits higher cleavage activity on D-loops and nicked HJs than intact HJs, Mus81–Mms4/Eme1 most likely processes an early strand exchange intermediate, prior to ligation to form a dHJ intermediate, to generate crossover products (Kaliraman *et al.* 2001; Osman *et al.* 2003; Mazon and Symington 2013; Mukherjee *et al.* 2014). Ho *et al.* (2010) found a significant decrease in the formation of DSB-induced CO products between homologs in the *mus81* diploid and a greater decrease in the *mus81 yen1* double mutant, suggesting Mus81–Mms4 is the primary activity to resolve recombination intermediates with Yen1 serving as a back up function. The partial redundancy between these activities is also observed for DNA damage sensitivity, but Yen1 is not able to counteract the lethality of the *mus81 sgs1* mutant unless it is constitutively activated (Blanco *et al.* 2010; Ho *et al.* 2010; Tay *et al.* 2010; Matos *et al.* 2013). Surprisingly, COs between ectopic repeats are only reduced by 50% in the *mus81 yen1* double mutant (Agmon *et al.* 2011). Persistent JMs containing a single HJ (sHJ) connecting the ectopic sequences were identified in the *mus81 yen1* mutant, and their formation, as well as generation of CO products, was dependent on *RAD1* (Mazon *et al.* 2012; Mazon and Symington 2013). However, Rad1–Rad10 has no apparent role in the formation of COs between chromosome homologs (Mazon *et al.* 2012). Rad1–Rad10 is proposed to facilitate ectopic CO formation by cleaving the leading edge of the captured D-loop at the heterology boundary creating a substrate for subsequent cleavage by Mus81–Mms4 or Yen1 (Mazon *et al.* 2012).

In contrast to *mus81*, the synthetic lethality of *slx1* or *slx4* with *sgs1* is not suppressed by *rad51* mutation, suggesting the lethality might be due to problems other than, or in addition to, unresolved recombination intermediates (Fabre *et al.* 2002; Bastin-Shanower *et al.* 2003). *In vitro*, the yeast Slx1–Slx4 complex preferentially cleaves 5′ flap structures; however, the human SLX1–SLX4 complex is reported to cleave intact HJs (Schwartz and Heyer 2011). The *slx1* mutant has no obvious defect in the formation of mitotic or meiotic crossovers, however, in an *slx4* mutant, spontaneous mitotic crossovers are reduced (Ho *et al.* 2010; De Muyt *et al.* 2012; Zakharyevich *et al.* 2012; Gritenaite *et al.* 2014).

Removal of heterologous flaps that can form during strand invasion or following strand annealing by the SDSA and SSA models requires the Rad1–Rad10 endonuclease, which cuts branched DNA structures at the transition between dsDNA and ssDNA (Fishman-Lobell and Haber 1992; Ivanov and Haber 1995; Mazon *et al.* 2012). The flap cleaving activity of Rad1–Rad10 requires Slx4, but not Slx1, and the mismatch repair proteins, Msh2 and Msh3 (Fishman-Lobell and Haber 1992; Ivanov and Haber 1995; Sugawara *et al.* 1997; Flott *et al.* 2007). Saw1, which was identified in a genome-wide screen for SSA defects, binds specifically to 3′ flap structures *in vitro* and recruits Rad1–Rad10 to ssDNA flaps *in vivo* (Li *et al.* 2013, 2008). Although Rad1–Rad10 is generally considered to be essential for heterologous flap removal, in an assay that detects chromosomal translocations formed by SSA, the *rad1* defect was suppressed by *rad51* (Manthey and Bailis 2010). This result raises the possibility that Rad51 binds to unrepaired ssDNA flaps and prevents access to nucleases other than Rad1–Rad10.

III. Genetic Assays of Mitotic Recombination

In this section, we describe the various assays that have been used over the years to both define mitotic recombination as well as to aid in the isolation of mutations in genes that affect the process. Further insight has been gained by the introducing of site-specific lesions in these assays, such as inserting the HO-cut site or site-specific nicking site (Nickoloff *et al.* 1986; Galli and Schiestl 1998; Cortes-Ledesma and Aguilera 2006; Nielsen *et al.* 2009). Concomitant expression of these nucleases has allowed researchers to induce DSBs and nicks to evaluate their role in many of these assays.

A. Allelic recombination in diploids

Allelic recombination refers to events that occur at allelic positions between homologous chromosomes. As shown in Figure 1C and Figure 7A, most recombination events occur in the G2 phase of the cell cycle and, although there is a strong preference for recombination between sister chromatids, numerous genetic studies have documented recombination between homologs. Conversion events can be selected by generation of a functional copy of a gene from different mutant alleles (heteroalleles). Use of heterozygous markers centromere (*CEN*) proximal to the recombining locus allows identification of associated crossovers by LOH, though half of the potential CO events are not detected because of random segregation of chromatids at mitosis (Figure 1C and Figure 7) (Chua and Jinks-Robertson 1991; Ho *et al.* 2010). The rate of spontaneous gene conversion is generally $\sim 10^{-6}$ events/cell/generation and 10–20% of events are associated with COs (Haber and Hearn 1985). Allelic recombination is stimulated by several orders of magnitude if cells are irradiated with IR or UV (Manney and Mortimer 1964; Esposito and Watsgaff

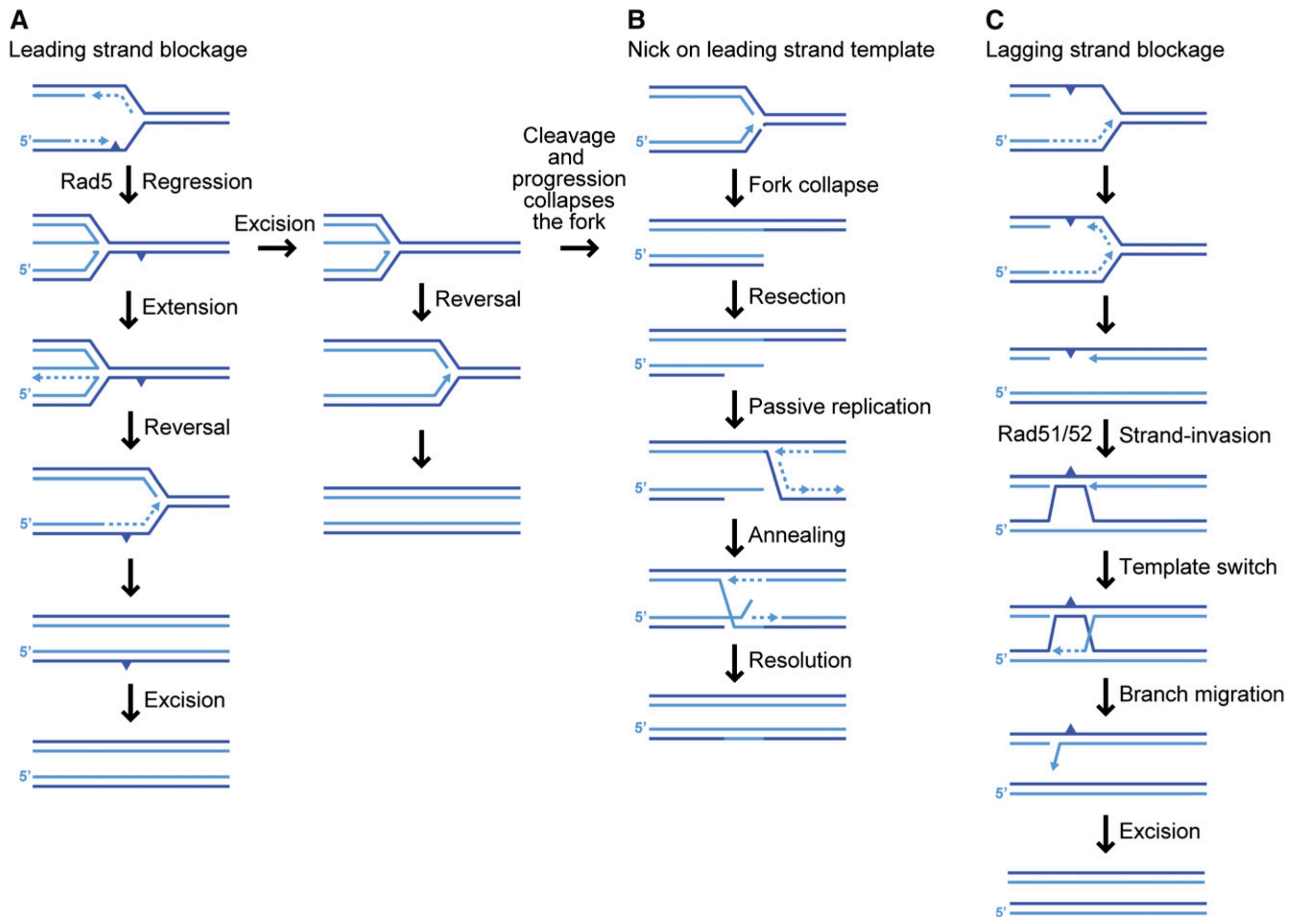

Figure 6 Recombination at replication forks. Parental strands are shown in dark blue and nascent strands in light blue. Polymerase-blocking DNA lesion indicated by a filled triangle. (A) Error-free bypass of leading strand blockage. The replication fork stalled at a DNA lesion on the leading strand template may be regressed in a Rad5-dependent manner to expose the lesion for excision repair after which the regressed fork is reversed and replication resumed. Alternatively, leading strand synthesis may transiently switch templates within the regressed fork. Upon fork reversal and reanneling of the extended leading strand to its parental template, the DNA lesion is bypassed and can subsequently be repaired by excision repair. (B) Fork collapse and rescue by passive replication. Fork collapse may result if the replication fork encounters a nick on the leading strand template or if a regressed fork is endonucleolytically cleaved to form a one-ended DSB, which is most often rescued by passive replication from an adjacent replication fork that can anneal to the end and be resolved into two intact sister chromatids. (C) Error-free bypass of lagging strand blockage. Lagging strand synthesis can be completed by postreplicative recombination to reestablish strand continuity at the lesion using the nascent sister chromatid as a template. The remaining lesion on the parental lagging strand can subsequently be removed by excision repair.

1981), or if one of the recombining loci has the recognition sequence for the HO or I-SceI rare-cutting endonucleases (Nickoloff *et al.* 1999; Palmer *et al.* 2003; Mozlin *et al.* 2008). The frequency of recombinants is sufficiently high when induced by a targeted DSB to analyze unselected events and to distinguish between a CO and BIR (Malkova *et al.* 1996; Ho *et al.* 2010) (Figure 7B).

Because spontaneous gene conversion events occur at low frequency and only one recombinant daughter cell is selected, reciprocal COs cannot be distinguished from BIR. Petes and colleagues developed a clever genetic assay to detect spontaneous reciprocal crossovers that occur between *CEN5* and the *CAN1* locus (Barbera and Petes 2006). The rate of spontaneous reciprocal exchange is 4×10^{-5} within this 120-kb interval. By using haploid strains with 0.5% sequence divergence to create the diploid, single nucleotide polymorphisms (SNPs) were used to map the site of exchange between *CEN5* and *CAN1* and determine the length of the conversion tracts associated with crossovers (Lee *et al.* 2009; Lee and Petes 2010). Unlike meiotic recombination, which occurs at nonrandom positions, the mitotic crossovers were evenly distributed and the median length of conversion tracts was ~12 kb, much longer than meiotic conversion tracts (Lee *et al.* 2009). Surprisingly, many of the conversion tracts associated with spontaneous crossovers showed 4:0 segregations of the heterozygous markers or hybrid tracts consisting of 3:1 adjacent to 4:0 segregations. Such events are best explained by the presence of a DSB in a G1 cell, replication of the broken chromosome and repair of the two broken sisters from the nonsister chromatids in G2, where one event would have to be associated with a CO to generate a sectored colony (Esposito 1978; Lee and Petes 2010).

The rate of spontaneous gene conversion is reduced >20-fold in *rad51* and *rad52* mutants, and by 5- to 10-fold in *rad54*

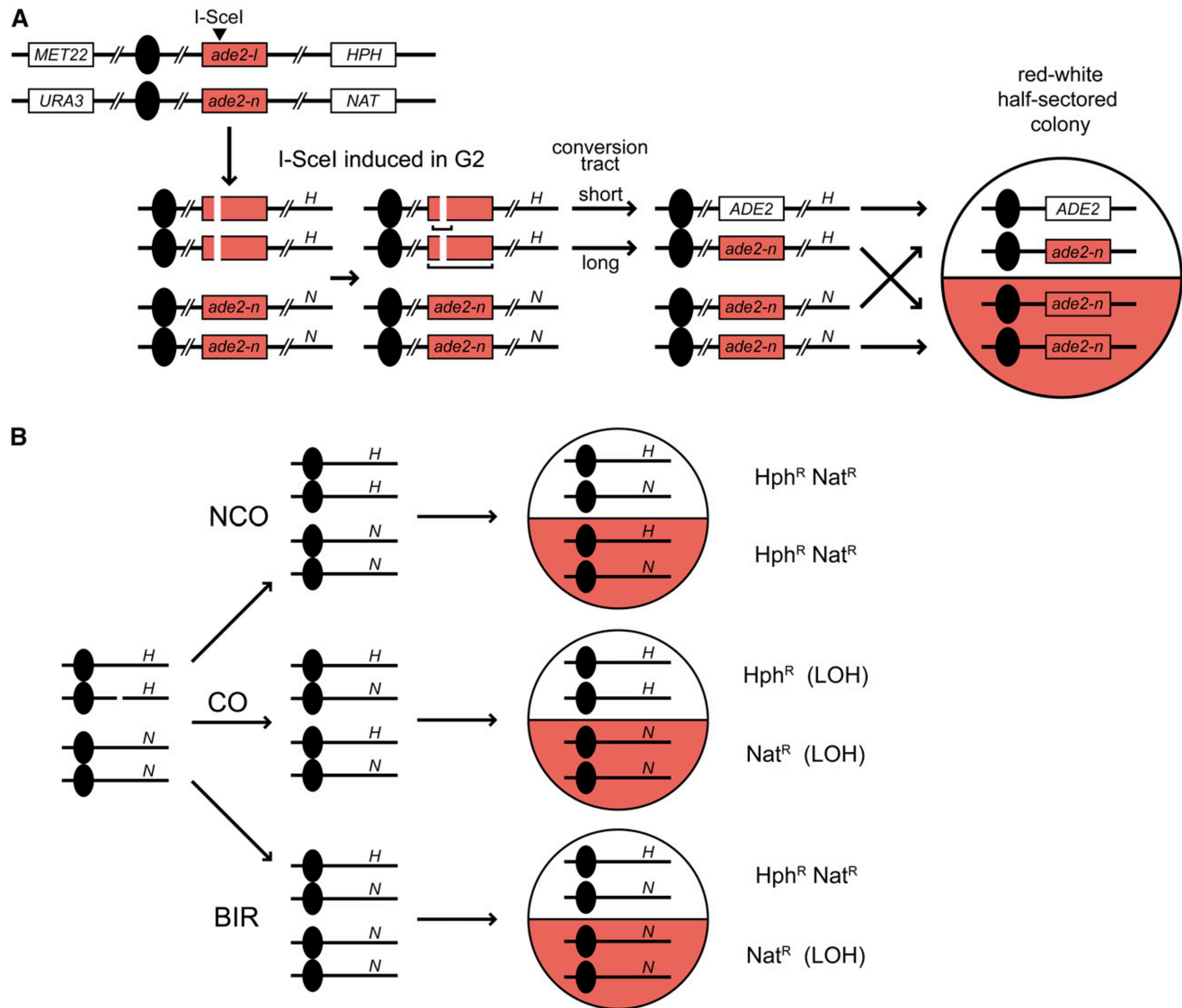

Figure 7 Assay for heteroallelic recombination. Nonfunctional *ade2-I* and *ade2-n* heteroalleles and wild-type *ADE2* give rise to red and white colonies, respectively. (A) I-SceI-induced heteroallelic recombination. A DSB induced by the I-SceI endonuclease in the *ade2-I* allele in G2 can be repaired from the intact homolog by short-tract or long-tract gene conversion to give rise to *ADE2* and *ade2-n*, respectively. Red-white half-sectored colonies are indicative of a recombination event that occurred in the first generation after plating. Markers *MET22* and *URA3* on the other side of the centromeres (filled circles) facilitate the scoring of chromosome nondisjunction events. Markers *HPH* and *NAT* adjacent to the *ade2* locus facilitate the scoring of CO events. (B) Scoring CO, NCO, and BIR events associated with gene conversion. Genotyping of red-white half-sectored colonies with respect to the *HPH* (H) and *NAT* (N) markers described in panel A allows the distinction of CO and BIR events as reciprocal and nonreciprocal LOH, respectively. The remaining events are NCOs.

and *rdh54* mutants as compared to wild type (Bai and Symington 1996; Klein 1997; Petukhova *et al.* 1999; Mortensen *et al.* 2002). In the absence of Rad52, aberrant products are recovered that are associated with chromosome loss and are thought to occur by a half crossover mechanism (Haber and Hearn 1985; Coic *et al.* 2008). Mutation of *MRE11*, *RAD50*, or *XRS2* results in higher rates of allelic recombination in diploids, but a slight reduction in the recombination rate between repeats (Alani *et al.* 1990; Ajimura *et al.* 1993; Rattray and Symington 1995). Cohesin loading at DSBs and stalled replication forks is dependent on MRX, suggesting recombinogenic lesions are channeled from sister chromatids to homologs in the absence of this complex (Strom *et al.* 2004; Unal *et al.* 2004; Tittel-Elmer *et al.* 2012). Allelic recombination is also higher in *rad59* diploids compared to wild type (Bai and Symington 1996). Rad59 physically interacts with the RSC chromatin-remodeling complex, which is required for cohesin loading at DSBs, and is important for sister chromatid recombination (Oum *et al.* 2011); thus, Rad59 may function to promote sister-chromatid recombination and in its absence, lesions might be channeled to nonsisters as suggested for the MRX complex.

B. Recombination between dispersed repeats

Ectopic or nonallelic homologous recombination (NAHR) refers to events occurring between homologous sequences located at

nonallelic positions (Figure 1, E and F). Naturally occurring repeats (for example, the rDNA array and Ty elements) and artificial duplications have been used as substrates for ectopic recombination, mainly in haploid cells. The repeated sequences can be located on different chromosomes or within the same chromosome. Closely spaced repeats in the same orientation (tandem or nontandem) or inverted relative to each other generally exhibit higher rates of recombination than repeats present on different chromosomes (dispersed) (Liefshitz *et al.* 1995). To a first approximation, the rate of spontaneous recombination between dispersed repeats and its genetic control are more similar to allelic recombination than to direct or inverted repeat recombination, suggesting heterologous chromosomes interact as frequently as homologous chromosomes in mitotic cells (Lichten and Haber 1989). However, global analyses of the yeast genome three-dimensional organization by chromosome conformation capture indicate that some loci are more prone to contact than others and these restricted chromosome territories influence the frequency of ectopic recombination (Burgess and Kleckner 1999; Duan *et al.* 2010; Agmon *et al.* 2013). Reciprocal exchange between dispersed repeats yields chromosome translocations that can only be detected if the orientation of the repeats with respect to their centromeres is the same. About 10–50% of gene conversion events between dispersed repeats have an associated crossover (Jinks-Robertson and Petes 1986; Liefshitz *et al.* 1995; Robert *et al.* 2006). However, the frequency of associated COs is generally less for DSB-induced ectopic recombination (Inbar and Kupiec 1999; Ira *et al.* 2003).

Direct-repeat recombination: Gene conversion between direct repeats can be detected using heteroalleles of a selectable gene, and can occur by intrachromatid or sister-chromatid interactions (Figure 8A). Gene conversion between misaligned sister chromatids can generate a triplication or deletion on one chromatid while retaining the direct repeat on the other, whereas a crossover between misaligned sister chromatids generates triplication and deletion products (Klein 1988) (Figure 1B). Intrachromatid gene conversion associated with a crossover generates a chromosomal deletion and an episomal circular product. Although deletions are formed at high frequency between direct repeats, the reciprocal product is only associated with ~7% of deletions, suggesting they arise primarily by a nonconservative mechanism (Schiestl *et al.* 1988; Santos-Rosa and Aguilera 1994).

Gene conversion events between direct repeats that maintain the intervening sequence require *RAD51*, *RAD54*, *RAD55*, and *RAD57*, whereas deletions arise independently of these genes, consistent with their formation by SSA (McDonald and Rothstein 1994; Liefshitz *et al.* 1995; Petukhova *et al.* 1999). By contrast, *rad52* mutants exhibit reduced frequencies of conversion and deletion events (Klein 1988; Thomas and Rothstein 1989b) likely due to the role of Rad52 as a Rad51 mediator and as a strand annealing protein, respectively.

The ribosomal genes in yeast naturally occur as a multiple tandem array (150–200 copies). Recombination within and between the repeats is important to maintain homogeneity of the cluster and is restricted to mitosis (Petes and Botstein 1977). Repeat homeostasis of rDNA is measured by determining the repeat length of the native array, which reveals either gain or loss of repeats. Recombination between repeats can also generate rDNA circles and the accumulation of rDNA circles in mother cells has been implicated in cellular aging (Sinclair and Guarente 1997; Kobayashi 2008). Due to their repetitive nature, rDNA have been excellent substrates for studying recombination. The insertion of selectable and counterselectable markers allows measurements for the effects of mutations on recombination outcomes—Rad51 is necessary for both deletion and duplication of the marker and Rad52 is necessary for duplication (Szostak and Wu 1980; Gangloff *et al.* 1996). In addition, the multiple copies of rDNA permit the detection of recombination intermediates after two-dimensional gel electrophoresis (Brewer and Fangman 1988; Zou and Rothstein 1997). Circles of rDNA are formed at high rates in mutants that are defective in DNA topoisomerases Top1 and Top2, indicating that regulating DNA topology is important for the stability of the array (Christman *et al.* 1988; Kim and Wang 1989). In addition, mutation of Top3, a eukaryotic type IA topoisomerase, also leads to increased rDNA instability (Wallis *et al.* 1989).

Importantly, to maintain sequence homogeneity, there is a genetic system in place that ensures recombination between the rDNA repeats. This system, controlled by the Fob1 replication block protein, is thought to help avoid collisions between transcription of rDNA and the bidirectional DNA synthesis that can be potentially initiated in every repeat (Takeuchi *et al.* 2003). At each repeat, Fob1 binds and inhibits DNA synthesis in one direction so that there are very few replication/transcription collisions. However, it is likely that the accumulation of blocked forks actually increases the amount of recombination that takes place naturally within this array, since in the absence of Fob1 protein, spontaneous recombination is reduced fivefold (Defossez *et al.* 1999). The increased recombination between the repeats stimulates homogenization of this multiple tandem array. The recent finding that spontaneous chromosomal fragile sites in yeast are enriched for motifs that correlate with paused replication forks supports the view that the Fob1 sites are playing this role in the rDNA array (Song *et al.* 2014).

Inverted-repeat recombination: Substrates with inverted repeats were designed to avoid formation of recombinants by SSA (Figure 8B). Gene conversion between inverted repeats retains the original configuration, whereas intrachromatid gene conversion associated with a CO or long tract conversion between misaligned sister chromatids results in inversion of the intervening sequence (Rothstein *et al.* 1987; Rattray and Symington 1994; Chen *et al.* 1998). Spontaneous recombination between inverted repeats is highly dependent on *RAD52*, but reduced only 5- to 10-fold

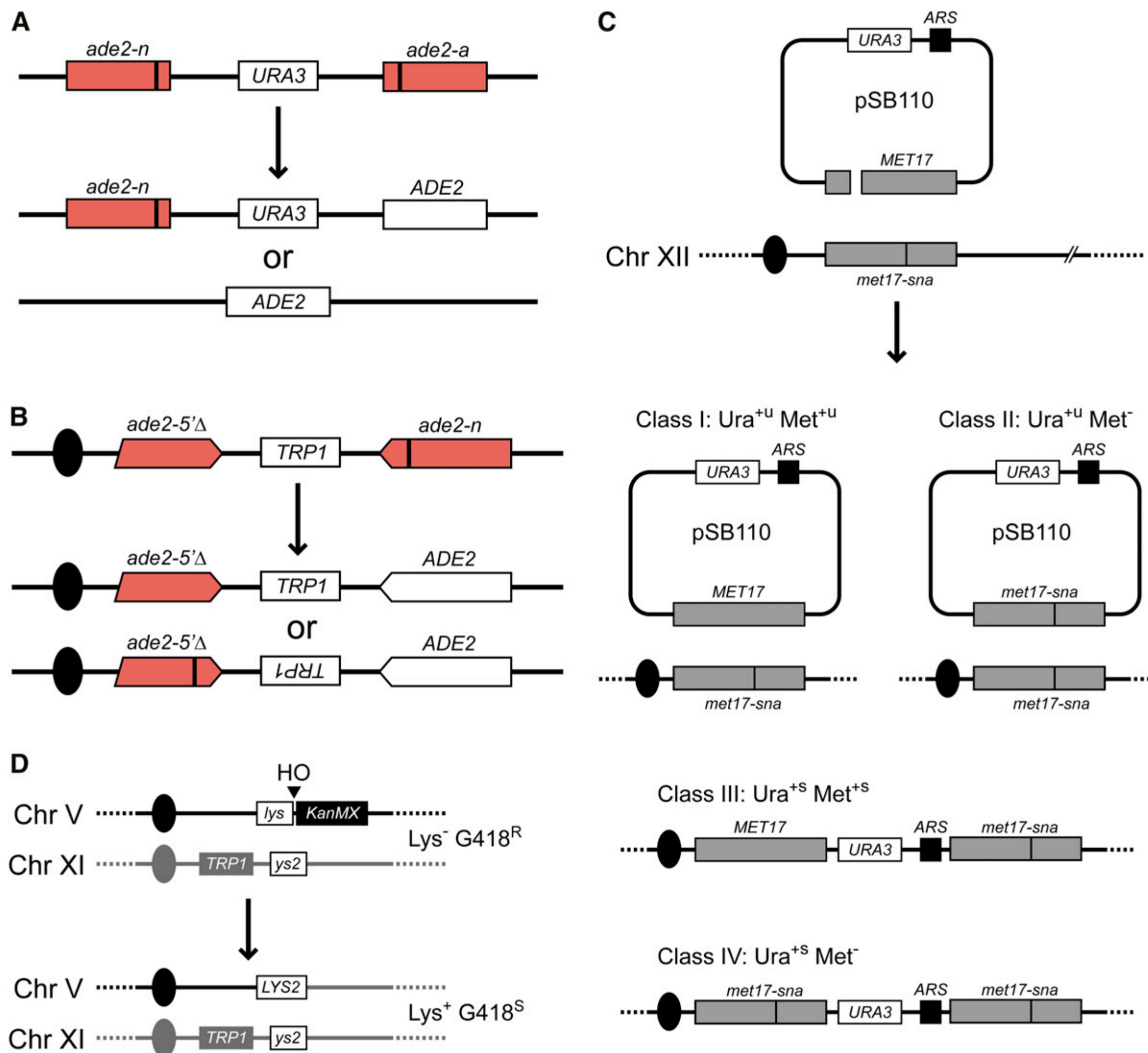

Figure 8 Genetic assays. (A) Direct-repeat recombination. Spontaneous homologous recombination between *ade2-n* and *ade2-a* alleles can occur by gene conversion to produce Ade+ Ura+ cells or by SSA to produce Ade+ Ura− cells (Fung *et al.* 2009). (B) Inverted repeat recombination. Inverted repeat recombination assays exclusively Ade+ recombinants arising from gene conversion since SSA will not produce viable recombinants. CO and NCO events will lead to inversion and noninversion of the *TRP1* marker, respectively (Mott and Symington 2011). (C) Plasmid gap repair assay. The efficiency of plasmid–chromosome recombination, crossover frequency, and conversion tract length is assayed by transformation of the gapped pSB110 plasmid into yeast containing the chromosomal *met17-sna* mutant allele in which a SnaBI site is eliminated 216 bp downstream of the gap in the plasmid (Symington *et al.* 2000). When the plasmid gap is repaired by noncrossover gene conversion, the result is unstable (u) Ura+ transformants, which will be Met+ (class I) or Met− (class II), depending on the absence or presence of co-conversion of the *met17-sna* mutation, respectively. If the gene conversion event is associated with a crossover, the result is a stable (s) Ura+ phenotype (classes III and IV). ARS, autonomously replicating sequence. (D) Break-induced replication. In this assay, BIR is initiated by induction of an HO-mediated DSB adjacent to a 3′ truncated *lys2* gene (*lys*) on chromosome V. The *lys* fragment has 2.1 kb of homology to a 5′ truncation of *lys2* (*ys2*) close to the telomere on chromosome XI (Donnianni and Symington 2013), which serves as a donor for BIR. BIR results in deletion of the *KanMX* gene and all nonessential genes telomere proximal to the HO cut site and loss of G418 resistance (G418R). The strain has the *MAT***a***-inc* allele to prevent cleavage at the endogenous *HO* cut site.

in the *rad51* mutant (Dornfeld and Livingston 1992; Rattray and Symington 1994; Gonzalez-Barrera *et al.* 2002). DSB-induced recombination between chromosomal inverted repeats is reduced by >1000-fold in the *rad51* mutant, but plasmid-borne inverted repeats exhibit less of a requirement for *RAD51* (Gonzalez-Barrera *et al.* 2002; Ira and Haber 2002; Rattray *et al.* 2002). *RAD59* is required for spontaneous *RAD51*-independent events, suggesting they occur by a strand annealing mechanism, possibly by template switching during DNA replication (Bai and Symington 1996; Gonzalez-Barrera *et al.* 2002; Mott and Symington 2011). The DSB-induced *RAD51*-independent events in the plasmid context might occur by BIR and SSA or by some other type of nonconservative event (Kang and Symington 2000; Ira and Haber 2002).

Naturally occurring inverted repeats, such as Ty elements and delta sequences, are also substrates for spontaneous recombination that can lead to genome instability and gross chromosomal rearrangements (Rothstein *et al.* 1987; Argueso *et al.* 2008; Casper *et al.* 2009; Paek *et al.* 2009; Chan

and Kolodner 2011). Many of these events are dependent on DSB repair pathways; however, notably, fusion of inverted repeats that lead to chromosome rearrangements are replication dependent (Paek *et al.* 2009). The rearrangements involving Ty elements and delta sequences are almost completely dependent on the *RAD51* and *RAD52* gene products (Rothstein *et al.* 1987; Liefshitz *et al.* 1995).

C. Plasmid gap repair

Transformation-based assays using plasmids linearized *in vitro* have been used extensively to study the mechanism and genetic control of DSB repair (Orr-Weaver *et al.* 1981, 1983; Bartsch *et al.* 2000; Mitchel *et al.* 2010; Tay *et al.* 2010). A plasmid containing a DSB or double-stranded gap within sequences that have homology to a chromosomal locus are introduced to cells by transformation and repair of the DSB or gap is templated by the homologous chromosomal sequence. In most assays, a second marker on the plasmid is used to select for transformants. In some respects, plasmid–chromosome recombination resembles ectopic recombination between dispersed repeats because both involve limited homology. If the plasmid contains no origin of replication, then only CO recombinants (integration of the plasmid at the chromosomal locus) are recovered; however, if the plasmid has an origin to allow stable maintenance as an episome then both NCO and CO products can be detected (Figure 8C). Use of a *CEN ARS* vector restricts events to NCOs (Bartsch *et al.* 2000). The frequency of COs recovered from an *ARS*-containing plasmid varies between assays, ranging from 20 to 50%, significantly higher than observed for DSB-induced chromosomal ectopic recombination (Orr-Weaver and Szostak 1983; Inbar and Kupiec 1999; Bartsch *et al.* 2000; Ira *et al.* 2003; Welz-Voegele and Jinks-Robertson 2008). Use of a plasmid substrate with SNPs located $\sim$100 bp apart to detect hDNA intermediates that persist in a mismatch repair defective background revealed that most NCO products formed by SDSA and only a few events were diagnostic of dHJ dissolution. Furthermore, the CO products were most consistent with resolution of a sHJ intermediate (Mitchel *et al.* 2010).

Early studies showed an essential role for *RAD52* in plasmid gap repair, and subsequently, *rad51*, *rad55*, and *rad57* mutations were shown to reduce the frequency of gap repair by $>$50-fold (Orr-Weaver *et al.* 1981; Bartsch *et al.* 2000). Elimination of *RAD1* reduces integration of an *ARS*-containing plasmid by 5- to 10-fold, whereas *mus81* and *yen1* mutations do not decrease integration. These confusing data were rationalized by studies showing the *RAD1*-dependent accumulation of a sHJ intermediate between ectopic sequences in the *mus81 yen1* mutant leading to the hypothesis that Rad1–Rad10 clips the D-loop intermediate when it encounters the heterology barrier creating a sHJ intermediate linking the plasmid to the chromosome; replication through the sHJ would then generate CO and NCO products (Mazon *et al.* 2012; Mazon and Symington 2013). This model explains the pattern of hDNA observed in CO recombinants and also the high frequency of plasmid integration (Figure 9).

D. Assays for BIR

BIR is most easily studied by creating a DSB where just one of the two ends can undergo homology-dependent strand invasion. Telomeres are a natural source of one-ended DSBs and maintenance of telomeres in the absence of telomerase provides a convenient genetic assay for BIR (see Wellinger and Zakian 2012 for review). Cells senesce in the absence of telomerase but survivors can arise by Rad52- and Pol32-dependent recombination (Lundblad and Blackburn 1993; Lydeard *et al.* 2007). Two pathways for generation of survivors have been defined: type I survivors are due to amplification of the Y′ subtelomeric repeats, have very short telomere repeat tracts, and are dependent on Rad51, Rad52, Rad54, Rad57, and Pol32; type II survivors have long heterogeneous telomere tracts and require the MRX complex, Pol32, Rad52, Rad59, and Sgs1 for their formation (see Wellinger and Zakian 2012).

To force repair of a chromosome-internal DSB by BIR, most assays restrict homology to only one side of the DSB to prevent gene conversion repair. Several systems have been developed using HO to create a chromosomal DSB, and a transformation-based system utilizing linear plasmid vectors has also been described (Morrow *et al.* 1997; Bosco and Haber 1998; Davis and Symington 2004; Malkova *et al.* 2005; Lydeard *et al.* 2007; Donnianni and Symington 2013). Malkova and colleagues use a haploid strain disomic for chromosome III to study BIR (Malkova *et al.* 2005; Deem *et al.* 2011; Saini *et al.* 2013a). The *MAT***a**-*inc* allele, which is refractory to HO cleavage, is present on one homolog and the HO-induced DSB on the other is forced to repair by BIR due to deletion of homology on the *CEN*-distal side of the DSB. Heterozygous markers present on both chromosome arms are used to differentiate between BIR, chromosome loss, and half COs. Two systems to study BIR in haploids make use of truncated partially overlapping fragments of the *CAN1* or *LYS2* gene to regenerate a wild-type copy of the gene by BIR (Lydeard *et al.* 2007; Donnianni and Symington 2013). The recipient cassette has an HO cut site between at the border of homology and a selectable marker located in a nonessential region of chromosome V; the donor cassette is located on another chromosome. After induction of the DSB, the recipient sequence invades the donor copying to the end of the chromosome, and the nonessential sequences *CEN*-distal to the DSB are lost (Figure 8D). Although BIR was originally reported to be very slow by physical monitoring assays, when the donor is close to the telomere and only 15–20 kb of DNA needs to be synthesized, the efficiency and kinetics are similar to other ectopic recombination assays (Jain *et al.* 2009; Donnianni and Symington 2013). Furthermore, cells synchronously released from G1 show a higher BIR efficiency than cells arrested in G2/M at the time of DSB induction.

BIR has also been proposed to explain Fob1-stimulated recombination in rDNA (Kobayashi *et al.* 1998). Strand invasion events occurring at the matching repeat on the sister chromatid preserves copy number while those occurring at

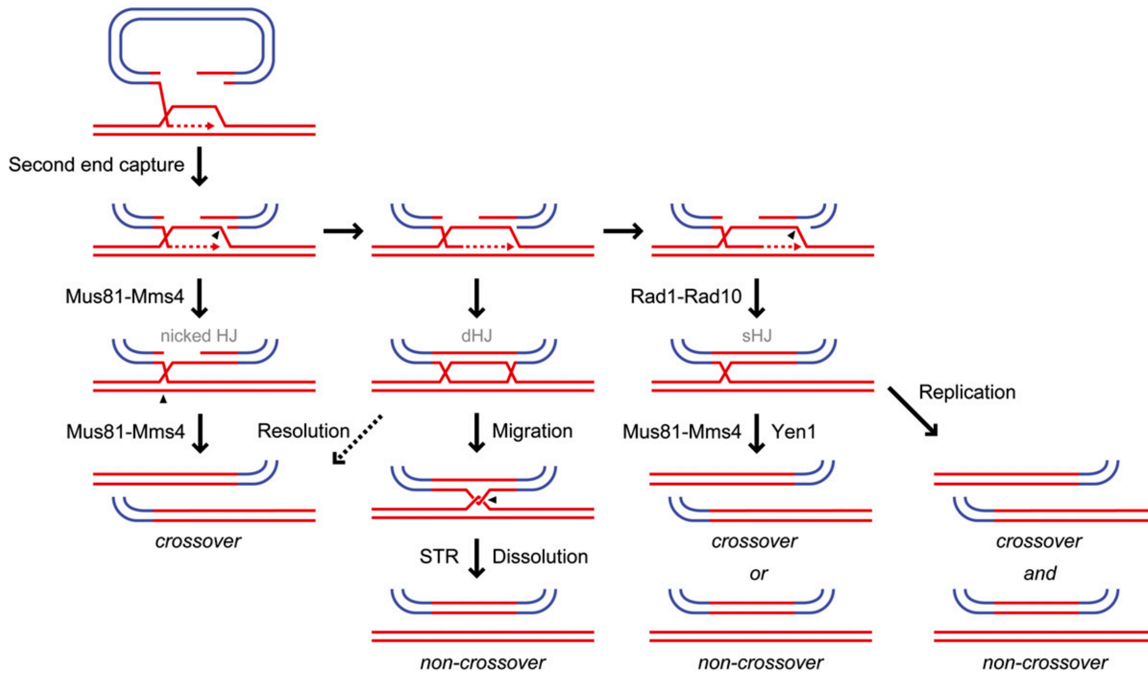

Figure 9 Model for the role of endonucleases in the resolution of recombination intermediates. Invasion by a 3′ end of a gapped vector into a chromosomal donor sequence generates a D-loop, which is extended by DNA synthesis. Initially, second end capture results in a structure that is a potential substrate for Mus81–Mms4 cleavage to produce a nicked HJ and subsequently a CO upon further cleavage. If, on the other hand, the captured D-loop is gap filled and ligated, a dHJ is formed, which in most cases is converted to a hemicatene and dissolved by STR to yield a NCO, but could also be resolved by Mus81–Mms4 to produce a CO or NCO. Alternatively, if resection and DNA synthesis proceed beyond the heterology boundary, the D-loop can branch migrate to create a region of single-stranded DNA adjacent to the branch point, which could be cleaved by Rad1–Rad10 to generate a single HJ (sHJ). The sHJ can be resolved by either Mus81–Mms4 or Yen1 cleavage to produce a CO or NCO, or converted to a CO and a NCO product during the next S phase.

unmatched repeats cause rDNA copy number changes. Cohesin restricts recombination to matched repeats and is regulated by transcription of non-coding RNA sequences near the replication fork block (Kobayashi and Ganley 2005). Transcription of these RNAs is regulated by the Sir2 histone deacetylase, explaining the increased rates of rDNA recombination observed in *sir2* mutant cells (Gottlieb and Esposito 1989; Kobayashi and Ganley 2005).

Although most of the genetic requirements for BIR are similar to other recombination reactions, *POL32*, which encodes a nonessential subunit of DNA Polδ, and *PIF1* are required for BIR but not for short tract gene conversion (Lydeard *et al.* 2007; Deem *et al.* 2008; Smith *et al.* 2009; Saini *et al.* 2013a; Wilson *et al.* 2013; Stafa *et al.* 2014). Interestingly, half crossovers are recovered at high frequency from *pol3-ct*, *pol32*, and *pif1* mutants (Deem *et al.* 2008; Smith *et al.* 2009; Saini *et al.* 2013a; Wilson *et al.* 2013), consistent with defective extension of the invading strand followed by cleavage of the D-loop intermediate. Half crossover products are also elevated in checkpoint mutants in agreement with the role of the DNA damage checkpoint in suppressing activation of structure-selective nucleases (Vasan *et al.* 2014).

IV. The Nature of the Recombinogenic DNA Lesion

This section describes the current thinking on the nature of the DNA lesion that results in defined recombination events. Many of our views on the exact kind of lesion that occurs have been influenced by studies of defined site-specific recombination events and the analysis of recombination mutants. Mating type switching is the "poster child" for this kind of study, having contributed greatly over the years to our understanding of the process.

A. Induction of recombination by DSBs and nicks

As described above, DSBs made by endonucleases serve as potent initiators of recombination. Furthermore, treatment of cells with ionizing radiation or radiomimetic drugs stimulates mitotic recombination. Testing the role of nicks as recombination initiators is more difficult because they can be healed by direct ligation, and any stimulation observed could be due to conversion to a DSB during replication (Figure 6). Insertion of the bacteriophage f1 gene *II* nick site between *trp1* and *his3* heteroalleles was shown to stimulate interchromosomal recombination when the gene II protein was expressed. Interestingly, there was a bias favoring conversion of the marker to the 5′ side of the nick site, in contrast to DSB initiated events that stimulate bidirectionally from the break site (Strathern *et al.* 1991). Conversion of the nick to a one-ended DSB during S-phase could account for the directionality of gene conversion observed. Aguilera and colleagues have shown a minimal HO site of 21 bp is cut by HO on one strand more frequently than on both strands, and the resulting nicks are converted to DSBs

as cells transition through S-phase (Cortes-Ledesma and Aguilera 2006). Although the frequency of cutting is lower than at the optimal HO cut site, it is sufficient to detect intermediates by physical methods. This system has proven extremely useful to study replication-associated DSBs and their repair by sister-chromatid recombination (Gonzalez-Barrera *et al.* 2003; Cortes-Ledesma and Aguilera 2006).

Studies with DNA damaging agents are used to gain insight in how particular lesions behave in different assays (reviewed in Kupiec 2000). For example, γ-rays and UV induce mainly DSBs or single-stranded gaps (Ma *et al.* 2013), respectively, while CPT leads to covalently bound topoisomerase I to the 3′ phosphate end at a nick (Pommier 2009). Replication of the unremoved adduct leads to a DSB in the next round of replication. In addition, the absence of topoisomerases I, II, or III themselves leads to increased recombination, especially in the rDNA multiple tandem array as discussed above. In these cases, it is thought that the absence of the topoisomerase causes topological problems such as catenanes and hemicatenates resulting in broken chromosomes during mitosis.

B. Replication-coupled recombination

Most spontaneous recombination is thought to occur during DNA replication, when the replisome encounters various challenges, such as reduced dNTP levels, DNA adducts on the template strand, DNA secondary structures, or tightly bound proteins that can stall the replication fork, and these impediments occasionally cause fork collapse resulting in a DSB (reviewed in Aguilera and Gomez-Gonzalez 2008). Fork collapse is thought to result from the replication fork running into a transient nick on the template strand, by fork reversal (pairing of the nascent strands) leading to the formation of Holliday junction or "chicken-foot" structure that can be cut by a HJ endonuclease or direct cleavage of the stalled fork by structure-selective nucleases, such as Mus81–Mms4 or Slx1–Slx4 (Figure 6). Fork regression requires Rad5 to facilitate either immediate excision repair or limited extension of the leading strand using the nascent lagging strand as a template (Sogo *et al.* 2002; Cotta-Ramusino *et al.* 2005; Blastyak *et al.* 2007), known as "template switching" (Higgins *et al.* 1976), followed by fork reversal and postreplicative excision repair (Figure 6A). Instead of fork reversal, the regressed fork may be cleaved, causing fork collapse (reviewed in Atkinson and McGlynn 2009). The one-ended DSB generated by fork collapse could be rescued by replication from an adjacent origin (Figure 6B). DNA adducts that block progression of the replicative polymerases result in ssDNA gaps on both lagging and leading strands, which can be acted on by TLS polymerases or by recombination (Figure 6C).

Most recombinogenic lesions formed during S-phase are expected to be repaired by sister-chromatid recombination; however, some lesions must be repaired by a nonsister to account for the increased interchromosomal recombination observed for mutants with replication defects or after treatment of cells with agents that stall replication, such as UV. These events are initiated from either ssDNA gaps or DSBs. Fabre and colleagues have argued against DSBs as the spontaneous recombination initiating lesion on the grounds that *rad52 yku70* double mutants (deficient for both HR and NHEJ) are viable, yet the *srs2 sgs1* double mutant is inviable but rescued by loss of HR function, suggesting lethal spontaneous recombination intermediates occur at high frequency (Fabre *et al.* 2002). Furthermore, certain *rad52* hypomorphic alleles confer high sensitivity to IR but are hyper-rec for spontaneous interchromosomal recombination (Lettier *et al.* 2006). Recently, whole chromosome analysis has revealed that single-stranded gaps are the intermediates of recombinational repair after UV irradiation (Ma *et al.* 2013). On the other hand, the pattern of conversion tracts associated with spontaneous mitotic crossovers is most compatible with initiation of recombination by a DSB present in a G1 cell (Lee and Petes 2010). Furthermore, analysis of conversion tracts associated with UV-induced mitotic crossovers showed they were similar to spontaneous events and those resulting from gamma-irradiation of G1 diploids (St Charles *et al.* 2012; Yin and Petes 2013).

C. Fragile sites and noncanonical structures

Fragile sites were first defined in mammalian cells as sequences that show gaps and breaks following inhibition of DNA synthesis and are associated with hotspots for genome rearrangements. Reducing the levels of DNA Polα or Polδ created a fragile site on yeast chromosome III detected by chromosome rearrangements between a pair of inverted Ty elements and other Ty elements located on other chromosomes (Lemoine *et al.* 2005, 2008). DSBs were detected at the inverted Ty elements when replication was compromised, analogous to mammalian fragile sites (Lemoine *et al.* 2005). Moreover, this fragile site experiences a high frequency of spontaneous BIR events leading to LOH on the right arm of chromosome III (Rosen *et al.* 2013).

Fragile sites are usually associated with DNA sequences that are difficult to replicate and prone to form secondary structures, such as certain trinucleotide repeats (TNRs), AT rich sequences, inverted repeats, and sequences with the potential to form G quadruplexes. Moreover, genome-wide mapping of fragile sites revealed a nonrandom distribution correlating with motifs that pause DNA replication forks, including replication-termination sites and binding sites for the helicase Rrm3 (Song *et al.* 2014). Insertion of inverted Alu elements, but not Alu direct repeats, was shown to stimulate ectopic recombination by 1000-fold in an MRX- and Sae2-dependent manner (Lobachev *et al.* 2002). A chromosomal DSB induced by the inverted Alu elements was detected by PFGE and shown to be hairpin capped in the absence of the MRX complex, the Mre11 nuclease, or Sae2. It was postulated that the Alu elements extrude into a cruciform, and the base, resembling a HJ, is cleaved by a structure-selective nuclease converting it into two hairpin-capped ends that must be opened by MRX and Sae2 to initiate recombination.

TNRs that are capable of forming hairpins when present in ssDNA show orientation-dependent replication fork stalling, increased chromosome fragility, and contractions and expansions when present on the lagging strand template (Freudenreich *et al.* 1997; Miret *et al.* 1998). Insertion of a long TNR tract between *CEN5* and *CAN1* was shown to stimulate mitotic crossovers by 30-fold, but the local stimulation was much greater and the majority of crossovers and conversion tracts were close to the TNR tract (Tang *et al.* 2011).

G-quadruplex (G4) DNA induces recombination by interfering with both replication and transcription. Interestingly, G4-induced recombination is observed only when the G-rich strand is the template for leading strand synthesis (Lopes *et al.* 2011) or when transcription of the G-rich DNA is oriented with the C-rich strand as the transcription template (Kim and Jinks-Robertson 2011). The effect of transcription orientation was enhanced in the absence of the type IB topoisomerase Top1, possibly due to enhanced R-loop formation (Kim and Jinks-Robertson 2011). Pif1 unwinds G4 structures *in vitro* and prevents replication fork stalling and DNA breakage at G4 motifs *in vivo* (Paeschke *et al.* 2011), which likely explains its suppression of recombination triggered by G-quadruplex forming tandem repeats (Ribeyre *et al.* 2009). Finally, Mre11 binds and cleaves G4 DNA *in vitro* (Ghosal and Muniyappa 2005).

D. Transcription-stimulated recombination

Early studies searching for hotspots of genetic recombination showed that promiscuous transcription by RNA polymerase I stimulates mitotic recombination (Keil and Roeder 1984; Voelkel-Meiman *et al.* 1987). In addition, a high level of transcription by RNA polymerase II resulted in increased repeat recombination (Thomas and Rothstein 1989a,b; Saxe *et al.* 2000). Topological changes induced by transcription may be responsible for creating recombinogenic lesions since mutations in topoisomerase I and II also lead to increased recombination especially in rDNA (Christman *et al.* 1988; Kim and Wang 1989; Wallis *et al.* 1989; El Hage *et al.* 2010). Studies of the THO/TREX complex indicate that an increased frequency of R-loop formation during transcription is likely the cause of transcription-stimulated recombination (Huertas and Aguilera 2003). In many cases, increased recombination can be suppressed by overexpressing RNaseH, which preferentially removes the RNA from the DNA–RNA hybrid (Huertas and Aguilera 2003; El Hage *et al.* 2010). Interestingly, it was recently reported that the Rad51 protein is involved in the formation of RNA–DNA hybrids and that Srs2 normally counteracts their potential for genome instability (Wahba *et al.* 2013). Although the precise lesion involved in stimultating recombination is not known, multiple lines of evidence suggest that the intermediate is a DSB (reviewed in Aguilera and Garcia-Muse 2012). Recently, the DNA damage checkpoint has been linked to transcription-associated R-loops that impede DNA replication (Bermejo *et al.* 2011). It is thought that highly transcribed genes associate with the nuclear periphery to aid in RNA export. When a replication fork is encountered head on with the transcribed gene, the resulting collision collapses the fork. This action activates the DNA damage checkpoint to release the transcription/replication unit from the nuclear pore to allow relief of topological stress.

V. Cell Biology of Recombination

Most recombination proteins can be expressed as functional fusions to genetically encoded fluorescent proteins such as GFP and mCherry (Lisby *et al.* 2004; Silva *et al.* 2012), which allows for the dynamic redistribution of these proteins to be monitored in real-time at the single-cell level during homologous recombination.

A. Recombination foci

Most homologous recombination proteins are recruited in many copies to the site of DNA damage during repair. The high local concentration of recombination proteins at the site of DNA damage can be visualized by fluorescence microscopy after immunostaining or by GFP-tagging of the proteins (Lisby *et al.* 2004; Eckert-Boulet *et al.* 2011; Silva *et al.* 2012). For example, a single DNA DSB is sufficient for the formation of a prominent focus containing 600–2100 molecules of Rad52 yielding a ≥50-fold higher local concentration of Rad52 at the DSB relative to the diffuse nuclear distribution in undamaged cells (Lisby *et al.* 2003b). Although the minimum number of Rad52 molecules required for mediating a single strand invasion is currently unknown, the high local concentration of recombination proteins within these foci may allow constitutively expressed proteins to be active only at the site of DNA damage, and therefore prevent untimely recombination or assembly of recombination complexes at undamaged DNA.

Recombination foci are highly dynamic in their protein composition and localization. Foci can assemble and disassemble within minutes. However, studies of Rad51, Rad52, and Rad54 foci in mammalian cells indicate that the residence time of individual molecules may vary between proteins and even subpopulations of proteins within foci (Essers *et al.* 2002). So far the dynamics of proteins within individual recombination foci has not been studied in yeast.

Although focus formation of recombination and checkpoint proteins is a useful tool for monitoring the cellular response to DSBs and a single DSB is sufficient to trigger focus formation (Lisby *et al.* 2003b), it is likely that some recombination events go undetected by this methodology. For example, recombinational restart of stalled replication forks, some sister chromatid events and intramolecular recombination may be too fast or require too few molecules of recombination proteins to be detected by current techniques.

B. Choreography of focus formation

HR starts with the recruitment of MRX, which binds directly to DNA ends (Chen *et al.* 2001; Hopfner *et al.* 2001; Lisby *et al.* 2004; Mimitou and Symington 2010). The two ends of

a DSB are held together by a mechanism that is partially dependent on MRX and Sae2 (Chen *et al.* 2001; Lisby *et al.* 2003a; Kaye *et al.* 2004; Lobachev *et al.* 2004; Clerici *et al.* 2005). For this reason, the two ends of a DSB give rise to a single Mre11 focus rather than two foci. Further, the MRX complex interacts with the Tel1 kinase and is required for its recruitment to foci at all phases of the cell cycle (Nakada *et al.* 2003; Lisby *et al.* 2004) (Figure 10). The Tel1 kinase phosphorylates histone H2A, which is a chromatin mark specific for damaged DNA in most eukaryotes (Rogakou *et al.* 1998, 1999; Redon *et al.* 2003). Importantly, the modification of chromatin by H2A phosphorylation facilitates binding of the checkpoint adaptor Rad9 to sites of DNA damage likely through a dual interaction of its BRCT domains with H2A-S129^{P} and its Tudor domain with histone H3 methylated at lysine 79 (H3-K79^{Me}) leading to subsequent recruitment and activation of Rad53 (Giannattasio *et al.* 2005; Javaheri *et al.* 2006; Toh *et al.* 2006; Grenon *et al.* 2007; Hammet *et al.* 2007; Germann *et al.* 2011). Notably, Rad53 foci are faint and transient, which is consistent with the notion from mammalian cells that Rad53/CHK2 must redistribute from the site of DNA damage upon phosphorylation to mediate a pannuclear checkpoint response (Lukas *et al.* 2003).

The proteins involved in resection have very different focal appearances giving clues to their function and regulation. Mre11 and Sae2 form prominent foci at all phases of the cell cycle in response to DSBs (Lisby *et al.* 2004; Barlow *et al.* 2008). Dna2 shuttles between the nucleus and cytoplasm in a cell-cycle-dependent manner, residing in the cytoplasm during G1 phase and relocalizing to the nucleus in S/G2 upon phosphorylation by Cdc28 (CDK) (Kosugi *et al.* 2009; Chen *et al.* 2011). Dna2 forms Rad52-colocalizing foci after DSB formation (Zhu *et al.* 2008). Sgs1 is a low abundance nuclear protein, which forms foci in S/G2/M (Frei and Gasser 2000 and M. Wagner, personal communication). Exo1 levels are cell cycle regulated gradually increasing through G1 and peaking in late S/G2 phase before it is degraded in anaphase (M. Lisby, unpublished data). Resection is accompanied by the dissociation of MRX, Sae2, and Tel1 from the DSB and binding of RPA to the 3′ single-stranded overhangs (Figure 10) (Lisby *et al.* 2004; Barlow *et al.* 2008). The intensity of Rfa1 foci can be used to estimate the extent of resection. This approach was used to demonstrate at the single-cell level that the rate of DSB end resection increases at the G1–S transition (Barlow *et al.* 2008). RPA is necessary for recruiting a number of checkpoint and HR proteins including the Dna2, Mec1–Ddc2/Lcd1, Rad24–RFC and 9–1–1 (Ddc1–Mec3–Rad17) complexes. Notably, Tel1 and Mec1 have many of the same phosphorylation targets, including histone H2A. As a consequence, Tel1-dependent checkpoint signaling is likely replaced by Mec1-dependent signaling upon resection of DSB ends. Consistent with a functional crosstalk between the Ddc2–Mec1 and 9–1–1 complexes during checkpoint signaling, there is a reported requirement for the 9–1–1 complex to stabilize Ddc2 foci in irradiated G1 cells (Barlow *et al.* 2008). Further, in S and G2 phases, the 9–1–1 complex and the Cdc28 kinase both contribute to the stabilization of DNA damage-induced Ddc2 foci (Barlow *et al.* 2008), demonstrating how checkpoint signaling is coordinated with cell cycle phase. The multifunctional Dpb11 protein is recruited to foci by the 9–1–1 complex, reflecting its role in mediating the DNA damage checkpoint through activation of the Mec1 kinase (Puddu *et al.* 2008; Germann *et al.* 2011). In contrast, the DNA replication and recombination functions of Dpb11 are independent of focus formation (Germann *et al.* 2011).

In S and G2 phases, RPA facilitates the recruitment of Rad52 to resected DSBs, likely via a direct physical interaction (Hays *et al.* 1995; Lisby *et al.* 2001, 2004; Plate *et al.* 2008). The recruitment is independent of DNA replication and requires B-type cyclin/Cdc28 activity (Barlow and Rothstein 2009). However, the cell cycle regulation of Rad52 focus formation can be circumvented at high doses of ionizing radiation at which Rad52 also forms foci in G1 phase, although it is unknown if these foci are productive for recombination (Lisby *et al.* 2003a). Rad52 interacts with the Rad51 recombinase and Rad59 to recruit these proteins to foci (Milne and Weaver 1993; Davis and Symington 2003; Lisby *et al.* 2004). In addition, Rad59 also requires Rad52 for its nuclear accumulation (Lisby *et al.* 2004). However, recent data indicate that Rad59 has Rad52-independent functions, indicating that some Rad59 enters the nucleus in a *rad52* mutant (Coic *et al.* 2008; Pannunzio *et al.* 2008; Pannunzio *et al.* 2012). Rad51 foci form, but are dimmer in the absence of Rad55–Rad57, consistent with the role of the Rad51 paralogs to stabilize Rad51 filaments. Interestingly, Rad55 focus formation requires Rad51 (Lisby *et al.* 2004; Fung *et al.* 2009). Formation of DNA damage-induced foci by Rad54 requires both Rad55–Rad57 and Rad51, suggesting that Rad54 recruitment to the site of DNA damage requires Rad51 nucleoprotein filament formation (Lisby *et al.* 2004). Interestingly, the Rad54 homolog, Rdh54, is recruited both to DSBs and to the kinetochore, although the functional significance of this dual localization is unknown. The recruitment of Rdh54 to DSBs is Rad52- and Rad51 dependent, while its localization to the kinetochore is independent of the recombination machinery. Interestingly, Rad54, which does not localize to the kinetochore in wild-type cells, localizes to the kinetochore in an *rdh54* mutant (Lisby *et al.* 2004), possibly explaining some of the functional redundancy between these two proteins (Shinohara *et al.* 1997).

Additional proteins that are recruited to recombination foci include the Pif1 helicase, which forms Rad52-colocalizing foci (Wagner *et al.* 2006), and the Srs2 helicase and anti-recombinase, which is recruited to two distinct classes of foci (Burgess *et al.* 2009). During S phase, Srs2 is recruited to sumoylated PCNA speckles, and in late S/G2 Srs2 is recruited to recombination foci marked by Rad52. The recruitment of Srs2 to recombination foci is independent of its SUMO-interacting motif (Burgess *et al.* 2009). The

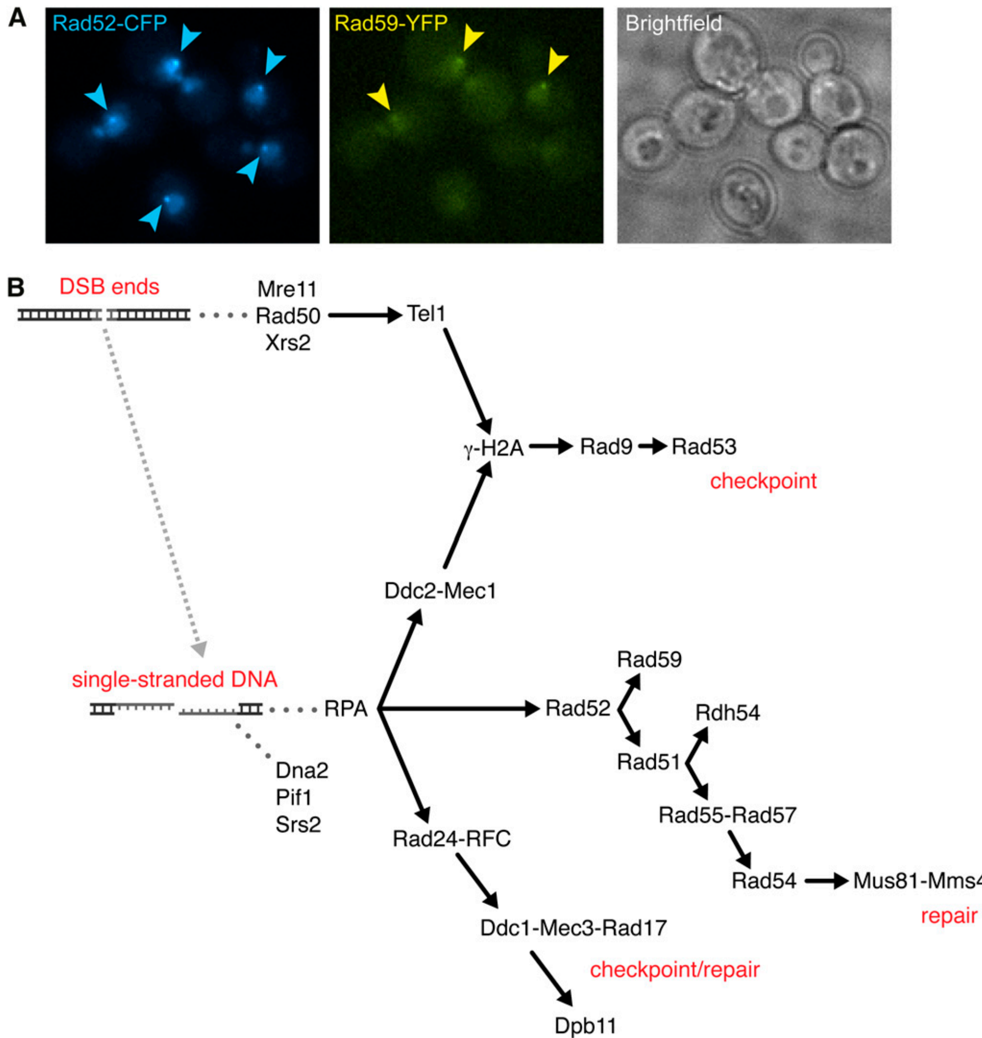

Figure 10 Choreography of HR focus assembly. (A) Focus formation of HR proteins. The high local concentration of Rad52 and Rad59 at DSBs induced by treatment with 200 μg/ml zeocin for 2 hr at 25°. Strain NEB110-25B is a *MAT***a** haploid containing *RAD52-CFP* and *RAD59-YFP*. Arrowheads mark foci. (B) Order of assembly of HR proteins at foci. Proteins are recruited from the left to right starting with MRX binding at DSB ends and later replaced by proteins recruited to ssDNA at resected DSBs.

Mus81–Mms4 structure-selective endonuclease forms foci, which are largely dependent on Rad54, consistent with Mus81–Mms4 acting downstream of the strand-invasion step of homologous recombination (Matulova *et al.* 2009). The SUMO-targeted ubiquitin ligase Slx5–Slx8 forms foci that partially overlap with Rad52 and Rad9 foci in response to DNA damage (Cook *et al.* 2009).

C. DSB dynamics and recombination centers

Work in both haploid and diploid yeast cells has found that, after DNA damage, the volume of the nucleus explored by the broken chromosome more than doubles from that seen in the absence of DSBs (Dion *et al.* 2012; Mine-Hattab and Rothstein 2012). The dynamics of unbroken chromosomes also increase depending on the number of DSBs (Mine-Hattab and Rothstein 2012; Seeber *et al.* 2013). The pairing of the homologs in diploid cells takes ~20 min before the repair center disassembles and the loci separate again (Mine-Hattab and Rothstein 2012). These studies suggest that increased chromosomal mobility facilitates the homology search, which is otherwise restricted by the proximity of donor and recipient loci (Agmon *et al.* 2013). Genetics and cell biological studies, in both haploid and diploid yeast cells, showed that increased DNA mobility depends on the Rad51 recombinase (Dion *et al.* 2012; Mine-Hattab and Rothstein 2012). In haploid yeast, increased mobility also depends on Rad54 and two checkpoint proteins, Rad9 and Mec1 (Dion *et al.* 2012; Seeber *et al.* 2013). In a *sae2* mutant, which has delayed appearance of single-stranded DNA, increased chromosome mobility is also delayed (Mine-Hattab and Rothstein 2012). In contrast to the increased mobility observed after IR or enzymatically induced DSBs, spontaneous Rad52 foci are constrained, which may reflect recombination between sister chromatids in the context of DNA replication (Dion *et al.* 2013).

The mobilization of DSBs may also allow multiple DSBs to interact. In fact, it has been shown that multiple DSBs in the same cell often come together at a single Rad52 focus (Lisby *et al.* 2003b). These recombinational repair centers are observed in both haploid and diploid cells. The aggregation of multiple DSBs takes place subsequent to recognition by the MRX complex and prior to recruitment of Rad52, which is indicated by the observation that cells exposed to 40 krad of ionizing radiation (equivalent to 20 DSBs per haploid cell) initially exhibit up to 20 Mre11 foci within

5 min, which transitions to 1–2 Rad52 foci within 20–30 min (M. Lisby and R. Rothstein, unpublished data). Most likely multiple DSBs are held together by the same scaffolding processes that hold together the two ends of a single DSB, but the molecular components of the scaffold have not been fully described, although a partial dependency on Sae2 and the MRX complex for tethering ends has been reported (Chen *et al.* 2001; Lisby *et al.* 2003a; Kaye *et al.* 2004; Lobachev *et al.* 2004; Clerici *et al.* 2005). Thus, the tethering of DNA ends may facilitate DSB repair but at the same time pose a risk for translocation between clustered DSBs.

D. Nuclear compartments

Some regions of the genome are more susceptible to deleterious recombination including repetitive elements such as the centromeres, telomeres, and Ty elements, and the highly transcribed rDNA and tRNA genes. Untimely recombination at these loci is prevented by compartmentalization of the nucleus into regions that suppress recombination and regions that allow or even stimulate recombination. The most prominent example is the nucleolus from which late-acting recombination and checkpoint proteins such as RPA, Rad52, Rad51, Rad59, Rad55, Rad24–RFC, 9–1–1 (Ddc1), Ddc2, and Rad9 are largely excluded even in the absence of DNA damage (Torres-Rosell *et al.* 2007). Although DSBs in the rDNA are initially recognized by the MRX complex within the nucleolus and resected (Torres-Rosell *et al.* 2007), they are only bound by Rad52 and downstream factors after exiting the nucleolus. The relocalization of rDNA breaks from the nucleolus to the nucleoplasm requires the Smc5–Smc6 complex and SUMO modification of Rad52 (see below), and mutants that disrupt the Smc5–Smc6 complex or prevent Rad52 sumoylation lead to Rad52 focus formation inside the nucleolus and rDNA instability (Torres-Rosell *et al.* 2007).

Similar to the rDNA, telomeres are compartmentalized. Telomeres associate into 6–8 clusters (Gotta *et al.* 1996), which are largely refractory to recombination and the DNA damage checkpoint response in general (Khadaroo *et al.* 2009; Ribeyre and Shore 2012). Importantly, all telomeres are generally in the vicinity of the nuclear envelope over long periods of time (Hediger *et al.* 2002). A component of the nuclear envelope in *Saccharomyces cerevisiae* is the SUN domain protein Mps3 (Antoniacci *et al.* 2007; Bupp *et al.* 2007). Several studies indicate that Mps3 is involved in anchoring telomeres at the nuclear envelope and shielding telomeres against spontaneous recombination (Antoniacci *et al.* 2007; Bupp *et al.* 2007; Schober *et al.* 2009) (reviewed in Taddei and Gasser 2012). Nevertheless, anchoring of telomeres at the nuclear periphery is essential for efficient DSB repair in subtelomeric DNA (Therizols *et al.* 2006).

In contrast to the nucleolus and telomere clusters, homologous recombination appears to be enhanced in the vicinity of nuclear pore complexes (NPCs). Persistent DSBs, collapsed replication forks, and eroded telomeres relocalize to NPCs, which stimulates recombinational repair at those loci (Nagai *et al.* 2008; Khadaroo *et al.* 2009). In an independent study, unrepaired DSBs were found to be enriched at the nuclear periphery in an Mps3-dependent but NPC-independent manner (Oza *et al.* 2009). Further, it was reported that spontaneous gene conversion is enhanced in a Nup84- and Slx8-dependent manner by tethering of a donor sequence to the nuclear pore complex (NPC) (Nagai *et al.* 2008). It was suggested that desumoylation of repair proteins by the SUMO-specific protease Ulp1, which associates with the NPC (Takahashi *et al.* 2000), could be responsible for the observed stimulation of gene conversion (Nagai *et al.* 2008) (see below).

VI. Regulation of Homologous Recombination

Homologous recombination is tightly regulated according to the type of DNA lesion, cell cycle phase, ploidy, and other environmental and development cues. The regulation of HR serves to ensure that the HR machinery does not interfere with DNA transactions such as transcription and replication on undamaged chromosomes and to fine tune the fidelity of repair. In budding yeast, the regulation of HR takes place mainly at the transcriptional level and by post-translational modification of HR proteins, while there is so far little evidence for post-transcriptional regulation.

A. Transcriptional regulation of homologous recombination

Many recombination proteins are constitutively expressed with some exceptions where expression is regulated with cell cycle or in response to DNA damage. Presumably, a constitutive basal expression of many recombination and checkpoint proteins allows for a rapid response to DNA damage. DNA damage-induced genes with relevance to mitotic recombination are the ribonucleotide reductase genes *RNR1–4*, which are required for reduction of ribonucleotides to their corresponding 2′-deoxyribonucleotides (dNTPs) necessary for DNA synthesis during recombination, the DNA damage checkpoint genes *RAD53* and *MEC1*, which form a positive feedback loop to increase their own expression, and the recombination genes *RFA1*, *RFA2*, *RFA3*, *RAD50*, *SRS2*, *RAD54*, and *RAD51* which are also induced in a *MEC1*-dependent manner (Cole *et al.* 1987; Elledge and Davis, 1989, 1990; Yagle and McEntee 1990; Basile *et al.* 1992; Kiser and Weinert 1996; Jelinsky and Samson 1999; Vallen and Cross 1999; Gasch *et al.* 2001; Mercier *et al.* 2001; Benton *et al.* 2006) (reviewed in Fu *et al.* 2008). In addition, many recombination genes exhibit cell cycle regulated expression, which peaks in late G1 to early S phase (Basile *et al.* 1992; Spellman *et al.* 1998 and reviewed in Mathiasen and Lisby 2014), although the functional importance of this regulation remains to be determined.

B. Regulation of homologous recombination by post-translational modifications

Homologous recombination proteins are acted upon by most known post-translational modifications (PTMs) including

phosphorylation, ubiquitylation, sumoylation, and acetylation, which are all reversible. Since the PTMs and their consequences are context dependent, this section will review the regulation by PTMs during DNA double-strand break repair and during recombinational restart of DNA replication (Table 2), while the regulation of specialized recombination events such as alternative lengthening of telomeres is described elsewhere (Wellinger and Zakian 2012). The following section will focus on the PTMs, for which a biological function has been described.

Regulation of DSB repair by PTMs: The initiating step of recombinational repair of a DSB end, resection to produce 3′ single-stranded overhangs, is regulated by cyclin-dependent kinase Cdc28 (CDK). Inhibition of Cdc28 using an analog-sensitive allele of *CDC28* or by overexpression of Sic1, an inhibitor of Cdc28, results in greatly reduced end resection (Aylon *et al.* 2004; Ira *et al.* 2004). As a consequence, resection of DSBs induced in G1 cells is greatly reduced compared with cycling or G2-arrested cells. Resection is regulated by PTMs at multiple levels. Initially, binding of the Ku complex to DSB ends blocks resection and its binding is inhibited by Cdc28 during S and G2 phase of the cell cycle (Clerici *et al.* 2008). G1 cells deficient for Ku show greater recruitment of Mre11 to an endonuclease-induced DSB and increased resection. As a consequence, HR can occur in G1 in *yku* mutants (Zhang *et al.* 2009; Trovesi *et al.* 2011). Overexpression of Exo1 is also able to overcome the inhibition to resection in G1 cells, consistent with other studies showing Ku is a barrier to Exo1-mediated end resection. The inhibitory effect on end resection was observed to a lesser extent in the *dnl4* (ligase IV deficient) mutant, suggesting the end binding function of Ku and ligation both contribute to protecting ends from degradation in G1 (Clerici *et al.* 2008; Zierhut and Diffley 2008). Interestingly, inhibition of CDK in G2 *yku80* cells fails to block short-range resection, similar to the situation in G1 *yku80* cells, and activation of CDK in G1 by overexpression of Clb2 restores both initiation and extensive resection (Clerici *et al.* 2008). Together, these results suggest that Ku (and to a lesser extent NHEJ) is the primary rate-limiting factor for the initiation of end resection in G1 by competing with MRX and Exo1 for end binding.

Resection is positively regulated by phosphorylation of Sae2 at serine 267 by Cdc28 during S and G2 phases (Huertas *et al.* 2008). Mutation of this site to a nonphosphorylatable residue, S267A, phenocopies *sae2*, including hypersensitivity to camptothecin, defective sporulation, reduced hairpin-induced recombination, impaired DSB processing, persistent Mre11 foci, and delayed Rad52 recruitment. Sae2 is phosphorylated at additional (S/T)Q motifs by Mec1 and Tel1 in reponse to DNA damage and mutation of these phosphorylation sites also impairs DNA repair (Baroni *et al.* 2004). These phosphorylation events activate Sae2 through a transition from an insoluble oligomeric state to active monomers/dimers, which allow the protein to be recruited to sites of DNA damage (Fu *et al.* 2014). Further, the stability of Sae2 is regulated by acetylation and treatment with the histone deacetylase inhibitor valproic acid causes accumulation of acetylated Sae2 and degradation of Sae2 (Robert *et al.* 2011). The mechanism for acetylation of Sae2 remains to be determined. Extensive resection is promoted in S and G2 phases by Cdc28-dependent phosphorylation of Dna2 at threonine 4 and serines 17 and 237, which is required for its recruitment to DSBs (Chen *et al.* 2011). Further, Dna2 shuttles from the cytoplasm to the nucleus upon phosphorylation on serine 17 by Cdc28 (Kosugi *et al.* 2009). Interestingly, resection in G2 is more dependent on MRX than in cycling cells, suggesting replication forks could serve to recruit Exo1 and/or STR–Dna2 in lieu of the MRX complex, possibly through RPA, which interacts directly with Dna2 (Bae *et al.* 2003; Chen *et al.* 2013). Loss of Rad9 can partially bypass the Cdc28 requirement for resection, suggesting that Rad9 could also be a target of the Cdc28-dependent regulation of resection (Lazzaro *et al.* 2008).

The nucleolytically produced 3′ single-stranded DNA ends are bound by RPA (Alani *et al.* 1992). The recruitment of Rad52 to ssDNA by RPA in S/G2 phase requires Cdc28 activity (Alabert *et al.* 2009; Barlow and Rothstein 2009), however the responsible phosphorylation sites have not been identified. Rad55 is subject to Mec1-dependent phosphorylation on serines 2, 8, and 14 in response to MMS or an HO-induced DSB (Bashkirov *et al.* 2000; Herzberg *et al.* 2006), and a *rad55-S2,8,14A* mutant exhibits reduced survival after DNA damage although the underlying mechanism remains to be established. The Rad51 recombinase itself is phosphorylated in a Mec1-dependent manner at serine 192 in response to DNA damage (Flott *et al.* 2011). Biochemical analysis indicates that serine 192 is required for Rad51 ATP hydrolysis and DNA binding, whereas mutation of serine 192 does not interfere with Rad51 multimerization. Srs2 is inhibited by Cdc28 phosphorylation at multiple sites to allow Rad51-dependent DSB repair via SDSA by controlling turnover of Srs2 at the invading strand (Saponaro *et al.* 2010).

Some aspects of recombination are also likely to be regulated by sumoylation of RPA, Rad52, Rad59, and Srs2 (Sacher *et al.* 2006; Burgess *et al.* 2007; Ohuchi *et al.* 2008; Saponaro *et al.* 2010; Cremona *et al.* 2012; Psakhye and Jentsch 2012). For Rad52, it was shown that sumoylation on lysines 43, 44, and 253 inhibits its ssDNA binding and annealing activities without affecting its interaction with Rad51 and RPA (Altmannova *et al.* 2010), and sumoylation of Rad52 protects it from proteasomal degradation (Sacher *et al.* 2006). Moreover, stimulating Rad52 sumoylation by overexpression of the Siz2 SUMO ligase or by fusing SUMO to the C terminus of Rad52 suppressed the DNA damage sensitivity of *srs2* cells (Esta *et al.* 2013). As a consequence, sumoylation of Rad52 improves the fidelity of recombinational repair by shifting DSB repair from SSA to gene conversion (Sacher *et al.* 2006; Altmannova *et al.* 2010). Sumoylation of Srs2 on lysines 1081, 1089, and 1142 appears to inhibit recombinational repair as mutation of these lysines to arginine partially suppresses the DNA repair

Table 2 Regulation of HR proteins by post-translational modifications

Target	PTM	Modifier(s)	Function	References
Sae2	S267^P	Cdc28	Promotes resection	Aylon *et al.* (2004); Huertas *et al.* (2008); Ira *et al.* (2004); Zierhut and Diffley (2008)
	P	Tel1/Mec1	Activation through solubilization	Baroni *et al.* (2004); Fu *et al.* (2014)
	Ac	?	Degradation	Robert *et al.* (2011)
Dna2	T4^P, S17^P, S237^P	Cdc28	Promotes long-range resection	Chen *et al.* (2011)
	S17^P	Cdc28	Nuclear localization	Kosugi *et al.* (2009)
Rad55	S2^P, S8^P, S14^P	Mec1	?	Bashkirov *et al.* (2000); Herzberg *et al.* (2006)
Rad51	S192^P	Mec1	ATPase regulation	Flott *et al.* (2011)
Srs2	P	CDC28	Inhibits displacement of Rad51	Saponaro *et al.* (2010)
	K1081^S, K1089^S, K1142^S	?	Promotes displacement of Rad51	Saponaro *et al.* (2010)
Rad52	K43^S, K44^S, K253^S	Siz2	Inhibits DNA binding and annealing of ssDNA	Altmannova *et al.* (2010); Sacher *et al.* (2006)
	Ub	Slx5–Slx8	?	(Ii *et al.* (2007)
Slx4	T113^P	Mec1/Tel1	Activation of Rad1-Rad10 nuclease	Toh *et al.* (2010)
	P	Mec1/Tel1	Interaction with Dpb11	Ohouo *et al.* (2010)
Mms4	P	CDC28, Cdc5	Activation in M phase	Loog and Morgan (2005); Matos *et al.* (2011)
Yen1	P	CDC28, Cdc5	Inhibition outside of anaphase	Loog and Morgan (2005); Matos *et al.* (2011)
	S655^P, S679^P	CDC28	Nuclear localization	Eissler *et al.* (2014); Kosugi *et al.* (2009)
Rad1	K32^S	Siz1, Siz2	Inhibition of DNA binding	Sarangi *et al.* (2014)
PCNA	K127^S, K164^S	?	Binding of Srs2	Hoege *et al.* (2002); Papouli *et al.* (2005)
	K164^{Ub}	Rad6–Rad18	Promotes translesion synthesis	Hoege *et al.* (2002)
	K164$^{Ub}{}_n$	Rad5–Ubc13–Mms2	Promotes error-free lesion bypass by template switching	Blastyak *et al.* (2007); Hoege *et al.* (2002)
Rtt107	P	Mec1 (Slx4)	Replication restart	Roberts *et al.* (2006)
Mcm2–7	S164^P, S170^P	Mec1 (Mrc1)	Replication restart	Randell *et al.* (2010); Stead *et al.* (2012)

P, phosphorylation. Ac, acetylation. S, sumoylation. Ub, ubiquitylation. Parentheses indicate partial dependency. ?, unknown.

defect of a Cdc28-phosphorylation deficient *srs2* mutant (Saponaro *et al.* 2010), suggesting that phosphorylation and sumoylation may have counteracting effects on Srs2 activity. Furthermore, sumoylation of Srs2 decreases its interaction with sumoylated PCNA and at the same time sumoylated PCNA inhibits Srs2 sumoylation (Kolesar *et al.* 2012). This observation suggests that sumoylation could make Srs2 association to the replication fork more dynamic. The effects of RPA and Rad59 sumoylation on recombination remain to be established in yeast. However, in human cells, RPA1 sumoylation facilitates recruitment of Rad51 to DNA damage-induced foci to initiate DNA repair through homologous recombination (Dou *et al.* 2010). It has also been suggested that desumoylation of repair proteins by the SUMO-specific protease Ulp1, which associates with the NPC (Takahashi *et al.* 2000), is responsible for the stimulation of spontaneous gene conversion observed at a locus artificially tethered to the nuclear envelope or to the NPC (Nagai *et al.* 2008). This notion is supported by changes in sumoylation patterns of RPA, Rad52, and Rad59 observed in nucleoporin mutants and in *slx8* (Burgess *et al.* 2007; Palancade *et al.* 2007).

The role of Rad1–Rad10 in processing heterologous flaps in collaboration with Slx4 requires phosphorylation of Slx4 on threonine 113 by Mec1 or Tel1 (Toh *et al.* 2010). Rad1 is sumoylated at lysine 32, which decreases the affinity of the Rad1–Rad10 for DNA without affecting its other activities, suggesting that Rad1 sumoylation promotes its disengagement from DNA after nuclease cleavage (Sarangi *et al.* 2014). Mus81–Mms4 and Yen1 activity are restricted to the G2/M transition and anaphase, respectively, by Cdc5- and Cdc28-dependent phosphorylation (Loog and Morgan 2005; Matos *et al.* 2011; Matos *et al.* 2013; Saugar *et al.* 2013). Premature activation of Mus81–Mms4 using a phosphomimetic Mms4 allele or by untimely activation of Cdc5 increases crossover-associated recombination events (Matos *et al.* 2013; Szakal and Branzei 2013). Yen1 is inactivated by Cdc28-dependent phosphorylation and activated at anaphase by the Cdc14 phosphatase (Blanco *et al.* 2014; Eissler *et al.* 2014). Further, to provide another level of regulation, Yen1 relocalizes from the cytoplasm to the nucleus upon phosphorylation on serines 655 and 679 by Cdc28 in G2/M phase (Kosugi *et al.* 2009; Blanco *et al.* 2014; Eissler *et al.* 2014).

Regulation of recombinational restart of replication by PTMs: A range of DNA lesions may cause stalling of DNA replication. Replication fork blockage activates the replication checkpoint, which is responsible for slowing of S-phase and cell cycle progression, down-regulation of late origin firing, activation of DNA repair proteins, and stabilization of replication forks (reviewed in Friedel *et al.* 2009). The replication checkpoint is mediated by the Mec1 kinase and its downstream effector kinase Rad53. In the absence of Mec1 or Rad53, stalled replication forks collapse and the replisome dissociates (Tercero and Diffley 2001; Cobb *et al.* 2003), which is likely due to the failure to phosphorylate functional targets at the replication fork such as Mrc1, Pol31, Rtt107, Dbf4, and Pol1 (Osborn and Elledge 2003; Roberts *et al.* 2006; Chen *et al.* 2010; Randell *et al.* 2010). In contrast to active or stalled replication forks, which are refractory to

recruitment of Rad52 (Lisby *et al.* 2004; Alabert *et al.* 2009), collapsed replication forks readily recruit Rad52 into foci.

PCNA is the master regulator of DNA damage tolerance pathways at the replication fork. During S phase and in response to replication stress by hydroxyurea, PCNA is sumoylated at lysines 127 and 164 (Hoege *et al.* 2002; Papouli *et al.* 2005). Sumoylated PCNA is bound by the Srs2 helicase (Papouli *et al.* 2005), which acts as an anti-recombinase by displacing Rad51 from single-stranded DNA (Krejci *et al.* 2003; Veaute *et al.* 2003). Accordingly, Rad51 is enriched at the replication fork in a nonsumoylatable *pol30-K127,164R* mutant (Papouli *et al.* 2005). In contrast, when DNA damage such as UV- or MMS-induced lesions are encountered by the replication fork, PCNA is first monoubiquitylated at lysine 164 by the Rad6–Rad18 pathway (Hoege *et al.* 2002), which promotes translesion synthesis by a number of error-prone polymerases (reviewed in Finley *et al.* 2012). Sumoylation and ubiquitylation of PCNA are independent processes (Papouli *et al.* 2005). Lysine 164 of PCNA can be further modified by K63-linked polyubiquitylation through the Rad5–Ubc13–Mms2 pathway (Hoege *et al.* 2002), which facilitates error-free repair of lesions on the leading strand template by fork regression and template switching using the Rad5 helicase (Blastyak *et al.* 2007), while Rad52 mediates error-free repair of lesions on the lagging strand template (Prakash 1981; Zhang and Lawrence 2005; Gangavarapu *et al.* 2007) (Figure 6). However, the majority of error-free lesion bypass was reported to be *RAD52* independent (Zhang and Lawrence 2005).

The role of Mec1-dependent phosphorylation during the restart of stalled replication forks is still poorly understood. Mec1-dependent phosphorylation of Slx4 facilitates assembly of an Rtt107–Slx4–Dpb11 complex at stalled forks (Ohouo *et al.* 2010). Further, Slx4-dependent phosphorylation of Rtt107 by Mec1 is critical for replication restart after alkylation damage (Roberts *et al.* 2006). Possibly, the Rtt107–Slx4–Dpb11 complex acts as a scaffold for the assembly of additional fork stabilizing and repair factors such as the Smc5–Smc6–Mms21 SUMO ligase (Ohouo *et al.* 2010; Leung *et al.* 2011). The Smc5–Smc6–Mms21 complex was shown to promote sister-chromatid junction-mediated intra-S repair (Branzei *et al.* 2006; De Piccoli *et al.* 2006; Sollier *et al.* 2009), although the relevant sumoylation targets remain to be identified. Finally, Mrc1 facilitates Mec1 phosphorylation of the S/T-Q motifs of chromatin-bound Mcm2–7 during S phase to facilitate replication restart during replication stress (Randell *et al.* 2010; Stead *et al.* 2012).

The Slx5–Slx8 (SUMO-targeted ubiquitin E3 ligase) is localized to replication foci and is important for suppressing recombination during DNA replication (Burgess *et al.* 2007). This may be explained by the observation that deletion of *SLX5*–*SLX8* results in reduced levels of Rad52, Rad59, and RPA sumoylation. In the case of Rad52, its sumoylation inhibits recombination (Sacher *et al.* 2006; Altmannova *et al.* 2010). *In vitro*, Rad52 and Rad57 are targets of the ubiquitylation activity of Slx5–Slx8 (Ii *et al.* 2007).

C. Role of chromatin in controlling mitotic recombination

Several lines of evidence suggest that homologous recombination is controlled by modification of chromatin structure (Chai *et al.* 2005; Shim *et al.* 2005; Tsukuda *et al.* 2005; Kent *et al.* 2007; Van Attikum *et al.* 2007; Sinha *et al.* 2009; Sinha and Peterson 2009; Tsukuda *et al.* 2009; Chen *et al.* 2012; Costelloe *et al.* 2012; Adkins *et al.* 2013). Similarly, capping of telomeres is likely a major barrier for recombination at telomere sequences either by inhibiting recombination proteins or by preventing resection of telomeres (Grossi *et al.* 2001; Dubois *et al.* 2002).

One of the principal and evolutionarily conserved chromatin marks associated with DNA damage that expose DNA ends or single-stranded regions is the phosphorylation of histone H2A on serine 129 by the Tel1 and Mec1 kinases (Rogakou *et al.* 1999; Downs *et al.* 2000; Shroff *et al.* 2004). An *hta-S129A* mutant is sensitive to DNA damage-inducing agents such as phleomycin, camptothecin, and methyl methanesulfonate (Redon *et al.* 2003; Downs *et al.* 2004). The modification of chromatin by H2A phosphorylation occurs preferentially at unresected DSB ends and in G1 phase, whereas the recruitment of chromatin modifiers NuA4, SWR1, RSC, SWI/SNF, and INO80 occurs in G2/M and correlates with homologous recombination (Downs *et al.* 2004; Morrison *et al.* 2004; Van Attikum *et al.* 2004; Bennett *et al.* 2013). The NuA4 complex contains an associated histone acetyltransferase, which targets histone H4 for acetylation and is important for DNA repair (Choy and Kron 2002; Downs *et al.* 2004). Histone H3-K56 acetylation, which is formed transiently by Rtt109 during DNA replication, is important for sister-chromatid repair of DSBs arising during replication (Munoz-Galvan *et al.* 2013). Other histone acetyltransferases, such as Gcn5 and Hat1, also contribute to the wave of chromatin acetylation that follows DSB formation (Qin and Parthun 2002; Tamburini and Tyler 2005). A number of histone deacetylases including Rpd3, Hda1, Sir2, and Hst1 are also recruited to sites of DNA damage presumably to remove DNA damage-induced chromatin marks after completion of repair (Robert *et al.* 2011; Tamburini and Tyler 2005). The acetylation marks may serve to stabilize the SWR1 complex at DSBs via binding of its Bdf1 subunit through its double bromodomain (Kobor *et al.* 2004). SWR1 mediates deposition of the histone variant Htz1 (H2A.Z) in place of H2A in chromatin (Kobor *et al.* 2004; Mizuguchi *et al.* 2004; Morillo-Huesca *et al.* 2010). The effects of these chromatin modifications are not fully understood, but it has been reported that deposition of Htz1 into chromatin is important for DSB end resection (Kalocsay *et al.* 2009). The checkpoint adaptor Rad9 is recruited to sites of DNA damage likely through a dual interaction of its BRCT domains with H2A-S129^{P} and its Tudor domain with histone H3 methylated at lysine 79 (H3-K79^{Me}) (Giannattasio *et al.* 2005; Javaheri *et al.* 2006; Toh *et al.* 2006; Hammet *et al.* 2007; Germann *et al.*

2011). Finally, H2A-S129 phosphorylation is also required for loading cohesins at a defined DSB (Unal *et al.* 2004).

ATP-dependent chromatin remodeling is equally important for efficient homologous recombination especially in heterochromatin (Sinha and Peterson 2009; Sinha *et al.* 2009). The RSC complex is one of the earliest factors recruited to a DSB along with the MRX and Ku complexes (Shim *et al.* 2005), but RSC also appears to have a later role in recombination following synapsis (Chai *et al.* 2005). The RSC complex produces a histone-free region of a few hundred nucleotides immediately adjacent to a DSB to promote binding of the MRX and Ku complexes and to facilitate resection (Kent *et al.* 2007; Shim *et al.* 2007; Adkins *et al.* 2013). The later role of RSC in recombination may be linked to its interaction with Rad59 and/or its involvement in loading of cohesin at DNA breaks (see below) (Oum *et al.* 2011). In contrast, the SWI/SNF chromatin-remodeling complex was found to play a role at or preceding the strand-invasion step of HR (Chai *et al.* 2005). The Fun30 nucleosome-remodeling factor is important for extensive resection by Exo1 and Sgs1–Dna2 (Chen *et al.* 2012; Costelloe *et al.* 2012; Eapen *et al.* 2012). A fourth ATP-dependent chromatin remodeling factor, Rad54, enhances DNA strand invasion by Rad51 on chromatin substrates *in vitro* (Alexiadis and Kadonaga 2002), and further plays a crucial role *in vivo* for the initiation of DNA synthesis after strand invasion likely by enhancing the accessibility to DNA within nucleosomal arrays (Jaskelioff *et al.* 2003; Sugawara *et al.* 2003; Ceballos and Heyer 2011). Rad54 interacts directly with histone H3 and its chromatin-remodeling activity is stimulated by Rad51 (Jaskelioff *et al.* 2003; Kwon *et al.* 2007).

D. Role of cohesin in regulating mitotic recombination

Cohesin is loaded and maintained at double-strand breaks independent of global DNA replication and is required for efficient sister-chromatid recombination (Sjogren and Nasmyth 2001). Loading of cohesin at DSBs requires H2A-S129^{P}, Mre11, and Scc2, a component of the cohesin loading machinery (Strom *et al.* 2004; Unal *et al.* 2004), and is further aided by the RSC chromatin remodeler and Rad59 (Oum *et al.* 2011). Replication-independent cohesion is induced genome-wide by the Eco1 acetyltransferase, which targets Smc3 in response to DNA damage (Strom *et al.* 2007; Unal *et al.* 2007; Heidinger-Pauli *et al.* 2009). The establishment of damage-induced cohesion by cohesin acetylation is further aided by Ctf4, Ctf18, Tof1, Csm3, Chl1, and Mrc1 (Borges *et al.* 2013). In contrast, cohesion must be relieved locally by separase in order for efficient resection of DSBs during postreplicative repair (McAleenan *et al.* 2013). Another structural maintenance of chromosomes (SMC) factor, which is loaded at DSBs by Scc2 is the Smc5–Smc6 complex in a manner dependent on the BRCT domain-containing protein Rtt107/Esc4 (Lindroos *et al.* 2006; Leung *et al.* 2011). The Smc5–Smc6 complex is required for MMS-induced recombination and DSB repair (Onoda *et al.* 2004; De Piccoli *et al.* 2006).

E. Ploidy/aneuploidy

Mitotic recombination is more efficient in heterozygous *MAT***a**/*MAT*α diploids than in homozygous *MAT***a**/*MAT***a** or *MAT*α/*MAT*α cells, both spontaneously and after UV irradiation (Friis and Roman 1968; Hopper *et al.* 1975; Esposito and Watsgaff 1981). In diploid yeast, NHEJ is severely disabled through the repression of *NEJ1*, a key component of NHEJ, by the transcriptional repressor, Mata1–Matα2 (Heude and Fabre 1993; Frank-Vaillant and Marcand 2001). As expected, diploids are more radioresistant than haploids due to the extra copy of the genome (Mortimer 1958). However, a further increase in ploidy leads to increased radiosensitivity and reliance on homologous recombination for survival (Storchova *et al.* 2006). In response to replication stress, haploid cells use the Rad6-dependent pathways that resume stalled forks, whereas diploid cells use homologous recombination (Li and Tye 2011). Indeed, the DNA damage sensitivity of *rad6* and *rad18* mutants is suppressed by mating-type heterozygosity in a *RAD52*-dependent manner (Yan *et al.* 1995). The IR sensitivity of *rad55* and *rad57* mutants is also suppressed by *MAT* heterozygosity by an unknown mechanism. Although the mechanism was reported to be due to loss of NHEJ, in other strain backgrounds *rad55 yku70* or *rad55 dnl4* mutants retain IR sensitivity (Valencia-Burton *et al.* 2006; Fung *et al.* 2009). Mutation of *SRS2* suppresses the IR sensitivity of *rad55* and *rad57* mutants consistent with the model that Srs2 and Rad55–Rad57 have opposing roles in Rad51 nucleoprotein filament stability (Fung *et al.* 2009; Liu *et al.* 2011). *SRS2* transcript levels vary in response to ploidy and might contribute to the *MAT* heterozygosity suppression of *rad6*, *rad18*, *rad55*, and *rad57* mutants. The only recombination gene that is clearly regulated at the transcriptional level by mating type is *RDH54*. There is a Mata1–Matα2 binding site within the *RDH54* promoter and Rdh54 protein level is reduced fivefold in diploids compared with haploids (de Godoy *et al.* 2008; Galgoczy *et al.* 2004). Surprisingly, the phenotype of *rdh54* mutants is most apparent in diploids (lethality with *rad54* and *srs2*), suggesting the reduced levels are nevertheless required for HR (Klein 1997).

VII. Postscript

The study of mitotic recombination in yeast has progressed immensely since the last time a chapter in the precursor of the YeastBook was written (Petes *et al.* 1991). Much has been learned about the many genes that act to preserve genome stability through genetics, biochemistry, and cell biology. However, there is still important work to be done. The *in vitro* reconstitution of many of the fundamental reactions has not yet been achieved. In addition, the emergence of single molecule techniques for defining biochemical reactions will greatly expand our detailed understanding of the steps for each of these reactions. Genetic analysis of combinations of mutants will be needed to push the boundaries to define new gene and pathway interactions. The underlying cell biology of these genes and pathways must also be

examined in detail. Finally, high throughput methods should be applied to more processes, which promises a greater depth of understanding of the relationships between the genetic, biochemical, and cell biological processes that are involved in this basic cellular function.

Acknowledgments

We thank Tom Petes, Peter Philippsen, Eric Bryant, Patrick Sung, and members of his laboratory and Patrick Ruff for insightful comments on the manuscript. We are grateful to the following funding agencies for financial support: Danish Council for Independent Research (FNU: 12-127136), the Villum Kann Rasmussen Foundation and the European Research Council (ERCStG, no. 242905) to M.L., and National Institutes of Health (GM50237 and GM67055 to R.R. and GM41784 and GM94386 to L.S.S.).

Literature Cited

Adkins, N. L., H. Niu, P. Sung, and C. L. Peterson, 2013 Nucleosome dynamics regulates DNA processing. Nat. Struct. Mol. Biol. 20: 836–842.

Agmon, N., B. Liefshitz, C. Zimmer, E. Fabre, and M. Kupiec, 2013 Effect of nuclear architecture on the efficiency of double-strand break repair. Nat. Cell Biol. 15: 694–699.

Agmon, N., M. Yovel, Y. Harari, B. Liefshitz, and M. Kupiec, 2011 The role of Holliday junction resolvases in the repair of spontaneous and induced DNA damage. Nucleic Acids Res. 39: 7009–7019.

Aguilera, A., and H. L. Klein, 1988 Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. Genetics 119: 779–790.

Aguilera, A., and B. Gomez-Gonzalez, 2008 Genome instability: a mechanistic view of its causes and consequences. Nat. Rev. 9: 204–217.

Aguilera, A., and T. Garcia-Muse, 2012 R loops: from transcription byproducts to threats to genome stability. Mol. Cell 46: 115–124.

Ajimura, M., S. H. Leem, and H. Ogawa, 1993 Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. Genetics 133: 51–66.

Aksenova, A. Y., P. W. Greenwell, M. Dominska, A. A. Shishkin, J. C. Kim *et al.*, 2013 Genome rearrangements caused by interstitial telomeric sequences in yeast. Proc. Natl. Acad. Sci. USA 110: 19866–19871.

Alabert, C., J. N. Bianco, and P. Pasero, 2009 Differential regulation of homologous recombination at DNA breaks and replication forks by the Mrc1 branch of the S-phase checkpoint. EMBO J. 28: 1131–1141.

Alani, E., S. Subbiah, and N. Kleckner, 1989 The yeast *RAD50* gene encodes a predicted 153-kD protein containing a purine nucleotide-binding domain and two large heptad-repeat regions. Genetics 122: 47–57.

Alani, E., R. Padmore, and N. Kleckner, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell 61: 419–436.

Alani, E., R. Thresher, J. D. Griffith, and R. D. Kolodner, 1992 Characterization of DNA-binding and strand-exchange stimulation properties of y-RPA, a yeast single-strand-DNA-binding protein. J. Mol. Biol. 227: 54–71.

Alexeev, A., A. Mazin, and S. C. Kowalczykowski, 2003 Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. Nat. Struct. Biol. 10: 182–186.

Alexiadis, V., and J. T. Kadonaga, 2002 Strand pairing by Rad54 and Rad51 is enhanced by chromatin. Genes Dev. 16: 2767–2771.

Altmannova, V., N. Eckert-Boulet, M. Arneric, P. Kolesar, R. Chaloupkova *et al.*, 2010 Rad52 SUMOylation affects the efficiency of the DNA repair. Nucleic Acids Res. 38: 4708–4721.

Alvaro, D., M. Lisby, and R. Rothstein, 2007 Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. PLoS Genet. 3: e228.

Andersen, M. P., Z. W. Nelson, E. D. Hetrick, and D. E. Gottschling, 2008 A genetic screen for increased loss of heterozygosity in *Saccharomyces cerevisiae*. Genetics 179: 1179–1195.

Antoniacci, L. M., M. A. Kenna, and R. V. Skibbens, 2007 The nuclear envelope and spindle pole body-associated Mps3 protein bind telomere regulators and function in telomere clustering. Cell Cycle 6: 75–79.

Argueso, J. L., J. Westmoreland, P. A. Mieczkowski, M. Gawel, T. D. Petes *et al.*, 2008 Double-strand breaks associated with repetitive DNA can reshape the genome. Proc. Natl. Acad. Sci. USA 105: 11845–11850.

Atkinson, J., and P. McGlynn, 2009 Replication fork reversal and the maintenance of genome stability. Nucleic Acids Res. 37: 3475–3492.

Aylon, Y., B. Liefshitz, and M. Kupiec, 2004 The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle. EMBO J. 23: 4868–4875.

Bae, K. H., H. S. Kim, S. H. Bae, H. Y. Kang, S. Brill *et al.*, 2003 Bimodal interaction between replication-protein A and Dna2 is critical for Dna2 function both *in vivo* and *in vitro*. Nucleic Acids Res. 31: 3006–3015.

Bai, Y., and L. S. Symington, 1996 A Rad52 homolog is required for *RAD51*-independent mitotic recombination in *Saccharomyces cerevisiae*. Genes Dev. 10: 2025–2037.

Ball, L. G., K. Zhang, J. A. Cobb, C. Boone, and W. Xiao, 2009 The yeast Shu complex couples error-free post-replication repair to homologous recombination. Mol. Microbiol. 73: 89–102.

Ballew, B. J., M. Yeager, K. Jacobs, N. Giri, J. Boland *et al.*, 2013 Germline mutations of regulator of telomere elongation helicase 1, RTEL1, in Dyskeratosis congenita. Hum. Genet. 132: 473–480.

Barbera, M. A., and T. D. Petes, 2006 Selection and analysis of spontaneous reciprocal mitotic cross-overs in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 103: 12819–12824.

Barlow, J. H., and R. Rothstein, 2009 Rad52 recruitment is DNA replication independent and regulated by Cdc28 and the Mec1 kinase. EMBO J. 28: 1121–1130.

Barlow, J. H., M. Lisby, and R. Rothstein, 2008 Differential regulation of the cellular response to DNA double-strand breaks in G1. Mol. Cell 30: 73–85.

Baroni, E., V. Viscardi, H. Cartagena-Lirola, G. Lucchini, and M. P. Longhese, 2004 The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. Mol. Cell. Biol. 24: 4151–4165.

Bartkova, J., Z. Horejsi, M. Sehested, J. M. Nesland, E. Rajpert-De Meyts *et al.*, 2007 DNA damage response mediators MDC1 and 53BP1: constitutive activation and aberrant loss in breast and lung cancer, but not in testicular germ cell tumours. Oncogene 26: 7414–7422.

Bartsch, S., L. E. Kang, and L. S. Symington, 2000 *RAD51* is required for the repair of plasmid double-stranded DNA gaps from either plasmid or chromosomal templates. Mol. Cell. Biol. 20: 1194–1205.

Bashkirov, V. I., J. S. King, E. V. Bashkirova, J. Schmuckli-Maurer, and W. D. Heyer, 2000 DNA repair protein Rad55 is a terminal substrate of the DNA damage checkpoints. Mol. Cell. Biol. 20: 4393–4404.
Basile, G., M. Aker, and R. K. Mortimer, 1992 Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51*. Mol. Cell. Biol. 12: 3235–3246.
Bastin-Shanower, S. A., W. M. Fricke, J. R. Mullen, and S. J. Brill, 2003 The mechanism of Mus81-Mms4 cleavage site selection distinguishes it from the homologous endonuclease Rad1-Rad10. Mol. Cell. Biol. 23: 3487–3496.
Bennett, G., M. Papamichos-Chronakis, and C. L. Peterson, 2013 DNA repair choice defines a common pathway for recruitment of chromatin regulators. Nat. Commun. 4: 2084.
Benton, M. G., S. Somasundaram, J. D. Glasner, and S. P. Palecek, 2006 Analyzing the dose-dependence of the *Saccharomyces cerevisiae* global transcriptional response to methyl methanesulfonate and ionizing radiation. BMC Genomics 7: 305.
Bermejo, R., T. Capra, R. Jossen, A. Colosio, C. Frattini *et al.*, 2011 The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. Cell 146: 233–246.
Bernstein, K. A., R. J. Reid, I. Sunjevaric, K. Demuth, R. C. Burgess *et al.*, 2011 The Shu complex, which contains Rad51 paralogues, promotes DNA repair through inhibition of the Srs2 anti-recombinase. Mol. Biol. Cell 22: 1599–1607.
Blanco, M. G., J. Matos, U. Rass, S. C. Ip, and S. C. West, 2010 Functional overlap between the structure-specific nucleases Yen1 and Mus81-Mms4 for DNA-damage repair in *S. cerevisiae*. DNA Repair (Amst.) 9: 394–402.
Blanco, M. G., J. Matos, and S. C. West, 2014 Dual control of Yen1 nuclease activity and cellular localization by Cdk and Cdc14 prevents genome instability. Mol. Cell 54: 94–106.
Blastyak, A., L. Pinter, I. Unk, L. Prakash, S. Prakash *et al.*, 2007 Yeast Rad5 protein required for postreplication repair has a DNA helicase activity specific for replication fork regression. Mol. Cell 28: 167–175.
Boddy, M. N., P. H. Gaillard, W. H. McDonald, P. Shanahan, J. R. Yates 3rd. *et al.*, 2001 Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell 107: 537–548.
Boiteux, S., and S. Jinks-Robertson, 2013 DNA repair mechanisms and the bypass of DNA damage in *Saccharomyces cerevisiae*. Genetics 193: 1025–1064.
Borges, V., D. J. Smith, I. Whitehouse, and F. Uhlmann, 2013 An Eco1-independent sister chromatid cohesion establishment pathway in *S. cerevisiae*. Chromosoma 122: 121–134.
Bosco, G., and J. E. Haber, 1998 Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture. Genetics 150: 1037–1047.
Boundy-Mills, K. L., and D. M. Livingston, 1993 A *Saccharomyces cerevisiae RAD52* allele expressing a C-terminal truncation protein: activities and intragenic complementation of missense mutations. Genetics 133: 33–49.
Branzei, D., J. Sollier, G. Liberi, X. Zhao, D. Maeda *et al.*, 2006 Ubc9- and mms21-mediated sumoylation counteracts recombinogenic events at damaged replication forks. Cell 127: 509–522.
Branzei, D., F. Vanoli, and M. Foiani, 2008 SUMOylation regulates Rad18-mediated template switch. Nature 456: 915–920.
Bressan, D. A., B. K. Baxter, and J. H. Petrini, 1999 The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19: 7681–7687.
Brewer, B. J., and W. L. Fangman, 1988 A replication fork barrier at the 3′ end of yeast ribosomal RNA genes. Cell 55: 637–643.
Brocas, C., J. B. Charbonnier, C. Dherin, S. Gangloff, and L. Maloisel, 2010 Stable interactions between DNA polymerase *delta* catalytic and structural subunits are essential for efficient DNA repair. DNA Repair (Amst.) 9: 1098–1111.
Bupp, J. M., A. E. Martin, E. S. Stensrud, and S. L. Jaspersen, 2007 Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3. J. Cell Biol. 179: 845–854.
Burgess, R. C., M. Lisby, V. Altmannova, L. Krejci, P. Sung *et al.*, 2009 Localization of recombination proteins and Srs2 reveals anti-recombinase function *in vivo*. J. Cell Biol. 185: 969–981.
Burgess, R. C., S. Rahman, M. Lisby, R. Rothstein, and X. Zhao, 2007 The Slx5-Slx8 complex affects sumoylation of DNA repair proteins and negatively regulates recombination. Mol. Cell. Biol. 27: 6153–6162.
Burgess, S. M., and N. Kleckner, 1999 Collisions between yeast chromosomal loci *in vivo* are governed by three layers of organization. Genes Dev. 13: 1871–1883.
Burkovics, P., M. Sebesta, A. Sisakova, N. Plault, V. Szukacsov *et al.*, 2013 Srs2 mediates PCNA-SUMO-dependent inhibition of DNA repair synthesis. EMBO J. 32: 742–755.
Bzymek, M., N. H. Thayer, S. D. Oh, N. Kleckner, and N. Hunter, 2010 Double Holliday junctions are intermediates of DNA break repair. Nature 464: 937–941.
Casper, A. M., P. W. Greenwell, W. Tang, and T. D. Petes, 2009 Chromosome aberrations resulting from double-strand DNA breaks at a naturally occurring yeast fragile site composed of inverted ty elements are independent of Mre11p and Sae2p. Genetics 183: 423–439, 421SI–426SI.
Ceballos, S. J., and W. D. Heyer, 2011 Functions of the Snf2/Swi2 family Rad54 motor protein in homologous recombination. Biochim. Biophys. Acta 1809: 509–523.
Cejka, P., E. Cannavo, P. Polaczek, T. Masuda-Sasa, S. Pokharel *et al.*, 2010a DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. Nature 467: 112–116.
Cejka, P., J. L. Plank, C. Z. Bachrati, I. D. Hickson, and S. C. Kowalczykowski, 2010b Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. Nat. Struct. Mol. Biol. 17: 1377–1382.
Chai, B., J. Huang, B. R. Cairns, and B. C. Laurent, 2005 Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. Genes Dev. 19: 1656–1661.
Chan, J. E., and R. D. Kolodner, 2011 A genetic and structural study of genome rearrangements mediated by high copy repeat Ty1 elements. PLoS Genet. 7: e1002089.
Chen, C., K. Umezu, and R. D. Kolodner, 1998 Chromosomal rearrangements occur in *S. cerevisiae rfa1* mutator mutants due to mutagenic lesions processed by double-strand-break repair. Mol. Cell 2: 9–22.
Chen, H., M. Lisby, and L. S. Symington, 2013 RPA coordinates DNA end resection and prevents formation of DNA hairpins. Mol. Cell 50: 589–600.
Chen, L., K. Trujillo, W. Ramos, P. Sung, and A. E. Tomkinson, 2001 Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. Mol. Cell 8: 1105–1115.
Chen, S. H., C. P. Albuquerque, J. Liang, R. T. Suhandynata, and H. Zhou, 2010 A proteome-wide analysis of kinase-substrate network in the DNA damage response. J. Biol. Chem. 285: 12803–12812.
Chen, X., H. Niu, W. H. Chung, Z. Zhu, A. Papusha *et al.*, 2011 Cell cycle regulation of DNA double-strand break end resection by Cdk1-dependent Dna2 phosphorylation. Nat. Struct. Mol. Biol. 18: 1015–1019.
Chen, X., D. Cui, A. Papusha, X. Zhang, C. D. Chu *et al*., 2012 The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. Nature 489: 576–580.

Choy, J. S., and S. J. Kron, 2002 NuA4 subunit Yng2 function in intra-S-phase DNA damage response. Mol. Cell. Biol. 22: 8215–8225.
Christman, M. F., F. S. Dietrich, and G. R. Fink, 1988 Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. Cell 55: 413–425.
Chua, P., and S. Jinks-Robertson, 1991 Segregation of recombinant chromatids following mitotic crossing over in yeast. Genetics 129: 359–369.
Clerici, M., D. Mantiero, G. Lucchini, and M. P. Longhese, 2005 The *Saccharomyces cerevisiae* Sae2 protein promotes resection and bridging of double strand break ends. J. Biol. Chem. 280: 38631–38638.
Clerici, M., D. Mantiero, I. Guerini, G. Lucchini, and M. P. Longhese, 2008 The Yku70-Yku80 complex contributes to regulate double-strand break processing and checkpoint activation during the cell cycle. EMBO Rep. 9: 810–818.
Cloud, V., Y. L. Chan, J. Grubb, B. Budke, and D. K. Bishop, 2012 Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. Science 337: 1222–1225.
Cobb, J. A., L. Bjergbaek, K. Shimada, C. Frei, and S. M. Gasser, 2003 DNA polymerase stabilization at stalled replication forks requires Mec1 and the RecQ helicase Sgs1. EMBO J. 22: 4325–4336.
Coic, E., T. Feldman, A. S. Landman, and J. E. Haber, 2008 Mechanisms of Rad52-independent spontaneous and UV-induced mitotic recombination in *Saccharomyces cerevisiae*. Genetics 179: 199–211.
Cole, G. M., D. Schild, S. T. Lovett, and R. K. Mortimer, 1987 Regulation of RAD54- and RAD52-lacZ gene fusions in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. 7: 1078–1084.
Cook, C. E., M. Hochstrasser, and O. Kerscher, 2009 The SUMO-targeted ubiquitin ligase subunit Slx5 resides in nuclear foci and at sites of DNA breaks. Cell Cycle 8: 1080–1089.
Cortes-Ledesma, F., and A. Aguilera, 2006 Double-strand breaks arising by replication through a nick are repaired by cohesin-dependent sister-chromatid exchange. EMBO Rep. 7: 919–926.
Costelloe, T., R. Louge, N. Tomimatsu, B. Mukherjee, E. Martini *et al.*, 2012 The yeast Fun30 and human SMARCAD1 chromatin remodellers promote DNA end resection. Nature 489: 581–584.
Cotta-Ramusino, C., D. Fachinetti, C. Lucca, Y. Doksani, M. Lopes *et al.*, 2005 Exo1 processes stalled replication forks and counteracts fork reversal in checkpoint-defective cells. Mol. Cell 17: 153–159.
Cremona, C. A., P. Sarangi, Y. Yang, L. E. Hang, S. Rahman *et al.*, 2012 Extensive DNA damage-induced sumoylation contributes to replication and repair and acts in addition to the *mec1* checkpoint. Mol. Cell 45: 422–432.
Crossan, G. P., L. van der Weyden, I. V. Rosado, F. Langevin, P. H. L. Gaillard *et al.*, 2011 Disruption of mouse Slx4, a regulator of structure-specific nucleases, phenocopies Fanconi anemia. Nature Genet. 43: 147–152.
Davis, A. P., and L. S. Symington, 2001 The yeast recombinational repair protein Rad59 interacts with Rad52 and stimulates single-strand annealing. Genetics 159: 515–525.
Davis, A. P., and L. S. Symington, 2003 The Rad52-Rad59 complex interacts with Rad51 and replication protein A. DNA Repair (Amst.) 2: 1127–1134.
Davis, A. P., and L. S. Symington, 2004 *RAD51*-dependent break-induced replication in yeast. Mol. Cell. Biol. 24: 2344–2351.
de Godoy, L. M., J. V. Olsen, J. Cox, M. L. Nielsen, N. C. Hubner *et al.*, 2008 Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. Nature 455: 1251–1254.
de Jager, M., J. van Noort, D. C. van Gent, C. Dekker, R. Kanaar *et al.*, 2001 Human Rad50/Mre11 is a flexible complex that can tether DNA ends. Mol. Cell 8: 1129–1135.
De Muyt, A., L. Jessop, E. Kolar, A. Sourirajan, J. Chen *et al.*, 2012 BLM helicase ortholog Sgs1 is a central regulator of meiotic recombination intermediate metabolism. Mol. Cell 46: 43–53.
De Piccoli, G., F. Cortes-Ledesma, G. Ira, J. Torres-Rosell, S. Uhle *et al.*, 2006 Smc5-Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. Nat. Cell Biol. 8: 1032–1034.
Decottignies, A., 2007 Microhomology-mediated end joining in fission yeast is repressed by *pku70* and relies on genes involved in homologous recombination. Genetics 176: 1403–1415.
Deem, A., K. Barker, K. Vanhulle, B. Downing, A. Vayl *et al.*, 2008 Defective break-induced replication leads to half-crossovers in *Saccharomyces cerevisiae*. Genetics 179: 1845–1860.
Deem, A., A. Keszthelyi, T. Blackgrove, A. Vayl, B. Coffey *et al.*, 2011 Break-induced replication is highly inaccurate. PLoS Biol. 9: e1000594.
Defossez, P. A., R. Prusty, M. Kaeberlein, S. J. Lin, P. Ferrigno *et al.*, 1999 Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell 3: 447–455.
Deng, S. K., B. Gibb, M. J. de Almeida, E. C. Greene, and L. S. Symington, 2014 RPA antagonizes microhomology-mediated repair of DNA double-strand breaks. Nat. Struct. Mol. Biol. 21: 405–412.
Dion, V., V. Kalck, C. Horigome, B. D. Towbin, and S. M. Gasser, 2012 Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. Nat. Cell Biol. 14: 502–509.
Dion, V., V. Kalck, A. Seeber, T. Schleker, and S. M. Gasser, 2013 Cohesin and the nucleolus constrain the mobility of spontaneous repair foci. EMBO Rep. 14: 984–991.
Donnianni, R. A., and L. S. Symington, 2013 Break-induced replication occurs by conservative DNA synthesis. Proc. Natl. Acad. Sci. USA 110: 13475–13480.
Dornfeld, K. J., and D. M. Livingston, 1992 Plasmid recombination in a *rad52* mutant of *Saccharomyces cerevisiae*. Genetics 131: 261–276.
Dou, H., C. Huang, M. Singh, P. B. Carpenter, and E. T. Yeh, 2010 Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. Mol. Cell 39: 333–345.
Downs, J. A., N. F. Lowndes, and S. P. Jackson, 2000 A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. Nature 408: 1001–1004.
Downs, J. A., S. Allard, O. Jobin-Robitaille, A. Javaheri, A. Auger *et al.*, 2004 Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Mol. Cell 16: 979–990.
Duan, Z., M. Andronescu, K. Schutz, S. McIlwain, Y. J. Kim *et al.*, 2010 A three-dimensional model of the yeast genome. Nature 465: 363–367.
DuBois, M. L., Z. W. Haimberger, M. W. McIntosh, and D. E. Gottschling, 2002 A quantitative assay for telomere protection in *Saccharomyces cerevisiae*. Genetics 161: 995–1013.
Eapen, V. V., N. Sugawara, M. Tsabar, W. H. Wu, and J. E. Haber, 2012 The *Saccharomyces cerevisiae* chromatin remodeler Fun30 regulates DNA end resection and checkpoint deactivation. Mol. Cell. Biol. 32: 4727–4740.
Eckert-Boulet, N., R. Rothstein, and M. Lisby, 2011 Cell biology of homologous recombination in yeast. Methods Mol. Biol. 745: 523–536.
Eggler, A. L., R. B. Inman, and M. M. Cox, 2002 The Rad51-dependent pairing of long DNA substrates is stabilized by replication protein A. J. Biol. Chem. 277: 39280–39288.

Eissler, C. L., G. Mazon, B. L. Powers, S. N. Savinov, L. S. Symington *et al.*, 2014 The cdk/cdc14 module controls activation of the yen1 holliday junction resolvase to promote genome stability. Mol. Cell 54: 80–93.

El Hage, A., S. L. French, A. L. Beyer, and D. Tollervey, 2010 Loss of Topoisomerase I leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis. Genes Dev. 24: 1546–1558.

Elledge, S. J., and R. W. Davis, 1989 DNA damage induction of ribonucleotide reductase. Mol. Cell. Biol. 9: 4932–4940.

Elledge, S. J., and R. W. Davis, 1990 Two genes differentially regulated in the cell cycle and by DNA-damaging agents encode alternative regulatory subunits of ribonucleotide reductase. Genes Dev. 4: 740–751.

Esposito, M., 1978 Evidence that spontaneous mitotic recombination occurs at the two-strand stage. Proc. Natl. Acad. Sci. USA 75: 4436–4440.

Esposito, M., and J. Watsgaff, 1981 Mechanisms of mitotic recombination, p. 29 in *Molecular Biology of the Yeast Saccharomyces*, edited by J. Strathern, E. W. Jones, and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Essers, J., A. B. Houtsmuller, L. van Veelen, C. Paulusma, A. L. Nigg *et al.*, 2002 Nuclear dynamics of *RAD52* group homologous recombination proteins in response to DNA damage. EMBO J. 21: 2030–2037.

Esta, A., E. Ma, P. Dupaigne, L. Maloisel, R. Guerois *et al.*, 2013 Rad52 sumoylation prevents the toxicity of unproductive Rad51 filaments independently of the anti-recombinase Srs2. PLoS Genet. 9: e1003833.

Fabre, F., A. Chan, W. D. Heyer, and S. Gangloff, 2002 Alternate pathways involving Sgs1/Top3, Mus81/Mms4, and Srs2 prevent formation of toxic recombination intermediates from single-stranded gaps created by DNA replication. Proc. Natl. Acad. Sci. USA 99: 16887–16892.

Ferguson, D. O., and W. K. Holloman, 1996 Recombinational repair of gaps in DNA is asymmetric in *Ustilago maydis* and can be explained by a migrating D-loop model. Proc. Natl. Acad. Sci. USA 93: 5419–5424.

Ferrari, S. R., J. Grubb, and D. K. Bishop, 2009 The Mei5-Sae3 protein complex mediates Dmc1 activity in *Saccharomyces cerevisiae*. J. Biol. Chem. 284: 11766–11770.

Finley, D., H. D. Ulrich, T. Sommer, and P. Kaiser, 2012 The ubiquitin-proteasome system of *Saccharomyces cerevisiae*. Genetics 192: 319–360.

Fishman-Lobell, J., and J. E. Haber, 1992 Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1*. Science 258: 480–484.

Fishman-Lobell, J., N. Rudin, and J. E. Haber, 1992 Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. Mol. Cell. Biol. 12: 1292–1303.

Flott, S., C. Alabert, G. W. Toh, R. Toth, N. Sugawara *et al.*, 2007 Phosphorylation of Slx4 by Mec1 and Tel1 regulates the single-strand annealing mode of DNA repair in budding yeast. Mol. Cell. Biol. 27: 6433–6445.

Flott, S., Y. Kwon, Y. Z. Pigli, P. A. Rice, P. Sung *et al.*, 2011 Regulation of Rad51 function by phosphorylation. EMBO Rep. 12: 833–839.

Fortin, G. S., and L. S. Symington, 2002 Mutations in yeast Rad51 that partially bypass the requirement for Rad55 and Rad57 in DNA repair by increasing the stability of Rad51-DNA complexes. EMBO J. 21: 3160–3170.

Frank-Vaillant, M., and S. Marcand, 2001 NHEJ regulation by mating type is exercised through a novel protein, Lif2p, essential to the ligase IV pathway. Genes Dev. 15: 3005–3012.

Frei, C., and S. M. Gasser, 2000 The yeast Sgs1p helicase acts upstream of Rad53p in the DNA replication checkpoint and colocalizes with Rad53p in S-phase-specific foci. Genes Dev. 14: 81–96.

Freudenreich, C. H., J. B. Stavenhagen, and V. A. Zakian, 1997 Stability of a CTG/CAG trinucleotide repeat in yeast is dependent on its orientation in the genome. Mol. Cell. Biol. 17: 2090–2098.

Fricke, W. M., and S. J. Brill, 2003 Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes Dev. 17: 1768–1778.

Friedel, A. M., B. L. Pike, and S. M. Gasser, 2009 ATR/Mec1: coordinating fork stability and repair. Curr. Opin. Cell Biol. 21: 237–244.

Friis, J., and H. Roman, 1968 The effect of the mating-type alleles on intragenic recombination in yeast. Genetics 59: 33–36.

Fu, Q., J. Chow, K. A. Bernstein, N. Makharashvili, S. Arora *et al.*, 2014 Phosphorylation-regulated transitions in an oligomeric state control the activity of the *sae2* DNA repair enzyme. Mol. Cell. Biol. 34: 778–793.

Fu, Y., L. Pastushok, and W. Xiao, 2008 DNA damage-induced gene expression in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 32: 908–926.

Fung, C. W., A. M. Mozlin, and L. S. Symington, 2009 Suppression of the double-strand-break-repair defect of the *Saccharomyces cerevisiae rad57* mutant. Genetics 181: 1195–1206.

Furuse, M., Y. Nagase, H. Tsubouchi, K. Murakami-Murofushi, T. Shibata *et al.*, 1998 Distinct roles of two separable *in vitro* activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J. 17: 6412–6425.

Galgoczy, D. J., A. Cassidy-Stone, M. Llinas, S. M. O'Rourke, I. Herskowitz *et al.*, 2004 Genomic dissection of the cell-type-specification circuit in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 101: 18069–18074.

Galli, A., and R. H. Schiestl, 1998 Effects of DNA double-strand and single-strand breaks on intrachromosomal recombination events in cell-cycle-arrested yeast cells. Genetics 149: 1235–1250.

Game, J. C., and B. S. Cox, 1971 Allelism tests of mutants affecting sensitivity to radiation in yeast and a proposed nomenclature. Mutat. Res. 12: 328–331.

Game, J. C., and R. K. Mortimer, 1974 A genetic study of x-ray sensitive mutants in yeast. Mutat. Res. 24: 281–292.

Gangavarapu, V., S. Prakash, and L. Prakash, 2007 Requirement of *RAD52* group genes for postreplication repair of UV-damaged DNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 27: 7758–7764.

Gangloff, S., H. Zou, and R. Rothstein, 1996 Gene conversion plays the major role in controlling the stability of large tandem repeats in yeast. EMBO J. 15: 1715–1725.

Garcia, V., S. E. Phelps, S. Gray, and M. J. Neale, 2011 Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. Nature 479: 241–244.

Gasch, A. P., M. Huang, S. Metzner, D. Botstein, S. J. Elledge *et al.*, 2001 Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. Mol. Biol. Cell 12: 2987–3003.

Gatti, R. A., S. Becker-Catania, H. H. Chun, X. Sun, M. Mitui *et al.*, 2001 The pathogenesis of ataxia-telangiectasia. Learning from a Rosetta Stone. Clin. Rev. Allergy Immunol. 20: 87–108.

Germann, S. M., V. H. Oestergaard, C. Haas, P. Salis, A. Motegi *et al.*, 2011 Dpb11/TopBP1 plays distinct roles in DNA replication, checkpoint response and homologous recombination. DNA Repair (Amst.) 10: 210–224.

Ghosal, G., and K. Muniyappa, 2005 *Saccharomyces cerevisiae* Mre11 is a high-affinity G4 DNA-binding protein and a G-rich DNA-specific endonuclease: implications for replication of telomeric DNA. Nucleic Acids Res. 33: 4692–4703.

Giannattasio, M., F. Lazzaro, P. Plevani, and M. Muzi-Falconi, 2005 The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6-Bre1 and H3 methylation by Dot1. J. Biol. Chem. 280: 9879–9886.

Godin, S., A. Wier, F. Kabbinavar, D. S. Bratton-Palmer, H. Ghodke *et al.*, 2013 The Shu complex interacts with Rad51 through the Rad51 paralogues Rad55-Rad57 to mediate error-free recombination. Nucleic Acids Res. 41: 4525–4534.

Gomez-Gonzalez, B., M. Garcia-Rubio, R. Bermejo, H. Gaillard, K. Shirahige *et al.*, 2011 Genome-wide function of THO/TREX in active genes prevents R-loop-dependent replication obstacles. EMBO J. 30: 3106–3119.

Gonzalez-Barrera, S., M. Garcia-Rubio, and A. Aguilera, 2002 Transcription and double-strand breaks induce similar mitotic recombination events in *Saccharomyces cerevisiae*. Genetics 162: 603–614.

Gonzalez-Barrera, S., F. Cortes-Ledesma, R. E. Wellinger, and A. Aguilera, 2003 Equal sister chromatid exchange is a major mechanism of double-strand break repair in yeast. Mol. Cell 11: 1661–1671.

Gotta, M., T. Laroche, A. Formenton, L. Maillet, H. Scherthan *et al.*, 1996 The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J. Cell Biol. 134: 1349–1363.

Gottlieb, S., and R. E. Esposito, 1989 A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. Cell 56: 771–776.

Gravel, S., J. R. Chapman, C. Magill, and S. P. Jackson, 2008 DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes Dev. 22: 2767–2772.

Gregg, S. Q., A. R. Robinson, and L. J. Niedernhofer, 2011 Physiological consequences of defects in ERCC1-XPF DNA repair endonuclease. DNA Repair (Amst.) 10: 781–791.

Grenon, M., T. Costelloe, S. Jimeno, A. O'Shaughnessy, J. Fitzgerald *et al.*, 2007 Docking onto chromatin via the *Saccharomyces cerevisiae* Rad9 Tudor domain. Yeast 24: 105–119.

Gritenaite, D., L. N. Princz, B. Szakal, S. C. Bantele, L. Wendeler *et al.*, 2014 A cell cycle-regulated Slx4-Dpb11 complex promotes the resolution of DNA repair intermediates linked to stalled replication. Genes Dev. 28: 1604–1619.

Grossi, S., A. Bianchi, P. Damay, and D. Shore, 2001 Telomere formation by rap1p binding site arrays reveals end-specific length regulation requirements and active telomeric recombination. Mol. Cell. Biol. 21: 8117–8128.

Haber, J. E., 2012 Mating-type genes and *MAT* switching in *Saccharomyces cerevisiae*. Genetics 191: 33–64.

Haber, J. E., and M. Hearn, 1985 *RAD52*-independent mitotic gene conversion in *Saccharomyces cerevisiae* frequently results in chromosomal loss. Genetics 111: 7–22.

Hammet, A., C. Magill, J. Heierhorst, and S. P. Jackson, 2007 Rad9 BRCT domain interaction with phosphorylated H2AX regulates the G1 checkpoint in budding yeast. EMBO Rep. 8: 851–857.

Hays, S. L., A. A. Firmenich, and P. Berg, 1995 Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. Proc. Natl. Acad. Sci. USA 92: 6925–6929.

Hediger, F., K. Dubrana, and S. M. Gasser, 2002 Myosin-like proteins 1 and 2 are not required for silencing or telomere anchoring, but act in the Tel1 pathway of telomere length control. J. Struct. Biol. 140: 79–91.

Heidinger-Pauli, J. M., E. Unal, and D. Koshland, 2009 Distinct targets of the Eco1 acetyltransferase modulate cohesion in S phase and in response to DNA damage. Mol. Cell 34: 311–321.

Herzberg, K., V. I. Bashkirov, M. Rolfsmeier, E. Haghnazari, W. H. McDonald *et al.*, 2006 Phosphorylation of Rad55 on serines 2, 8, and 14 is required for efficient homologous recombination in the recovery of stalled replication forks. Mol. Cell. Biol. 26: 8396–8409.

Heude, M., and F. Fabre, 1993 a/alpha-control of DNA repair in the yeast *Saccharomyces cerevisiae*: genetic and physiological aspects. Genetics 133: 489–498.

Hicks, W. M., M. Kim, and J. E. Haber, 2010 Increased mutagenesis and unique mutation signature associated with mitotic gene conversion. Science 329: 82–85.

Hicks, W. M., M. Yamaguchi, and J. E. Haber, 2011 Real-time analysis of double-strand DNA break repair by homologous recombination. Proc. Natl. Acad. Sci. USA 108: 3108–3115.

Higgins, N. P., K. Kato, and B. Strauss, 1976 A model for replication repair in mammalian cells. J. Mol. Biol. 101: 417–425.

Ho, C. K., G. Mazon, A. F. Lam, and L. S. Symington, 2010 Mus81 and Yen1 promote reciprocal exchange during mitotic recombination to maintain genome integrity in budding yeast. Mol. Cell 40: 988–1000.

Hoege, C., B. Pfander, G. L. Moldovan, G. Pyrowolakis, and S. Jentsch, 2002 *RAD6*-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419: 135–141.

Hohl, M., Y. Kwon, S. M. Galvan, X. Xue, C. Tous *et al.*, 2011 The Rad50 coiled-coil domain is indispensable for Mre11 complex functions. Nat. Struct. Mol. Biol. 18: 1124–1131.

Holbeck, S. L., and J. N. Strathern, 1997 A role for *REV3* in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae*. Genetics 147: 1017–1024.

Holzen, T. M., P. P. Shah, H. A. Olivares, and D. K. Bishop, 2006 Tid1/Rdh54 promotes dissociation of Dmc1 from nonrecombinogenic sites on meiotic chromatin. Genes Dev. 20: 2593–2604.

Hopfner, K. P., A. Karcher, L. Craig, T. T. Woo, J. P. Carney *et al.*, 2001 Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell 105: 473–485.

Hopfner, K. P., L. Craig, G. Moncalian, R. A. Zinkel, T. Usui *et al.*, 2002 The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature 418: 562–566.

Hopper, A. K., J. Kirsch, and B. D. Hall, 1975 Mating type and sporulation in yeast. II. Meiosis, recombination, and radiation sensitivity in an alpha-alpha diploid with altered sporulation control. Genetics 80: 61–76.

Huang, D., and D. Koshland, 2003 Chromosome integrity in *Saccharomyces cerevisiae*: the interplay of DNA replication initiation factors, elongation factors, and origins. Genes Dev. 17: 1741–1754.

Huang, M. E., A. G. Rio, A. Nicolas, and R. D. Kolodner, 2003 A genomewide screen in *Saccharomyces cerevisiae* for genes that suppress the accumulation of mutations. Proc. Natl. Acad. Sci. USA 100: 11529–11534.

Huertas, P., and A. Aguilera, 2003 Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol. Cell 12: 711–721.

Huertas, P., F. Cortes-Ledesma, A. A. Sartori, A. Aguilera, and S. P. Jackson, 2008 CDK targets Sae2 to control DNA-end resection and homologous recombination. Nature 455: 689–692.

Ii, T., J. Fung, J. R. Mullen, and S. J. Brill, 2007 The yeast Slx5-Slx8 DNA integrity complex displays ubiquitin ligase activity. Cell Cycle 6: 2800–2809.

Inbar, O., and M. Kupiec, 1999 Homology search and choice of homologous partner during mitotic recombination. Mol. Cell. Biol. 19: 4134–4142.

Ip, S. C., U. Rass, M. G. Blanco, H. R. Flynn, J. M. Skehel *et al.*, 2008 Identification of Holliday junction resolvases from humans and yeast. Nature 456: 357–361.

Ira, G., and J. E. Haber, 2002 Characterization of *RAD51*-independent break-induced replication that acts preferentially with short homologous sequences. Mol. Cell. Biol. 22: 6384–6392.
Ira, G., A. Malkova, G. Liberi, M. Foiani, and J. E. Haber, 2003 Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. Cell 115: 401–411.
Ira, G., A. Pellicioli, A. Balijja, X. Wang, S. Fiorani *et al.*, 2004 DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature 431: 1011–1017.
Ivanov, E. L., and J. E. Haber, 1995 *RAD1* and *RAD10*, but not other excision repair genes, are required for double-strand break-induced recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15: 2245–2251.
Ivanov, E. L., N. Sugawara, C. I. White, F. Fabre, and J. E. Haber, 1994 Mutations in *XRS2* and *RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 14: 3414–3425.
Jain, S., N. Sugawara, J. Lydeard, M. Vaze, N. Tanguy Le Gac *et al.*, 2009 A recombination execution checkpoint regulates the choice of homologous recombination pathway during DNA double-strand break repair. Genes Dev. 23: 291–303.
Jaskelioff, M., S. Van Komen, J. E. Krebs, P. Sung, and C. L. Peterson, 2003 Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. J. Biol. Chem. 278: 9212–9218.
Javaheri, A., R. Wysocki, O. Jobin-Robitaille, M. Altaf, J. Cote *et al.*, 2006 Yeast G1 DNA damage checkpoint regulation by H2A phosphorylation is independent of chromatin remodeling. Proc. Natl. Acad. Sci. USA 103: 13771–13776.
Jelinsky, S. A., and L. D. Samson, 1999 Global response of *Saccharomyces cerevisiae* to an alkylating agent. Proc. Natl. Acad. Sci. USA 96: 1486–1491.
Jensen, R. E., and I. Herskowitz, 1984 Directionality and regulation of cassette substitution in yeast. Cold Spring Harb. Symp. Quant. Biol. 49: 97–104.
Jinks-Robertson, S., and T. D. Petes, 1986 Chromosomal translocations generated by high-frequency meiotic recombination between repeated yeast genes. Genetics 114: 731–752.
Johnson, R. D., and L. S. Symington, 1995 Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57. Mol. Cell. Biol. 15: 4843–4850.
Kagawa, W., H. Kurumizaka, R. Ishitani, S. Fukai, O. Nureki *et al.*, 2002 Crystal structure of the homologous-pairing domain from the human Rad52 recombinase in the undecameric form. Mol. Cell 10: 359–371.
Kaliraman, V., J. R. Mullen, W. M. Fricke, S. A. Bastin-Shanower, and S. J. Brill, 2001 Functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease. Genes Dev. 15: 2730–2740.
Kalocsay, M., N. J. Hiller, and S. Jentsch, 2009 Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Mol. Cell 33: 335–343.
Kaneko, H., and N. Kondo, 2004 Clinical features of Bloom syndrome and function of the causative gene, BLM helicase. Expert Rev. Mol. Diagn. 4: 393–401.
Kang, L. E., and L. S. Symington, 2000 Aberrant double-strand break repair in *rad51* mutants of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 20: 9162–9172.
Kans, J. A., and R. K. Mortimer, 1991 Nucleotide sequence of the *RAD57* gene of *Saccharomyces cerevisiae*. Gene 105: 139–140.
Karppinen, S. M., H. Erkko, K. Reini, H. Pospiech, K. Heikkinen *et al.*, 2006 Identification of a common polymorphism in the TopBP1 gene associated with hereditary susceptibility to breast and ovarian cancer. Eur. J. Cancer 42: 2647–2652.
Kaye, J. A., J. A. Melo, S. K. Cheung, M. B. Vaze, J. E. Haber *et al.*, 2004 DNA breaks promote genomic instability by impeding proper chromosome segregation. Curr. Biol. 14: 2096–2106.
Kaytor, M. D., M. Nguyen, and D. M. Livingston, 1995 The complexity of the interaction between *RAD52* and *SRS2*. Genetics 140: 1441–1442.
Keil, R. L., and G. S. Roeder, 1984 Cis-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell 39: 377–386.
Keil, R. L., and A. D. McWilliams, 1993 A gene with specific and global effects on recombination of sequences from tandemly repeated genes in *Saccharomyces cerevisiae*. Genetics 135: 711–718.
Kent, N. A., A. L. Chambers, and J. A. Downs, 2007 Dual chromatin remodeling roles for RSC during DNA double strand break induction and repair at the yeast *MAT* locus. J. Biol. Chem. 282: 27693–27701.
Khadaroo, B., M. T. Teixeira, P. Luciano, N. Eckert-Boulet, S. M. Germann *et al.*, 2009 The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat. Cell Biol. 11: 980–987.
Kim, N., and S. Jinks-Robertson, 2011 Guanine repeat-containing sequences confer transcription-dependent instability in an orientation-specific manner in yeast. DNA Repair (Amst.) 10: 953–960.
Kim, R. A., and J. C. Wang, 1989 A subthreshold level of DNA topoisomerases leads to the excision of yeast rDNA as extrachromosomal rings. Cell 57: 975–985.
Kim, Y., F. P. Lach, R. Desetty, H. Hanenberg, A. D. Auerbach *et al.*, 2011 Mutations of the *SLX4* gene in Fanconi anemia. Nature Genet. 43: 142–146.
Kiser, G. L., and T. A. Weinert, 1996 Distinct roles of yeast *MEC* and *RAD* checkpoint genes in transcriptional induction after DNA damage and implications for function. Mol. Biol. Cell 7: 703–718.
Klein, H. L., 1988 Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. Genetics 120: 367–377.
Klein, H. L., 1997 *RDH54*, a *RAD54* homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. Genetics 147: 1533–1543.
Kobayashi, T., 2008 A new role of the rDNA and nucleolus in the nucleus–rDNA instability maintains genome integrity. BioEssays 30: 267–272.
Kobayashi, T., and A. R. Ganley, 2005 Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. Science 309: 1581–1584.
Kobayashi, T., D. J. Heck, M. Nomura, and T. Horiuchi, 1998 Expansion and contraction of ribosomal DNA repeats in *Saccharomyces cerevisiae*: requirement of replication fork blocking (Fob1) protein and the role of RNA polymerase I. Genes Dev. 12: 3821–3830.
Kobor, M. S., S. Venkatasubrahmanyam, M. D. Meneghini, J. W. Gin, J. L. Jennings *et al.*, 2004 A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol. 2: E131.
Kolesar, P., P. Sarangi, V. Altmannova, X. Zhao, and L. Krejci, 2012 Dual roles of the SUMO-interacting motif in the regulation of Srs2 sumoylation. Nucleic Acids Res. 40: 7831–7843.
Kosugi, S., M. Hasebe, M. Tomita, and H. Yanagawa, 2009 Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs. Proc. Natl. Acad. Sci. USA 106: 10171–10176.
Kraus, E., W. Y. Leung, and J. E. Haber, 2001 Break-induced replication: a review and an example in budding yeast. Proc. Natl. Acad. Sci. USA 98: 8255–8262.

Krejci, L., B. Song, W. Bussen, R. Rothstein, U. H. Mortensen *et al.*, 2002 Interaction with Rad51 is indispensable for recombination mediator function of Rad52. J. Biol. Chem. 277: 40132–40141.

Krejci, L., S. Van Komen, Y. Li, J. Villemain, M. S. Reddy *et al.*, 2003 DNA helicase Srs2 disrupts the Rad51 presynaptic filament. Nature 423: 305–309.

Kupiec, M., 2000 Damage-induced recombination in the yeast *Saccharomyces cerevisiae*. Mutat. Res. 451: 91–105.

Kwon, Y., P. Chi, D. H. Roh, H. Klein, and P. Sung, 2007 Synergistic action of the *Saccharomyces cerevisiae* homologous recombination factors Rad54 and Rad51 in chromatin remodeling. DNA Repair (Amst.) 6: 1496–1506.

Lammens, K., D. J. Bemeleit, C. Mockel, E. Clausing, A. Schele *et al.*, 2011 The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. Cell 145: 54–66.

Lao, J. P., S. D. Oh, M. Shinohara, A. Shinohara, and N. Hunter, 2008 Rad52 promotes postinvasion steps of meiotic double-strand-break repair. Mol. Cell 29: 517–524.

Lazzaro, F., V. Sapountzi, M. Granata, A. Pellicioli, M. Vaze *et al.*, 2008 Histone methyltransferase Dot1 and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. EMBO J. 27: 1502–1512.

Lee, K., Y. Zhang, and S. E. Lee, 2008 *Saccharomyces cerevisiae* ATM orthologue suppresses break-induced chromosome translocations. Nature 454: 543–546.

Lee, P. S., and T. D. Petes, 2010 From the Cover: mitotic gene conversion events induced in G1-synchronized yeast cells by gamma rays are similar to spontaneous conversion events. Proc. Natl. Acad. Sci. USA 107: 7383–7388.

Lee, P. S., P. W. Greenwell, M. Dominska, M. Gawel, M. Hamilton *et al.*, 2009 A fine-structure map of spontaneous mitotic crossovers in the yeast *Saccharomyces cerevisiae*. PLoS Genet. 5: e1000410.

Lemoine, F. J., N. P. Degtyareva, K. Lobachev, and T. D. Petes, 2005 Chromosomal translocations in yeast induced by low levels of DNA polymerase a model for chromosome fragile sites. Cell 120: 587–598.

Lemoine, F. J., N. P. Degtyareva, R. J. Kokoska, and T. D. Petes, 2008 Reduced levels of DNA polymerase *delta* induce chromosome fragile site instability in yeast. Mol. Cell. Biol. 28: 5359–5368.

Lengsfeld, B. M., A. J. Rattray, V. Bhaskara, R. Ghirlando, and T. T. Paull, 2007 Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. Mol. Cell 28: 638–651.

Lettier, G., Q. Feng, A. A. de Mayolo, N. Erdeniz, R. J. Reid *et al.*, 2006 The role of DNA double-strand breaks in spontaneous homologous recombination in *S. cerevisiae*. PLoS Genet. 2: 1773–1786.

Leung, G. P., L. Lee, T. I. Schmidt, K. Shirahige, and M. S. Kobor, 2011 Rtt107 is required for recruitment of the SMC5/6 complex to DNA double strand breaks. J. Biol. Chem. 286: 26250–26257.

Lewis, L. K., G. Karthikeyan, J. W. Westmoreland, and M. A. Resnick, 2002 Differential suppression of DNA repair deficiencies of Yeast *rad50*, *mre11* and *xrs2* mutants by *EXO1* and *TLC1* (the RNA component of telomerase). Genetics 160: 49–62.

Li, F., J. Dong, X. Pan, J. H. Oum, J. D. Boeke *et al.*, 2008 Microarray-based genetic screen defines *SAW1*, a gene required for Rad1/Rad10-dependent processing of recombination intermediates. Mol. Cell 30: 325–335.

Li, F., J. Dong, R. Eichmiller, C. Holland, E. Minca *et al.*, 2013 Role of Saw1 in Rad1/Rad10 complex assembly at recombination intermediates in budding yeast. EMBO J. 32: 461–472.

Li, X., and W. D. Heyer, 2009 *RAD54* controls access to the invading 3′-OH end after *RAD51*-mediated DNA strand invasion in homologous recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res. 37: 638–646.

Li, X. C., and B. K. Tye, 2011 Ploidy dictates repair pathway choice under DNA replication stress. Genetics 187: 1031–1040.

Liberi, G., G. Maffioletti, C. Lucca, I. Chiolo, A. Baryshnikova *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.

Lichten, M., and J. E. Haber, 1989 Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae*. Genetics 123: 261–268.

Liefshitz, B., A. Parket, R. Maya, and M. Kupiec, 1995 The role of DNA repair genes in recombination between repeated sequences in yeast. Genetics 140: 1199–1211.

Lim, H. S., J. S. Kim, Y. B. Park, G. H. Gwon, and Y. Cho, 2011 Crystal structure of the Mre11-Rad50-ATP*gamma*S complex: understanding the interplay between Mre11 and Rad50. Genes Dev. 25: 1091–1104.

Limbo, O., C. Chahwan, Y. Yamada, R. A. de Bruin, C. Wittenberg *et al.*, 2007 Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. Mol. Cell 28: 134–146.

Lindroos, H. B., L. Strom, T. Itoh, Y. Katou, K. Shirahige *et al.*, 2006 Chromosomal association of the Smc5/6 complex reveals that it functions in differently regulated pathways. Mol. Cell 22: 755–767.

Lisby, M., R. Rothstein, and U. H. Mortensen, 2001 Rad52 forms DNA repair and recombination centers during S phase. Proc. Natl. Acad. Sci. USA 98: 8276–8282.

Lisby, M., A. Antúnez de Mayolo, U. H. Mortensen, and R. Rothstein, 2003a Cell cycle-regulated centers of DNA double-strand break repair. Cell Cycle 2: 479–483.

Lisby, M., U. H. Mortensen, and R. Rothstein, 2003b Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nat. Cell Biol. 5: 572–577.

Lisby, M., J. H. Barlow, R. C. Burgess, and R. Rothstein, 2004 Choreography of the DNA damage response; spatiotemporal relationships among checkpoint and repair proteins. Cell 118: 699–713.

Liu, J., L. Renault, X. Veaute, F. Fabre, H. Stahlberg *et al.*, 2011 Rad51 paralogues Rad55-Rad57 balance the antirecombinase Srs2 in Rad51 filament formation. Nature 479: 245–248.

Llorente, B., C. E. Smith, and L. S. Symington, 2008 Break-induced replication: What is it and what is it for? Cell Cycle 7: 859–864.

Lloyd, J., J. R. Chapman, J. A. Clapperton, L. F. Haire, E. Hartsuiker *et al.*, 2009 A supramodular FHA/BRCT-repeat architecture mediates Nbs1 adaptor function in response to DNA damage. Cell 139: 100–111.

Lobachev, K. S., D. A. Gordenin, and M. A. Resnick, 2002 The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. Cell 108: 183–193.

Lobachev, K., E. Vitriol, J. Stemple, M. A. Resnick, and K. Bloom, 2004 Chromosome fragmentation after induction of a double-strand break is an active process prevented by the RMX repair complex. Curr. Biol. 14: 2107–2112.

Loog, M., and D. O. Morgan, 2005 Cyclin specificity in the phosphorylation of cyclin-dependent kinase substrates. Nature 434: 104–108.

Lopes, J., A. Piazza, R. Bermejo, B. Kriegsman, A. Colosio *et al.*, 2011 G-quadruplex-induced instability during leading-strand replication. EMBO J. 30: 4033–4046.

Lopes, M., C. Cotta-Ramusino, G. Liberi, and M. Foiani, 2003 Branch migrating sister chromatid junctions form at rep-

lication origins through Rad51/Rad52-independent mechanisms. Mol. Cell 12: 1499–1510.
Lorenz, A., F. Osman, W. Sun, S. Nandi, R. Steinacher *et al.*, 2012 The fission yeast FANCM ortholog directs non-crossover recombination during meiosis. Science 336: 1585–1588.
Lovett, S. T., 1994 Sequence of the *RAD55* gene of *Saccharomyces cerevisiae*: similarity of *RAD55* to prokaryotic RecA and other RecA-like proteins. Gene 142: 103–106.
Lukas, C., J. Falck, J. Bartkova, J. Bartek, and J. Lukas, 2003 Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. Nat. Cell Biol. 5: 255–260.
Luke-Glaser, S., and B. Luke, 2012 The Mph1 helicase can promote telomere uncapping and premature senescence in budding yeast. PLoS ONE 7: e42028.
Lundblad, V., and E. H. Blackburn, 1993 An alternative pathway for yeast telomere maintenance rescues *est1*- senescence. Cell 73: 347–360.
Lydeard, J. R., S. Jain, M. Yamaguchi, and J. E. Haber, 2007 Break-induced replication and telomerase-independent telomere maintenance require Pol32. Nature 448: 820–823.
Ma, J. L., E. M. Kim, J. E. Haber, and S. E. Lee, 2003 Yeast Mre11 and Rad1 proteins define a Ku-independent mechanism to repair double-strand breaks lacking overlapping end sequences. Mol. Cell. Biol. 23: 8820–8828.
Ma, W., J. W. Westmoreland, and M. A. Resnick, 2013 Homologous recombination rescues ssDNA gaps generated by nucleotide excision repair and reduced translesion DNA synthesis in yeast G2 cells. Proc. Natl. Acad. Sci. USA 110: E2895–E2904.
Malkova, A., L. Ross, D. Dawson, M. F. Hoekstra, and J. E. Haber, 1996 Meiotic recombination initiated by a double-strand break in *rad50Δ* yeast cells otherwise unable to initiate meiotic recombination. Genetics 143: 741–754.
Malkova, A., M. L. Naylor, M. Yamaguchi, G. Ira, and J. E. Haber, 2005 *RAD51*-dependent break-induced replication differs in kinetics and checkpoint responses from *RAD51*-mediated gene conversion. Mol. Cell. Biol. 25: 933–944.
Maloisel, L., F. Fabre, and S. Gangloff, 2008 DNA polymerase *delta* is preferentially recruited during homologous recombination to promote heteroduplex DNA extension. Mol. Cell. Biol. 28: 1373–1382.
Mankouri, H. W., H. P. Ngo, and I. D. Hickson, 2007 Shu proteins promote the formation of homologous recombination intermediates that are processed by Sgs1-Rmi1-Top3. Mol. Biol. Cell 18: 4062–4073.
Manney, T. R., and R. K. Mortimer, 1964 Allelic mapping in yeast by X-ray-induced mitotic reversion. Science 143: 581–583.
Manthey, G. M., and A. M. Bailis, 2010 Rad51 inhibits translocation formation by non-conservative homologous recombination in *Saccharomyces cerevisiae*. PLoS ONE 5: e11889.
Mathiasen, D. P., and M. Lisby, 2014 Cell cycle regulation of homologous recombination in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 38: 172–184.
Matos, J., M. G. Blanco, S. Maslen, J. M. Skehel, and S. C. West, 2011 Regulatory control of the resolution of DNA recombination intermediates during meiosis and mitosis. Cell 147: 158–172.
Matos, J., M. G. Blanco, and S. C. West, 2013 Cell-cycle kinases coordinate the resolution of recombination intermediates with chromosome segregation. Cell Reports 4: 76–86.
Matulova, P., V. Marini, R. C. Burgess, A. Sisakova, Y. Kwon *et al.*, 2009 Cooperativity of Mus81·Mms4 with Rad54 in the resolution of recombination and replication intermediates. J. Biol. Chem. 284: 7733–7745.
Mazin, A. V., C. J. Bornarth, J. A. Solinger, W. D. Heyer, and S. C. Kowalczykowski, 2000 Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. Mol. Cell 6: 583–592.
Mazon, G., and L. S. Symington, 2013 Mph1 and Mus81-Mms4 prevent aberrant processing of mitotic recombination intermediates. Mol. Cell 52: 63–74.
Mazon, G., A. F. Lam, C. K. Ho, M. Kupiec, and L. S. Symington, 2012 The Rad1-Rad10 nuclease promotes chromosome translocations between dispersed repeats. Nat. Struct. Mol. Biol. 19: 964–971.
McAleenan, A., A. Clemente-Blanco, V. Cordon-Preciado, N. Sen, M. Esteras *et al.*, 2013 Post-replicative repair involves separase-dependent removal of the kleisin subunit of cohesin. Nature 493: 250–254.
McDonald, J. P., and R. Rothstein, 1994 Unrepaired heteroduplex DNA in *Saccharomyces cerevisiae* is decreased in *RAD1 RAD52*-independent recombination. Genetics 137: 393–405.
Meetei, A. R., A. L. Medhurst, C. Ling, Y. Xue, T. R. Singh *et al.*, 2005 A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. Nat. Genet. 37: 958–963.
Mercier, G., Y. Denis, P. Marc, L. Picard, and M. Dutreix, 2001 Transcriptional induction of repair genes during slowing of replication in irradiated *Saccharomyces cerevisiae*. Mutat. Res. 487: 157–172.
Milne, G. T., and D. T. Weaver, 1993 Dominant negative alleles of *RAD52* reveal a DNA repair/recombination complex including Rad51 and Rad52. Genes Dev. 7: 1755–1765.
Mimitou, E. P., and L. S. Symington, 2008 Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455: 770–774.
Mimitou, E. P., and L. S. Symington, 2009 DNA end resection: many nucleases make light work. DNA Repair (Amst.) 8: 983–995.
Mimitou, E. P., and L. S. Symington, 2010 Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. EMBO J. 29: 3358–3369.
Mine-Hattab, J., and R. Rothstein, 2012 Increased chromosome mobility facilitates homology search during recombination. Nat. Cell Biol. 14: 510–517.
Miret, J. J., L. Pessoa-Brandao, and R. S. Lahue, 1998 Orientation-dependent and sequence-specific expansions of CTG/CAG trinucleotide repeats in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 95: 12438–12443.
Mitchel, K., H. Zhang, C. Welz-Voegele, and S. Jinks-Robertson, 2010 Molecular structures of crossover and noncrossover intermediates during gap repair in yeast: implications for recombination. Mol. Cell 38: 211–222.
Mitchel, K., K. Lehner, and S. Jinks-Robertson, 2013 Heteroduplex DNA position defines the roles of the Sgs1, Srs2, and Mph1 helicases in promoting distinct recombination outcomes. PLoS Genet. 9: e1003340.
Mizuguchi, G., X. Shen, J. Landry, W. H. Wu, S. Sen *et al.*, 2004 ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303: 343–348.
Moreau, S., J. R. Ferguson, and L. S. Symington, 1999 The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. Mol. Cell. Biol. 19: 556–566.
Moreau, S., E. A. Morgan, and L. S. Symington, 2001 Overlapping functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 nucleases in DNA metabolism. Genetics 159: 1423–1433.
Morillo-Huesca, M., M. Clemente-Ruiz, E. Andujar, and F. Prado, 2010 The SWR1 histone replacement complex causes genetic instability and genome-wide transcription misregulation in the absence of H2A. Z. PLoS One 5: e12143.
Morrison, A. J., J. Highland, N. J. Krogan, A. Arbel-Eden, J. F. Greenblatt *et al.*, 2004 INO80 and *gamma*-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119: 767–775.

Morrow, D. M., C. Connelly, and P. Hieter, 1997 "Break copy" duplication: a model for chromosome fragment formation in *Saccharomyces cerevisiae*. Genetics 147: 371–382.

Mortensen, U. H., C. Bendixen, I. Sunjevaric, and R. Rothstein, 1996 DNA strand annealing is promoted by the yeast Rad52 protein. Proc. Natl. Acad. Sci. USA 93: 10729–10734.

Mortensen, U. H., N. Erdeniz, Q. Feng, and R. Rothstein, 2002 A molecular genetic dissection of the evolutionarily conserved N terminus of yeast Rad52. Genetics 161: 549–562.

Mortimer, R. K., 1958 Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of *Saccharomyces cerevisiae*. Radiat. Res. 9: 312–326.

Mott, C., and L. S. Symington, 2011 *RAD51*-independent inverted-repeat recombination by a strand-annealing mechanism. DNA Repair (Amst.) 10: 408–415.

Mozlin, A. M., C. W. Fung, and L. S. Symington, 2008 Role of the *Saccharomyces cerevisiae* Rad51 paralogs in sister chromatid recombination. Genetics 178: 113–126.

Mukherjee, S., W. D. Wright, K. T. Ehmsen, and W. D. Heyer, 2014 The Mus81-Mms4 structure-selective endonuclease requires nicked DNA junctions to undergo conformational changes and bend its DNA substrates for cleavage. Nucleic Acids Res. 42: 6511–6522.

Mullen, J. R., V. Kaliraman, S. S. Ibrahim, and S. J. Brill, 2001 Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. Genetics 157: 103–118.

Munoz-Galvan, S., S. Jimeno, R. Rothstein, and A. Aguilera, 2013 Histone H3K56 acetylation, Rad52, and non-DNA repair factors control double-strand break repair choice with the sister chromatid. PLoS Genet. 9: e1003237.

Nagai, S., K. Dubrana, M. Tsai-Pflugfelder, M. B. Davidson, T. M. Roberts *et al.*, 2008 Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 322: 597–602.

Nakada, D., K. Matsumoto, and K. Sugimoto, 2003 ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. Genes Dev. 17: 1957–1962.

Nassif, N., J. Penney, S. Pal, W. R. Engels, and G. B. Gloor, 1994 Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. Mol. Cell. Biol. 14: 1613–1625.

New, J. H., T. Sugiyama, E. Zaitseva, and S. C. Kowalczykowski, 1998 Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature 391: 407–410.

Nickoloff, J. A., E. Y. Chen, and F. Heffron, 1986 A 24-base-pair DNA sequence from the *MAT* locus stimulates intergenic recombination in yeast. Proc. Natl. Acad. Sci. USA 83: 7831–7835.

Nickoloff, J. A., D. B. Sweetser, J. A. Clikeman, G. J. Khalsa, and S. L. Wheeler, 1999 Multiple heterologies increase mitotic double-strand break-induced allelic gene conversion tract lengths in yeast. Genetics 153: 665–679.

Nicolette, M. L., K. Lee, Z. Guo, M. Rani, J. M. Chow *et al.*, 2010 Mre11-Rad50-Xrs2 and Sae2 promote 5′ strand resection of DNA double-strand breaks. Nat. Struct. Mol. Biol. 17: 1478–1485.

Nielsen, I., I. B. Bentsen, M. Lisby, S. Hansen, K. Mundbjerg *et al.*, 2009 A Flp-nick system to study repair of a single protein-bound nick *in vivo*. Nat. Methods 6: 753–757.

Nissar, S., S. M. Baba, T. Akhtar, R. Rasool, Z. A. Shah *et al.*, 2014 RAD51 G135C gene polymorphism and risk of colorectal cancer in Kashmir. Eur. J. Cancer Prev. 23: 264–268.

Niu, H., W. H. Chung, Z. Zhu, Y. Kwon, W. Zhao *et al.*, 2010 Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. Nature 467: 108–111.

O'Driscoll, M., V. L. Ruiz-Perez, C. G. Woods, P. A. Jeggo, and J. A. Goodship, 2003 A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. Nat. Genet. 33: 497–501.

Ogawa, T., X. Yu, A. Shinohara, and E. H. Egelman, 1993 Similarity of the yeast *RAD51* filament to the bacterial RecA filament. Science 259: 1896–1899.

Ohouo, P. Y., F. M. Bastos de Oliveira, B. S. Almeida, and M. B. Smolka, 2010 DNA damage signaling recruits the Rtt107-Slx4 scaffolds via Dpb11 to mediate replication stress response. Mol. Cell 39: 300–306.

Ohuchi, T., M. Seki, D. Branzei, D. Maeda, A. Ui *et al.*, 2008 Rad52 sumoylation and its involvement in the efficient induction of homologous recombination. DNA Repair (Amst.) 7: 879–889.

Onoda, F., M. Takeda, M. Seki, D. Maeda, J. Tajima *et al.*, 2004 *SMC6* is required for MMS-induced interchromosomal and sister chromatid recombinations in *Saccharomyces cerevisiae*. DNA Repair (Amst.) 3: 429–439.

Orr-Weaver, T. L., and J. W. Szostak, 1983 Yeast recombination: the association between double-strand gap repair and crossing-over. Proc. Natl. Acad. Sci. USA 80: 4417–4421.

Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA 78: 6354–6358.

Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein, 1983 Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. 101: 228–245.

Osborn, A. J., and S. J. Elledge, 2003 Mrc1 is a replication fork component whose phosphorylation in response to DNA replication stress activates Rad53. Genes Dev. 17: 1755–1767.

Osman, F., J. Dixon, C. L. Doe, and M. C. Whitby, 2003 Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol. Cell 12: 761–774.

Oum, J. H., C. Seong, Y. Kwon, J. H. Ji, A. Sid *et al.*, 2011 RSC facilitates Rad59-dependent homologous recombination between sister chromatids by promoting cohesin loading at DNA double-strand breaks. Mol. Cell. Biol. 31: 3924–3937.

Oza, P., S. L. Jaspersen, A. Miele, J. Dekker, and C. L. Peterson, 2009 Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes Dev. 23: 912–927.

Paek, A. L., S. Kaochar, H. Jones, A. Elezaby, L. Shanks *et al.*, 2009 Fusion of nearby inverted repeats by a replication-based mechanism leads to formation of dicentric and acentric chromosomes that cause genome instability in budding yeast. Genes Dev. 23: 2861–2875.

Paeschke, K., J. A. Capra, and V. A. Zakian, 2011 DNA replication through G-quadruplex motifs is promoted by the *Saccharomyces cerevisiae* Pif1 DNA helicase. Cell 145: 678–691.

Palancade, B., X. Liu, M. Garcia-Rubio, A. Aguilera, X. Zhao *et al.*, 2007 Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. Mol. Biol. Cell 18: 2912–2923.

Palmer, S., E. Schildkraut, R. Lazarin, J. Nguyen, and J. A. Nickoloff, 2003 Gene conversion tracts in *Saccharomyces cerevisiae* can be extremely short and highly directional. Nucleic Acids Res. 31: 1164–1173.

Pannunzio, N. R., G. M. Manthey, and A. M. Bailis, 2008 *RAD59* is required for efficient repair of simultaneous double-strand breaks resulting in translocations in *Saccharomyces cerevisiae*. DNA Repair (Amst.) 7: 788–800.

Pannunzio, N. R., G. M. Manthey, L. C. Liddell, B. X. Fu, C. M. Roberts *et al.*, 2012 Rad59 regulates association of Rad52 with DNA double-strand breaks. MicrobiologyOpen 1: 285–297.

Papouli, E., S. Chen, A. A. Davies, D. Huttner, L. Krejci *et al.*, 2005 Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. Mol. Cell 19: 123–133.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 63: 349–404.

Paques, F., W. Y. Leung, and J. E. Haber, 1998 Expansions and contractions in a tandem repeat induced by double-strand break repair. Mol. Cell. Biol. 18: 2045–2054.

Payen, C., R. Koszul, B. Dujon, and G. Fischer, 2008 Segmental duplications arise from Pol32-dependent repair of broken forks through two alternative replication-based mechanisms. PLoS Genet. 4: e1000175.

Petes, T. D., and D. Botstein, 1977 Simple mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc. Natl. Acad. Sci. USA 74: 5091–5095.

Petes, T. D., R. E. Malone, and L. S. Symington, 1991 Recombination in yeast, pp. 407–521 in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis and Energetics*, edited by J. R. Broach, J. R. Pringle, and E. W. Jones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Petrucelli, N., M. B. Daly, and G. L. Feldman, 2010 Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2. Genet. Med. 12: 245–259.

Petukhova, G., S. A. Stratton, and P. Sung, 1999 Single strand DNA binding and annealing activities in the yeast recombination factor Rad59. J. Biol. Chem. 274: 33839–33842.

Petukhova, G., P. Sung, and H. Klein, 2000 Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. Genes Dev. 14: 2206–2215.

Pfander, B., G. L. Moldovan, M. Sacher, C. Hoege, and S. Jentsch, 2005 SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. Nature 436: 428–433.

Plate, I., S. C. Hallwyl, I. Shi, L. Krejci, C. Muller *et al.*, 2008 Interaction with RPA is necessary for Rad52 repair center formation and for its mediator activity. J. Biol. Chem. 283: 29077–29085.

Plessis, A., A. Perrin, J. E. Haber, and B. Dujon, 1992 Site-specific recombination determined by I-*Sce*I, a mitochondrial group I intron-encoded endonuclease expressed in the yeast nucleus. Genetics 130: 451–460.

Pommier, Y., 2009 DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition. Chem. Rev. 109: 2894–2902.

Prakash, L., 1981 Characterization of postreplication repair in *Saccharomyces cerevisiae* and effects of *rad6*, *rad18*, *rev3* and *rad52* mutations. Mol. Gen. Genet. 184: 471–478.

Prakash, R., D. Satory, E. Dray, A. Papusha, J. Scheller *et al.*, 2009 Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. Genes Dev. 23: 67–79.

Psakhye, I., and S. Jentsch, 2012 Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. Cell 151: 807–820.

Puddu, F., M. Granata, L. Di Nola, A. Balestrini, G. Piergiovanni *et al.*, 2008 Phosphorylation of the budding yeast 9–1-1 complex is required for Dpb11 function in the full activation of the UV-induced DNA damage checkpoint. Mol. Cell. Biol. 28: 4782–4793.

Putnam, C. D., S. R. Allen-Soltero, S. L. Martinez, J. E. Chan, T. K. Hayes *et al.*, 2012 Bioinformatic identification of genes suppressing genome instability. Proc. Natl. Acad. Sci. USA 109: E3251–E3259.

Putnam, C. D., V. Pennaneach, and R. D. Kolodner, 2005 *Saccharomyces cerevisiae* as a model system to define the chromosomal instability phenotype. Mol. Cell. Biol. 25: 7226–7238.

Qin, S., and M. R. Parthun, 2002 Histone H3 and the histone acetyltransferase Hat1p contribute to DNA double-strand break repair. Mol. Cell. Biol. 22: 8353–8365.

Randell, J. C., A. Fan, C. Chan, L. I. Francis, R. C. Heller *et al.*, 2010 Mec1 is one of multiple kinases that prime the Mcm2–7 helicase for phosphorylation by Cdc7. Mol. Cell 40: 353–363.

Rapakko, K., K. Heikkinen, S. M. Karppinen, H. Erkko, and R. Winqvist, 2007 Germline alterations in the 53BP1 gene in breast and ovarian cancer families. Cancer Lett. 245: 337–340.

Rattray, A. J., and L. S. Symington, 1994 Use of a chromosomal inverted repeat to demonstrate that the *RAD51* and *RAD52* genes of *Saccharomyces cerevisiae* have different roles in mitotic recombination. Genetics 138: 587–595.

Rattray, A. J., and L. S. Symington, 1995 Multiple pathways for homologous recombination in *Saccharomyces cerevisiae*. Genetics 139: 45–56.

Rattray, A. J., B. K. Shafer, C. B. McGill, and J. N. Strathern, 2002 The roles of *REV3* and *RAD57* in double-strand-break-repair-induced mutagenesis of *Saccharomyces cerevisiae*. Genetics 162: 1063–1077.

Redon, C., D. R. Pilch, E. P. Rogakou, A. H. Orr, N. F. Lowndes *et al.*, 2003 Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. EMBO Rep. 4: 678–684.

Ribeyre, C., and D. Shore, 2012 Anticheckpoint pathways at telomeres in yeast. Nat. Struct. Mol. Biol. 19: 307–313.

Ribeyre, C., J. Lopes, J. B. Boule, A. Piazza, A. Guedin *et al.*, 2009 The yeast Pif1 helicase prevents genomic instability caused by G-quadruplex-forming CEB1 sequences *in vivo*. PLoS Genet. 5: e1000475.

Robert, T., D. Dervins, F. Fabre, and S. Gangloff, 2006 Mrc1 and Srs2 are major actors in the regulation of spontaneous crossover. EMBO J. 25: 2837–2846.

Robert, T., F. Vanoli, I. Chiolo, G. Shubassi, K. A. Bernstein *et al.*, 2011 HDACs link the DNA damage response, processing of double-strand breaks and autophagy. Nature 471: 74–79.

Roberts, S. A., J. Sterling, C. Thompson, S. Harris, D. Mav *et al.*, 2012 Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. Mol. Cell 46: 424–435.

Roberts, T. M., M. S. Kobor, S. A. Bastin-Shanower, M. Ii, S. A. Horte *et al.*, 2006 Slx4 regulates DNA damage checkpoint-dependent phosphorylation of the BRCT domain protein Rtt107/Esc4. Mol. Biol. Cell 17: 539–548.

Rogakou, E. P., D. R. Pilch, A. H. Orr, V. S. Ivanova, and W. M. Bonner, 1998 DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. 273: 5858–5868.

Rogakou, E. P., C. Boon, C. Redon, and W. M. Bonner, 1999 Megabase chromatin domains involved in DNA double-strand breaks *in vivo*. J. Cell Biol. 146: 905–916.

Rong, L., F. Palladino, A. Aguilera, and H. L. Klein, 1991 The hyper-gene conversion *hpr5–1* mutation of *Saccharomyces cerevisiae* is an allele of the *SRS2/RADH* gene. Genetics 127: 75–85.

Rosen, D. M., E. M. Younkin, S. D. Miller, and A. M. Casper, 2013 Fragile site instability in *Saccharomyces cerevisiae* causes loss of heterozygosity by mitotic crossovers and break-induced replication. PLoS Genet. 9: e1003817.

Rothstein, R., C. Helms, and N. Rosenberg, 1987 Concerted deletions and inversions are caused by mitotic recombination between *delta* sequences in *Saccharomyces cerevisiae*. [published erratum appears in Mol Cell Biol 1989 Aug;9(8):3592] Mol. Cell. Biol. 7: 1198–1207.

Ruiz, J. F., B. Gomez-Gonzalez, and A. Aguilera, 2009 Chromosomal translocations caused by either *p*ol32-dependent or *p*ol32-independent triparental break-induced replication. Mol. Cell. Biol. 29: 5441–5454.

Sacher, M., B. Pfander, C. Hoege, and S. Jentsch, 2006 Control of Rad52 recombination activity by double-strand break-induced SUMO modification. Nat. Cell Biol. 8: 1284–1290.

Saini, N., S. Ramakrishnan, R. Elango, S. Ayyar, Y. Zhang *et al.*, 2013a Migrating bubble during break-induced replication drives conservative DNA synthesis. Nature 502: 389–392.

Saini, N., Y. Zhang, Y. Nishida, Z. Sheng, S. Choudhury *et al.*, 2013b Fragile DNA motifs trigger mutagenesis at distant chromosomal loci in *saccharomyces cerevisiae*. PLoS Genet. 9: e1003551.

Santos-Rosa, H., and A. Aguilera, 1994 Increase in incidence of chromosome instability and non-conservative recombination between repeats in *Saccharomyces cerevisiae hpr1 delta* strains. Mol. Gen. Genet. 245: 224–236.

Saponaro, M., D. Callahan, X. Zheng, L. Krejci, J. E. Haber *et al.*, 2010 Cdk1 targets Srs2 to complete synthesis-dependent strand annealing and to promote recombinational repair. PLoS Genet. 6: e1000858.

Sarangi, P., Z. Bartosova, V. Altmannova, C. Holland, M. Chavdarova *et al.*, 2014 Sumoylation of the Rad1 nuclease promotes DNA repair and regulates its DNA association. Nucleic Acids Res. 42: 6393–6404.

Sasanuma, H., M. S. Tawaramoto, J. P. Lao, H. Hosaka, E. Sanda *et al.*, 2013 A new protein complex promoting the assembly of Rad51 filaments. Nat. Commun. 4: 1676.

Saugar, I., M. V. Vazquez, M. Gallo-Fernandez, M. A. Ortiz-Bazan, M. Segurado *et al.*, 2013 Temporal regulation of the Mus81-Mms4 endonuclease ensures cell survival under conditions of DNA damage. Nucleic Acids Res. 41: 8943–8958.

Saxe, D., A. Datta, and S. Jinks-Robertson, 2000 Stimulation of mitotic recombination events by high levels of RNA polymerase II transcription in yeast. Mol. Cell. Biol. 20: 5404–5414.

Schiestl, R. H., S. Igarashi, and P. J. Hastings, 1988 Analysis of the mechanism for reversion of a disrupted gene. Genetics 119: 237–247.

Schiller, C. B., K. Lammens, I. Guerini, B. Coordes, H. Feldmann *et al.*, 2012 Structure of Mre11-Nbs1 complex yields insights into ataxia-telangiectasia-like disease mutations and DNA damage signaling. Nat. Struct. Mol. Biol. 19: 693–700.

Schober, H., H. Ferreira, V. Kalck, L. R. Gehlen, and S. M. Gasser, 2009 Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. Genes Dev. 23: 928–938.

Scholes, D. T., M. Banerjee, B. Bowen, and M. J. Curcio, 2001 Multiple regulators of Ty1 transposition in *Saccharomyces cerevisiae* have conserved roles in genome maintenance. Genetics 159: 1449–1465.

Schwartz, E. K., and W. D. Heyer, 2011 Processing of joint molecule intermediates by structure-selective endonucleases during homologous recombination in eukaryotes. Chromosoma 120: 109–127.

Sebesta, M., P. Burkovics, L. Haracska, and L. Krejci, 2011 Reconstitution of DNA repair synthesis *in vitro* and the role of polymerase and helicase activities. DNA Repair (Amst.) 10: 567–576.

Seeber, A., V. Dion, and S. M. Gasser, 2013 Checkpoint kinases and the INO80 nucleosome remodeling complex enhance global chromatin mobility in response to DNA damage. Genes Dev. 27: 1999–2008.

Sehorn, M. G., S. Sigurdsson, W. Bussen, V. M. Unger, and P. Sung, 2004 Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. Nature 429: 433–437.

Shah, K. A., A. A. Shishkin, I. Voineagu, Y. I. Pavlov, P. V. Shcherbakova *et al.*, 2012 Role of DNA polymerases in repeat-mediated genome instability. Cell Reports 2: 1088–1095.

Shah, P. P., X. Zheng, A. Epshtein, J. N. Carey, D. K. Bishop *et al.*, 2010 Swi2/Snf2-related translocases prevent accumulation of toxic Rad51 complexes during mitotic growth. Mol. Cell 39: 862–872.

Sheridan, S. D., X. Yu, R. Roth, J. E. Heuser, M. G. Sehorn *et al.*, 2008 A comparative analysis of Dmc1 and Rad51 nucleoprotein filaments. Nucleic Acids Res. 36: 4057–4066.

Shim, E. Y., J. L. Ma, J. H. Oum, Y. Yanez, and S. E. Lee, 2005 The yeast chromatin remodeler RSC complex facilitates end joining repair of DNA double-strand breaks. Mol. Cell. Biol. 25: 3934–3944.

Shim, E. Y., S. J. Hong, J. H. Oum, Y. Yanez, Y. Zhang *et al.*, 2007 RSC mobilizes nucleosomes to improve accessibility of repair machinery to the damaged chromatin. Mol. Cell. Biol. 27: 1602–1613.

Shim, E. Y., W. H. Chung, M. L. Nicolette, Y. Zhang, M. Davis *et al.*, 2010 *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. EMBO J. 29: 3370–3380.

Shinohara, A., H. Ogawa, and T. Ogawa, 1992 Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell 69: 457–470.

Shinohara, A., and T. Ogawa, 1998 Stimulation by Rad52 of yeast Rad51-mediated recombination. Nature 391: 404–407.

Shinohara, M., E. Shita-Yamaguchi, J. M. Buerstedde, H. Shinagawa, H. Ogawa *et al.*, 1997 Characterization of the roles of the *Saccharomyces cerevisiae RAD54* gene and a homologue of *RAD54*, *RDH54/TID1*, in mitosis and meiosis. Genetics 147: 1545–1556.

Shinohara, A., M. Shinohara, T. Ohta, S. Matsuda, and T. Ogawa, 1998 Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. Genes Cells 3: 145–156.

Shor, E., S. Gangloff, M. Wagner, J. Weinstein, G. Price *et al.*, 2002 Mutations in homologous recombination genes rescue *top3* slow growth in *Saccharomyces cerevisiae*. Genetics 162: 647–662.

Shor, E., J. Weinstein, and R. Rothstein, 2005 A genetic screen for *top3* suppressors in *Saccharomyces cerevisiae* identifies *SHU1*, *SHU2*, *PSY3* and *CSM2*: four genes involved in error-free DNA repair. Genetics 169: 1275–1289.

Shroff, R., A. Arbel-Eden, D. Pilch, G. Ira, W. M. Bonner *et al.*, 2004 Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr. Biol. 14: 1703–1711.

Silva, S., I. Gallina, N. Eckert-Boulet, and M. Lisby, 2012 Live cell microscopy of DNA damage response in *Saccharomyces cerevisiae*. Methods Mol. Biol. 920: 433–443.

Silva, S. N., M. Tomar, C. Paulo, B. C. Gomes, A.P. Azevedo *et al.*, 2010 Breast cancer risk and common single nucleotide polymorphisms in homologous recombination DNA repair pathway genes *XRCC2*, *XRCC3*, *NBS1* and *RAD51*. Cancer Epidemiol. 34: 85–92.

Sinclair, D. A., and L. Guarente, 1997 Extrachromosomal rDNA circles: a cause of aging in yeast. Cell 91: 1033–1042.

Singleton, M. R., L. M. Wentzell, Y. Liu, S. C. West, D. B. Wigley *et al.*, 2002 Structure of the single-strand annealing domain of human RAD52 protein crystal structure of the homologous-pairing domain from the human Rad52 recombinase in the undecameric form. Proc. Natl. Acad. Sci. USA 99: 13492–13497.

Sinha, M., and C. L. Peterson, 2009 Chromatin dynamics during repair of chromosomal DNA double-strand breaks. Epigenomics 1: 371–385.

Sinha, M., S. Watanabe, A. Johnson, D. Moazed, and C. L. Peterson, 2009 Recombinational repair within heterochromatin requires ATP-dependent chromatin remodeling. Cell 138: 1109–1121.

Sjogren, C., and K. Nasmyth, 2001 Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*. Curr. Biol. 11: 991–995.

Smith, C. E., B. Llorente, and L. S. Symington, 2007 Template switching during break-induced replication. Nature 447: 102–105.

Smith, C. E., A. F. Lam, and L. S. Symington, 2009 Aberrant double-strand break repair resulting in half crossovers in mutants defective for Rad51 or the DNA polymerase *delta* complex. Mol. Cell. Biol. 29: 1432–1441.

Smith, J., and R. Rothstein, 1999 An allele of *RFA1* suppresses *RAD52*-dependent double-strand break repair in *Saccharomyces cerevisiae*. Genetics 151: 447–458.
Smith, S., J. Y. Hwang, S. Banerjee, A. Majeed, A. Gupta *et al.*, 2004 Mutator genes for suppression of gross chromosomal rearrangements identified by a genome-wide screening in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 101: 9039–9044.
Sogo, J. M., M. Lopes, and M. Foiani, 2002 Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. Science 297: 599–602.
Solinger, J. A., K. Kiianitsa, and W. D. Heyer, 2002 Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. Mol. Cell 10: 1175–1188.
Sollier, J., R. Driscoll, F. Castellucci, M. Foiani, S. P. Jackson *et al.*, 2009 The *Saccharomyces cerevisiae* Esc2 and Smc5–6 proteins promote sister chromatid junction-mediated intra-S repair. Mol. Biol. Cell 20: 1671–1682.
Song, W., M. Dominska, P. W. Greenwell, and T. D. Petes, 2014 Genome-wide high-resolution mapping of chromosome fragile sites in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 111: E2210–E2218.
Spellman, P. T., G. Sherlock, M. Q. Zhang, V. R. Iyer, K. Anders *et al.*, 1998 Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol. Biol. Cell 9: 3273–3297.
St Charles, J., E. Hazkani-Covo, Y. Yin, S. L. Andersen, F. S. Dietrich *et al.*, 2012 High-resolution genome-wide analysis of irradiated (UV and gamma-rays) diploid yeast cells reveals a high frequency of genomic loss of heterozygosity (LOH) events. Genetics 190: 1267–1284.
Stafa, A., R. A. Donnianni, L. A. Timashev, A. F. Lam, and L. S. Symington, 2014 Template switching during break-induced replication is promoted by the Mph1 helicase in *Saccharomyces cerevisiae*. Genetics 196: 1017–1028.
Stead, B. E., C. J. Brandl, M. K. Sandre, and M. J. Davey, 2012 Mcm2 phosphorylation and the response to replicative stress. BMC Genet. 13: 36.
Stoepker, C., K. Hain, B. Schuster, Y. Hilhorst-Hofstee, M. A. Rooimanst *et al.*, 2011 SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. Nature Genet. 43: 138–141.
Storchova, Z., A. Breneman, J. Cande, J. Dunn, K. Burbank *et al.*, 2006 Genome-wide genetic analysis of polyploidy in yeast. Nature 443: 541–547.
Strathern, J. N., K. G. Weinstock, D. R. Higgins, and C. B. McGill, 1991 A novel recombinator in yeast based on gene II protein from bacteriophage f1. Genetics 127: 61–73.
Strom, L., H. B. Lindroos, K. Shirahige, and C. Sjogren, 2004 Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol. Cell 16: 1003–1015.
Strom, L., C. Karlsson, H. B. Lindroos, S. Wedahl, Y. Katou *et al.*, 2007 Postreplicative formation of cohesion is required for repair and induced by a single DNA break. Science 317: 242–245.
Sugawara, N., and J. E. Haber, 1992 Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. Mol. Cell. Biol. 12: 563–575.
Sugiyama, T., and S. C. Kowalczykowski, 2002 Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation. J. Biol. Chem. 277: 31663–31672.
Sugawara, N., F. Paques, M. Colaiacovo, and J. E. Haber, 1997 Role of *Saccharomyces cerevisiae* Msh2 and Msh3 repair proteins in double-strand break-induced recombination. Proc. Natl. Acad. Sci. USA 94: 9214–9219.
Sugawara, N., G. Ira, and J. E. Haber, 2000 DNA length dependence of the single-strand annealing pathway and the role of *Saccharomyces cerevisiae RAD59* in double-strand break repair. Mol. Cell. Biol. 20: 5300–5309.
Sugawara, N., X. Wang, and J. E. Haber, 2003 *In vivo* roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol. Cell 12: 209–219.
Sugiyama, T., E. M. Zaitseva, and S. C. Kowalczykowski, 1997 A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. J. Biol. Chem. 272: 7940–7945.
Sugiyama, T., J. H. New, and S. C. Kowalczykowski, 1998 DNA annealing by Rad52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. Proc. Natl. Acad. Sci. USA 95: 6049–6054.
Sugiyama, T., N. Kantake, Y. Wu, and S. C. Kowalczykowski, 2006 Rad52-mediated DNA annealing after Rad51-mediated DNA strand exchange promotes second ssDNA capture. EMBO J. 25: 5539–5548.
Sun, H., J. Bai, F. Chen, Y. Jin, Y. Yu *et al.*, 2011 RAD51 G135C polymorphism is associated with breast cancer susceptibility: a meta-analysis involving 22,399 subjects. Breast Cancer Res. Treat. 125: 157–161.
Sun, W., S. Nandi, F. Osman, J. S. Ahn, J. Jakovleska *et al.*, 2008 The FANCM ortholog Fml1 promotes recombination at stalled replication forks and limits crossing over during DNA double-strand break repair. Mol. Cell 32: 118–128.
Sung, P., 1994 Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science 265: 1241–1243.
Sung, P., 1997a Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. J. Biol. Chem. 272: 28194–28197.
Sung, P., 1997b Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. Genes Dev. 11: 1111–1121.
Sung, P., L. Krejci, S. Van Komen, and M. G. Sehorn, 2003 Rad51 recombinase and recombination mediators. J. Biol. Chem. 278: 42729–42732.
Symington, L. S., 2002 Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. 66: 630–670.
Symington, L. S., L. E. Kang, and S. Moreau, 2000 Alteration of gene conversion tract length and associated crossing over during plasmid gap repair in nuclease-deficient strains of *Saccharomyces cerevisiae*. Nucleic Acids Res. 28: 4649–4656.
Szakal, B., and D. Branzei, 2013 Premature Cdk1/Cdc5/Mus81 pathway activation induces aberrant replication and deleterious crossover. EMBO J. 32: 1155–1167.
Szostak, J. W., and R. Wu, 1980 Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*. Nature 284: 426–430.
Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl, 1983 The double-strand-break repair model for recombination. Cell 33: 25–35.
Taddei, A., and S. M. Gasser, 2012 Structure and function in the budding yeast nucleus. Genetics 192: 107–129.
Takahashi, Y., J. Mizoi, E. A. Toh, and Y. Kikuchi, 2000 Yeast Ulp1, an Smt3-specific protease, associates with nucleoporins. J. Biochem. 128: 723–725.
Takeuchi, Y., T. Horiuchi, and T. Kobayashi, 2003 Transcription-dependent recombination and the role of fork collision in yeast rDNA. Genes Dev. 17: 1497–1506.
Tamburini, B. A., and J. K. Tyler, 2005 Localized histone acetylation and deacetylation triggered by the homologous recombina-

tion pathway of double-strand DNA repair. Mol. Cell. Biol. 25: 4903–4913.
Tang, W., M. Dominska, P. W. Greenwell, Z. Harvanek, K. S. Lobachev *et al.*, 2011 Friedreich's ataxia (GAA)n*(TTC)n repeats strongly stimulate mitotic crossovers in *Saccharomyces cerevisae*. PLoS Genet. 7: e1001270.
Tang, W., M. Dominska, M. Gawel, P. W. Greenwell, and T. D. Petes, 2013 Genomic deletions and point mutations induced in *Saccharomyces cerevisiae* by the trinucleotide repeats (GAA. TTC) associated with Friedreich's ataxia. DNA Repair (Amst.) 12: 10–17.
Tao, Y., X. Li, Y. Liu, J. Ruan, S. Qi *et al.*, 2012 Structural analysis of Shu proteins reveals a DNA binding role essential for resisting damage. J. Biol. Chem. 287: 20231–20239.
Tay, Y. D., J. M. Sidebotham, and L. Wu, 2010 Mph1 requires mismatch repair-independent and -dependent functions of MutSalpha to regulate crossover formation during homologous recombination repair. Nucleic Acids Res. 38: 1889–1901.
Tercero, J. A., and J. F. Diffley, 2001 Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint. Nature 412: 553–557.
Therizols, P., C. Fairhead, G. G. Cabal, A. Genovesio, J. C. Olivo-Marin *et al.*, 2006 Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol. 172: 189–199.
Thomas, B. J., and R. Rothstein, 1989a Elevated recombination rates in transcriptionally active DNA. Cell 56: 619–630.
Thomas, B. J., and R. Rothstein, 1989b The genetic control of direct-repeat recombination in Saccharomyces: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. Genetics 123: 725–738.
Tittel-Elmer, M., A. Lengronne, M. B. Davidson, J. Bacal, P. Francois *et al.*, 2012 Cohesin association to replication sites depends on rad50 and promotes fork restart. Mol. Cell 48: 98–108.
Toh, G. W., A. M. O'Shaughnessy, S. Jimeno, I. M. Dobbie, M. Grenon *et al.*, 2006 Histone H2A phosphorylation and H3 methylation are required for a novel Rad9 DSB repair function following checkpoint activation. DNA Repair (Amst.) 5: 693–703.
Toh, G. W., N. Sugawara, J. Dong, R. Toth, S. E. Lee *et al.*, 2010 Mec1/Tel1-dependent phosphorylation of Slx4 stimulates Rad1-Rad10-dependent cleavage of non-homologous DNA tails. DNA Repair (Amst.) 9: 718–726.
Torres-Rosell, J., I. Sunjevaric, G. De Piccoli, M. Sacher, N. Eckert-Boulet *et al.*, 2007 The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. Nat. Cell Biol. 9: 923–931.
Trovesi, C., M. Falcettoni, G. Lucchini, M. Clerici, and M. P. Longhese, 2011 Distinct Cdk1 requirements during single-strand annealing, noncrossover, and crossover recombination. PLoS Genet. 7: e1002263.
Trujillo, K. M., and P. Sung, 2001 DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50*Mre11 complex. J. Biol. Chem. 276: 35458–35464.
Tsubouchi, H., and H. Ogawa, 2000 Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in *Saccharomyces cerevisiae*. Mol. Biol. Cell 11: 2221–2233.
Tsukamoto, Y., C. Mitsuoka, M. Terasawa, H. Ogawa, and T. Ogawa, 2005 Xrs2p regulates Mre11p translocation to the nucleus and plays a role in telomere elongation and meiotic recombination. Mol. Biol. Cell 16: 597–608.
Tsukuda, T., A. B. Fleming, J. A. Nickoloff, and M. A. Osley, 2005 Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. Nature 438: 379–383.
Tsukuda, T., Y. C. Lo, S. Krishna, R. Sterk, M. A. Osley *et al.*, 2009 INO80-dependent chromatin remodeling regulates early and late stages of mitotic homologous recombination. DNA Repair (Amst.) 8: 360–369.
Unal, E., A. Arbel-Eden, U. Sattler, R. Shroff, M. Lichten *et al.*, 2004 DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol. Cell 16: 991–1002.
Unal, E., J. M. Heidinger-Pauli, and D. Koshland, 2007 DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). Science 317: 245–248.
Usui, T., T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa *et al.*, 1998 Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell 95: 705–716.
Valencia-Burton, M., M. Oki, J. Johnson, T. A. Seier, R. Kamakaka *et al.*, 2006 Different mating-type-regulated genes affect the DNA repair defects of Saccharomyces *RAD51*, *RAD52* and *RAD55* mutants. Genetics 174: 41–55.
Vallen, E. A., and F. R. Cross, 1999 Interaction between the *MEC1*-dependent DNA synthesis checkpoint and G1 cyclin function in *Saccharomyces cerevisiae*. Genetics 151: 459–471.
van Attikum, H., O. Fritsch, and S. M. Gasser, 2007 Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. EMBO J. 26: 4113–4125.
van Attikum, H., O. Fritsch, B. Hohn, and S. M. Gasser, 2004 Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119: 777–788.
Van Komen, S., G. Petukhova, S. Sigurdsson, S. Stratton, and P. Sung, 2000 Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54. Mol. Cell 6: 563–572.
Varon, R., C. Vissinga, M. Platzer, K. M. Cerosaletti, K. H. Chrzanowska *et al.*, 1998 Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell 93: 467–476.
Vasan, S., A. Deem, S. Ramakrishnan, J. L. Argueso, and A. Malkova, 2014 Cascades of genetic instability resulting from compromised break-induced replication. PLoS Genet. 10: e1004119.
Vaze, M. B., A. Pellicioli, S. E. Lee, G. Ira, G. Liberi *et al.*, 2002 Recovery from checkpoint-mediated arrest after repair of a double-strand break requires Srs2 helicase. Mol. Cell 10: 373–385.
Veaute, X., J. Jeusset, C. Soustelle, S. C. Kowalczykowski, E. Le Cam *et al.*, 2003 The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature 423: 309–312.
Villarreal, D. D., K. Lee, A. Deem, E. Y. Shim, A. Malkova *et al.*, 2012 Microhomology directs diverse DNA break repair pathways and chromosomal translocations. PLoS Genet. 8: e1003026.
Voelkel-Meiman, K., R. L. Keil, and G. S. Roeder, 1987 Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. Cell 48: 1071–1079.
Vuorela, M., K. Pylkas, J. M. Hartikainen, K. Sundfeldt, A. Lindblom *et al.*, 2011 Further evidence for the contribution of the *RAD51C* gene in hereditary breast and ovarian cancer susceptibility. Breast Cancer Res. Treat. 130: 1003–1010.
Wagner, M., G. Price, and R. Rothstein, 2006 The absence of Top3 reveals an interaction between the Sgs1 and Pif1 DNA helicases in *Saccharomyces cerevisiae*. Genetics 174: 555–573.
Wahba, L., S. K. Gore, and D. Koshland, 2013 The homologous recombination machinery modulates the formation of RNA-DNA hybrids and associated chromosome instability. eLife 2: e00505.
Wallis, J. W., G. Chrebet, G. Brodsky, M. Rolfe, and R. Rothstein, 1989 A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. Cell 58: 409–419.
Wang, X., G. Ira, J. A. Tercero, A. M. Holmes, J. F. Diffley *et al.*, 2004 Role of DNA replication proteins in double-strand break-induced recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 24: 6891–6899.
Watt, P. M., I. D. Hickson, R. H. Borts, and E. J. Louis, 1996 *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is

required for maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics 144: 935–945.
Wellinger, R. J., and V. A. Zakian, 2012 Everything you ever wanted to know about *Saccharomyces cerevisiae* telomeres: beginning to end. Genetics 191: 1073–1105.
Welz-Voegele, C., and S. Jinks-Robertson, 2008 Sequence divergence impedes crossover more than noncrossover events during mitotic gap repair in yeast. Genetics 179: 1251–1262.
Westmoreland, T. J., S. M. Wickramasekara, A. Y. Guo, A. L. Selim, T. S. Winsor *et al.*, 2009 Comparative genome-wide screening identifies a conserved doxorubicin repair network that is diploid specific in *Saccharomyces cerevisiae*. PLoS ONE 4: e5830.
Williams, G. J., R. S. Williams, J. S. Williams, G. Moncalian, and A. S. Arvai *et al*., 2011 ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. Nat. Struct. Mol. Biol. 18: 423–431.
Wilson, M. A., Y. Kwon, Y. Xu, W. H. Chung, P. Chi *et al.*, 2013 Pif1 helicase and Pol*delta* promote recombination-coupled DNA synthesis via bubble migration. Nature 502: 393–396.
Wiltzius, J. J., M. Hohl, J. C. Fleming, and J. H. Petrini, 2005 The Rad50 hook domain is a critical determinant of Mre11 complex functions. Nat. Struct. Mol. Biol. 12: 403–407.
Wright, W. D., and W. D. Heyer, 2014 Rad54 functions as a heteroduplex DNA pump modulated by its DNA substrates and Rad51 during D loop formation. Mol. Cell 53: 420–432.
Wu, L., and I. D. Hickson, 2003 The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426: 870–874.
Wu, Y., T. Sugiyama, and S. C. Kowalczykowski, 2006 DNA annealing mediated by Rad52 and Rad59 proteins. J. Biol. Chem. 281: 15441–15449.
Yagle, K., and K. McEntee, 1990 The DNA damage-inducible gene *DIN1* of *Saccharomyces cerevisiae* encodes a regulatory subunit of ribonucleotide reductase and is identical to *RNR3*. Mol. Cell. Biol. 10: 5553–5557.
Yan, Y. X., R. H. Schiestl, and L. Prakash, 1995 Mating-type suppression of the DNA-repair defect of the yeast *rad6 delta* mutation requires the activity of genes in the *RAD52* epistasis group. Curr. Genet. 28: 12–18.
Yang, R., B. Chen, K. Pfutze, S. Buch, V. Steinke *et al.*, 2014 Genome-wide analysis associates familial colorectal cancer with increases in copy number variations and a rare structural variation at 12p12.3. Carcinogenesis 35: 315–323.
Yin, Y., and T. D. Petes, 2013 Genome-wide high-resolution mapping of UV-induced mitotic recombination events in *Saccharomyces cerevisiae*. PLoS Genet. 9: e1003894.
Zakharyevich, K., Y. Ma, S. Tang, P. Y. Hwang, S. Boiteux *et al.*, 2010 Temporally and biochemically distinct activities of Exo1 during meiosis: double-strand break resection and resolution of double Holliday junctions. Mol. Cell 40: 1001–1015.
Zakharyevich, K., S. Tang, Y. Ma, and N. Hunter, 2012 Delineation of joint molecule resolution pathways in meiosis identifies a crossover-specific resolvase. Cell 149: 334–347.
Zhang, H., and C. W. Lawrence, 2005 The error-free component of the *RAD6/RAD18* DNA damage tolerance pathway of budding yeast employs sister-strand recombination. Proc. Natl. Acad. Sci. USA 102: 15954–15959.
Zhang, Y., E. Y. Shim, M. Davis, and S. E. Lee, 2009 Regulation of repair choice: Cdk1 suppresses recruitment of end joining factors at DNA breaks. DNA Repair (Amst.) 8: 1235–1241.
Zhang, Y., A. A. Shishkin, Y. Nishida, D. Marcinkowski-Desmond, N. Saini *et al.*, 2012 Genome-wide screen identifies pathways that govern GAA/TTC repeat fragility and expansions in dividing and nondividing yeast cells. Mol. Cell 48: 254–265.
Zhang, Y., N. Saini, Z. Sheng, and K. S. Lobachev, 2013 Genome-wide screen reveals replication pathway for quasi-palindrome fragility dependent on homologous recombination. PLoS Genet. 9: e1003979.
Zhu, Z., W. H. Chung, E. Y. Shim, S. E. Lee, and G. Ira, 2008 Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell 134: 981–994.
Zierhut, C., and J. F. Diffley, 2008 Break dosage, cell cycle stage and DNA replication influence DNA double strand break response. EMBO J. 27: 1875–1885.
Zou, H., and R. Rothstein, 1997 Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. Cell 90: 87–96.

Communicating editor: J. Boeke