Chapter 8

Homologous Recombination and Nonhomologous End-Joining Repair in Yeast

R.E. Jones, T.C. Humphrey
University of Oxford, Oxford, United Kingdom

Chapter Outline

1. INTRODUCTION

DNA double-strand breaks (DSBs) are the most cytotoxic of DNA lesions a cell can incur, with a single unrepaired break being potentially lethal [1]. Further, misrepaired DSBs can result in various genetic alterations such as deletions, translocations, loss of heterozygosity (LOH), and chromosome loss, all of which can cause genetic diseases such as cancer [2]. DSBs occur when breaks are formed proximally on both strands of a DNA duplex simultaneously and the two ends of a DSB become physically separated. These lesions can arise through programmed biological events such as mating-type switching, class-switch recombination, restriction enzyme digestion, V(D) J recombination, and meiotic recombination, which are responsible for genetic variation [3]. DSBs can also arise from endogenous metabolic errors, such as stalled replication forks or reactive oxygen species (a byproduct of respiration), or from exposure to exogenous agents such as ionizing radiation (IR) and IR mimetics (eg, bleomycin), ultraviolet radiation (UV), alkylating agents, or other clastogens [4]. Indeed, generating irreparable DSBs through exposure to such exogenous agents is the aim of both radiotherapy and many forms of chemotherapy. Moreover, introducing targeted DSBs through the use of zinc finger nucleases or more promisingly CRISPR-CAS9 technology is the basis of genome editing, and holds considerable biomedical promise [5]. For these reasons, there is considerable interest in understanding the mechanisms of DSB repair (DSBR) and recombination.

Here we describe DSBR in budding and fission yeasts, the study of which has provided unparalleled insights into these processes. The yeasts have provided excellent models for studying repair pathways due to their simplicity, the ease with which

Genome Stability. http://dx.doi.org/10.1016/B978-0-12-803309-8.00008-2

they can be cultured, and because the functions of genes can be elucidated by screening for various phenotypes [6]. As such, findings made in yeast, and in particular the budding yeast *Saccharomyces cerevisiae*, have formed the basis for much of our understanding of DSBR pathways. Models for homologous recombination (HR) repair were first proposed based on observations in yeast and when the proteins involved were subsequently identified, many were found to be evolutionarily conserved in higher eukaryotes [7]. In this chapter, we describe the two DSBR pathways, HR and nonhomologous end-joining (NHEJ), together with their sub-pathways. Furthermore, we discuss the proteins that underpin the various steps in these repair pathways. While we cite original papers, we also highlight helpful reviews throughout the chapter where further details can be obtained.

1.1 A Brief History

In 1949, Latarjet and Ephrussi found a correlation between the level of ploidy of yeast and their sensitivity to radiation [8]. This suggested that when a sister chromosome was available for repair cells were less susceptible to damage by radiation. However, it was not until the 1960s that radiation-sensitive mutants in yeast were isolated, and the 1970s when studies in yeast began to provide an extensive genetic analysis of DNA repair [9]. In 1967, Nakai and Matsumoto discovered yeast mutants that were sensitive to ultraviolet (UV) radiation [10], and Snow [11] and Resnick [12] isolated further UV-sensitive mutants. Resnick also identified X-ray-sensitive yeast mutants [12], some of these caused by mutations in the same genes identified by Rodarte et al. as conferring recombination deficiency when mutated [10,12–14]. At the International Conference on Yeast Genetics in 1970, it was decided that all of the radiation-sensitive mutants in yeast should be labeled as "*rad*" and that mutants that are UV sensitive or UV and IR sensitive should be numbered 1–49. It was also agreed that Rad50 and onwards would be used to describe mutants that primarily affect IR sensitivity [9]. In fact, many of the genes involved in recombination were initially identified as causing sensitivity to IR when mutated [15].

2. HOMOLOGOUS RECOMBINATION MODELS

HR provides a high-fidelity form of DSBR that uses homologous DNA sequences as a template. It is ubiquitous to all organisms and can be initiated by various DNA lesions, including DSBs and interstrand crosslinks (ICLs) [16,17]. It is used to increase genetic diversity during meiosis, but in mitotic cells the main purpose of HR is to repair DSBs [18,19]. In yeast, HR is also required for mating-type switching, a process by which yeast can switch their mating type in order for haploid cells of opposite mating type to mate with each other, forming diploid cells that undergo meiosis [20].

2.1 Holliday Model

The current models for HR were developed from models of meiotic recombination in yeast from over half-a-century ago. In 1964, Robin Holliday proposed a molecular model of HR that was based on observations during meiosis in the fungus *Ustilago maydis* [21]. Based on the structure of DNA, he proposed that unraveling DNA strands anneal with complementary bases within the homologous chromosome. A key feature of this model was the prediction that the homologous chromosomes would become covalently bound through a DNA structure at this exchange point that would form a symmetrical four-way junction. Importantly, this model accounted for the genetic phenotype of crossovers as these eponymously termed "Holliday" junctions (HJs) could be resolved in either of two orientations resulting in DNA molecules with either parental (noncrossover, NCO), or recombinant (crossover, CO) configurations with respect to distal genetic markers (Fig. 8.1, double Holliday junction (dHJ)). Moreover, if crossovers occurred at sites where parental molecules differed, this could result in gene conversion, following correction of the mismatched region.

Evidence supporting the Holliday model materialized in the early 1970s from studies involving bacteriophages where HJs were visualized using electron microscopy [22–24]. In 1979, Bell and Byers provided evidence of HJs forming in *S. cerevisiae* during recombination [25,26]. Significantly, this model formed the basis of the prevailing HR models proposed to accommodate new genetic data generated over the next half-century.

2.2 Double-Strand Break Repair Model

A mechanism of DSBR using HR was first proposed by Resnick [14] and was based on direct studies of repair of chromosomal DSBs [1] that were induced by X-rays. The model extended that of Holliday in two significant ways: the initiating event was a DSB rather than a single-strand break, and it proposed that when a DSB occurs in DNA the broken DNA ends are resected to produce 3′ single-stranded tails which could then engage a homologous chromosome or sister chromatid. Another DSB-based HR model, the DSBR model proposed by Szostak and Stahl in 1983 [27], arose from studies where

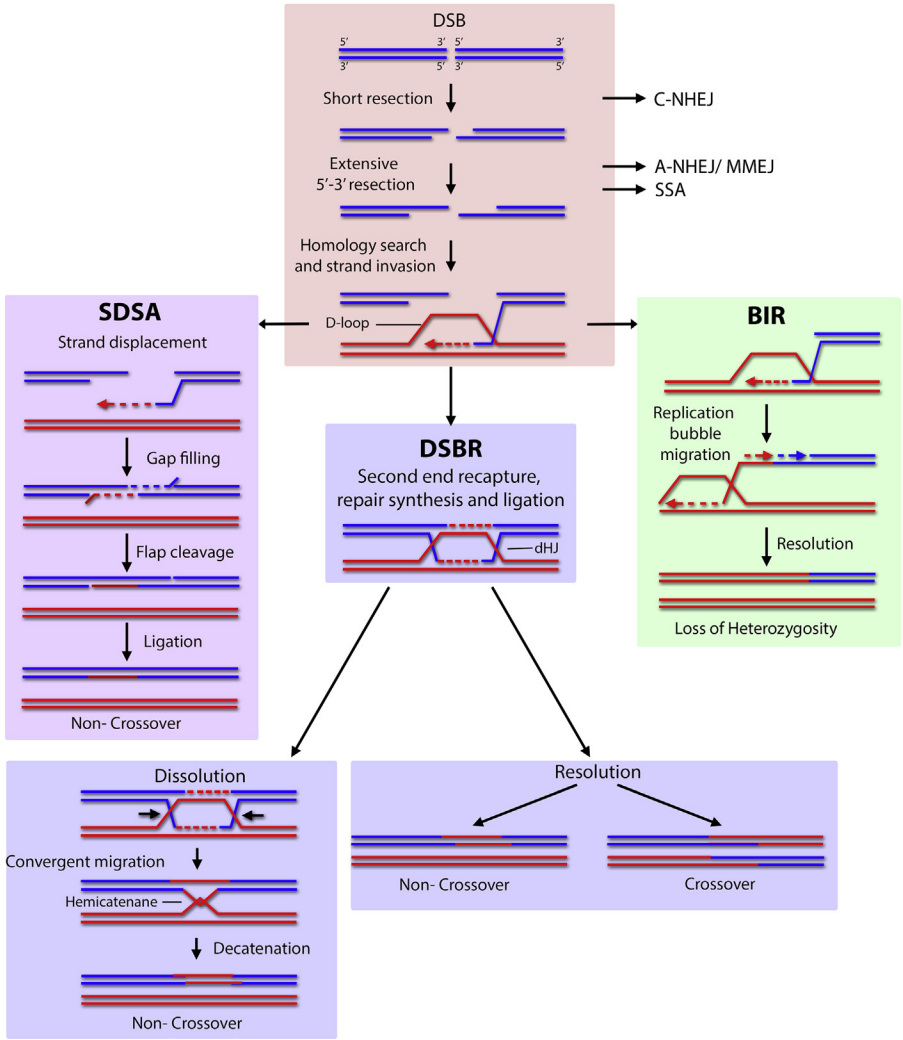

FIGURE 8.1 The various models of homologous recombination (HR) and nonhomologous end-joining (NHEJ)-mediated double-strand break (DSB) repair in yeast. After a DSB has formed in DNA, ends can be repaired via classical NHEJ (C-NHEJ) if no resection occurs. Alternatively, short resection can occur which enables repair via the alternative NHEJ and single-strand annealing (SSA) pathways. If extensive 5′–3′ resection is carried out, repair is committed to HR pathways. Homology search and strand invasion lead to D-loop formation. The main models of HR repair in yeast are double-strand break repair (DSBR), synthesis-dependent strand annealing (SDSA), and break-induced replication (BIR). These homologous recombination pathways have various outcomes. If repair is carried out via SDSA, after repair synthesis, the invading strand is displaced from the D-loop. The ends of the DSB are then annealed, possibly with flaps of excess DNA copied from the homologous template. Flap cleavage occurs to remove these, followed by ligation of the two DNA ends. This HR repair pathway results in noncrossover (NCO). In the DSBR model, once the D-loop has formed and repair synthesis carried out, second end recapture occurs forming a double Holliday junction (dHJ). The dHJ can be resolved to produce either crossover (CO) or NCO products. Alternatively, dissolution of the dHJ can take place via convergent migration of the HJs resulting in a hemicatenane, leading to noncrossover. BIR repair occurs when only one end of the DSB is available, such as at stalled replication forks. BIR involves migration of the D-loop replication "bubble" along the chromosome until another replication fork or the end of the chromosome is reached. This form of repair causes both strands of the homologous donor sequence to be copied resulting in loss of heterozygosity (LOH).

it was observed that, when plasmids containing yeast genes were linearized to simulate a DSB, increased integration of the genetic material into the yeast genome was observed compared to nonlinearized plasmids [27–29]. The DSBR model included elements of both the Holliday and Resnick models to explain these observations, and suggested that when a DSB occurs in DNA, the broken DNA ends are resected to produce 3′ single-stranded tails [30] (Fig. 8.1). Through subsequent findings, the specific role of the 3′ tail was elaborated as well as downstream events leading to HJs and resolution of recombinant structures. RPA (replication protein A), a heterotrimeric single-stranded DNA (ssDNA)-binding protein, binds to the 3′ ssDNA tails. RPA is then dissociated from the single-stranded tail by Rad51 [18]. Rad51 binds to ssDNA and forms a helical nuclear protein filament that seeks out a homologous sequence and binds to it [31]. The ssDNA nucleoprotein filament invades the homologous sequence and base pairs (bps) with the template strand, displacing the complementary

homologous strand in a process known as strand invasion. The structure formed is called a displacement loop (D-loop) [27]. DNA synthesis occurs via DNA polymerase to extend the invading strand, copying a portion of the homologous sequence, and allowing the invading strand to rejoin the other side of the break (second end recapture). Further DNA synthesis occurs and the broken ends are ligated to form a dHJ. The DSBR model revolves around the generation of dHJs [32]. A dHJ can be resolved by either cutting the same pair of strands at each junction resulting in NCO, or by cutting different strands at each junction resulting in CO [33]. Resolution of dHJs can also occur by dissolution resulting in NCO (Fig. 8.1). The DSBR model is supported by studies demonstrating that the formation of DSBs initiates HR during yeast meiosis [34], as well as experiments using Southern blotting and an RNA probe that detects ssDNA that show end resection resulting in 3′ ssDNA tails [30]. Additionally, evidence for the existence of dHJs was found in yeast using 2D gel electrophoresis [35].

It was initially thought that the DSBR model could account for the formation of all HR products based on the observation that both CO and NCO products are formed at approximately the same time during meiosis [36]. However, later studies indicated that there are separate mechanisms for forming NCO DSB products. For example, studies in *S. cerevisiae* during meiosis have demonstrated that NCO products can be detected more than 30 min before CO products, and at the same time as dHJs are present. Furthermore, yeast strains carrying a mutation in Ndt80, a meiosis-specific transcription factor required for dHJ resolution in meiotic *S. cerevisiae*, had no COs but still formed NCO products [37]. These findings suggested that NCOs could also arise by a distinct pathway that is independent of the dHJ intermediates formed as predicted by the DSBR model. Moreover, during mitotic recombination, COs are infrequent and can give rise to extensive LOH, suggesting that the DSBR model may not be able to fully explain mitotic HR outcomes. These studies indicate that recombination resulting in NCOs may arise through a distinct pathway [38].

2.3 Synthesis-Dependent Strand Annealing Model

An alternative model for HR, known as synthesis-dependent strand annealing (SDSA), explains the experimental evidence indicating that NCOs can arise before and independently of COs [37,39] which comprise only approximately 5% of mitotic recombination events in *S. cerevisiae* [40]. The SDSA model of recombination was supported by studies in the T4 phage, *S. cerevisiae*, and other organisms [41–43]. The initial steps of the SDSA model are analogous to that of the DSBR model in the processing of DSB ends and strand invasion. However, once repair synthesis has occurred, displacement of the invading strand takes place followed by second end recapture with the other end of the DSB. The displacement of the invading strand can occur either by migration of the D-loop causing continuous displacement of the newly synthesized DNA [44], or through the activity of DNA helicases [45]. Unlike in the DSBR model, no HJs are formed (Fig. 8.1). When the displaced strand reanneals with the other end of the DSB, this may result in a flap of excess nonhomologous ssDNA that is removed by the Rad1–Rad10 endonuclease [46] and the nick that is formed is sealed by DNA ligase [47]. In addition to being responsible for meiotic NCOs, it is thought that yeast mating-type switching occurs via SDSA [17]. Furthermore, the SDSA model is thought to be the predominant mechanism of repair during mitotic recombination, which has a fundamentally different purpose [48].

2.4 Break-Induced Replication Model

Break-induced replication (BIR) (also known as recombination-dependent DNA replication) is similar in the initial steps to the DSBR and SDSA models but occurs when only one end of the DSB is available for recombination or the two ends of a DSB are not coordinated. It is primarily used to reestablish stalled replication forks and repair degraded telomeres, as in both of these instances the DSBs are one-ended and so the other repair pathways cannot be used. The recombination-dependent DNA replication model was initially described in the bacteriophage T4 [49], but was later described in yeast as the BIR model using a chromosome fragmentation vector [50]. The BIR model predicts that after strand invasion, DNA synthesis extends the invading strand, copying the template via a unidirectional replication fork or replication "bubble" that migrates along the template sequence [50] (Fig. 8.1). Repair synthesis and bubble migration continue until it encounters a converging replication fork or reaches the end of the chromosome [51]. This results in an extensive nonreciprocal transfer of genetic information from the template chromosome to the invading chromosome [45], which can cause extensive LOH and is highly mutagenic [51–53]. Unlike SDSA, which uses leading strand DNA synthesis, BIR requires both leading and lagging strand synthesis. BIR restoration of collapsed replication forks is Rad51 dependent [54]; however, Rad51-independent BIR has been reported in both budding and fission yeast [52,55,56].

2.5 Single-Strand Annealing Model

Single-strand annealing (SSA) is a form of HR repair that occurs when repetitive DNA sequences lay either side of the break, and differs from most other HR pathways in that it is independent of Rad51 in yeast [57]. As in HR, 5′–3′ resection

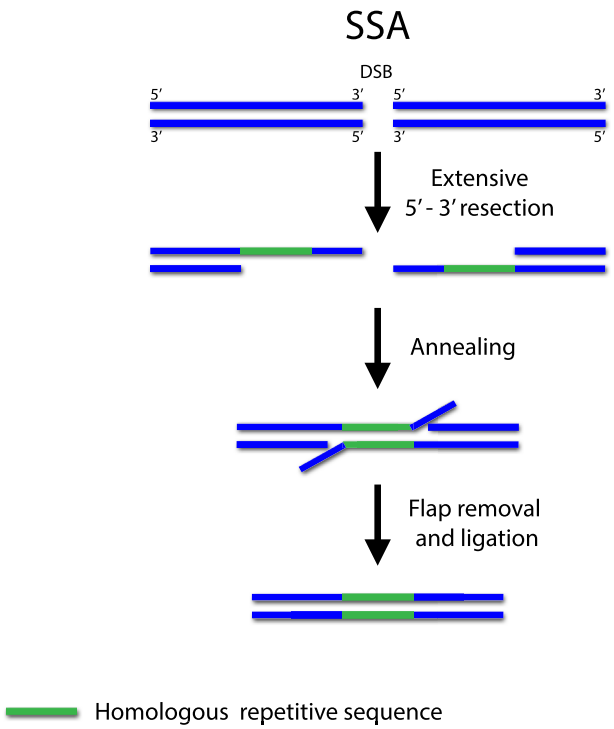

FIGURE 8.2 **The single-strand annealing (SSA) pathway of homologous recombination (HR) repair.** Extensive 5′–3′ resection of double-strand break (DSB) ends reveals homologous repetitive sequences on either side of the DSB. These sequences anneal and flaps of nonhomologous DNA can form. The flaps are cleaved and the annealed ends ligated. This model of repair differs from other HR pathways, as it does not involve a homologous donor sequence as a template.

occurs but SSA takes place when resection exposes repetitive homologous sequences on either side of the break site, leaving 3′ ssDNA tails. The two complementary ends of the DSB anneal and any unique sequence present between the repeats forms 3′ overhanging flaps [45], which are cleaved by the Rad1–Rad10 endonuclease. Ligation occurs, sealing the break, resulting in deletion of the DNA that existed between the repeats (Fig. 8.2). Thus, SSA is a nonconservative HR pathway, but it is an efficient form of DSBR when the repeats are greater than 200 bp in length [58]. Indeed, SSA remains efficient even if up to 15 kb of unique sequence lies between the repeats [17]. However, SSA is likely to occur at far higher rates in higher eukaryotes than in yeast, because the yeast genome contains comparatively low levels of repetitive sequences, and the other HR mechanisms in *S. cerevisiae* are extremely efficient [59].

3. COMMON HOMOLOGOUS RECOMBINATION STEPS

HR can be divided into several functionally distinct steps, which occur before or after synapsis (ie, the formation of joint molecule intermediates as a result of homology search and strand invasion [60]). Presynaptic steps include end resection, nucleofilament formation, and homology search. Postsynaptic steps include DNA synthesis, branch migration, HJ resolution/dissolution, and strand annealing [61]. Here we describe these key steps, together with the relevant repair factors that facilitate them (Table 8.1).

3.1 End Resection

End resection, or end processing, refers to the removal of bases from the 5′ end to reveal a 3′ overhang. This is a two-step process, the first of which requires the MRX complex [comprised of the proteins Mre11, Rad50, and Xrs2 (Nbs1 in *Schizosaccharomyces pombe* and *Homo sapiens*) [62]] in conjunction with Sae2 to initiate end resection of approximately 100 nucleotides, resulting in a short 3′ ssDNA overhang [63]. Accordingly, null mutants of Mre11, Rad50, or Xrs2 in yeast are highly sensitive to IR and exhibit slowed DSB processing [7]. Mre11 is an 83 kDa protein with phosphoesterase motifs that are involved in its 3′–5′ double-stranded DNA (dsDNA) exonuclease activity and require manganese as a cofactor [64,65]. Sae2 and the nuclease activity of Mre11 are required to remove covalent adducts from DSB ends or "dirty ends," but are

TABLE 8.1 Proteins Involved in the Homologous Recombination (HR) Repair Pathways in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*

Saccharomyces cerevisiae	*Schizosaccharomyces pombe*[b]	Function
(a) Presynaptic[a]		
Rad52	Rad52/Rad22	Facilitates nucleoprotein filament formation.
Mre11	Mre11/Rad32	Part of the MRX complex and involved in end resection. This is known as the MRN complex in *S. pombe*.
Rad50	Rad50	Rad50 is required for formation of the MRX/MRN complex and is vital for end resection.
Xrs2	Nbs1	A subunit of the MRX complex.
Sae2	Ctp1	Works with MRX to resect 5′ ends of a DSB.
Exo1	Exo1	5′–3′ Exonuclease required for extensive end resection.
Dna2	Dna2	DNA-dependent ATPase required for extensive resection.
RPA (Replication protein A)	Rpa1	Single-stranded DNA-binding protein. RPA coats ssDNA preventing secondary structures forming. It is required for Rad51 filament formation.
Rad51	Rhp51	Forms helical filaments with single-stranded DNA. Involved in strand exchange and homology seeking.
Rad55	Rhp55	Paralogue of Rad51 and forms a heterodimer with Rad57. This complex stabilizes Rad51 filaments.
Rad57	Rad57	Paralogue of Rad51 and forms a heterodimer with Rad55. This complex stabilizes Rad51 filaments.
Rad54	Rhp54	Facilitates strand exchange and D-loop formation.
Rdh54	Rdh54	Facilitates strand exchange and D-loop formation.
Srs2	Srs2	DNA helicase and DNA-dependent ATPase. Srs2 is anti-recombinogenic by interrupting Rad51 filaments and inhibiting DNA strand exchange.
Shu1	No known homolog	Shu1 is a component of the Shu complex, consisting of Psy3, Csm2, Shu2 and Shu1. It is involved in inhibiting Srs2 and stabilizing Rad51 filaments.
Shu2	Sws1	Shu2 is a component of the Shu complex, consisting of Psy3, Csm2, Shu2 and Shu1. It is involved in inhibiting Srs2 and stabilizing Rad51 filaments.
Psy3	Rld1	Psy3 is a component of the Shu complex, consisting of Psy3, Csm2, Shu2, and Shu1. It is involved in inhibiting Srs2 and stabilizing Rad51 filaments.
Csm2	Swi3	Csm2 is a component of the Shu complex, consisting of Psy3, Csm2, Shu2, and Shu1. It is involved in inhibiting Srs2 and stabilizing Rad51 filaments.
Rad59	No known homolog	Part of the Rad52 epistasis group.
(b) Postsynaptic		
Pol3/DNA polymerase δ	Cdc6	DNA polymerase δ is used during leading strand repair synthesis.
Pol2/DNA polymerase ε	Cdc20	DNA polymerase ε is used during leading strand repair synthesis.
Pol1/DNA polymerase α	Pol1	DNA polymerase α is used in BIR as both leading and lagging strand synthesis is required.

TABLE 8.1 **Proteins Involved in the Homologous Recombination (HR) Repair Pathways in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*—cont'd**

Saccharomyces cerevisiae	*Schizosaccharomyces pombe*[b]	Function
PCNA complex	PCNA complex	Acts as a sliding clamp for DNA polymerase δ.
Sgs1	Rqh1	DNA helicase that works with Dna2 in extensive resection. It is also required for dHJ dissolution and forms a complex with Top3 and Rmi1 in *Saccharomyces cerevisiae*.
Top3	Top3	Topoisomerase III is a type IA topoisomerase that unwinds single stranded negatively supercoiled DNA. Required for dHJ dissolution.
Rmi1	Rmi1	Subunit of the Sgs1–Top3–Rmi1 complex. It stimulates decatenation.
Yen1	No known homolog	Holliday junction resolvase.
Ndt80	No known homolog	Meiosis-specific transcription factor implicated in dHJ resolution to form COs.
Rad1	Rad16	Forms a structure-specific endonuclease with Rad10 (Rad1–Rad10) that is involved in removing excess nonhomologous ssDNA after recombination resolution.
Rad10	Swi10	Forms a structure-specific endonuclease with Rad1 (Rad10–Rad10).
Mms4	Eme1	Forms a structure-specific nuclease with Mus81. Involved in resolution of HR-intermediates.
Mus81	Mus81	Forms a structure-specific nuclease with Mms4. Involved in resolution of HR-intermediates.
Mph1	Fml1 and Fml2 are orthologs	A helicase involved in strand displacement during SDSA.

CO, Crossover; *dHJ*, double Holliday junction; *DSB*, double-strand break; *SDSA*, synthesis-dependent strand annealing; *ssDNA*, single-stranded DNA.
[a]*The proteins have been categorized into presynaptic (a) and postsynaptic (b).*
[b]*The* S. pombe *homologs were found by searching for the systematic name.*
Taken from http://www.yeastgenome.org/ on http://www.pombase.org/.

not required to resect "clean ends." Similarly, the Sae2 ortholog, Ctp1 in fission yeast is required for removal of covalently bound Topoisomerase I and II from DNA [66]. The nuclease activities of Mre11 are enhanced by the binding of Rad50 [67], which is part of the structural maintenance of chromosomes (SMC) family and has a long coiled-coil domain that is necessary for Mre11 binding and MRX complex function. Mre11 binds to Rad50 at the coiled-coil in a zinc-dependent manner forming an MR subcomplex [67]. A Rad50 mutation (Rad50S) causes 3′ ssDNA tails to be abolished, indicating that it is also required for end resection [30]. The Xrs2 subunit of MRX binds Mre11 but not Rad50 and translocates Mre11 to the nucleus, which is necessary for mitotic DNA repair [62]. Xrs2 also enhances the exonuclease activity of Mre11 and the MR subcomplex [67]. The MRX complex is also able to stimulate resection in an indirect manner by recruiting Dna2 and/or Exo1 nucleases [68].

Dna2 together with Sgs1, or Exo1 alone, can catalyze extensive resection. They can directly process and resect DSB ends, but not those that contain covalent adducts. Additionally, Top3 and Rmi1, which interact with Sgs1, are necessary for end resection; however, their functions are not catalytic but structural. A model has been proposed based on studies in meiosis that MRX and Sae2 create a nick in the 5′ strand at a point distant from the DSB end that is resected in both directions by Mre11, which has 3′–5′ exonuclease activity, and Exo1, which is a 5′–3′ exonuclease. This theory is based on the observation that the Mre11 3′–5′ exonuclease has a role in end resection, which would not be otherwise possible as it is unable to resect in the 5′–3′ direction [69]. Experiments have demonstrated that the short 3′ ssDNA tails that result from MRX/Sae2-dependent resection are adequate for HR to occur. Because this occurs without Exo1 or Sgs1–Dna2, it suggests that extensive resection may not be required for HR. Additionally, a lack of MRX, Mre11, or Sae2 causes complete inhibition of end resection and cell death. However, despite extensive end resection not being necessary for HR to occur, it is required for the DNA damage checkpoint to be activated [45].

3.2 Nucleofilament Formation

Once nucleases begin to resect DSB ends, RPA binds to the resulting ssDNA. RPA is needed to prevent ssDNA tail degradation by MRX-Sae2 and the formation of hairpin-capped ends that would hinder HR repair [70]. RPA is also required to stimulate Rad51 nucleoprotein filament formation by preventing secondary structures from forming in the 3′ ssDNA tails [71]. Although RPA can inhibit Rad51 filament formation if both bind to ssDNA simultaneously, mediator proteins (eg, Rad52) can overcome this inhibition [72–74].

Rad51 nucleoprotein filaments are important for homology searching and strand invasion [3]. Rad51 is part of the Rad52 epistasis group of proteins and polymerizes to form helical filaments on ssDNA in an ATP-dependent manner [45,75,76]. Rad51 has been shown to catalyze strand exchange alone in vitro, but other proteins are required for this to occur efficiently in vivo. These mediator proteins have various roles such as facilitating Rad51 filament formation, filament stabilization, and strand exchange [77,78]. The Rad51 paralogues Rad55 and Rad57 form a heterodimer (Rad55–Rad57) that has been shown to form a complex with Rad51 and Srs2 simultaneously in vitro and may be incorporated into Rad51 filaments which acts to stabilize the Rad51 filament and inhibit displacement of Rad51 from ssDNA by Srs2 [79]. Srs2 is a translocase/helicase that interacts with Rad51 and activates Rad51's ATPase activity, causing Rad51 filament disassembly and acting as a negative regulator of HR [76,79]. The Shu proteins Shu1, Shu2, Psy3, and Csm2 form a complex that is structurally similar to the Rad51 dimer and it has been suggested that the Shu complex is integrated into Rad51 filaments in a manner similar to the Rad55–Rad57 heterodimer. Furthermore, the Shu complex has been shown to stabilize Rad51 filaments on ssDNA and to inhibit Srs2 anti-recombination activity [80].

3.3 Homology Search and Strand Invasion

Homology seeking is the process by which the nucleoprotein filament searches for a homologous sequence within the genome to use as a template for repair. Sister chromatids are preferred over homologs as substrates for recombinational repair in *S. cerevisiae* [81]. This is due to cohesins providing physical links between sister chromatids [82]. Despite chromatid cohesion, chromatin immunoprecipitation (ChIP) experiments demonstrated that Rad51 signals can be found at great distances from the site of a DSB and it was suggested that these signals are caused by homology probing by the Rad51 nucleoprotein filament transiently interacting with distant and nonhomologous sequences. However, these signals were lower in regions of the genome more distant to where the DSB occurred, suggesting that homology search/genome probing is not equally efficient throughout the genome [83]. In addition, physical proximity of homologous regions within the nucleus and the DSB site is an important factor in efficient and fast repair. For example, if both a DSB and a homologous sequence lie close to a centromere, homology search and repair occur more rapidly because in yeast the centromeres of chromosomes cluster in the nucleus and are tethered to the spindle pole bodies. The three-dimensional (3D) organization of chromosomes within the nucleus is not random and this organization greatly affects the efficiency of recombination events [83,84].

Homology search is also stimulated by the dsDNA-dependent ATPases Rad54 and Rdh54 (a Rad54 homolog) [85], and when both are absent genome probing is abolished. These proteins stimulate D-loop formation by interacting with Rad51 [83]. When bound to dsDNA, Rad54 can trigger conformational changes in the DNA, resulting in positive and negative supercoils. The negative supercoiling (or unwinding) of DNA may allow transient separation of homologous dsDNA strands, facilitating strand invasion and D-loop formation [85,86]. Rad54 is also important for displacement of Rad51 once strand invasion takes place, thereby facilitating DNA synthesis by allowing DNA polymerases to access and extend the invading strand [87].

3.4 DNA Repair Synthesis

DNA repair synthesis is important for creating a homologous sequence to which the second end can anneal. Using a homologous chromatid or chromosome as its replication template facilitates the high fidelity of HR repair [88]. DNA repair synthesis involves extension of the 3′ end of the invading strand within the D-loop by DNA polymerase δ and/or ε. DNA pol δ and ε are redundant with each other as studies have demonstrated that repair synthesis can occur when either is deleted but not when both are absent [47]. However, experiments studying *MAT* locus recombination have demonstrated that repair synthesis does not require a number of the proteins necessary for "standard" DNA replication. For example, some of the proteins required for initiating replication, such as ORC (origin recognition complex) and Cdc7–Dbf4 kinase, are not needed. Furthermore, DNA pol α and Okazaki fragment processing proteins are mostly not essential for HR repair synthesis, presumably because many HR pathways use only leading strand synthesis [45,51,89]. However, BIR requires DNA pol δ, ε, and α (the latter being the primary polymerase responsible for replication) due to both leading and lagging strand synthesis taking place in this repair pathway [90,91].

Additionally, the trimeric PCNA complex is required and forms a ring around DNA, acting as a sliding clamp for DNA pol δ. It has been demonstrated that posttranslational modification of PCNA affects its function. For example, polyubiquitination of PCNA is necessary for its role in error-free HR replication synthesis, whereas SUMOylation (attachment of a small ubiquitin-related modifier to the protein) inhibits its repair replication functions. Both SUMO and ubiquitin competitively bind to PCNA, thereby acting as a molecular switch to either activate or antagonize its repair synthesis activity [47,88].

In addition to repair synthesis within the D-loop, DNA synthesis also occurs to fill any gaps after recombination intermediates have been resolved. This requires deoxyribonucleotide triphosphate (dNTP) synthesis, which is induced by the DNA damage checkpoint in response to DNA damage to promote HR [92].

3.5 Strand Annealing

Rad52 has been shown to stimulate ssDNA annealing in vitro [93] which may be important for second end recapture in DSBR and SDSA and for recombination via SSA. It has been demonstrated that SUMOylation of Rad52 promotes repair via HR but suppress SSA and BIR in yeast [94]. RPA ensures that SSA is not able to occur in the absence of Rad52 by preventing annealing between short microhomologies that cannot be annealed in a Rad52-dependent manner [45,95]. Once annealing takes place, there may be flaps of excess DNA where the strands have annealed, which are cleaved by the Rad1–Rad10 endonuclease [46].

3.6 Resolution and Dissolution of Recombination Intermediates

The SDSA model predicts that only NCO products are created via this pathway, by resolving D-loop intermediates via displacement of the extended invading strand. This is now understood to be facilitated through the activities of RecQ, Srs2, Fbh1, Mph1, and possibly other helicases.

In *S. cerevisiae*, the Mph1 helicase has been shown in vitro to dissociate D-loop intermediates generated by Rad51. Furthermore, mutations in *MPH1* lead to an increase in CO products formed in response to DSBR, indicating that D-loop dissociation is impaired in these mutants, likely causing dHJ formation. Furthermore, 2D gel electrophoresis experiments have demonstrated that when Mph1 is not present, HJs transiently accumulate. Overexpressing Mph1 suppresses BIR and mutation of Mph1 causes an increase in the incidence of BIR. This is likely due to Mph1-dependent D-loop intermediate dissociation. However, BIR does require strand displacement via Pif1 during repair synthesis, which facilitates D-loop migration rather than dissociation [45].

The helicase Srs2 has been shown to facilitate NCO formation, more specifically by promoting SDSA [96]. Srs2 has also been shown to be capable of resolving D-loop intermediates in vitro; however, it is less efficient than Mph1 [97]. Additionally, the *S. pombe* Srs2 ortholog Fbh1 has been shown to suppress CO formation [98]. The budding yeast RecQ helicase Sgs1 is an ortholog to human BLM and, as well as having a role in end resection, has been shown to promote repair via the SDSA pathway. Human BLM has been shown to dissociate D-loops but the primary role of Sgs1 in resolving HR intermediates is in dissolution [96].

dHJs can be resolved via dissolution, resulting in NCO. Dissolution involves convergent migration of the two HJs, forming a hemicatenane (the joining of two DNA duplexes where one strand of a DNA duplex is wound around the strand of another duplex), followed by unlinking (decatenation) of the two duplexes without CO (Fig. 8.1). The Sgs1–Top3–Rmi1 complex has been shown to dissolve dHJs in vitro [99]. Sgs1 is a helicase that causes branch migration of the HJs leading to a hemicatenane and creating ssDNA as a substrate for Top3. RPA binds the ssDNA generated by Sgs1 and stabilizes it, facilitating unlinking. Top3 is a type IA topoisomerase that is responsible for decatenation. Rmi1 acts to stimulate Sgs1–Top3-mediated dissolution, specifically the decatenation stage [100].

Resolution of HR intermediates can also occur via endonucleolytic cleavage.

Mus81 forms a structure-specific nuclease with Mms4, becoming Mus81–Mms4 (Mus81–Eme1 in *S. pombe*), and has been implicated in the production of CO products [38]. However, Mus81–Mms4 uses nicked HJs or 3′ flaps and replication fork substrates preferentially and it has therefore been suggested that it does not act on dHJs, but instead cleaves D-loops. This type of HR intermediate resolution differs from both the SDSA and DSBR models and it has been proposed that dHJs are not formed [101,102]. Another resolvase, Yen1, has been implicated in resolution of dHJs, which can result in both CO and NCO products [103]. This type of resolution is consistent with the DSBR model of recombination. However, *yen1*Δ mutants do not exhibit severe deficiencies in recombination in both mitotic and meiotic cells compared to when both Yen1 and Mms4 are deleted. If dHJs were the primary form of HR intermediates in mitotic cells, it would be expected that deletion of Yen1 would have a greater effect on recombination efficiency. Experimental data has demonstrated that Mms4 is

able to compensate for the activity of Yen1 in *yen1*Δ strains, but Yen1 cannot replace the activity of Mms4. This further supports the existence of alternative recombination intermediates to dHJs [101].

4. NONHOMOLOGOUS END-JOINING

The term NHEJ was coined by Moore and Haber in 1996 when they used it to describe DSBR in yeast that occurs when a homologous donor sequence is not present [104]. It is the process by which the two ends of a DSB are re-ligated together and is considered to be a more error-prone method of repair compared to HR [105]. In contrast to HR, most of the research relating to NHEJ has come from mammalian studies, which reflects the finding that NHEJ is the predominant repair pathway in higher eukaryotes. In yeast, however, HR is the predominant method used for DSBR. This has made identifying the proteins involved in NHEJ in yeast more difficult, because if NHEJ genes are simply deleted in a background where HR is possible, it is harder to detect repair defects [106]. NHEJ is the predominant form of repair during G1 phase of the cell cycle in yeast [107]. This further complicates the identification of yeast NHEJ repair genes as yeast are usually grown under exponential growth conditions where G1 is short, and because DSBs incurred in G1 are fixed via HR pathways in S phase. Furthermore, fission yeast lack a G1/S checkpoint, which would be expected to delay cell cycle progression in response to DSBs in other organisms. Despite these complexities, many NHEJ genes have been identified in yeast.

The basic process of NHEJ involves the processing and alignment of the two ends of a DSB, followed by ligation. In brief, NHEJ is initiated by the binding of Yku to DSB ends, which then facilitates the recruitment of nucleases to remove damaged DNA, polymerases to facilitate repair, and ligase to ligate the ends together [108].

The initial steps of C-NHEJ (classical-NHEJ) are evolutionarily conserved and involve binding of Yku (the yeast equivalent of Ku) and MRX to the DSB ends in yeast. Yku is a heterodimeric protein, and its binding to DNA ends prevents resection of the DSB ends by Exo1, which would otherwise result in a loss of genetic material [45]. Additionally, extensive 5′ resection of DSB ends inhibits NHEJ by irreversibly committing that DSB to be repaired via HR [109]. In order for the NHEJ reaction to occur, the two DSB ends need to be in close proximity to one another. It has been suggested that end-bridging occurs, whereby protein–protein interactions between each DSB end physically connect the two DNA molecules. The MRX complex is involved in NHEJ as well as HR and has been shown to have end-bridging activity. While such end-bridging is thought to result from the formation of Rad50 dimers via its zinc hooks, experimental data indicate that all the subunits of the MRX complex are required for end-bridging [109–111]. Perhaps surprisingly, *S. pombe* does not require MRN (the MRX homolog) for C-NHEJ [112].

4.1 Core Nonhomologous End-Joining Machinery

The core machinery of NHEJ are the factors considered essential for C-NHEJ (Table 8.2) [109]. The Yku heterodimer, made up of Yku70 and Yku80, is vital for NHEJ. It binds and forms a ring around DNA at DSB ends with the Yku80 subunit oriented toward the DSB end to enable it to interact with Dnl4. Dnl4 also forms a ring structure around DNA and creates a complex with Lif1, making up DNA ligase IV; interestingly, *S. pombe* lacks Lif1. DNA ligase IV joins together the two broken DNA ends in a DSB by creating a phosphodiester bond between the 3′ end of one end of the DSB and the 5′ end of the other via adenylation [112–114]. Dnl4 has two tandem BRCT (BRCA1 C-terminal) domains that are connected by a linker that allows Dnl4 to bind to Lif1. Interaction between the linker and Lif1 is required for NHEJ and without Lif1, Dnl4 is not recruited to DSB ends. It was thought that this interaction between the BRCT linker of Dnl4 and Lif1 is what made Lif1 and the BRCT domains of Dnl4 necessary for NHEJ but, in reality, interaction between Lif1 and the BRCT domains themselves (not just their linker) is required [114]. Lif1 is predicted to have a coiled-coil domain that allows it to bind DNA and interact with the BRCT linker of Dnl4. Xrs2 of the MRX complex recruits and interacts with Lif1 via the forkhead-associated (FHA) domain of Xrs2. FHA domains bind phosphorylated threonine residues, indicating that Lif1 may be phosphorylated; similarly, the human homolog of Lif1, XRCC4, is phosphorylated during NHEJ. The FHA domain of Xrs2 is specific to its NHEJ activity and is not required for its role in HR [109,111,115].

Nej1 interacts with the Dnl4–Lif1 complex and is essential for efficient NHEJ in yeast. It has been shown to facilitate nuclear localization of Lif1 and to promote efficient adenylation of Dnl4–Lif1. Furthermore, it has a greater role in the deadenylation of Dnl4–Lif1, which is required for Dnl4–Lif1 molecules to be able to catalyze more than one ligation reaction. Without this deadenylation activity, the efficiency of NHEJ would be limited by the abundance of Dnl4–Lif1 molecules. It has also been suggested that Nej1 may have a role earlier on in NHEJ by stabilizing the binding of Yku70–Yku80 to DSB ends [109,116,117].

4.1.1 End Processing During Nonhomologous End-Joining

Although many of the end-processing components in mammalian NHEJ have been identified, only some homologs have been discovered in yeast. For example, the main nucleases in mammalian C-NHEJ (eg, Artemis/DNA-PKc complex) have

TABLE 8.2 Core Proteins Involved in the Classical NHEJ (C-NHEJ) Pathway in *Saccharomyces cerevisiae* and *Saccharomyces pombe* [109]

Saccharomyces cerevisiae	*Schizosaccharomyces pombe*[a]	Function
Yku70	Pku70	Subunit of the Ku complex, binds to double-strand break (DSB) ends.
Yku80	Pku80	Subunit of the Ku complex, binds to DSB ends.
Lif1	Xrc4	Forms a heterodimer with Dnl4.
Dnl4	Lig4	Forms a heterodimer with Lif1 and is required for ligation.
Nej1	Xlf1	Interacts with Dnl4–Lif1 complex to localize Lif1 to the nucleus.
Mre11	-	Forms MRX complex which functions similarly to DNA-PKc in mammalian cells. N/A in *S. pombe*.
Rad50	–	Forms MRX complex which functions similarly to DNA-PKc in mammalian cells. N/A in *S. pombe*.
Xrs2	–	Forms MRX complex which functions similarly to DNA-PKc in mammalian cells. N/A in *S. pombe*.
Pol4	Pol4	Required for filling gaps in the DNA where DSB ends have been misaligned.
Rad27	Rad2/FEN1	5′ Flap endonuclease recruited by Nej1 and Dnl4–Lif1 to remove 5′ flaps of noncomplementary DNA after DSB end annealing.

[a]*The* S. pombe *homologs were found by searching for the systematic name.*
Taken from http://www.yeastgenome.org/ on http://www.pombase.org/.

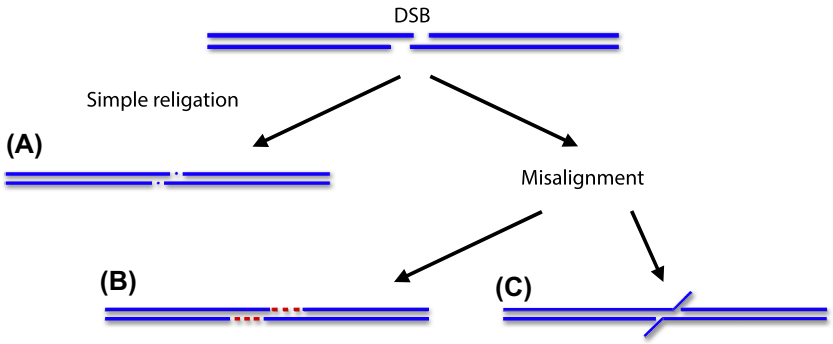

FIGURE 8.3 **The classical NHEJ (C-NHEJ) repair pathway.** Double-strand break (DSB) ends are bound by Yku70–80 heterodimer that protects the ends from resection, thus facilitating C-NHEJ. (A) Simple re-ligation of DSB ends requires Yku, MRX, and Dnl4/Lif1/Nej1. (B) Misalignment of DSB ends requires Yku, MRX, Dnl4/Lif1/Nej1, and Pol4 to fill gaps. This gap filling is error prone. (C) Misalignment of DSB ends resulting in flaps of excess DNA. This requires Yku, MRX, Dnl4/Lif1/Nej1, and Rad27 to cleave flaps, resulting in deletions.

no homologs in yeast. However, it has been suggested that MRX may not only have a role in early NHEJ, but may also be involved in the removal of adducts from DSB ends in NHEJ [118]. Pol4 is a DNA polymerase that has been implicated in NHEJ in yeast and is required to fill gaps in the DNA where DSB ends have been misaligned. This acts to stabilize the annealed DNA ends before ligation takes place [118]. Pol4 also reads through mismatched bases, even when the priming 3′ base is mismatched, as it lacks proofreading exonuclease activity [119]. This contributes to the error-prone nature of NHEJ. Rad27 is a 5′ flap endonuclease that interacts with and is recruited by Nej1 and Dnl4–Lif1. It is involved in cleaving 5′ flaps of noncomplementary DNA that can form when DSB ends anneal [120]. The combined activity of Rad27 and Pol4 facilitates efficient gap filling and end processing in NHEJ [109]. However, whenever Rad27 is required to cleave 5′ flaps of excess DNA or Pol4 is required to fill in gaps, it is possible that the misalignment of the two DSB ends has occurred, resulting in deletions (former) or insertions (latter) (see Fig. 8.3).

4.1.2 Ligation

If the ends of a DSB are "clean" with complementary overhangs (such as the DSBs caused by nucleases), NHEJ can occur via simple re-ligation. However, DSBs in DNA often result in "dirty" DNA ends, meaning that the ends lack 5′ phosphates and 3′ hydroxyls and thus cannot simply be re-ligated [109]. Furthermore, DSBs that occur as a result of DNA-damaging agents such as IR or bleomycin (a radiomimetic) may have further damage such as adducts or missing, damaged, or altered bases, making re-ligation more difficult. If simple re-ligation is not possible, microhomologies (short region of complementary bases at either end of a DSB) at each of the DSB ends can be aligned, but this can lead to deletions and occasionally small insertions [109,118,121,122].

4.2 Alternative End-Joining

Alternative NHEJ (A-EJ) pathways have been proposed based on observations that end joining can occur independently of Yku, and were first discovered in yeast [123]. Curiously, in *yku70*Δ cells, joining of complementary ends (such as those generated by HO endonuclease) is inefficient while noncomplementary ends are joined with great efficiency. However, these joins require greater microhomology, have more extensive deletions [124], and are prone to base substitutions [112]. This type of repair is termed microhomology-mediated end-joining (MMEJ) (see Fig. 8.4), and falls under the category of A-EJ. MMEJ differs from SSA, in that it is Rad52 independent. However, if the microhomologies are greater than 8 bp in length, Rad52 is required [45,122]. Additionally, evidence suggests that the Rad1–Rad10 endonuclease is required for MMEJ, similar to SSA [109]. Studies have demonstrated that while MMEJ can occur independently of MRX, the majority of MMEJ events are MRX dependent [122]. In *S. cerevisiae*, while DNA ligase IV is required for all C-NHEJ events, it is only partially required for A-EJ, where ligase I can also be employed, albeit less efficiently [105]. However, in *S. pombe* (as in mammals), A-EJ can occur entirely independently of ligase IV [112].

Evidence suggests that blunt ends (DSBs that do not result in overhangs) may be repaired by a different mechanism from C-NHEJ. Unlike in mammalian cells, blunt end joining via NHEJ in yeast is very inefficient and occurs independently of Yku70 [105]. This form of repair is inaccurate, requires Rad50 and Rad52, and is partially dependent on Srs2. However, when Yku70 is present, blunt end repair is not dependent on Srs2 or Rad52 and occurs with greater accuracy [125].

It has been argued that these A-EJ pathways are not separate pathways to C-NHEJ, but are simply the result of cells utilizing other analogous proteins when the core NHEJ machinery is not available, resulting in less-efficient and more error-prone repair [118]. In wild-type *S. pombe*, only 1 in 48 NHEJ events occurred via MMEJ in an extrachromosomal DSBR assay where HO endonuclease was used to induce DSBs. This suggests that repair by MMEJ is repressed in the presence of core C-NHEJ factors (Yku and Dnl4) [112]. These findings support the argument that A-EJ pathways are redundant mechanisms used only when the core NHEJ machinery is not available. Additionally, the dependence on Rad1–Rad10 in some MMEJ events raises the possibility that MMEJ is a class of SSA and not a separate pathway. However, Yku-dependent end joining (C-NHEJ) occurs when microhomologies are less than 5 bp, whereas Rad52-dependent end joining occurs only

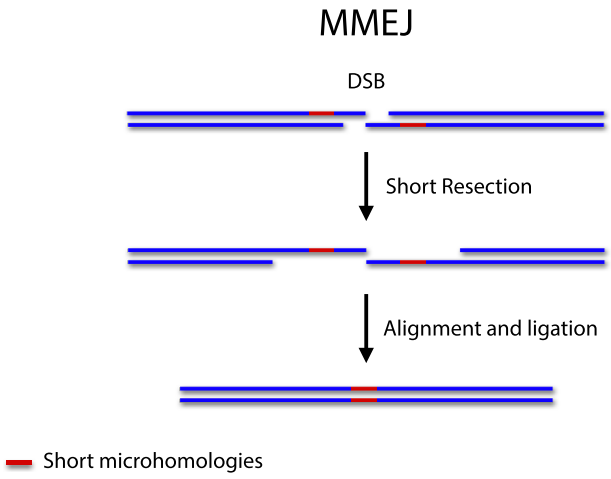

FIGURE 8.4 The microhomology-mediated end-joining (MMEJ) repair pathway. Unlike classical NHEJ, this alternative NHEJ pathway involves short resection of double-strand break (DSB) ends to reveal short homologous sequences of 5–25 bp [122]. These microhomologies are used to align the DSB ends before ligation.

when microhomologies are greater than 8 bp. When homologies of 6–8 bp exist between DSB ends, end joining is Yku and Rad52 independent. These findings support the notion that MMEJ is a separate pathway from SSA and C-NHEJ [122].

5. CELL CYCLE REGULATION OF HOMOLOGOUS RECOMBINATION AND NONHOMOLOGOUS END-JOINING

The cell cycle plays an important role in regulating DSBR pathway choice. In yeast, NHEJ repair pathways are restricted to G1 phase, while HR repair takes place in the S and G2 phases of the cell cycle. This is logical since, due to the haploid nature of yeast, sister chromatids are only available as a homologous template during S and G2.

A key mechanism by which DSBR pathways are regulated through the cell cycle is through CDK1-dependent phosphorylation and activation of Sae2, which facilitates DSB end-resection. Moreover, CDK1 activation is required for RPA recruitment and Rad51 nucleoprotein filament formation, and is thus required for HR to occur in yeast [126]. As CDK activity is low during G1 but increases in S-phase and G2, this helps ensure DSBR pathway choice.

Another mechanism of pathway choice is employed during meiosis, where Nej1, a negative regulator of DNA ligase IV, is transcriptionally repressed by *MAT*a1/α2. This repressor is induced in meiotic diploid cells, where both alleles of the *MAT* locus are expressed, thus, HR repair is favored as a result of DNA ligase IV inhibition [116].

Chromatin remodeling also influences DSBR pathway choice. Histones that form chromatin with DNA can be methylated or acetylated, affecting the structure of chromatin [127]. Posttranslational methylation of histone H3 on lysine 36 (H3K36) by Set2 has been shown to reduce the accessibility of chromatin, diminish resection of DSB ends, and promote the recruitment of Ku to DSBs, thereby promoting NHEJ. In contrast, Gcn5-dependent H3K36 acetylation increases accessibility of DNA within chromatin, enhances end resection, and encourages repair via HR. Accordingly, trimethylated H3K36 is increased in G1, while acetylated H3K36 is increased in S phase [128]. Thus, cell cycle regulation of DSBR pathway choice is regulated by multiple factors.

The importance of regulating DSBR pathway choice during the cell cycle is likely to reflect the consequences of inappropriate choice on genome stability. HR in G1 in haploid yeast may result in duplication of inappropriate genetic material or chromosomal rearrangements if HR repair is associated with a crossover. Furthermore, it has been suggested that HR occurring during G1 phase results in LOH events [129]. Moreover, NHEJ repair of a one-ended break resulting from replication fork collapse will result in deletions or chromosomal rearrangements.

6. CONCLUSION

DSBs can be caused by a multitude of events and the pathways that repair them, which are outlined in this chapter, are vital for maintaining both cell viability and genomic stability [130]. In yeast, HR pathways are of particular importance as they are the primary mechanism by which DSBs are repaired and are thus vital for maintaining the integrity of the genome during replication, where DSBs can arise following replication fork collapse [131]. Furthermore, HR plays an important role in meiosis, where it not only promotes genetic diversity by creating COs, but also provides physical connections between sister chromatids which are vital for proper chromosome segregation and the prevention of aneuploidy [132]. The advances in our understanding of DSBR using yeast model systems have contributed to our understanding of DSBR in all organisms. This is particularly important in the field of cancer research where, in mammalian cells, chromosomal rearrangements contribute to the transformation of normal cells to cancerous cells via tumor-suppressor gene loss or oncogene activation [133]. Indeed, many orthologs of both the C-NHEJ and HR pathways function as tumor suppressors in mammals.

While considerable advances have been made in elucidating the factors required for C-NHEJ and HR repair mechanisms, there are a number of issues that remain to be resolved. There is still a lack of clarity regarding the mechanisms leading to A-EJ, and the functional relationship between C-NHEJ and A-EJ pathways. Moreover, while the impact of chromatin on DSBR is becoming more apparent, a detailed mechanistic understanding of the role of chromatin remodeling and histone variants, or their modifications on DSBR, and how these events influence pathway choice, requires further elucidation. A comprehensive understanding of DSBR in yeast and higher eukaryotes will be expected to impact on a range of related disciplines, including cancer therapy and genome editing, and thus remains an important goal.

GLOSSARY

Aneuploidy An abnormal number of chromosomes within a cell.
Bubble migration Movement of the D-loop and accompanying replication machinery along a chromosome.
Clastogen A mutagenic agent that causes chromosome breaks.

Clean ends The two DNA ends of a double-strand break where each end has a complementary overhang and no lesions.
Covalent adduct A type of DNA lesion where a chemical is covalently bound to DNA.
Crossover Reciprocal exchange of a region of DNA between homologous chromosomes as a result of homologous recombination.
Decatenation Unlinking of two DNA duplexes where one strand of a DNA duplex is wound around a strand of another duplex (unlinking of a hemicatenane).
Dirty ends DNA ends of a double-strand break that lack 5′ phosphates and/or 3′ hydroxyls or exhibit other forms of DNA damage.
End bridging Physical connection of two DNA molecules (each end of a double-strand break) via protein–protein interactions between each molecule.
Endonuclease Enzyme that cleaves a polynucleotide molecule by cleaving nucleotides within the polynucleotide chain.
Endonucleolytic cleavage The process by which an endonuclease cuts a polynucleotide sequence.
Exonuclease Enzyme that cleaves nucleotides from the end of a polynucleotide molecule.
Helicase Enzyme that unwinds the double-stranded helical structure of polynucleotide molecules.
Hemicatenane Joining of two DNA duplexes where one strand of a DNA duplex is wound around a strand of another duplex.
Holliday junctions Homologous recombination intermediate containing four double-stranded arms joined together to form a joint molecule.
Homologous recombination A high-fidelity form of DNA damage repair that makes use of a homologous DNA sequence as a template.
Interstrand crosslinks A type of DNA lesion that prevents separation of the strands within a DNA duplex.
Loss of heterozygosity An event where either one copy of a gene is lost or is replaced with the allele from the sister chromosome.
Microhomology-mediated end-joining A subset of alternative end-joining repair that relies on regions of microhomology on either side of the break, which anneal following limited resection.
Noncrossover The resulting structure when recombination intermediates are resolved with no reciprocal exchange of regions of DNA between homologous chromosomes.
Nonhomologous end-joining A form of double-strand break repair whereby two broken DNA ends are ligated together without the need for a homologous template sequence.
Nucleoprotein filament A complex comprised of helical chains of protein(s) bound to DNA.
Okazaki fragment Short segments of DNA newly synthesized on the lagging template strand as part of DNA replication.
Ploidy The number of pairs of chromosomes within a cell.
Postsynapsis The steps of homologous recombination following joint molecule formation.
Presynapsis The steps of homologous recombination preceding strand invasion and joint molecule formation.
Replication fork A point in a DNA duplex where the strands are separated to allow replication to occur.
Resolvase In respect to homologous recombination, an enzyme that acts to resolve Holliday junctions and recombination intermediates.
SUMOylation A type of posttranslational modification by which a small ubiquitin-related modifier is attached to a protein.
Synapsis The process of strand invasion and joint molecule formation as part of homologous recombination.
Translocase Protein that assists in the movement of another molecule.
Translocation Transfer of part of a chromosome to a different position such as a nonhomologous chromosome.

LIST OF ACRONYMS AND ABBREVIATIONS

2D Two-dimensional
3D Three-dimensional
A-EJ Alternative end-joining
ATP Adenosine triphosphate
BIR Break-induced replication
BLM Bloom syndrome protein
CDK Cyclin-dependent kinase
ChIP Chromatin immunoprecipitation
C-NHEJ Classical nonhomologous end-joining
CO Crossover
CRISPR Clustered regularly interspaced short palindromic repeat
dHJ Double Holliday junction
D-loop Displacement loop
DNA pol DNA polymerase
dNTP Deoxyribonucleotide triphosphate
DSB Double-strand break
DSBR Double-strand break repair
dsDNA Double-stranded DNA
FHA Forkhead associated
G1 phase Growth 1 phase of the cell cycle
G2 phase Growth 2 phase of the cell cycle
H3K36 Histone 3 lysine 36

HJ Holliday junction
HR Homologous recombination
ICL Interstrand crosslinks
IR Ionizing radiation
LOH Loss of heterozygosity
MAT Mating locus
MMEJ Microhomology-mediated end-joining
MR Subcomplex comprised of Mre11 and Rad50
MRX Complex comprised of Mre11, Rad50, and Xrs2
NCO Noncrossover
NHEJ Nonhomologous end-joining
ORC Origin recognition complex
PCNA Proliferation cell nuclear antigen
RPA Replication protein A
S phase Synthesis phase of the cell cycle
SDSA Synthesis-dependent strand-annealing
SMC Structural maintenance of chromosomes
ssDNA Single-stranded DNA
Top3 Topoisomerase III
UV Ultraviolet

REFERENCES

[1] Resnick MA, Martin P. The repair of double-strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. MGG 1976;143(2):119–29.
[2] Kasparek TR, Humphrey TC. DNA double-strand break repair pathways, chromosomal rearrangements and cancer. Sem Cell Dev Biol 2011;22(8):886–97.
[3] Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Res 2008;18:134–47.
[4] Pfeiffer P, Goedecke W, Obe G. Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations. Mutagenesis 2000;15(4):289–302.
[5] Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 2014;346(6213):1258096.
[6] Aylon Y, Kupiec M. DSB repair: the yeast paradigm. DNA Repair 2004;3:797–815.
[7] Symington LS. Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol Mol Biol Rev 2002;66:630–70.
[8] Latarjet R, Ephrussi B. Courbes de survie de levures haploides et diploides soumises aux rayons-X. C R Acad Sci 1949;229(4):306–8.
[9] Friedberg EC. Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. Microbiol Rev 1988;52:70–102.
[10] Nakai S, Matsumoto S. Two types of radiation-sensitive mutant in yeast. Mutat Res 1967;4(2):129–36.
[11] Snow R. Mutants of yeast sensitive to ultraviolet light. J Bacteriol 1967;94(3):571–5.
[12] Resnick MA. Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics 1969;62(3):519–31.
[13] Rodarte U, Fogel S, Mortimer R, editors. Detection of Recombination Defective Mutants in *Saccharomyces cerevisiae*. Genetics 1968. 428 EAST Preston St, Baltimore, MD 21202.
[14] Resnick MA. The repair of double-strand breaks in DNA; a model involving recombination. J Theor Biol 1976;59(1):97–106.
[15] Moore CW. Responses of radiation-sensitive mutants of *Saccharomyces cerevisiae* to lethal effects of bleomycin. Mutat Res 1978;51:165–80.
[16] Li X, Heyer W-D. Homologous recombination in DNA repair and DNA damage tolerance. Cell Res 2008;18:99–113.
[17] Pâques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 1999;63:349–404.
[18] Dupaigne P, Le Breton C, Fabre F, Gangloff S, Le Cam E, Veaute X. The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: implications for crossover incidence during mitotic recombination. Mol Cell 2008;29:243–54.
[19] Yeeles JTP, Poli J, Marians KJ, Pasero P. Rescuing stalled or damaged replication forks. Cold Spring Harb Perspect Biol 2013;5:a012815.
[20] Haber JE. Mating-type genes and MAT switching in *Saccharomyces cerevisiae*. Genetics 2012;191:33–64.
[21] Holliday R. A mechanism for gene conversion in fungi. Genet Res 1964;5:282–304.
[22] Doniger J, Warner RC, Tessma I. Role of circular dimer DNA in the primary recombination mechanism of bacteriophage S13. Nat New Biol 1973;242(114):9–12.
[23] Thompson BJ, Escarmis C, Parker B, Slater WC, Doniger J, Tessman I, et al. Figure-8 configuration of dimers of S13 and phiX174 replicative form DNA. J Mol Biol 1975;91(4):409–19.
[24] Benbow RM, Zuccarelli AJ, Sinsheimer RL. Recombinant DNA molecules of bacteriophage phi chi174. Proc Natl Acad Sci USA 1975;72(1):235–9.
[25] Bell L, Byers B. Occurrence of crossed strand-exchange forms in yeast DNA during meiosis. Proc Natl Acad Sci USA 1979;76:3445–9.
[26] Liu Y, West SC. Happy Hollidays: 40th anniversary of the Holliday junction. Nat Rev Mol Cell Biol 2004;5:937–44.

[27] Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. The double-strand-break repair model for recombination. Cell 1983;33:25–35.
[28] Orr-Weaver TL, Szostak JW, Rothstein RJ. Yeast transformation: a model system for the study of recombination. Proc Natl Acad Sci USA 1981;78:6354–8.
[29] Orr-Weaver TL, Szostak JW. Yeast recombination: the association between double-strand gap repair and crossing-over. Proc Natl Acad Sci USA 1983;80:4417–21.
[30] Sun H, Treco D, Szostak JW. Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. Cell 1991;64:1155–61.
[31] Mazin AV, Bornarth CJ, Solinger JA, Heyer W-D, Kowalczykowski SC. Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. Mol Cell 2000;6:583–92.
[32] Sung P, Klein H. Mechanism of homologous recombination: mediators and helicases take on regulatory functions. Nat Rev Mol Cell Biol 2006;7:739–50.
[33] Whitby MC. Making crossovers during meiosis. Biochem Soc Trans 2005;33:1451.
[34] Cao L, Alani E, Kleckner N. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell 1990;61:1089–101.
[35] Schwacha A, Kleckner N. Identification of double Holliday junctions as intermediates in meiotic recombination. Cell 1995;83:783–91.
[36] Storlazzi A, Xu L, Cao L, Kleckner N. Crossover and noncrossover recombination during meiosis: timing and pathway relationships. Proc Natl Acad Sci USA 1995;92:8512–6.
[37] Allers T, Lichten M. Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 2001;106:47–57.
[38] Mitchel K, Zhang H, Welz-Voegele C, Jinks-Robertson S. Molecular structures of crossover and noncrossover intermediates during gap repair in yeast: implications for recombination. Mol Cell 2010;38(2):211–22.
[39] Nassif N, Penney J, Pal S, Engels WR, Gloor GB. Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. Mol Cell Biol 1994;14:1613–25.
[40] Ira G, Malkova A, Liberi G, Foiani M, Haber JE. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. Cell 2003;115:401–11.
[41] Mueller JE, Clyman J, Huang YJ, Parker MM, Belfort M. Intron mobility in phage T4 occurs in the context of recombination-dependent DNA replication by way of multiple pathways. Genes Dev 1996;10:351–64.
[42] McGill C, Shafer B, Strathern J. Coconversion of flanking sequences with homothallic switching. Cell 1989;57:459–67.
[43] Nelson HH, Sweetser DB, Nickoloff JA. Effects of terminal nonhomology and homeology on double-strand-break-induced gene conversion tract directionality. Mol Cell Biol 1996;16:2951–7.
[44] Formosa T, Alberts BM. DNA synthesis dependent on genetic recombination: characterization of a reaction catalyzed by purified bacteriophage T4 proteins. Cell 1986;47:793–806.
[45] Symington LS, Rothstein R, Lisby M. Mechanisms and regulation of mitotic recombination in *Saccharomyces cerevisiae*. Genetics 2014;198:795–835.
[46] Lyndaker AM, Goldfarb T, Alani E. Mutants defective in Rad1-Rad10-Slx4 exhibit a unique pattern of viability during mating-type switching in *Saccharomyces cerevisiae*. Genetics 2008;179:1807–21.
[47] Wang X, Ira G, Tercero JA, Holmes AM, Diffley JFX, Haber JE. Role of DNA replication proteins in double-strand break-induced recombination in *Saccharomyces cerevisiae*. Mol Cell Biol 2004;24:6891–9.
[48] Andersen SL, Sekelsky J. Meiotic versus mitotic recombination: two different routes for double-strand break repair. BioEssays 2010;32:1058–66.
[49] Luder A, Mosig G. Two alternative mechanisms for initiation of DNA replication forks in bacteriophage T4: priming by RNA polymerase and by recombination. Proc Natl Acad Sci USA 1982;79(4):1101–5.
[50] Morrow DM, Connelly C, Hieter P. "Break copy" duplication: a model for chromosome fragment formation in *Saccharomyces cerevisiae*. Genetics 1997;147:371–82.
[51] Llorente B, Smith CE, Symington LS. Break-induced replication: what is it and what is it for? Cell Cycle 2008;7:859–64.
[52] Cullen JK, Hussey SP, Walker C, Prudden J, Wee BY, Dave A, et al. Break-induced loss of heterozygosity in fission yeast: dual roles for homologous recombination in promoting translocations and preventing de novo telomere addition. Mol Cell Biol 2007;27(21):7745–57.
[53] Sakofsky CJ, Roberts SA, Malc E, Mieczkowski PA, Resnick MA, Gordenin DA, et al. Break-induced replication is a source of mutation clusters underlying kataegis. Cell Rep 2014;7(5):1640–8.
[54] Davis AP, Symington LS. RAD51-dependent break-induced replication in yeast. Mol Cell Biol 2004;24:2344–51.
[55] Anand RP, Lovett ST, Haber JE. Break-induced DNA replication. Cold Spring Harb Perspect Biol 2013;5(12):a010397.
[56] Tinline-Purvis H, Savory AP, Cullen JK, Dave A, Moss J, Bridge WL, et al. Failed gene conversion leads to extensive end processing and chromosomal rearrangements in fission yeast. EMBO J 2009;28(21):3400–12.
[57] Ivanov EL, Sugawara N, Fishman-Lobell J, Haber JE. Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. Genetics 1996;142(3):693–704.
[58] Sugawara N, Ira G, Haber JE. DNA length dependence of the single-strand annealing pathway and the role of *Saccharomyces cerevisiae* RAD59 in double-strand break repair. Mol Cell Biol 2000;20(14):5300–9.
[59] Perez C, Guyot V, Cabaniols J-P, Gouble A, Micheaux B, Smith J, et al. Factors affecting double-strand break-induced homologous recombination in mammalian cells. Biotechniques 2005;39:109–15.
[60] Liu J, Ehmsen KT, Heyer WD, Morrical SW. Presynaptic filament dynamics in homologous recombination and DNA repair. Crit Rev Biochem Mol Biol 2011;46(3):240–70.

[61] Schipler A, Iliakis G. DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. Nucleic Acids Res 2013;41(16):7589–605.
[62] Tsukamoto Y, Mitsuoka C, Terasawa M, Ogawa H, Ogawa T. Xrs2p regulates Mre11p translocation to the nucleus and plays a role in telomere elongation and meiotic recombination. Mol Biol Cell 2005;16:597–608.
[63] Mimitou EP, Symington LS. DNA end resection: many nucleases make light work. DNA Repair 2009;8:983–95.
[64] Furuse M, Nagase Y, Tsubouchi H, Murakami-Murofushi K, Shibata T, Ohta K. Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J 1998;17:6412–25.
[65] Sharples GJ, Leach DR. Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. Mol Microbiol 1995;17:1215–7.
[66] Hartsuiker E, Neale MJ, Carr AM. Distinct requirements for the Rad32(Mre11) nuclease and Ctp1(CtIP) in the removal of covalently bound topoisomerase I and II from DNA. Mol Cell 2009;33(1):117–23.
[67] Trujillo KM, Roh DH, Chen L, Komen SV, Tomkinson A, Sung P. Yeast Xrs2 binds DNA and helps target Rad50 and Mre11 to DNA ends. J Biol Chem 2003;278:48957–64.
[68] Shim EY, Chung WH, Nicolette ML, Zhang Y, Davis M, Zhu Z, et al. *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. EMBO J 2010;29(19):3370–80.
[69] Paull TT, Gellert M. The 3′ to 5′ exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. Mol Cell 1998;1(7):969–79.
[70] Chen H, Lisby M, Symington LS. RPA coordinates DNA end resection and prevents formation of DNA hairpins. Mol Cell 2013;50(4):589–600.
[71] Sugiyama T, Zaitseva EM, Kowalczykowski SC. A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. J Biol Chem 1997;272(12):7940–5.
[72] New JH, Sugiyama T, Zaitseva E, Kowalczykowski SC. Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature 1998;391(6665):407–10.
[73] Shinohara A, Ogawa T. Stimulation by Rad52 of yeast Rad51-mediated recombination. Nature 1998;391(6665):404–7.
[74] Sung P. Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. J Biol Chem 1997;272(45):28194–7.
[75] Sung P, Robberson DL. DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. Cell 1995;82:453–61.
[76] Antony E, Tomko EJ, Xiao Q, Krejci L, Lohman TM, Ellenberger T. Srs2 disassembles Rad51 filaments by a protein-protein interaction triggering ATP turnover and dissociation of Rad51 from DNA. Mol Cell 2009;35:105–15.
[77] Sung P. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science 1994;265(5176):1241–3.
[78] Sheridan SD, Yu X, Roth R, Heuser JE, Sehorn MG, Sung P, et al. A comparative analysis of Dmc1 and Rad51 nucleoprotein filaments. Nucleic Acids Res 2008;36(12):4057–66.
[79] Liu J, Renault L, Veaute X, Fabre F, Stahlberg H, Heyer W-D. Rad51 paralogues Rad55-Rad57 balance the antirecombinase Srs2 in Rad51 filament formation. Nature 2011;479:245–8.
[80] Bernstein KA, Reid RJ, Sunjevaric I, Demuth K, Burgess RC, Rothstein R. The Shu complex, which contains Rad51 paralogues, promotes DNA repair through inhibition of the Srs2 anti-recombinase. Mol Biol Cell 2011;22(9):1599–607.
[81] Kadyk LC, Hartwell LH. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics 1992;132(2):387–402.
[82] Sjogren C, Nasmyth K. Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*. Curr Biol 2001;11(12):991–5.
[83] Renkawitz J, Lademann Claudio A, Kalocsay M, Jentsch S. Monitoring homology search during DNA double-strand break repair in vivo. Mol Cell 2013;50:261–72.
[84] Renkawitz J, Lademann CA, Jentsch S. Mechanisms and principles of homology search during recombination. Nat Rev Mol Cell Biol 2014;15:369–83.
[85] Petukhova G, Komen SV, Vergano S, Klein H, Sung P. Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. J Biol Chem 1999;274:29453–62.
[86] Petukhova G, Sung P, Klein H. Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. Genes Dev 2000;14:2206–15.
[87] Li X, Heyer WD. RAD54 controls access to the invading 3′-OH end after RAD51-mediated DNA strand invasion in homologous recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res 2009;37(2):638–46.
[88] Matunis MJ. On the road to repair: PCNA encounters SUMO and ubiquitin modifications. Mol Cell 2002;10:441–2.
[89] Owens JC, Detweiler CS, Li JJ. CDC45 is required in conjunction with CDC7/DBF4 to trigger the initiation of DNA replication. Proc Natl Acad Sci USA 1997;94(23):12521–6.
[90] Bhaumik D, Wang TS-F. Mutational effect of fission yeast Polα on cell cycle events. Mol Biol Cell 1998;9:2107–23.
[91] Lydeard JR, Jain S, Yamaguchi M, Haber JE. Break-induced replication and telomerase-independent telomere maintenance require Pol32. Nature 2007;448:820–3.
[92] Moss J, Tinline-Purvis H, Walker CA, Folkes LK, Stratford MR, Hayles J, et al. Break-induced ATR and Ddb1-Cul4(Cdt)(2) ubiquitin ligase-dependent nucleotide synthesis promotes homologous recombination repair in fission yeast. Genes Dev 2010;24(23):2705–16.
[93] Mortensen UH, Bendixen C, Sunjevaric I, Rothstein R. DNA strand annealing is promoted by the yeast Rad52 protein. Proc Natl Acad Sci USA 1996;93(20):10729–34.
[94] Krejci L, Altmannova V, Spirek M, Zhao X. Homologous recombination and its regulation. Nucleic Acids Res 2012;40(13):5795–818. http://dx.doi.org/10.1093/nar/gks270.

[95] Deng SK, Gibb B, de Almeida MJ, Greene EC, Symington LS. RPA antagonizes microhomology-mediated repair of DNA double-strand breaks. Nat Struct Mol Biol 2014;21(4):405–12.
[96] Mitchel K, Lehner K, Jinks-Robertson S. Heteroduplex DNA position defines the roles of the Sgs1, Srs2, and Mph1 helicases in promoting distinct recombination outcomes. PLoS Genet 2013;9(3):e1003340.
[97] Sebesta M, Burkovics P, Haracska L, Krejci L. Reconstitution of DNA repair synthesis in vitro and the role of polymerase and helicase activities. DNA Repair (Amst) 2011;10(6):567–76.
[98] Tsutsui Y, Kurokawa Y, Ito K, Siddique MS, Kawano Y, Yamao F, et al. Multiple regulation of Rad51-mediated homologous recombination by fission yeast Fbh1. PLoS Genet 2014;10(8):e1004542.
[99] Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 2003;426(6968):870–4.
[100] Cejka P, Plank JL, Bachrati CZ, Hickson ID, Kowalczykowski SC. Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1–Top3. Nat Struct Mol Biol 2010;17:1377–82.
[101] Osman F, Dixon J, Doe CL, Whitby MC. Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol Cell 2003;12(3):761–74.
[102] Agmon N, Yovel M, Harari Y, Liefshitz B, Kupiec M. The role of Holliday junction resolvases in the repair of spontaneous and induced DNA damage. Nucleic Acids Res 2011;39(16):7009–19.
[103] Ip SC, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC. Identification of Holliday junction resolvases from humans and yeast. Nature 2008;456(7220):357–61.
[104] Moore JK, Haber JE. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. Mol Cell Biol 1996;16:2164–73.
[105] Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 2010;79:181–211.
[106] Jazayeri A, Jackson SP. Screening the yeast genome for new DNA-repair genes. Genome Biol 2002;3. reviews1009.
[107] Ferreira MG, Cooper JP. Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. Genes Dev 2004;18(18):2249–54.
[108] Lieber MR. The mechanism of human nonhomologous DNA end joining. J Biol Chem 2008;283(1):1–5.
[109] Daley JM, Palmbos PL, Wu D, Wilson TE. Nonhomologous end joining in yeast. Annu Rev Genet 2005;39:431–51.
[110] Hefferin ML, Tomkinson AE. Mechanism of DNA double-strand break repair by non-homologous end joining. DNA Repair 2005;4:639–48.
[111] Chen L, Trujillo K, Ramos W, Sung P, Tomkinson AE. Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. Mol Cell 2001;8:1105–15.
[112] Li P, Li J, Li M, Dou K, Zhang MJ, Suo F, et al. Multiple end joining mechanisms repair a chromosomal DNA break in fission yeast. DNA Repair (Amst) 2012;11(2):120–30.
[113] Lehnman IR. DNA ligase: structure, mechanism, and function. Science 1974;186:790–7.
[114] Chiruvella KK, Renard BM, Birkeland SR, Sunder S, Liang Z, Wilson TE. Yeast DNA ligase IV mutations reveal a nonhomologous end joining function of BRCT1 distinct from XRCC4/Lif1 binding. DNA Repair 2014;24:37–45.
[115] Durocher D, Jackson SP. The FHA domain. FEBS Lett 2002;513:58–66.
[116] Valencia M, Bentele M, Vaze MB, Herrmann G, Kraus E, Lee SE, et al. NEJ1 controls non-homologous end joining in *Saccharomyces cerevisiae*. Nature 2001;414:666–9.
[117] Chen X, Tomkinson AE. Yeast Nej1 is a key participant in the initial end binding and final ligation steps of nonhomologous end joining. J Biol Chem 2011;286:4931–40.
[118] Pannunzio NR, Li S, Watanabe G, Lieber MR. NHEJ often uses microhomology: implications for alternative end joining. DNA Repair 2014;17:74–80.
[119] Pardo B, Ma E, Marcand S. Mismatch tolerance by DNA polymerase Pol4 in the course of nonhomologous end joining in *Saccharomyces cerevisiae*. Genetics 2006;172(4):2689–94.
[120] Yang H, Matsumoto Y, Trujillo KM, Lees-Miller SP, Osley MA, Tomkinson AE. Role of the yeast DNA repair protein Nej1 in end processing during the repair of DNA double strand breaks by non-homologous end joining. DNA Repair 2015;31:1–10.
[121] McKinney JS, Sethi S, Tripp JD, Nguyen TN, Sanderson BA, Westmoreland JW, et al. A multistep genomic screen identifies new genes required for repair of DNA double-strand breaks in *Saccharomyces cerevisiae*. BMC Genomics 2013;14:251.
[122] McVey M, Lee SE. MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. Trends Genet 2008;24(11):529–38.
[123] Boulton SJ, Jackson SP. *Saccharomyces cerevisiae* Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. EMBO J 1996;15(18):5093–103.
[124] Ma JL, Kim EM, Haber JE, Lee SE. Yeast Mre11 and Rad1 proteins define a Ku-independent mechanism to repair double-strand breaks lacking overlapping end sequences. Mol Cell Biol 2003;23(23):8820–8.
[125] Hegde V, Klein H. Requirement for the SRS2 DNA helicase gene in non-homologous end joining in yeast. Nucleic Acids Res 2000;28(14):2779–83.
[126] Ira G, Pellicioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, et al. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature 2004;431(7011):1011–7.

[127] Wurtele H, Verreault A. Histone post-translational modifications and the response to DNA double-strand breaks. Curr Opin Cell Biol 2006;18(2): 137–44.
[128] Pai CC, Deegan RS, Subramanian L, Gal C, Sarkar S, Blaikley EJ, et al. A histone H3K36 chromatin switch coordinates DNA double-strand break repair pathway choice. Nat Commun 2014;5:4091.
[129] Choi YE, Pan Y, Park E, Konstantinopoulos P, De S, D'Andrea A, et al. MicroRNAs down-regulate homologous recombination in the G1 phase of cycling cells to maintain genomic stability. Elife 2014;3:e02445.
[130] Burma S, Chen BP, Chen DJ. Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA Repair (Amst) 2006; 5(9–10):1042–8.
[131] Haber JE. DNA recombination: the replication connection. Trends Biochem Sci 1999;24(7):271–5.
[132] Roeder GS. Meiotic chromosomes: it takes two to tango. Genes Dev 1997;11:2600–21.
[133] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646–74.