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DNA double-strand break repair: how to fix a broken relationship

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Abstract. DNA double-strand breaks (DSBs) arise in cells from endogenous and exogenous attacks on the DNA backbone, but also as a direct consequence of replication failures. Proper repair of all these DSBs is essential for genome stability. Repair of broken chromosomes is a challenge for dividing cells that need to distribute equal genetic information to daughter cells. Consequently, eukaryotic organisms have evolved multi-potent and efficient mechanisms to repair DSBs that are primarily divided into two types of pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). Here we briefly describe how eukaryotic cells sense DSBs and trigger cell cycle arrest to allow repair, and we review the mechanisms of both NHEJ and HR pathways and the choice between them. (Part of a Multi-author Review)

Keywords. DNA repair, genome stability, checkpoint, nonhomologous end joining, homologous recombination, repair pathway choice.

Introduction

Eukaryotic organisms have evolved multiple molecular mechanisms to ensure the integrity of their genetic information carried by the DNA molecule (reviewed by other authors in this issue). Among DNA lesions, the most harmful seems to come from the breakage of both DNA strands, since a single unrepaired DNA double-strand break (DSB) can induce cell death [1].

DSBs arise in cells from endogenous as well as from exogenous attacks on the DNA backbone. Inside cells, DNA faces nucleases and metabolic products such as reactive oxygen species. These products introduce chemical modifications in the DNA, including modified bases and sugars, DNA-protein adducts, base-free sites and tandem lesions. From the outside, ionizing radiation (IR) from the background and ultraviolet (UV) light alter the chemical composition of the DNA backbone. A large panel of chemical agents and DNA topoisomerase inhibitors used in anti-cancer therapy also modify DNA. If unrepaired, all these modifications can impede DNA replication in dividing cells and provoke DSB formation. Finally, it is believed that DSBs are created by physical stress when chromosomes are pulled to opposite poles during mitosis.

Apart from DSBs arising 'accidentally', eukaryotic cells also produce DSBs by programmed expression of specific endonucleases for their own benefit. For instance, their repair is used for mating-type switching in yeast [2], for genetic mixing and proper chromosome segregation during meiosis in all eukaryotes [3] and for producing a diverse immune repertoire in the context of V(D)J and class-switch recombination in vertebrate cells [4].

Proper repair of DSBs is essential for genome stability. Repair of broken chromosomes is a challenge for dividing cells that need to distribute equal genetic information to daughter cells. Alteration or loss of chromosome fragments can lead to apoptosis but also to carcinogenesis with the activation of oncogenes or inactivation of tumor suppressor genes. Consequently, eukaryotic organisms have evolved multi-potent and

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efficient DSB-repair mechanisms. DSB repair occurs primarily by two pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR).

Here we review the so-called checkpoints of eukaryotic cells that sense DSBs and trigger cell cycle arrest to allow repair, followed by a the mechanistic description of both the NHEJ and HR pathways. Finally, the choice between the use of these pathways is discussed.

The cellular response to DSBs: DNA-damage checkpoints

Checkpoints were originally defined as a delay in cell cycle transition to allow time for repair to take place [5]. However, they have since been related to other functions such as transcriptional induction of DNA repair genes and post-translational modification of several other proteins. In multi-cellular organisms, DNA damage signalling can also induce apoptosis and prevent the dangerous proliferation of damaged cells, thus preserving genetic integrity in the whole organism. Among the different checkpoints, those that sense genome integrity are crucial in allowing eukaryotic cells safe progression through the cell cycle. Here we briefly review current knowledge on the factors and mechanisms of DNA-damage checkpoints.

Factors involved in the DNA-damage checkpoints

DNA-damage checkpoints consist of complex phosphorylation cascades in which DNA-damage sensors can detect unrepaired DSBs and recruit transducer kinases (Fig. 1). These are key players in the DNA damage response because they mediate the phosphorylation of several effector kinases and ultimately activate the appropriate effectors. Among the checkpoint players, the mammalian transducer kinases ATM (ataxia telangiectasia-mutated) and ATR (ataxia telangiectasia and Rad3-related) together with DNA-PKcs (DNA-dependent protein kinase catalytic subunit) play a central role in triggering the checkpoint response through activation of the effector kinases CHK1 and CHK2, which propagate the signal. Likewise, in yeast *Saccharomyces cerevisiae*, DNA lesions trigger the recruitment of ATM and ATR orthologs Tel1 and, more important, Mec1, responsible for the phosphorylation of the effector kinases Rad53, Chk1 and Dun1. In addition, several mediator proteins modulate the activity of the transducer kinases by interactions with multiple components of the DNA-damage response pathway. Thus, the *S. cerevisiae* Rad9 protein is a mediator of the checkpoint response for DNA damage occurring all over the cell cycle. Rad9 orthologs that act as ATM mediators in vertebrates include MDC1 and the breast and ovarian cancer-specific tumor suppressor BRCA1 (for an extended review on the DNA-damage response see [6]).

The checkpoint-signalling cascade

When a DSB occurs, it is primarily detected by the direct interaction of the DNA ends with a complex called MR(X)N, composed of Mre11, Rad50 and Xrs2 in yeast (Xrs2 is substituted by NBS1 in mammalian cells) (Fig. 1A) [7, 8]. This complex has multiple functions as it is involved in DSB repair and telomere maintenance. Further, being the first recognizer of the DSB, MR(X)N is involved in DNA-damage sensing. It recruits the Tel1/ATM transducer kinase triggering checkpoint activation (Fig. 1B) [9–12]. Tel1/ATM is thus recognizing and signalling unprocessed DSBs.

In yeast, the signal generated by Tel1 seems sufficient to generate a checkpoint response and mediate a cell cycle arrest only when multiple unprocessed DSBs are present, but the persistence of a single unrepaired DSB leads to a G2 cell cycle arrest that depends on activation of the checkpoint-response pathway triggered by Mec1 [12].

The activation of Mec1/ATR relies on the formation of single-stranded DNA (ssDNA) [13]. DSB processing involves several nucleases (see below) that catalyze the generation of ssDNA (Fig. 1D). DSB processing is stimulated by Tel1/ATM [14–16], although it does not require the checkpoint to be activated. ssDNA is first coated by the ssDNA-binding factor RPA, which is recognized by the checkpoint transducer kinase Mec1/ATR via the cofactor Ddc2/ATRIP (Fig. 1E–F) [13, 17, 18]. In addition to Ddc2/ATRIP, full ATR activation requires RPA-mediated recruitment of a complex composed of RAD9-RAD1-HUS1 in humans and Rad17-Mec3-Ddc1 in *S. cerevisiae* (Fig. 1F) [19, 20]. This complex is structurally similar to the replication-sliding clamp PCNA (PCNA-like) and is loaded onto ssDNA by a replication factor C-like complex (RFC-like) that consists of yeast Rad24 (RAD17 in humans) in association with Rfc2, Rfc3, Rfc4 and Rfc5 replication proteins (Fig. 1F) [21].

The DNA-damage response

Once the checkpoint has been activated, transducer kinases convey a DNA-damage response through the

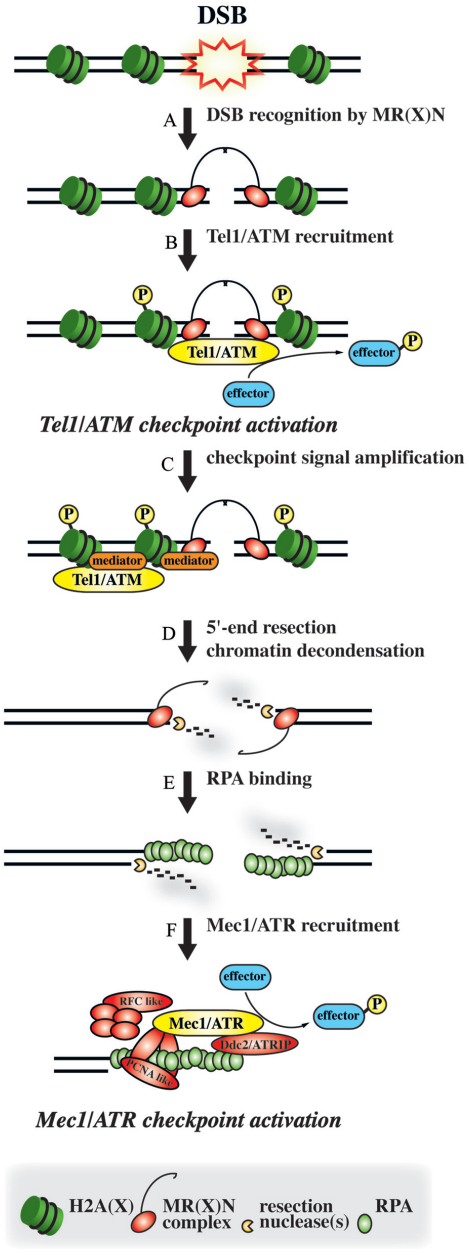

Figure 1. Simplified model of DSB signalling and checkpoint activation. DNA undergoing a DSB is represented by a close pair of black lines. Sensors involved in checkpoint signalling are depicted in red, transducer kinases in yellow, effector kinases in light blue and mediators in orange. (*A*) DSB ends are recognized by the MR(X)N complex. (*B*) MR(X)N recruits Tel1/ATM. Tel1/ATM phosphorylates H2A/H2AX histones (γH2AX) and effector kinases that propagate the signal. (*C*) Mediator proteins engender checkpoint factor propagation around the breakpoint, generating γH2AX large chromatin domains. (*D*) DSB 5′-ends are resected, involving MR(X)N and other nucleases and chromatin decondensates. (*E*) RPA binds to single-stranded overhangs generated by resection. (*F*) RPA-coated single-stranded DNA recruits Mec1/ATR through its cofactor Ddc2/ATRIP. It also recruits a PCNA-like complex via an RFC-like complex, which contribute to full Mec1/ATR activation.

activation of effector kinases. Ultimately, several proteins are targets of this complex DNA-damage response, such as transcription factors, cell cycle-regulation proteins and DSB-repair factors (Fig. 1). Checkpoint transducers are also responsible for one of the earliest events that occur after the formation of a DSB, that is histone H2AX C-terminal tail phosphorylation, referred to as γH2AX (Fig. 1B) [22]. In mammals, γH2AX interacts with the mediator protein MDC1, which, at the same time, interacts with ATM and MR(X)N, thus generating further γH2AX and amplifying the signal (Fig. 1C) [23]. The spreading of γH2AX to larger chromatin domains on either side of a DSB is responsible for the stable accumulation of MDC1, MR(X)N, ATM and several other proteins implied in the DSB response [24]. Similarly, yeast H2A is phosphorylated by the checkpoint kinases [25]. These chromatin modifications occurring around a DSB have been described as recruiting chromatin-remodelling complexes that may have a role in DSB repair [26, 27]. Although the exact mechanism is unknown, chromatin decondensation seems to be required for efficient DSB repair (Fig. 1D) (reviewed in [28]).

The checkpoint generates a broad spectrum of responses after DNA damage, and possibly it also controls DNA repair pathways. The checkpoint is responsible for the phosphorylation of many of the proteins involved in DSB repair such as Srs2, Mus81, Mre11, Xrs2/NBS1, Rad51, Rad55-Rad57 and RPA. Recent reports showing a checkpoint-dependent phosphorylation of yeast proteins involved in NHEJ and HR [29, 30] suggests, indeed, that checkpoints may enhance the efficiency of both pathways of DSB repair. Therefore, checkpoint factors are continuously signalling the appearance of DNA damage. Although checkpoint activation is not needed for normal DSB repair, in situations in which many DSBs are occurring or a single DSB persists, it mediates a general response that consists of cell cycle arrest and promotion of the DSB repair itself.

Nonhomologous end joining

NHEJ is the pathway that repairs DSBs by re-ligating their two ends together. It appears to be the most powerful DSB-repair pathway because it has the potential to ligate any kind of DSB ends without the requirement for a homologous sequence, as opposed to the alternative HR DSB-repair pathway.

NHEJ has frequently been considered to be the error-prone DSB-repair pathway because it was observed to generate small insertions and deletions [31, 32]. However, these observations came from analyses of

NHEJ events at a unique DSB or repair of DSBs with incompatible ends that did not derive from the same break [31, 32]. In fact, repair fidelity should only be considered for DSBs showing complementary or blunt ends that come from the same parental DNA duplex. In this context, NHEJ is a highly faithful mechanism, with an error rate of about 10^{-3} per joining event between fully compatible DSB ends created by the continuous expression of an endonuclease in yeast [31, 33]. This rate has most likely been overestimated because it does not account for multiple undetectable cycles of cleavage and precise repair. If DSB ends are not fully compatible, NHEJ may attempt to stabilize both ends together using minimum base pairing. Indeed, base pairing of DSB overhangs seems essential since ligation of blunt ends is not efficient, as shown in yeast [34]. In order to optimize base pairing, terminal base degradation may be necessary, but this processing should not be extensive since NHEJ only efficiently joins DSB ends with overhangs of less than four bases [35]. Gap filling by DNA synthesis may also be required prior to ligation. The final reaction may result in small insertions or deletions of DNA sequences at the DSB location.

To perform such reactions, the NHEJ machinery relies on many protein factors that carry structural stabilization functions as well as DNA degradation, polymerization and ligation functions. All NHEJ reactions require the core NHEJ machinery that is composed of three complexes: MR(X)N, Ku and the DNA ligase complexes (see [36] for a more detailed review on end joining proteins). The order of action of these complexes has not been fully established. It is thought that MR(X)N and Ku complexes bind DSB ends shortly after DSB formation. They appear to bridge DSB ends together and to inhibit their degradation (Fig. 2A–B). Ku and MRX also play crucial roles in recruiting, stabilizing and stimulating the ligase complex at DSBs (Fig. 2C). Different alignments and base pairing of DSB overhangs should take place and ligation may be attempted (Fig. 2D). If DSB-end processing is necessary to allow ligation, the ligase and Ku complexes have the ability to recruit a large panel of DNA-modifying enzymes. DSB end-processing reactions and ligation attempts may be intertwined until ligation succeeds (Fig. 2D), showing that NHEJ is a highly dynamic reaction. All the NHEJ factors and processes are presented below.

Tethering DSB ends and 5′-end resection inhibition: the MR(X)N and Ku complexes

MRX is the only protein complex shared by NHEJ and HR DSB-repair pathways in yeast. In vertebrates, the MRN complex is analogous to yeast MRX, although there is no definitive evidence that MRN is involved in NHEJ. Yeast MRX has been shown to be one of the first complexes to bind DSB ends after their occurrence [37]. MR(X)N is composed of Rad50/RAD50, Mre11/MRE11 and Xrs2/NBS1 proteins. Rad50 shares the structural assembly of SMC (structural maintenance of chromosomes) proteins. Rad50 has a high-affinity DNA binding domain and seems to be able to bridge DNA molecules together [8]. This property could help maintain DSB ends close to each other to facilitate their paring and ligation in the NHEJ reaction [8]. This hypothesis has been confirmed in wild-type yeast cells where DSB ends remain associated after DSB induction and this association depends on the MRX complex [38, 39]. Mre11 shows *in vitro* DNA nuclease activities [40, 41]. It cleaves DNA structures that can be found at DSB ends such as hairpin structures and 3′ single-strand overhangs at the single-/double-stranded transition. It also carries a 3′ to 5′ exonuclease activity. The nucleolytic repertoire of Mre11 could participate in the processing of DSB ends to facilitate their overhang pairing, but it is not essential [40]. Finally, Xrs2 in yeast also binds DNA and, with Rad50, influences MRX substrate binding [42]. Xrs2 also seems to be the regulatory subunit of the yeast MRX complex. It is phosphorylated in response to DSB formation as part of the signalling pathway triggering cell cycle arrest [43]. MRX seems to participate to the formation of a stable NHEJ complex as demonstrated by the interactions of Xrs2 with the Lif1 cofactor of the Lig4 ligase and Mre11 with the yKu80 subunit of the yeast Ku complex [7, 44]. *In vitro*, the MRX complex stimulates the ligation reaction by the Lig4 complex [7]. Although MRX complex carries a nuclease activity, its essential function during NHEJ appears to be structural in tethering DSB ends together and recruiting the ligase complex.

yKu70/KU70 and yKu80/KU80 form a heterodimeric complex (Ku) essential to NHEJ [34]. In vertebrates Ku is part of a larger complex called DNA-dependent protein kinase (DNA-PK) whose catalytic subunit DNA-PK_{cs} is also required for NHEJ [45]. DNA-PK functions as a DNA end-bridging factor in NHEJ [46]. This analogy of function relative to yeast MRX complex may explain the non-absolute requirement of the MRN complex during NHEJ in higher eukaryotes.

Ku binds double-stranded DNA and also makes direct contacts with Lig4 [44, 47]. Defective binding of Ku to Lig4 is additive to those of MRX with Ku and Lif1, demonstrating that this network of interactions may allow the creation of a structure that could stabilize or stimulate the final ligation step of NHEJ [44]. In yeast,

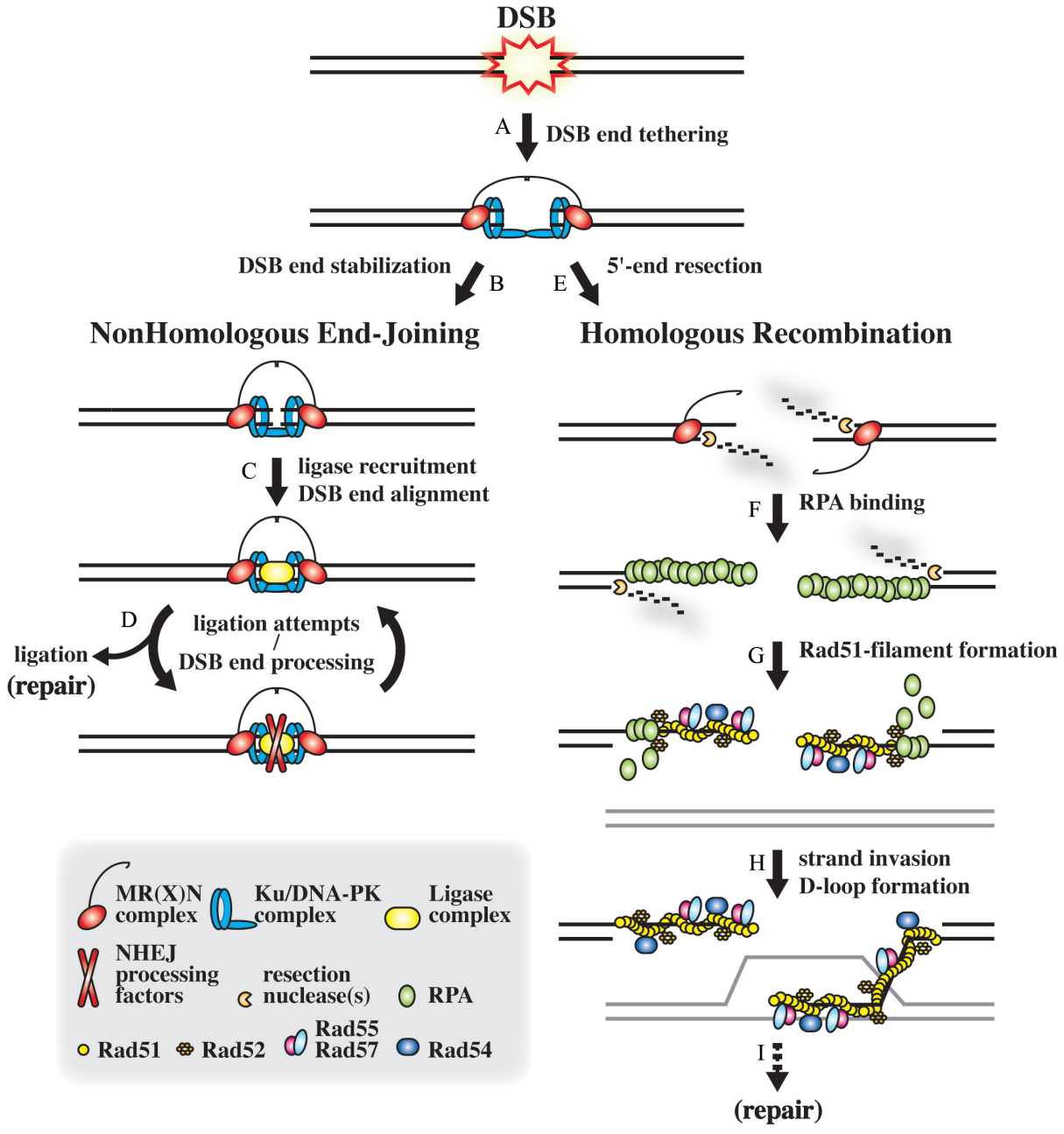

Figure 2. Model of DSB repair by NHEJ and HR pathways. DNA undergoing a DSB and the homologous template used for repair are respectively represented by a close pair of black and grey lines. (*A*) DSB ends are tethered by MR(X)N and Ku/DNA-PK complexes. (*B*) In NHEJ, DSB ends are further stabilized by MR(X)N and Ku/DNA-PK. (*C*) MR(X)N and Ku/DNA-PK recruit the ligase complex and DSB ends are aligned. (*D*) DSB ends are ligated or are processed prior to ligation (repair). (*E*) In HR, 5′ DSB ends are resected by MR(X)N and other nucleases. (*F*) RPA binds to single-stranded overhangs generated by resection. (*G*) RPA-coated single-stranded DNA is a substrate for Rad51-filament formation, involving Rad52, Rad55-Rad57 and Rad54. (*H*) Rad51-filament homology search and strand invasion lead to the formation of a D-loop. (*I*) From the D-loop, different HR pathways can result in DSB repair.

the absence of yKu70 accelerates DSB end degradation, suggesting that Ku functions in NHEJ to protect DSB ends before their ligation [48, 49]. DNA 5′-end resection is a prerequisite for repair by HR (see next section), and thus Ku acts to channel DSB repair to NHEJ. In summary, the Ku complex seems to participate in NHEJ by stabilizing DSB ends and by preventing 5′-end resection.

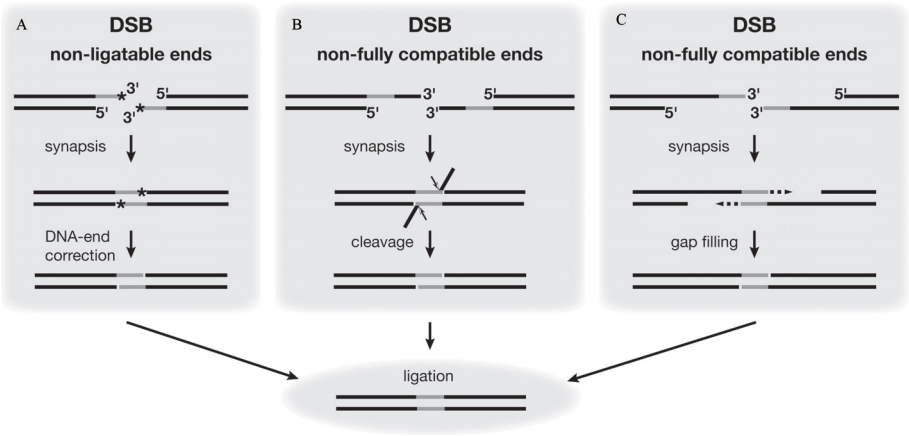

Figure 3. Processing of DSB ends during NHEJ. DNA undergoing a DSB is represented by a close pair of black lines containing a region of complementary bases (grey lines). 3′ and 5′ DSB ends are indicated. Only end joining of DSBs containing 3′ overhangs are shown for clarity. (*A*) Non-ligatable DSB ends are corrected to allow end joining. (*B*) Non-fully compatible DSB ends could require DNA nuclease cleavage (arrows with black and white arrowheads) or (*C*) or gap filling (dashed black arrows) by a DNA polymerase prior to ligation.

Ligating DSB ends: the NHEJ ligase complex

The DNA ligase involved in NHEJ is Lig4/Ligase IV [32, 50]. On its unique C-terminal part, Lig4 is bound by an obligatory cofactor to the ligation reaction called Lif1/XRCC4 [51, 52]. Nej1/XLF (also known as Lif2 in yeast and Cernunnos in humans) is the third essential component of the NHEJ ligase complex [53, 54].

Most studies have addressed the possible biochemical function of Lif1 and Nej1 using their vertebrate counterparts XRCC4 and XLF, and have shown that both stimulate the ligation reaction performed by DNA Ligase IV [50, 55]. Interestingly, the DNA Ligase IV in a complex with XRCC4 or XLF has a high degree of substrate flexibility. It is able to ligate one strand of a DNA duplex independent of the other as well as two DNA strands across small gaps [56]. In the additional presence of the Ku complex, it can perform the ligation of mismatched and incompatible DNA ends [56, 57]. This latter function promotes DNA sequence conservation at the break location. All of this flexibility accounts for the high potential of NHEJ to repair every kind of DSB.

Processing DSB ends prior to ligation: a plethora of possibilities

DSB ends may need to be processed prior to ligation. This is the case of incompatible DSB ends, in which NHEJ may only proceed after mismatch correction and/or single-stranded gap filling. Thus, ligation can be prevented if terminal nucleotides are damaged or modified and do not present ligatable 5′ phosphates and 3′ hydroxyls (Fig. 3a). This would be the case, for example, of ionizing radiation-induced DSBs, whose ends contain non-ligatable 5′ hydroxyls and 3′ phosphates. During NHEJ, the mammalian polynucleotide kinase (PNK) is recruited through its interaction with the XRCC4 protein to correct these modifications [58, 59]. This enzyme is well-suited to correct both modifications because it contains 5′ kinase and 3′ phosphatase activities. Another enzyme, Aprataxin, is involved in removing adenylate groups from 5′ phosphates and also interacts with XRCC4 [60, 61]. DNA nucleases may be required for eliminating damaged bases, correcting mismatches and substantially resecting DSB ends to allow their pairing (Fig. 3B). Yeast Rad27 has been clearly involved in NHEJ reactions where non-paired 5′ flaps need to be removed and physically interacts with Lig4-Lif1 [62, 63]. The identification of an equivalent function for processing 3′ overhangs is missing in yeast. Mre11 is a good candidate, but the mutation of its nuclease activity does not cause any defect in NHEJ [40]. In human cells, the nuclease Artemis interacts with DNA-PK [64]. Conformational change of DNA-PK following DNA binding may also alter the conformation of Artemis, which acts as a 3′ or 5′ flap endonuclease at DSB overhangs [64, 65].

Finally, DNA polymerases are mobilized by NHEJ to fill in some single-stranded gaps after pairing of partial complementary overhangs or nuclease processing (Fig. 3C). NHEJ involves DNA polymerases belonging to the PolX family: yeast Pol4 and its vertebrate counterparts Polλ, Polμ and the terminal deoxynucleotidyl transferase (TdT) [66–68]. The TdT role is restricted to V(D)J recombination occurring during maturation of B cells of the immune system, but Polλ and Polμ can perform template-independent DNA synthesis in all somatic cells [69]. Random terminal addition of few nucleotides appears useful to allow pairing of DSB ends that do not carry microhomology. Together with Pol4, Polλ and Polμ can also perform the joining of incompatible DSB ends by extending 3′ overhangs that are mispaired or even unpaired [66, 70, 71]. This ability bypasses terminal-nucleotide degra-

dation for annealing and promotes DNA sequence conservation. As described earlier for the NHEJ ligase complex, DNA polymerases involved in NHEJ also show a remarkable degree of flexibility regarding their substrates which allow the joining of every configuration of DSB ends.

Microhomology-mediated end joining

The observation of rare Ku-independent end-joining events using imperfect microhomology of about 5–20 nucleotides has led to the proposal of the existence of a new DSB-repair pathway related to NHEJ. Consequently, this pathway has been termed microhomology-mediated end joining (MMEJ) [72]. MMEJ events are observed in the absence of core NHEJ factors and imply much larger sequence deletions than the ones occurring after NHEJ. Hence, MMEJ requires extensive resection of DSB ends, which is a prerequisite of the HR DSB-repair pathway (see below). Moreover, depending on the genetic context and the organism studied, MMEJ requires components of both NHEJ (MRX, Ku, Lig4) and HR (MRX, Rad1-Rad10, Rad52) pathways [72–75]. Therefore, MMEJ appears to represent the ultimate way to repair a DSB using every biochemical activity available, when NHEJ and HR have failed. Nevertheless, the physiological relevance and mechanisms responsible for these types of DSB-repair events are still scarce and will not be discussed further.

Homologous recombination

DSB repair by HR corresponds to an exchange or a transfer of identical or quasi-identical sequences between the DNA molecule carrying the DSB and another intact DNA molecule. HR is a faithful mechanism if the DNA template used for repair is identical to the original DNA sequence present at the DSB. Otherwise, HR repair of DSBs can lead to local mutations or even more deleterious genome rearrangements.

HR mostly involves proteins encoded by genes of the RAD52 epistasis group composed of RAD50, RAD51, RAD52, RAD54, RAD55, RAD57, RAD59, RDH54, MRE11 and XRS2 (see [76] for a more detailed review on recombination proteins). In addition to these genes, DNA nucleases (Exo1, Sae2, Rad1-Rad10), helicases (Sgs1, Srs2), topoisomerases (Top3), polymerases (Pol32) and ligases may also be required to complete specific HR reactions.

Classical HR is mainly characterized by three successive steps: 1) resection of the 5′-ended DNA strand at break ends, followed by 2) strand invasion into a homologous DNA duplex and strand exchange, and 3) resolution of recombination intermediates. Depending on the ability of both DSB ends to perform strand invasion and on the outcome of the strand invasion intermediate, different HR pathways can complete DSB repair. These include the synthesis-dependent strand-annealing (SDSA) pathway, the classical double-strand break repair (DSBR) model for HR as defined by Szostak et al. [77] and break-induced replication (BIR). Alternatively, 5′ resection of DSB ends can eventually uncover repetitive DNA that could channel repair through the single-strand annealing (SSA) pathway. All HR mechanisms are interconnected and share a large number of enzymatic steps. As described in the following section, each protein used during HR has a preponderant role in one or several of these HR pathways, which will be described together with the different HR enzymatic steps.

A prerequisite for HR: 5′ resection of DSB ends

The first and critical step of all HR reactions at a DSB corresponds to the nucleolytic degradation of the 5′-ended DNA strand (Fig. 2e) [78, 79]. 5′-end resection generates a long 3′ single-stranded end that is able to perform a sequence homology search (Fig. 2F – G), to invade the duplex containing the homologous sequence (Fig. 2 H) and to prime DNA synthesis. DNA synthesis will allow the recovery of DNA sequences lost at the break location as well as reconstituting the loss of sequence present on the 5′ degraded strand.

The data accumulated during the past few years provide evidence that all members of the yeast MR(X)N complex are involved in the processing of both meiotic and mitotic DSBs [48, 80, 81]. Regarding 5′-end resection *per se*, Mre11/MRE11 is a DNA nuclease [40, 41]. However, Mre11 carries an *in vitro* exonuclease activity with a 3′ to 5′ polarity that is the opposite of the 5′ to 3′ exonuclease activity inferred as a requirement for resection of DSB ends. Several studies have shown that nuclease activities of Mre11 are needed for processing of meiotic or mitotic induced DSBs by DNA-damaging agents [40, 80–82]. A model has emerged that proposes that Mre11 would be required at DSBs showing modified ends which cannot be cleaved by other nucleases [83]. This could be the case of IR-induced DSBs, whose ends can contain damaged bases or sugar moieties as well as possibly DNA-protein adducts. In other cases, DSBs induced by the DNA topoisomerase inhibitor camptothecin or during meiosis show one strand covalently linked to a specific protein. Thus, the Mre11-nuclease

function would be needed in HR to remove modified DSB ends to allow 5′-ended strand resection.

Resection of non-modified DSB ends is slowed but not abolished in cells lacking MRX, demonstrating the importance of MRX to perform efficient resection [84]. Here again, as in NHEJ, MRX function can be structural because Rad50 carries the ability of bridging DNA ends [38, 39], thus maintaining DSB ends close to each other or closer to a homologous template sequence to facilitate repair [8]. As in NHEJ, Xrs2 does not seem to play a particular role in the HR reaction apart from its function in DNA checkpoint signalling. In summary, MR(X)N complex may not be directly involved in the 5′ strand degradation but seems to fulfill an important role in the first steps of HR, combining a checkpoint function with DSB-end tethering and nucleolytic cleaning.

Sae2/CtIP is also involved with MR(X)N in the processing of DSB ends [85, 86]. Sae2 itself exhibits endonuclease activity on single-stranded DNA and single-strand/double-strand transitions *in vitro* [87]. As a complex, it cooperates with MRX to cleave hairpin DNA structures. This nuclease activity seems to be required to process hairpin structures and meiotic DSBs *in vivo* [87]. As for Mre11, it appears that Sae2 does not directly perform the 5′-ended strand resection at DSBs. One possibility is that MRX-Sae2 prepares the substrate and then activates or recruits another nuclease to perform 5′-ended strand degradation. One identified candidate is the $5'-3'$ exonuclease Exo1/EXO1 that is active in this process [83, 88]. Exo1 over-expression partially suppresses DSB-repair defects of MRX-depleted cells, suggesting that increasing Exo1 amounts could bypass recruitment by MRX but also that MRX is still required to process certain types of DSBs [82]. Moreover, DSB 5′-ended strand resection is still occurring in the absence of both Exo1 and Mre11 nuclease activities, meaning that another nuclease that remains to be identified can substitute for these enzymes [82]. Interestingly, a recent study reported that the Tel1 kinase also promotes DSB-end resection, connecting HR DSB repair and the DNA damage checkpoint [12]. In conclusion, Mre11/MRE11, Rad50/RAD50, Xrs2/NBS1, Sae2/CtIP and Exo1/EXO1 are clearly involved in HR initiation by processing DSB ends. It results in the generation of a 3′ single-stranded DNA end that is competent for searching a homologous template and performing its invasion (Fig. 4A – C).

The central steps of HR mechanisms: D-loop formation and strand exchange

The homologous DNA duplex invasion involves the displacement of one strand of the duplex by the invasive strand and pairing with the other. It results in the formation a heteroduplex or hybrid DNA called displacement-loop (D-loop). Further pairing of the 3′ single-stranded DSB end with the homologous duplex involves a reaction called strand exchange. These reactions are mostly performed by a nucleoprotein filament composed of the 3′ single-stranded DSB end coated with the Rad51/RAD51 recombinase protein. The Rad51-filament assembly requires the binding of RPA to the 3′ single-stranded DSB ends [89]. RPA is a heterotrimeric complex that binds ssDNA with high affinity and is believed to remove its secondary structures [90]. RPA binds DSB ends shortly after resection and directly interacts with Rad52 [37, 91]. Rad52 is the factor needed for almost all recombination mechanisms [92]. Yeast Rad52 interacts also with Rad51 and is thought to facilitate Rad51 loading on single-strand DNA by displacing RPA molecules [89]. In vertebrates it appears that BRCA2, rather than RAD52, fulfills the multiple roles of yeast Rad52 in the strand invasion and exchange reaction during HR [93, 94]. Yeast Rad51 paralogs (Rad55 and Rad57) and Rad54 are also mediators of Rad51 nucleofilament assembly, but their precise role in this process is still unclear. It is thought that yeast Rad55 and Rad57 form a heterodimer that stabilizes the filament and stimulates the subsequent strand exchange step [95, 96]. Rad54/RAD54 appears to participate in multiple steps of HR as Rad51 loading on single strand DNA, homology search and pairing to homologous sequence, and maturation of recombination intermediates after D-loop formation [95, 97, 98].

Details of the strand invasion and exchange reactions have not been well characterized yet, but a new set of single-molecule experiments have recently emerged to fill this knowledge gap. Briefly, the search for homology seems to occur by random collisions between the two DNA molecules. Rad51 filament is a dynamic structure and the observed association and dissociation of Rad51 along the DNA filament may have a preponderant role in the strand exchange reaction [99]. It has been estimated that a Rad51 nucleofilament of at least 100 bp is required for efficient strand exchange [100]. Rad54 has also been described as a chromatin-remodeling complex and could move nucleosomes in order to facilitate duplex invasion by the Rad51 nucleofilament [101]. Then pairing occurs notably thanks to the annealing function of Rad52 [102].

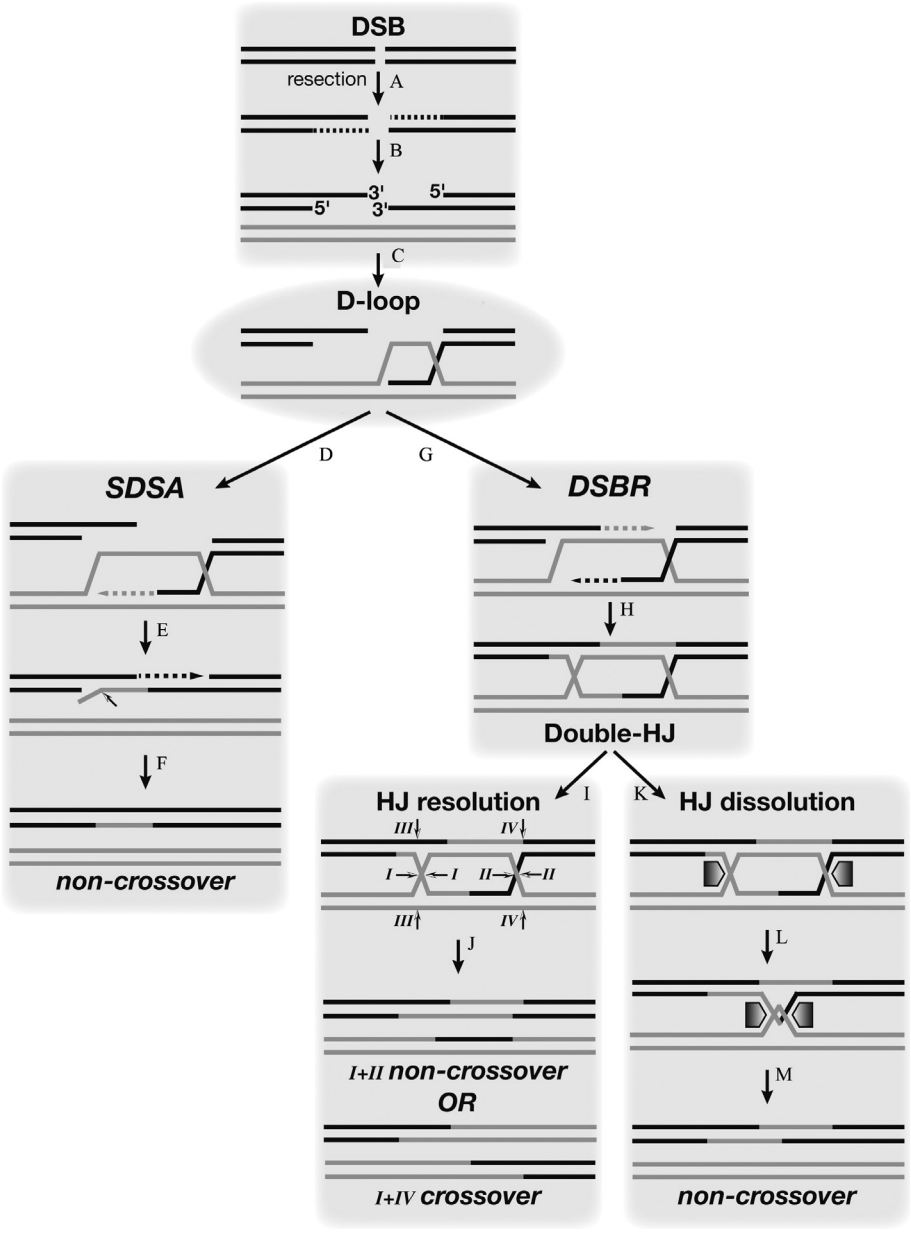

Figure 4. Recombination-mediated DSB repair by SDSA and DSBR. (*A*) DSB 5′-end resection (dashed black lines). (*B*) Search for a homologous template (shown in grey). (*C*) Strand invasion and D-loop formation. (*D*) SDSA pathway. (*E*) After its elongation (dashed grey arrow), strand displacement of the invasive strand and annealing to the other DSB end. (*F*) Cleavage of non-homologous sequence (arrow with black and white arrowhead), gap filling (dashed black arrow) and ligation produce a non-crossover. (*G*) DSBR pathway. (*H*) Elongation (dashed black and grey arrows) and ligation of invasive strands: double-HJ formation. (*I*) HJ resolution. (*J*) Differential cleavages *I*, *II*, *III* and *IV* are indicated (arrows with black and white arrowheads). Cleavages *I*+*II* (or *III*+*IV*, not represented) produce a non-crossover. Cleavages *I*+*IV* (or *II*+*III*, not represented) produce a crossover. (*K*) HJ dissolution. (*L*) HJ branch migration and (*M*) strand decatenation (pentagonal shapes) produce a non-crossover.

From this stage of recombination, several HR pathways can complete the repair. All of these pathways will end with the replacement of the sequence surrounding the DSB by the homologous sequence used for repair. HR repair may or may not be associated with reciprocal exchanges (crossovers) between the DSB-containing DNA molecule and the homologous template, depending on the HR mechanism used. Because the homologous template used for repair can notably be found on the homologous chromosome or at an ectopic location, crossovers may have adverse genetic consequences, such as loss of heterozygosity or genome rearrangements (translocations, duplications or inversions). For these reasons, crossovers are efficiently suppressed in mitotic cells [103, 104].

Repair of two-ended DSBs by HR: SDSA and DSBR mechanisms

If the initial DSB is two-ended and if both sides of the break share homology to the repair template, both resected 5′-ends coated with Rad51 and other recombination factors can engage their homology regions in a homologous template separately and independently. It is likely that one DSB end will perform strand invasion and form a D-loop before the other one does (Fig. 4C). DNA synthesis primed from the invasive

strand will extend the D-loop and allow the recovery of DNA sequences lost at the DSB.

Displacement of the elongated DSB end out of the D-loop offers the possibility of re-annealing DSB ends together through the newly synthesized complementary region (Fig. 4D – E). Sequences not involved in annealing are cleaved and repair can be completed by gap filling synthesis and ligation (Fig. 4E – F). This pathway, named SDSA [105], does not lead to crossovers (Fig. 4F) and seems to account for genome stability by avoiding crossovers in mitotic cells. SDSA appears to be promoted by the DNA helicases Sgs1 and Srs2 in yeast and some RecQ helicases in mammals, such as BLM [103, 104, 106]. Srs2 and BLM are able to dismantle Rad51 nucleofilaments by displacing Rad51 from single-stranded DNA [107, 108]. Moreover Srs2, BLM as well as RAD54 can disrupt D-loops *in vitro* by promoting DNA branch migration that rejects the invasive strand from the duplex [97, 107, 109].

Alternatively, elongation of the invasive strand and resultant displacement of the homologous duplex strands will extend the loop that can then capture the second DSB end by annealing (Fig. 4 G). The second DSB end can also be elongated by DNA synthesis. Gap filling and ligation will result in the formation of two four-branched DNA structures called Holliday junctions (HJs) (Fig. 4 H). Differential resolutions (cleavages) of both HJs lead to the DSBR model (Fig. 4I) [77]. This pathway yields crossover or non-crossover products, depending on cleavage (Fig. 4J). Identification of the 'resolvase' enzyme capable of achieving HJ cleavages is still under intense investigation. A resolvase activity has been purified from human cells but no gene encoding it has yet been identified. Interestingly, this activity is reduced in the absence of two RAD51 paralogs, RAD51C and XRCC3 [110]. It is likely that the resolvase is a combination of different factor activities and that involvement of these activities could depend on the context where HR occurs.

It has been demonstrated recently that double-HJ intermediates can also be 'dissoluted' by the concerted action of a DNA helicase and a DNA topoisomerases (Fig. 4K). Dissolution involves the human BLM helicase that promotes branch migration of the two HJs resulting in a hemicatenane structure that can be untangled by the TOPO III alpha topoisomerases (Fig. 4L – M) [106]. HJ dissolution yields a non-crossover product (Fig. 4 M). A third component that may recruit the BLM-TOPO III alpha complex to branched DNA structures is BLAP75 [111]. A similar situation may be true in yeast since all these factors are conserved (Sgs1, Top3 and Rmi1) and individual mutants share the same phenotypes [112, 113].

Finally, D-loop intermediates have been described as also being cleaved by the Mus81-Mms4 nuclease complex, which has a preference for branched DNA structures [114]. In this case, due to specific endonucleolytic cleavages by Mus81, the final repair product is always a crossover (see [115] for more details). This type of recombination intermediate resolution has been observed during programmed DSB repair in meiotic cells where crossovers are promoted.

Break-induced replication

Under some circumstances, only one end of a DSB can be used for repair. This is the case when only one of the DSB ends shares homology with another region in the genome or when one end of a broken DNA molecule is lost. Telomeres that have lost their protective telomeric repeats also generate one-ended DSBs that can be repaired by recombination [116]. Importantly, it is thought that one-ended DSBs can also arise by replication fork collapse. For instance, the replication fork encountering a DNA single-stranded gap or a nick will convert it into a one-ended DSB on one sister-chromatid. It is not known if these one-ended DSBs are repaired immediately in eukaryotic cells in order to restart replication. Alternatively, since DNA replication is initiated at multiple origins in these cells, the collapsed replication fork can be held until the arrival of the oncoming fork. It would produce a two-ended DSB that can be repaired after replication by other HR pathways that appear to be less deleterious in terms of repair fidelity.

In context of one-ended DSBs, repair occurs through the 'break-induced replication' (BIR) pathway (Fig. 5) [117, 118]. The DSB end invades a homologous sequence (Fig. 5A), initiates a unidirectional DNA synthesis from the site of strand invasion (Fig. 5B) and replicates the chromosome template. The simplest situation would involve a DSB-containing fragment carrying a centromere that invades and replicates a chromosome template until reaching its nearest telomere (Fig. 5C) [117, 119]. One BIR model involves the formation of a single HJ, and its cleavage results in DSB repair associated with a complete duplication of the chromosome arm used as a template (Fig. 5D). Consequently, BIR possibly results in a large-scale loss of heterozygosity if it occurs between homologous chromosomes. More complex genome rearrangements can happen through multiple strand invasions and dissociations into disperse repeated sequences during BIR [120]. This may reflect anti-recombination activities described earlier at two-ended DSBs that disrupt the D-loop intermediate to

promote SDSA by capturing the displaced invasive strand.

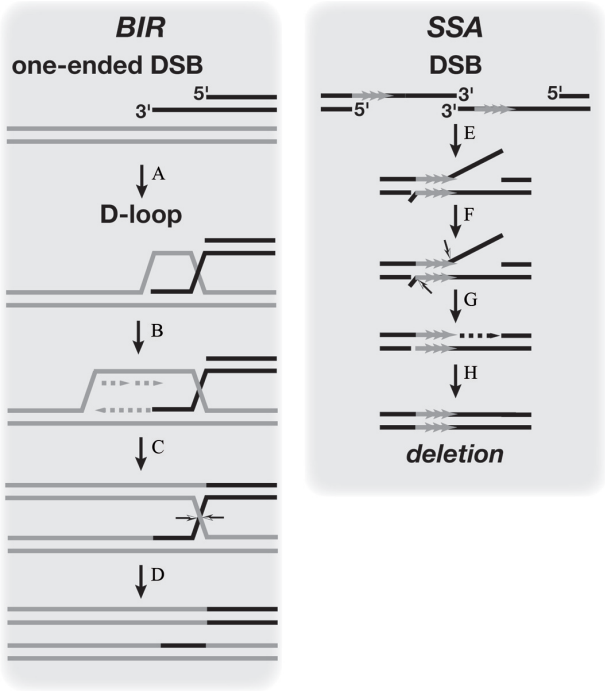

Figure 5. Recombination-mediated DSB repair by BIR and SSA. (*A*) BIR: Strand invasion by a single DSB end and D-loop formation. (*B*) Complete replication-fork formation and DNA synthesis (dashed grey arrows). (*C*) Replication of the entire homologous template arm and strand ligation: HJ formation. (*D*) HJ resolution (arrows with black and white arrowheads) results in the DSB repair associated with the duplication of the template chromosome arm. (*E*) SSA: DSB 5′-end resection and annealing of complementary DNA repeats (grey arrow arrays). (*F*) Cleavages of non-homologous sequences (arrows with black and white arrowheads). (*G*) Gap filling (dashed black arrows). (*H*) Ligation. SSA produces a deletion.

BIR is largely dependent on the Rad51 recombinase [121, 122]. This seems logical since the initiating event of BIR is the formation of a D-loop by strand invasion. For this purpose, Rad51-dependent BIR also requires Rad52, Rad54, Rad55 and Rad57 [121, 122]. Recently, it was shown that BIR operates on both leading- and lagging-strand DNA synthesis and requires the DNA polymerase δ subunit Pol32 [123]. It suggests that Pol32, which is not required by gene conversion, may help establish a full replication fork at the strand invasion site.

Rare events of DSB repair in the absence of Rad51 involving DNA repeats have been interpreted as BIR events [124]. These repair events require Rad52, the MRX complex, Rad59 and Rdh54 [124, 125]. This dependency is reminiscent of DSB repair by single-strand annealing (SSA; see below) suggesting that SSA between repeated DNA accounts, at least in part, for these repair events [126]. Another type of BIR independent of Rad51 has been observed when looking at inversions between inverted repeats [127–129]. Although it is unclear how strand invasion can be performed without Rad51, it is likely that local relaxation of DNA surrounding a DSB located between DNA repeats can allow on rare occasions Rad51-independent strand invasion to occur (see [130]).

Single-strand annealing

As long as no homologous template for repair is found, 5′ to 3′ strand resection can extend for many kilobases. In cases where resection uncovers direct repeat sequences, both single-stranded DSB ends can anneal together to repair the break (Fig. 5E). Single-stranded terminal sequences that are not involved in annealing can be removed by nucleases (Fig. 5F) and resulting gaps or nicks are filled in by DNA synthesis and ligation (Fig. 5G – H). This mechanism of DSB repair is called single-strand annealing (SSA) [131]. Mechanistically, the uncovering of repeated sequences by 5′ resection would involve Rad52, recruited by RPA [91], through its ssDNA annealing properties [102, 132]. It also involves the Rad52 homolog Rad59, but the latter seems to intervene only when the region of homology is short or interrupted by regions of heterology [127]. Nucleolytic cleavages are performed by Rad1-Rad10 specialized 3′ flap endonuclease and also involve Msh2, Msh3 and Slx4 [133–135]. Finally, the replication machinery likely undertakes final steps of DNA synthesis and ligation.

SSA causes the deletion of one DNA repeat plus the sequence located between the repeats. It is, therefore, considered to be a mutagenic pathway of HR. Nevertheless, DSB repair by SSA does not appear to be of minor importance since eukaryotic genomes carry a large amount of repeated elements susceptible to repair of this kind.

The choice between DSB-repair pathways

NHEJ and HR are the two main types of DSB-repair pathways in eukaryotic cells, but vertebrate cells seem to use NHEJ more frequently than yeast. One can argue that it may be due to the fact that higher eukaryotes have large and complex genomes that make the homology search required for HR inefficient. Higher eukaryote genomes also contain a high level of repetitiveness that can constitute a dangerous potential source of genetic instability if DSB repair occurs by ectopic recombination. Another argument

could be that vertebrates possess more NHEJ factors than yeast (such as DNA-PKcs or Artemis) and that can make NHEJ more efficient in vertebrates.

However, the belief about the different relevance of HR and NHEJ between yeast and vertebrates mostly comes from the observation that NHEJ-deficient vertebrate cells are hypersensitive to IR, whereas yeast NHEJ-deficient cell sensitivity to IR can only be seen in the absence of HR [34, 50, 136, 137]. In fact, this apparent difference seems to come from the cell cycle stage at which DSB repair occurs because IR sensitivity in yeast has mostly been assayed on dividing cells. It has been known for some time that DSB-repair pathways are regulated differently during cell cycle stages, but the mechanistic explanation for this regulation was missing. It is clear now that the DSB-repair pathway choice depends, at least, on the fact that 5′-ended DSB resection initiates HR and inhibits further possibilities of NHEJ. In that sense, recent studies about DSB-end resection regulation have begun to clarify how eukaryotic cells use DSB-repair pathways differentially during the cell cycle.

Cell cycle regulation of DSB repair pathway choice

NHEJ substrates are double-stranded ends that have undergone limited processing whereas HR substrates are 3′ single-stranded tails produced by extensive 5′-end resection. This 5′-ended DSB resection is irreversible, at least in yeast, which makes it a good mechanistic stage for the repair pathway choice that channels DSB repair to HR [138]. DSB-end resection has been demonstrated to be tightly regulated through the cell cycle [139–141].

In the G1 cell cycle phase of yeast, DSB-end resection has been shown to be inefficient, and HR events are consequently rare [139, 140]. According to these results, few RPA foci are observed in G1 yeast cells after IR irradiation [37]. Rad52 foci are not observed unless cells are subjected to high doses of IR [38]. This downregulation of HR favors NHEJ to repair DSBs in G1. It is reflected by the increased IR sensitivity of Ku-deficient cells in vertebrates during G1 [142] and in haploid yeast during the stationary phase (considered as G1-like) [137]. In diploid yeast cells, NHEJ is repressed [54, 143], and it is still unclear how DSBs are repaired in G1.

DSB-end resection is active in S and G2 phases, thus activating HR. The biological significance for this up-regulation of resection is related to the presence of a sister chromatid only during S and G2. The sister chromatid is the preferred HR template because it promotes an error-free repair, while use of the homologous chromosome or an ectopic region can compromise genome stability. Cohesins are among the factors that determine the choice of the sister as the main template to repair DSBs by HR in S and G2 [144]. After DSB formation γH2AX recruits cohesins *de novo*, thus maintaining the two sister chromatids in close proximity to facilitate sister-chromatid recombination [145, 146]. Consistently, cohesins have been observed to colocalize with sites of IR-induced DNA damage in mammals [147].

When a DSB occurs, transient stability of its DNA ends allows NHEJ to precede HR during all cell cycle stages with a higher stability in G1 and G2 [138]. Hence there is a competition between NHEJ and HR to repair DSBs in S and G2 phases. Consistently, Ku binding to the DSB interferes with HR factors by inhibiting the DSB resection step [48]. Other NHEJ factors such as Lig4 and its cofactor Lif1 can also suppress HR by stabilizing Ku binding to the DSB ends [49]. Overall, NHEJ may act before HR in S and G2 if the repair can be achieved rapidly [138, 140].

However, NHEJ repair of DSBs during S phase seems deleterious for cell survival, as shown by the increased resistance of Ku- and Ligase IV-deficient mammalian cells to camptothecin [148]. Replication through DNA lesions can make the replication fork stall or collapse [149–151]. It has recently been proposed that Mus81 endonuclease contributes to replication restart by cleaving stalled forks and thus generating DSBs in S phase [152]. HR appears to represent the most adequate DSB-repair pathway to restart stalled or collapsed replication forks, so NHEJ might be down-regulated during S phase to favor HR. In this view the poly(ADP-ribose) polymerase PARP-1 and the post-replicative repair protein RAD18 suppress synergistically the toxic effects of NHEJ during the HR reaction at stalled replication forks, but the mechanisms involved remain unknown [153, 154]. Moreover, Mre11 associates with chromatin in S phase even in the absence of induced DNA damage, which may favor repair by HR before Ku binding [155].

Finally, HR can be regulated by the cell cycle at a level other than DSB-end resection in mammalian cells. BRCA2 modulates the assembly of the RAD51-filament after resection [93, 94]. BRCA2 is phosphorylated by cyclin-dependent kinases (CDKs), and this phosphorylation blocks BRCA2 interaction with RAD51 [156]. This phosphorylation is low in S phase when HR is active but increases as cells progress toward mitosis. Thus, BRCA2 also has a role in the cell cycle regulation of the repair pathway choice by controlling the Rad51-filament formation when cells are leaving the G2 phase.

Mechanistic control of DSB resection

Our actual comprehension of the DSB-repair pathway choice appears to depend mainly on a cell cycle regulation of the DSB resection step in both yeast and mammals [14, 139–141]. A role for CDKs in cell cycle regulation of the DSB resection is reasonably well-established [139–141]. Cdc28, the yeast CDK that is active in S and G2 phases, has been shown to promote DSB resection, channelling repair to HR [139–141]. However, the downstream targets of the CDK have remained elusive until now. Recently, a new human protein called CtIP (ortholog of *S. cerevisiae* Sae2 and *Schizosaccharomyces pombe* Ctp1) is emerging to be a key regulator of the DSB-repair pathway choice at the molecular level. Hence, CtIP cell amounts are low in G1 and peak during S phase in fission yeast and humans [157, 158]. Human CtIP has been shown to functionally interact with the MRN complex and regulate DSB resection in mammalian cells [86].

Human CtIP, as well as yeast Sae2, is also a target of the checkpoint transducer kinases [159]. Phosphorylated CtIP interacts with several factors in human cells, such as BRCA1 [160]. BRCA1 is a large protein that contains several protein-protein interaction domains, and it is involved in many cellular processes such as the DNA damage response, although its exact function is unknown. BRCA1 is recruited to DNA-damage sites by MRN and can form a complex with MRN and CtIP in S and G2 [161]. BRCA1-mediated recruitment of CtIP to DNA damage sites would be important to facilitate DSB resection and subsequent HR repair.

Altogether, these data suggest that the regulatory network of the DSB-repair pathway choice might rely on the cell cycle regulation of factors involved directly in DSB resection, such as CtIP as well as on their post-translational modifications, although the exact pathways that lead to these modifications are still not fully understood. For instance, the molecular mechanism by which CtIP/Sae2 promotes MR(X)N resection of mitotic DSBs is still an open question.

Conclusion and future perspectives

Knowledge about DSB repair has advanced notably since it was shown in the early 1980s that double-stranded gaps could be efficiently repaired via HR in yeast [162]. Not only do we know now that there are several alternative mechanisms for the repair of DSBs by HR, but also that NHEJ constitutes a ubiquitous and efficient manner of DSB repair, especially in cells at the non-replicative stage. Far from the time in which we had only a few genes involved in DSB repair and a number of mechanistic models originating from genetic data, we now have fairly inclusive information on enzymatic activities of a large number of DSB-repair proteins, some of which have been identified in the last two decades. As we started acquiring a rational picture of the mechanisms of DSB, especially at the genetic and biochemical levels, the number of genes and activities that control DSB repair has turned out to be beyond anyone's original expectations. This has proved crucial in drawing a better map of DSB-repair mechanisms and their conservation in eukaryotes from yeast to humans. There are likely to be other functions still to be uncovered, but we now have a biochemical and cellular scenario in which to view DSB repair.

Nevertheless, important questions are still begging to be solved to have a complete understanding of DSB repair at both molecular and cellular levels. These include the role of chromatin in DSB repair, how each type of DSB repair is related to checkpoints, the cellular localization of DSB-repair reactions and their likely connection to other nuclear metabolic functions and structures, as well as the relevance of DSB repair in cell proliferation and differentiation and its role in the origin of cancer and human genetic syndromes. In addition to the classical biochemical and genetic approaches that still have a lot to provide, additional approaches based on structural biology, single-molecule analysis, genome-wide analyses and cell biology, as well as the use of genetically modified vertebrate models are likely to be in the coming years the major contributors to our knowledge of DSB repair as one of the most physiologically relevant process of the eukaryotic cell.

Note added in proof: After this manuscript was sent to the Editorial a number of relevant papers have been published some of which add a new twist to our understanding of HR DSB repair. Among these, we would like to select the identification of a new and conserved HJ resolvase protein both in yeast and humans [163], the identification of two alternative ways for 5'-end resection of DSBs necessary for HR mediated by Sgs1, Exo1, Dna2 and Sae2 [164–166], and the relevance of Sae2 in the control of 5'-end resection via its phosphorylation by CDK [167].

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1 Rich, T., Allen, R. L. and Wyllie, A. H. (2000) Defying death after DNA damage. Nature 407, 777–783.
2 Paques, F. and Haber, J. E. (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 63, 349–404.
3 Keeney, S. and Neale, M. J. (2006) Initiation of meiotic recombination by formation of DNA double-strand breaks: mechanism and regulation. Biochem. Soc. Trans. 34, 523–525.
4 Soulas-Sprauel, P., Rivera-Munoz, P., Malivert, L., Le Guyader, G., Abramowski, V., Revy, P. and de Villartay, J. P. (2007) V(D)J and immunoglobulin class switch recombinations: a paradigm to study the regulation of DNA end-joining. Oncogene 26, 7780–7791.
5 Weinert, T. A. and Hartwell, L. H. (1988) The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science 241, 317–322.
6 Su, T. T. (2006) Cellular responses to DNA damage: one signal, multiple choices. Annu. Rev. Genet. 40, 187–208.
7 Chen, L., Trujillo, K., Ramos, W., Sung, P. and Tomkinson, A. E. (2001) Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. Mol. Cell 8, 1105–1115.
8 Hopfner, K. P., Craig, L., Moncalian, G., Zinkel, R. A., Usui, T., Owen, B. A., Karcher, A., Henderson, B., Bodmer, J.L., McMurray, C.T. et al. (2002) The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature 418, 562–566.
9 Grenon, M., Gilbert, C. and Lowndes, N. F. (2001) Checkpoint activation in response to double-strand breaks requires the Mre11/Rad50/Xrs2 complex. Nat. Cell. Biol. 3, 844–847.
10 Lee, J. H. and Paull, T. T. (2005) ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science 308, 551–554.
11 Paull, T. T. and Lee, J. H. (2005) The Mre11/Rad50/Nbs1 complex and its role as a DNA double-strand break sensor for ATM. Cell Cycle 4, 737–740.
12 Mantiero, D., Clerici, M., Lucchini, G. and Longhese, M. P. (2007) Dual role for *Saccharomyces cerevisiae* Tel1 in the checkpoint response to double-strand breaks. EMBO Rep. 8, 380–387.
13 Zou, L. and Elledge, S. J. (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300, 1542–1548.
14 Jazayeri, A., Falck, J., Lukas, C., Bartek, J., Smith, G. C., Lukas, J. and Jackson, S. P. (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. Nat. Cell. Biol. 8, 37–45.
15 Adams, K. E., Medhurst, A. L., Dart, D. A. and Lakin, N. D. (2006) Recruitment of ATR to sites of ionising radiation-induced DNA damage requires ATM and components of the MRN protein complex. Oncogene 25, 3894–3904.
16 Dubrana, K., van Attikum, H., Hediger, F. and Gasser, S. M. (2007) The processing of double-strand breaks and binding of single-strand-binding proteins RPA and Rad51 modulate the formation of ATR-kinase foci in yeast. J. Cell. Sci. 120, 4209–4220.
17 Paciotti, V., Clerici, M., Lucchini, G. and Longhese, M. P. (2000) The checkpoint protein Ddc2, functionally related to *S. pombe* Rad26, interacts with Mec1 and is regulated by Mec1-dependent phosphorylation in budding yeast. Genes Dev. 14, 2046–2059.
18 Rouse, J. and Jackson, S. P. (2000) LCD1: an essential gene involved in checkpoint control and regulation of the MEC1 signalling pathway in *Saccharomyces cerevisiae*. EMBO J. 19, 5801–5812.
19 Melo, J. A., Cohen, J. and Toczyski, D. P. (2001) Two checkpoint complexes are independently recruited to sites of DNA damage in vivo. Genes Dev. 15, 2809–2821.
20 Zou, L., Liu, D. and Elledge, S. J. (2003) Replication protein A-mediated recruitment and activation of Rad17 complexes. Proc. Natl. Acad. Sci. USA 100, 13827–13832.
21 Naiki, T., Shimomura, T., Kondo, T., Matsumoto, K. and Sugimoto, K. (2000) Rfc5, in cooperation with rad24, controls DNA damage checkpoints throughout the cell cycle in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 20, 5888–5896.
22 Rogakou, E. P., Pilch, D.R., Orr, A. H., Ivanova, V. S. and Bonner, W. M. (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. 273, 5858–5868.
23 Stucki, M., Clapperton, J. A., Mohammad, D., Yaffe, M. B., Smerdon, S. J. and Jackson, S. P. (2005) MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 123, 1213–1226.
24 Celeste, A., Fernandez-Capetillo, O., Kruhlak, M. J., Pilch, D. R., Staudt, D. W., Lee, A., Bonner, R. F., Bonner, W. M. and Nussenzweig, A. (2003) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat. Cell. Biol. 5, 675–679.
25 Downs, J. A., Lowndes, N. F. and Jackson, S. P. (2000) A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. Nature 408, 1001–1004.
26 Morrison, A. J., Highland, J., Krogan, N. J., Arbel-Eden, A., Greenblatt, J. F., Haber, J. E. and Shen, X. (2004) INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119, 767–775.
27 van Attikum, H., Fritsch, O., Hohn, B. and Gasser, S. M. (2004) Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119, 777–788.
28 Bao, Y. and Shen, X. (2007) Chromatin remodeling in DNA double-strand break repair. Curr. Opin. Genet. Dev. 17, 126–131.
29 Herzberg, K., Bashkirov, V.I., Rolfsmeier, M., Haghnazari, E., McDonald, W. H., Anderson, S., Bashkirova, E. V., Yates, J.R., 3rd and Heyer, W. D. (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.
30 Ahnesorg, P. and Jackson, S. P. (2007) The non-homologous end-joining protein Nej1p is a target of the DNA damage checkpoint. DNA Repair (Amst) 6, 190–201.
31 Moore, J. K. and Haber, J. E. (1996) Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 16, 2164–2173.
32 Wilson, T. E., Grawunder, U. and Lieber, M. R. (1997) Yeast DNA ligase IV mediates non-homologous DNA end joining. Nature 388, 495–498.
33 Wilson, T. E. and Lieber, M. R. (1999) Efficient processing of DNA ends during yeast nonhomologous end joining. Evidence for a DNA polymerase beta (Pol4)-dependent pathway. J. Biol. Chem. 274, 23599–23609.
34 Boulton, S. J. and Jackson, S. P. (1996) *Saccharomyces cerevisiae* Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. EMBO J. 15, 5093–5103.
35 Daley, J. M. and Wilson, T. E. (2005) Rejoining of DNA double-strand breaks as a function of overhang length. Mol. Cell. Biol. 25, 896–906.
36 Daley, J. M., Palmbos, P. L., Wu, D. and Wilson, T. E. (2005) Nonhomologous end joining in yeast. Annu. Rev. Genet. 39, 431–451.
37 Lisby, M., Barlow, J. H., Burgess, R. C. and Rothstein, R. (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell 118, 699–713.
38 Lisby, M., Antunez de Mayolo, A., Mortensen, U. H. and Rothstein, R. (2003) Cell cycle-regulated centers of DNA double-strand break repair. Cell Cycle 2, 479–483.
39 Lobachev, K., Vitriol, E., Stemple, J., Resnick, M. A. and Bloom, K. (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.

40 Moreau, S., Ferguson, J. R. and Symington, L. S. (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.

41 Trujillo, K. M., Yuan, S. S., Lee, E. Y. and Sung, P. (1998) Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. J. Biol. Chem. 273, 21447–21450.

42 Trujillo, K. M., Roh, D. H., Chen, L., Van Komen, S., Tomkinson, A. and Sung, P. (2003) Yeast xrs2 binds DNA and helps target rad50 and mre11 to DNA ends. J. Biol. Chem. 278, 48957–48964.

43 D'Amours, D. and Jackson, S. P. (2001) The yeast Xrs2 complex functions in S phase checkpoint regulation. Genes Dev. 15, 2238–2249.

44 Palmbos, P. L., Daley, J. M. and Wilson, T. E. (2005) Mutations of the Yku80 C terminus and Xrs2 FHA domain specifically block yeast nonhomologous end joining. Mol. Cell. Biol. 25, 10782–10790.

45 Gottlieb, T. M. and Jackson, S. P. (1993) The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell 72, 131–142.

46 Spagnolo, L., Rivera-Calzada, A., Pearl, L. H. and Llorca, O. (2006) Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. Mol. Cell 22, 511–519.

47 Ribes-Zamora, A., Mihalek, I., Lichtarge, O. and Bertuch, A. A. (2007) Distinct faces of the Ku heterodimer mediate DNA repair and telomeric functions. Nat. Struct. Mol. Biol. 14, 301–307.

48 Lee, S. E., Moore, J. K., Holmes, A., Umezu, K., Kolodner, R. D. and Haber, J. E. (1998) *Saccharomyces* Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell 94, 399–409.

49 Zhang, Y., Hefferin, M. L., Chen, L., Shim, E. Y., Tseng, H. M., Kwon, Y., Sung, P., Lee, S. E. and Tomkinson, A. E. (2007) Role of Dnl4-Lif1 in nonhomologous end-joining repair complex assembly and suppression of homologous recombination. Nat. Struct. Mol. Biol. 14, 639–646.

50 Grawunder, U., Wilm, M., Wu, X., Kulesza, P., Wilson, T. E., Mann, M. and Lieber, M. R. (1997) Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. Nature 388, 492–495.

51 Herrmann, G., Lindahl, T. and Schar, P. (1998) *Saccharomyces cerevisiae* LIF1: a function involved in DNA double-strand break repair related to mammalian XRCC4. EMBO J. 17, 4188–4198.

52 Sibanda, B. L., Critchlow, S. E., Begun, J., Pei, X. Y., Jackson, S. P., Blundell, T. L. and Pellegrini, L. (2001) Crystal structure of an Xrcc4-DNA ligase IV complex. Nat. Struct. Biol. 8, 1015–1019.

53 Ahnesorg, P., Smith, P. and Jackson, S. P. (2006) XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 124, 301–313.

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

55 Lu, H., Pannicke, U., Schwarz, K. and Lieber, M. R. (2007) Length-dependent binding of human XLF to DNA and stimulation of XRCC4.DNA ligase IV activity. J. Biol. Chem. 282, 11155–11162.

56 Gu, J., Lu, H., Tsai, A. G., Schwarz, K. and Lieber, M. R. (2007) Single-stranded DNA ligation and XLF-stimulated incompatible DNA end ligation by the XRCC4-DNA ligase IV complex: influence of terminal DNA sequence. Nucleic Acids Res. 35, 5755–5762.

57 Tsai, C. J., Kim, S. A. and Chu, G. (2007) Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends. Proc. Natl. Acad. Sci. USA 104, 7851–7856.

58 Chappell, C., Hanakahi, L.A., Karimi-Busheri, F., Weinfeld, M. and West, S. C. (2002) Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. EMBO J. 21, 2827–2832.

59 Koch, C. A., Agyei, R., Galicia, S., Metalnikov, P., O'Donnell, P., Starostine, A., Weinfeld, M. and Durocher, D. (2004) Xrcc4 physically links DNA end processing by polynucleotide kinase to DNA ligation by DNA ligase IV. EMBO J. 23, 3874–3885.

60 Ahel, I., Rass, U., El-Khamisy, S. F., Katyal, S., Clements, P. M., McKinnon, P. J., Caldecott, K. W. and West, S. C. (2006) The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates. Nature 443, 713–716.

61 Clements, P. M., Breslin, C., Deeks, E. D., Byrd, P. J., Ju, L., Bieganowski, P., Brenner, C., Moreira, M. C., Taylor, A. M. and Caldecott, K. W. (2004) The ataxia-oculomotor apraxia 1 gene product has a role distinct from ATM and interacts with the DNA strand break repair proteins XRCC1 and XRCC4. DNA Repair (Amst) 3, 1493–1502.

62 Tseng, H. M. and Tomkinson, A. E. (2004) Processing and joining of DNA ends coordinated by interactions among Dnl4/Lif1, Pol4, and FEN-1. J. Biol. Chem. 279, 47580–47588.

63 Wu, X., Wilson, T. E. and Lieber, M. R. (1999) A role for FEN-1 in nonhomologous DNA end joining: the order of strand annealing and nucleolytic processing events. Proc. Natl. Acad. Sci. USA 96, 1303–1308.

64 Ma, Y., Pannicke, U., Schwarz, K. and Lieber, M. R. (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell 108, 781–794.

65 Niewolik, D., Pannicke, U., Lu, H., Ma, Y., Wang, L. C., Kulesza, P., Zandi, E., Lieber, M. R. and Schwarz, K. (2006) DNA-PKcs dependence of Artemis endonucleolytic activity, differences between hairpins and 5′ or 3′ overhangs. J. Biol. Chem. 281, 33900–33909.

66 Daley, J. M., Laan, R. L., Suresh, A. and Wilson, T .E. (2005) DNA joint dependence of pol X family polymerase action in nonhomologous end joining. J. Biol. Chem. 280, 29030–29037.

67 Lee, J. W., Blanco, L., Zhou, T., Garcia-Diaz, M., Bebenek, K., Kunkel, T. A., Wang, Z. and Povirk, L. F. (2004) Implication of DNA polymerase lambda in alignment-based gap filling for nonhomologous DNA end joining in human nuclear extracts. J. Biol. Chem. 279, 805–811.

68 Mahajan, K. N., Nick McElhinny, S. A., Mitchell, B. S. and Ramsden, D. A. (2002) Association of DNA polymerase mu (pol mu) with Ku and ligase IV: role for pol mu in end-joining double-strand break repair. Mol. Cell. Biol. 22, 5194–5202.

69 Moon, A. F., Garcia-Diaz, M., Batra, V. K., Beard, W. A., Bebenek, K., Kunkel, T. A., Wilson, S. H. and Pedersen, L. C. (2007) The X family portrait: structural insights into biological functions of X family polymerases. DNA Repair (Amst) 6, 1709–1725.

70 Nick McElhinny, S. A., Havener, J. M., Garcia-Diaz, M., Juarez, R., Bebenek, K., Kee, B. L., Blanco, L., Kunkel, T. A. and Ramsden, D. A. (2005) A gradient of template dependence defines distinct biological roles for family x polymerases in nonhomologous end joining. Mol. Cell 19, 357–366.

71 Pardo, B., Ma, E. and Marcand, S. (2006) Mismatch tolerance by DNA polymerase Pol4 in the course of nonhomologous end joining in *Saccharomyces cerevisiae*. Genetics 172, 2689–2694.

72 Ma, J. L., Kim, E. M., Haber, J. E. and Lee, S. E. (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.

73 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.

74 Katsura, Y., Sasaki, S., Sato, M., Yamaoka, K., Suzukawa, K., Nagasawa, T., Yokota, J. and Kohno, T. (2007) Involvement of Ku80 in microhomology-mediated end joining for DNA

double-strand breaks in vivo. DNA Repair (Amst) 6, 639–648.
75 Lee, K. and Lee, S. E. (2007) S*accharomyces cerevisiae* Sae2- and Tel1-dependent single-strand DNA formation at DNA break promotes microhomology-mediated end joining. Genetics 176, 2003–2014.
76 Krogh, B. O. and Symington, L. S. (2004) Recombination proteins in yeast. Annu. Rev. Genet. 38, 233–271.
77 Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. and Stahl, F. W. (1983) The double-strand-break repair model for recombination. Cell 33, 25–35.
78 Sun, H., Treco, D. and Szostak, J. W. (1991) Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. Cell 64, 1155–1161.
79 White, C. I. and Haber, J. E. (1990) Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. EMBO J. 9, 663–673.
80 Bressan, D. A., Baxter, B. K. and Petrini, J. H. (1999) The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19, 7681–7687.
81 Johzuka, K. and Ogawa, H. (1995) Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. Genetics 139, 1521–1532.
82 Moreau, S., Morgan, E. A. and Symington, L. S. (2001) Overlapping functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 nucleases in DNA metabolism. Genetics 159, 1423–1433.
83 Llorente, B. and Symington, L. S. (2004) The Mre11 nuclease is not required for 5′ to 3′ resection at multiple HO-induced double-strand breaks. Mol. Cell. Biol. 24, 9682–9694.
84 Ivanov, E. L., Sugawara, N., White, C. I., Fabre, F. and Haber, J. E. (1994) Mutations in XRS2 and RAD50 delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 14, 3414–3425.
85 McKee, A. H. and Kleckner, N. (1997) A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene SAE2. Genetics 146, 797–816.
86 Sartori, A. A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J. and Jackson, S. P. (2007) Human CtIP promotes DNA end resection. Nature 450, 509–514.
87 Lengsfeld, B. M., Rattray, A. J., Bhaskara, V., Ghirlando, R. and Paull, T. T. (2007) Sae2 Is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. Mol. Cell 28, 638–651.
88 Tsubouchi, H. and Ogawa, H. (2000) Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in *Saccharomyces cerevisiae*. Mol. Biol. Cell. 11, 2221–2233.
89 Song, B. and Sung, P. (2000) Functional interactions among yeast Rad51 recombinase, Rad52 mediator, and replication protein A in DNA strand exchange. J. Biol. Chem. 275, 15895–15904.
90 Alani, E., Thresher, R., Griffith, J. D. and Kolodner, R. D. (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.
91 Hays, S. L., Firmenich, A. A., Massey, P., Banerjee, R. and Berg, P. (1998) Studies of the interaction between Rad52 protein and the yeast single-stranded DNA binding protein RPA. Mol. Cell. Biol. 18, 4400–4406.
92 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, table of contents.
93 Davies, O. R. and Pellegrini, L. (2007) Interaction with the BRCA2 C terminus protects RAD51-DNA filaments from disassembly by BRC repeats. Nat. Struct. Mol. Biol. 14, 475–483.
94 Esashi, F., Galkin, V. E., Yu, X., Egelman, E. H. and West, S. C. (2007) Stabilization of RAD51 nucleoprotein filaments by the C-terminal region of BRCA2. Nat. Struct. Mol. Biol. 14, 468–474.
95 Sugawara, N., Wang, X. and Haber, J. E. (2003) *In vivo* roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol. Cell 12, 209–219.
96 Sung, P. (1997) 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.
97 Bugreev, D. V., Hanaoka, F. and Mazin, A. V. (2007) Rad54 dissociates homologous recombination intermediates by branch migration. Nat. Struct. Mol. Biol. 14, 746–753.
98 Heyer, W. D., Li, X., Rolfsmeier, M. and Zhang, X. P. (2006) Rad54: the Swiss Army knife of homologous recombination? Nucleic Acids Res. 34, 4115–4125.
99 Ristic, D., Modesti, M., van der Heijden, T., van Noort, J., Dekker, C., Kanaar, R. and Wyman, C. (2005) Human Rad51 filaments on double- and single-stranded DNA: correlating regular and irregular forms with recombination function. Nucleic Acids Res. 33, 3292–3302.
100 Ira, G. and Haber, J. E. (2002) Characterization of RAD51-independent break-induced replication that acts preferentially with short homologous sequences. Mol. Cell. Biol. 22, 6384–6392.
101 Amitani, I., Baskin, R. J. and Kowalczykowski, S. C. (2006) Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. Mol. Cell 23, 143–148.
102 Mortensen, U. H., Bendixen, C., Sunjevaric, I. and Rothstein, R. (1996) DNA strand annealing is promoted by the yeast Rad52 protein. Proc. Natl. Acad. Sci. USA 93, 10729–10734.
103 Ira, G., Malkova, A., Liberi, G., Foiani, M. and Haber, J. E. (2003) Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. Cell 115, 401–411.
104 Robert, T., Dervins, D., Fabre, F. and Gangloff, S. (2006) Mrc1 and Srs2 are major actors in the regulation of spontaneous crossover. EMBO J. 25, 2837–2846.
105 Nassif, N., Penney, J., Pal, S., Engels, W. R. and Gloor, G. B. (1994) Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. Mol. Cell. Biol. 14, 1613–1625.
106 Wu, L. and Hickson, I. D. (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426, 870–874.
107 Bugreev, D. V., Yu, X., Egelman, E. H. and Mazin, A. V. (2007) Novel pro- and anti-recombination activities of the Bloom's syndrome helicase. Genes Dev. 21, 3085–3094.
108 Veaute, X., Jeusset, J., Soustelle, C., Kowalczykowski, S. C., Le Cam, E. and Fabre, F. (2003) The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature 423, 309–312.
109 Dupaigne, P., Le Breton, C., Fabre, F., Gangloff, S., Le Cam, E. and Veaute, X. (2008) The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: implications for crossover incidence during mitotic recombination. Mol. Cell 29, 243–254.
110 Liu, Y., Masson, J. Y., Shah, R., O'Regan, P. and West, S. C. (2004) RAD51C is required for Holliday junction processing in mammalian cells. Science 303, 243–246.
111 Wu, L., Bachrati, C. Z., Ou, J., Xu, C., Yin, J., Chang, M., Wang, W., Li, L., Brown, G. W. and Hickson, I. D. (2006) BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. Proc. Natl. Acad. Sci. USA 103, 4068–4073.
112 Kaliraman, V., Mullen, J. R., Fricke, W. M., Bastin-Shanower, S. A. and Brill, S. J. (2001) Functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease. Genes Dev. 15, 2730–2740.
113 Mullen, J. R., Nallaseth, F. S., Lan, Y. Q., Slagle, C. E. and Brill, S. J. (2005) Yeast Rmi1/Nce4 controls genome stability

as a subunit of the Sgs1-Top3 complex. Mol. Cell. Biol. 25, 4476–4487.
114 Fricke, W. M., Bastin-Shanower, S. A. and Brill, S. J. (2005) Substrate specificity of the *Saccharomyces cerevisiae* Mus81-Mms4 endonuclease. DNA Repair (Amst) 4, 243–251.
115 Osman, F., Dixon, J., Doe, C. L. and Whitby, M. C. (2003) Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol. Cell 12, 761–774.
116 Lundblad, V. and Blackburn, E. H. (1993) An alternative pathway for yeast telomere maintenance rescues est1- senescence. Cell 73, 347–360.
117 Morrow, D. M., Connelly, C. and Hieter, P. (1997) 'Break copy' duplication: a model for chromosome fragment formation in *Saccharomyces cerevisiae*. Genetics 147, 371–382.
118 Voelkel-Meiman, K. and Roeder, G. S. (1990) Gene conversion tracts stimulated by HOT1-promoted transcription are long and continuous. Genetics 126, 851–867.
119 Bosco, G. and Haber, J. E. (1998) Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture. Genetics 150, 1037–1047.
120 Smith, C. E., Llorente, B. and Symington, L. S. (2007) Template switching during break-induced replication. Nature 447, 102–105.
121 Davis, A. P. and Symington, L. S. (2004) RAD51-dependent break-induced replication in yeast. Mol. Cell. Biol. 24, 2344–2351.
122 Malkova, A., Naylor, M. L., Yamaguchi, M., Ira, G. and Haber, J. E. (2005) RAD51-dependent break-induced replication differs in kinetics and checkpoint responses from RAD51-mediated gene conversion. Mol. Cell. Biol. 25, 933–944.
123 Lydeard, J. R., Jain, S., Yamaguchi, M. and Haber, J. E. (2007) Break-induced replication and telomerase-independent telomere maintenance require Pol32. Nature 448, 820–823.
124 Malkova, A., Ivanov, E. L. and Haber, J. E. (1996) Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. Proc. Natl. Acad. Sci. USA 93, 7131–7136.
125 Signon, L., Malkova, A., Naylor, M. L., Klein, H. and Haber, J. E. (2001) Genetic requirements for RAD51- and RAD54-independent break-induced replication repair of a chromosomal double-strand break. Mol. Cell. Biol. 21, 2048–2056.
126 VanHulle, K., Lemoine, F. J., Narayanan, V., Downing, B., Hull, K., McCullough, C., Bellinger, M., Lobachev, K., Petes, T. D. and Malkova, A. (2007) Inverted DNA repeats channel repair of distant double-strand breaks into chromatid fusions and chromosomal rearrangements. Mol. Cell. Biol. 27, 2601–2614.
127 Bai, Y. and Symington, L. S. (1996) A Rad52 homolog is required for RAD51-independent mitotic recombination in *Saccharomyces cerevisiae*. Genes Dev. 10, 2025–2037.
128 Kang, L. E. and Symington, L. S. (2000) Aberrant double-strand break repair in rad51 mutants of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 20, 9162–9172.
129 Malagon, F. and Aguilera, A. (2001) Yeast spt6–140 mutation, affecting chromatin and transcription, preferentially increases recombination in which Rad51p-mediated strand exchange is dispensable. Genetics 158, 597–611.
130 Aguilera, A. (2001) Double-strand break repair: are Rad51/RecA–DNA joints barriers to DNA replication? Trends Genet. 17, 318–321.
131 Lin, F. L., Sperle, K. and Sternberg, N. (1984) Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. 4, 1020–1034.
132 Fishman-Lobell, J., Rudin, N. and Haber, J. E. (1992) Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. Mol. Cell. Biol. 12, 1292–1303.
133 Fishman-Lobell, J. and Haber, J. E. (1992) Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene RAD1. Science 258, 480–484.
134 Flott, S., Alabert, C., Toh, G. W., Toth, R., Sugawara, N., Campbell, D. G., Haber, J.E., Pasero, P. and Rouse, J. (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.
135 Saparbaev, M., Prakash, L. and Prakash, S. (1996) Requirement of mismatch repair genes MSH2 and MSH3 in the RAD1-RAD10 pathway of mitotic recombination in *Saccharomyces cerevisiae*. Genetics 142, 727–736.
136 Schar, P., Herrmann, G., Daly, G. and Lindahl, T. (1997) A newly identified DNA ligase of *Saccharomyces cerevisiae* involved in RAD52-independent repair of DNA double-strand breaks. Genes Dev. 11, 1912–1924.
137 Siede, W., Friedl, A. A., Dianova, I., Eckardt-Schupp, F. and Friedberg, E. C. (1996) The *Saccharomyces cerevisiae* Ku autoantigen homologue affects radiosensitivity only in the absence of homologous recombination. Genetics 142, 91–102.
138 Frank-Vaillant, M. and Marcand, S. (2002) Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. Mol. Cell 10, 1189–1199.
139 Aylon, Y., Liefshitz, B. and Kupiec, M. (2004) The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle. EMBO J. 23, 4868–4875.
140 Ira, G., Pellicioli, A., Balijja, A., Wang, X., Fiorani, S., Carotenuto, W., Liberi, G., Bressan, D., Wan, L., Hollingsworth, N. M., Haber, J. E. et al. (2004) DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature 431, 1011–1017.
141 Ferreira, M. G. and Cooper, J. P. (2004) Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. Genes Dev. 18, 2249–2254.
142 Takata, M., Sasaki, M. S., Sonoda, E., Morrison, C., Hashimoto, M., Utsumi, H., Yamaguchi-Iwai, Y., Shinohara, A. and Takeda, S. (1998) Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO J. 17, 5497–5508.
143 Astrom, S. U., Okamura, S. M. and Rine, J. (1999) Yeast cell-type regulation of DNA repair. Nature 397, 310.
144 Cortes-Ledesma, F. and Aguilera, A. (2006) Double-strand breaks arising by replication through a nick are repaired by cohesin-dependent sister-chromatid exchange. EMBO Rep. 7, 919–926.
145 Strom, L., Lindroos, H. B., Shirahige, K. and Sjogren, C. (2004) Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol. Cell 16, 1003–1015.
146 Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J. E. and Koshland, D. (2004) DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol. Cell 16, 991–1002.
147 Kim, J. S., Krasieva, T. B., LaMorte, V., Taylor, A. M. and Yokomori, K. (2002) Specific recruitment of human cohesin to laser-induced DNA damage. J. Biol. Chem. 277, 45149–45153.
148 Adachi, N., So, S. and Koyama, H. (2004) Loss of non-homologous end joining confers camptothecin resistance in DT40 cells. Implications for the repair of topoisomerase I-mediated DNA damage. J. Biol. Chem. 279, 37343–37348.
149 Tercero, J. A. and Diffley, J. F. (2001) Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint. Nature 412, 553–557.
150 Lopes, M., Cotta-Ramusino, C., Pellicioli, A., Liberi, G., Plevani, P., Muzi-Falconi, M., Newlon, C. S. and Foiani, M. (2001) The DNA replication checkpoint response stabilizes stalled replication forks. Nature 412, 557–561.

151 Sogo, J. M., Lopes, M. and Foiani, M. (2002) Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. Science 297, 599–602.
152 Hanada, K., Budzowska, M., Davies, S. L., van Drunen, E., Onizawa, H., Beverloo, H. B., Maas, A., Essers, J., Hickson, I. D. and Kanaar, R. (2007) The structure-specific endonuclease Mus81 contributes to replication restart by generating double-strand DNA breaks. Nat. Struct. Mol. Biol. 14, 1096–1104.
153 Hochegger, H., Dejsuphong, D., Fukushima, T., Morrison, C., Sonoda, E., Schreiber, V., Zhao, G. Y., Saberi, A., Masutani, M., Adachi, N. et al. (2006) Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells. EMBO J. 25, 1305–1314.
154 Saberi, A., Hochegger, H., Szuts, D., Lan, L., Yasui, A., Sale, J. E., Taniguchi, Y., Murakawa, Y., Zeng, W., Yokomori, K. et al. (2007) RAD18 and poly(ADP-ribose) polymerase independently suppress the access of nonhomologous end joining to double-strand breaks and facilitate homologous recombination-mediated repair. Mol. Cell. Biol. 27, 2562–2571.
155 Mirzoeva, O. K. and Petrini, J. H. (2003) DNA replication-dependent nuclear dynamics of the Mre11 complex. Mol. Cancer. Res. 1, 207–218.
156 Esashi, F., Christ, N., Gannon, J., Liu, Y., Hunt, T., Jasin, M. and West, S. C. (2005) CDK-dependent phosphorylation of BRCA2 as a regulatory mechanism for recombinational repair. Nature 434, 598–604.
157 Limbo, O., Chahwan, C., Yamada, Y., de Bruin, R. A., Wittenberg, C. and Russell, P. (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.
158 Yu, X. and Baer, R. (2000) Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. J. Biol. Chem. 275, 18541–9.
159 Li, S., Ting, N. S., Zheng, L., Chen, P. L., Ziv, Y., Shiloh, Y., Lee, E. Y. and Lee, W. H. (2000) Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. Nature 406, 210–215.
160 Wong, A. K., Ormonde, P. A., Pero, R., Chen, Y., Lian, L., Salada, G., Berry, S., Lawrence, Q., Dayananth, P., Ha, P. et al. (1998) Characterization of a carboxy-terminal BRCA1 interacting protein. Oncogene 17, 2279–2285.
161 Chen, L., Nievera, C., Lee, A. Y. and Wu, X. (2008) Cell cycle-dependent complex formation of BRCA1/CtIP/MRN is important for DNA double-strand break repair. J. Biol. Chem. 283, 7713–7720.
162 Orr-Weaver, T. L., Szostak, J. W. and Rothstein, R. J. (1981) Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA 78, 6354–6358.
163 Ip, S.C., Rass, U., Blanco, M.G., Flynn, H.R., Skehel, J.M. and West, S.C. (2008). Identification of Holliday junction resolvases from humans and yeast. Nature 456, 357–61.
164 Mimitou, E.P. and Symington, L.S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455, 770–4.
165 Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E. and Ira, G. (2008). Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell 134, 981–94.
166 Gravel, S., Chapman, J.R., Magill, C. and Jackson, S.P. (2008). DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes Dev. 22, 2767–72.
167 Huertas, P., Cortes-Ledesma, F., Sartori, A.A., Aguilera, A. and Jackson, S.P. (2008). CDK targets Sae2 to control DNA-end resection and homologous recombination. Nature 455, 689–92.