

Nucleases and helicases take center stage in homologous recombination

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Homologous recombination (HR)-mediated DNA double-strand break repair maintains genome integrity. Although long-studied, an understanding of two essential steps in this process – the resection of DNA ends to produce recombinogenic 3' single-stranded DNA tails and the resolution of recombination intermediates – has remained elusive. Recent findings show an unexpected role for the Sgs1 (BLM) helicase and Dna2 nuclease in end resection, and provide mechanistic insight into the initiation of 5'–3' resection as well as its regulation by the cell cycle and the DNA damage response. Moreover, the identification of a novel Holliday junction resolvase, Yen1 (GEN1), and several helicases that dismantle strand invasion intermediates has increased the repertoire of nucleases and helicases capable of resolving recombination intermediates.

Homologous recombination mechanisms

Homologous recombination (HR) is an important repair mechanism in mitotic cells to eliminate DNA double-strand breaks (DSBs), and it is essential during meiosis to promote pairing and segregation of chromosome homologs at the first meiotic division. If left unrepaired, or repaired inappropriately, DSBs can trigger mutagenic events such as chromosome loss, deletion, duplication or translocation, events that can lead to carcinogenesis. HR relies on the presence of a homologous duplex to template repair of the broken chromosome; this is generally the sister chromatid in mitotic cells and the chromosome homolog in meiosis.

HR is proposed to occur in several discrete stages. First, DNA ends are processed by 5' to 3' degradation to create 3' single-stranded DNA (ssDNA) tails (Figure 1). The resulting ssDNA is initially bound by replication protein A (RPA, the eukaryotic ssDNA-binding protein) and then replaced by Rad51 (radiation sensitive 51) with the assistance of accessory factors [1]. The nucleoprotein filament formed by Rad51 searches a second DNA molecule for homology, resulting in pairing of the Rad51–ssDNA complex to a complementary ssDNA region within the homologous duplex. The 3' invading end from the broken chromosome is used to prime DNA synthesis templated by the donor duplex. The DSB repair (DSBR) model predicts that the other end of the break is captured by the displaced strand from the donor duplex (D-loop) and is used to prime a second round of leading strand DNA synthesis [2]. A double Holliday junction (dHJ) intermediate is formed that can be resolved to form crossover or non-crossover products.

During synthesis-dependent strand annealing (SDSA), the invading strand that has been extended by DNA synthesis is displaced and anneals to complementary sequences exposed by 5'–3' resection of the other side of the break [2]. The remaining gaps can be filled by DNA synthesis and the nicks ligated. The SDSA model forms only non-crossover products, and there is no alteration to the donor duplex during this mode of repair.

Although great progress has been made in characterizing Rad51-mediated strand exchange and its associated accessory factors [1,3], our knowledge of the first and last steps has lagged. Here, we review recent findings that have shed light on the mechanism of 5'–3' resection and identified a novel HJ resolvase. We also discuss new insights into how these activities are regulated during the cell cycle and in response to DNA damage.

Role of the MRX complex and Sae2 in DSB processing

Mre11 (meiotic recombination 11) and Rad50 form an evolutionarily conserved complex with DNA binding, 3'–5' exonuclease and ssDNA endonuclease activities (Table 1) [2]. In eukaryotes, Mre11 and Rad50 interact with Xrs2 (X-ray sensitive 2, the *Saccharomyces cerevisiae* NBS1 [Nijmegen breakage syndrome 1] ortholog) to form a complex (MRX [Mre11–Rad50–Xrs2], MRN [MRE11–RAD50–NBS1] in mammals) that is involved in several aspects of DNA metabolism, including DNA end binding, Tel1 (telomere maintenance 1, the yeast ATM [ataxia telangiectasia mutated] ortholog) activation, 5'–3' end resection, tethering of DNA ends and telomere maintenance [4]. Null mutations in components of the MRX complex confer sensitivity to ionizing radiation (IR), failure to induce meiosis-specific DSBs and defects in mitotic recombination [2]. The first indication of a role for the MRX complex in DSB processing came from the analysis of budding yeast separation-of-function alleles of *RAD50* (*rad50S*) that accumulate unprocessed DSBs in meiosis [5,6]. Spo11 (sporulation defective 11), the catalytic subunit of the meiotic DSB-forming complex, becomes covalently attached to the 5' ends at break sites and must be removed from the ends for subsequent steps of recombination to proceed [7,8]. Spo11 remains covalently attached to the 5' ends in *rad50S* mutants. Mutants defective for the Mre11 nuclease activity (*mre11-nd*), and *sae2* (sporulation in the absence of Spo11)-null mutants, also accumulate unprocessed meiotic DSBs [2]. In *Schizosaccharomyces pombe*, null mutants in MRN components form meiosis-specific DSBs but fail to remove Spo11 from the ends [9]. Neale *et al.*

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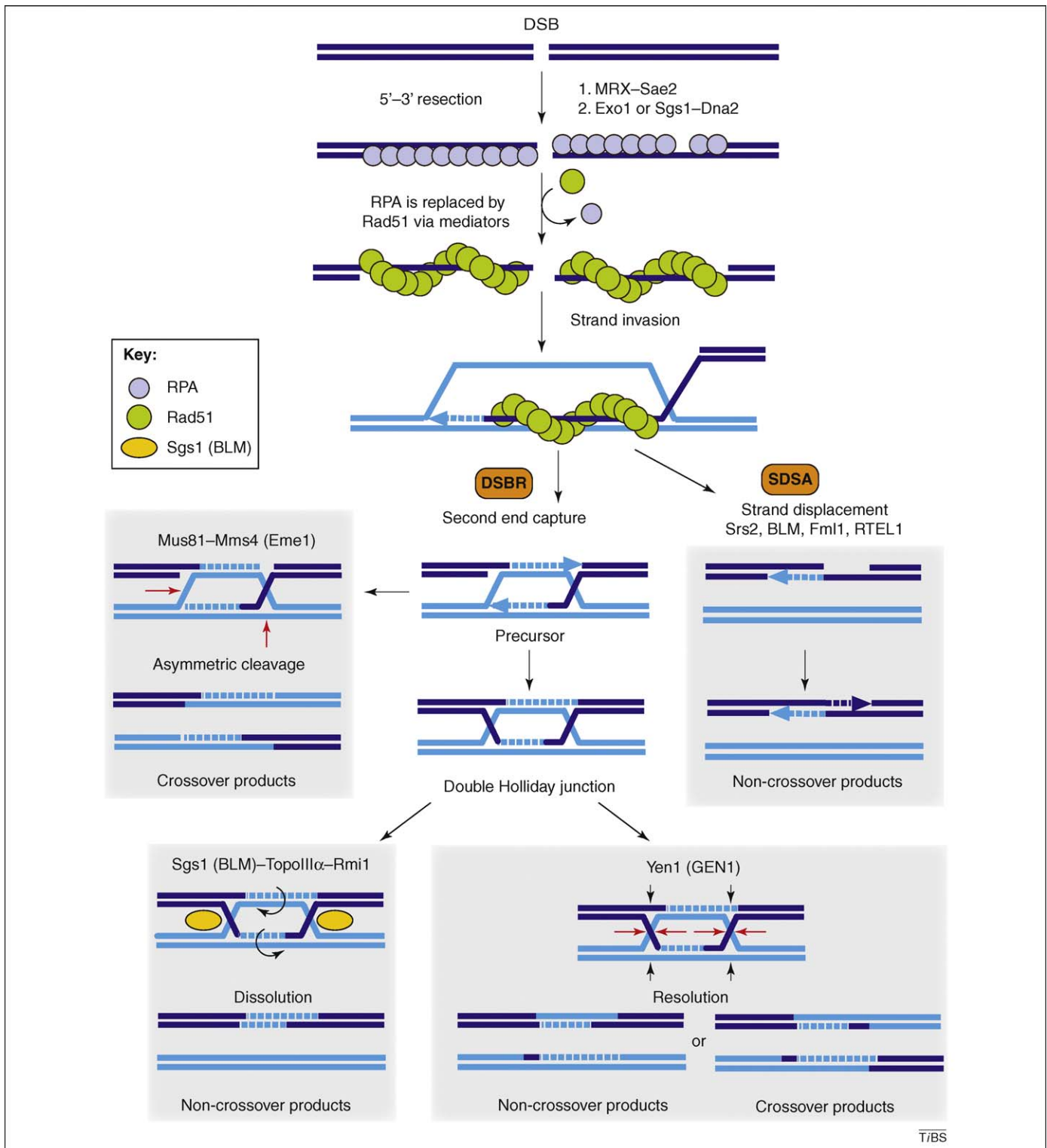


Figure 1. Models and outcomes of the homologous recombination pathway. 5'-3' resection of the broken ends creates 3' ssDNA tails that are rapidly coated by RPA (light blue). RPA is replaced by Rad51 (green) to form the nucleoprotein filament, which can initiate pairing and strand invasion with the homologous duplex DNA. The 3' end of the invading strand is extended by DNA synthesis using the donor duplex as a template. In the SDSA model, the invading strand is displaced and pairs with the other 3' single stranded tail, allowing DNA synthesis to complete repair. In the DSBR model, second end capture forms an early strand exchange intermediate. Processing of this precursor by Mus81-Mms4 (Eme1) generates crossover products, and ligation of this precursor creates a dHJ. Dissolution of the dHJ (via Sgs1 [BLM]-TopoIII α -Rmi1) gives rise to non-crossover products, whereas resolution (via Yen1 [GEN1]) can lead to either crossover or non-crossover products.

[10] made the important discovery that Spo11 is removed endonucleolytically from DSB sites with a short oligonucleotide (10–40 nucleotides) attached. This cleavage step is eliminated by *rad50S* or *sae2 Δ* mutations, which is consistent with the view that MRX functions as an

endonuclease with Sae2 to remove Spo11 concomitant with resection of the 5' strand. Sae2 was recently shown to possess a ssDNA endonuclease activity that cleaves hairpin DNA cooperatively with the MRX complex [11]. However, unlike Mre11, there are no obvious nuclease

Table 1. Homologous recombination and DNA damage checkpoint proteins

<i>S. cerevisiae</i>	<i>S. pombe</i>	Human	Biochemical function	Refs
RPA	RPA	RPA	Heterotrimeric ssDNA-binding protein	[1]
Rad51	Rad51 (Rhp51)	RAD51	RecA homolog. Binds ssDNA and dsDNA, promotes homologous pairing and strand exchange	[1]
Mre11–Rad50–Xrs2 (MRX complex)	Mre11 (Rad32)–Rad50–Nbs1 (MRN complex)	MRE11–RAD50–NBS1 (MRN complex)	Mre11, ssDNA endonuclease and 3'–5' exonuclease; Rad50, DNA binding and tethering activity; Xrs2 (Nbs1), interacts with Tel1 (ATM)	[2,4]
Spo11	Rec12	SPO11	Catalyzes the formation of meiosis-specific DSBs by a topoisomerase-like transesterase mechanism	[7,8]
Sae2	Ctp1	CtIP	Interacts with MRX (MRN) complex on DNA; <i>S. cerevisiae</i> protein has ssDNA endonuclease	[11]
Exo1	Exo1	EXO1	5'–3' exonuclease and flap endonuclease	[27]
Sgs1	Rqh1	BLM	3'–5' helicase of the RecQ family	[32,34]
Top3	Top3	TopoIII α	Type I topoisomerase	[76]
Rmi1	Rmi1	RMI1	Interacts with Sgs1–Top3 complex	[60,61,76]
Dna2	Dna2	DNA2	ssDNA endonuclease; ATPase; helicase	[56]
Mec1–Ddc2	Rad3–Rad26	ATR–ATRIP	PIKK required for DNA damage and replication checkpoint	[47]
Tel1	Tel1	ATM	PIKK required for DNA damage checkpoint and telomere maintenance	[47]
Rad53	Cds1	CHK2	Effector kinase	[47]
Rad9	Crb2	53BP1	DNA damage sensor; interacts with modified histones and Rad53	[47]
Ddc1–Rad17–Mec3 (9-1-1 complex)	Rad9–Rad1–Hus1	RAD9–RAD1–HUS1	PCNA-like clamp	[47]
Rad24	Rad17	RAD17	Alternative large subunit of the RFC clamp loader complex	[47]
Yku70–Yku80	Pku70–Pku80	KU70–KU80	Heterodimeric DNA end binding protein	[40]
Mus81	Mus81	MUS81	Catalytic subunit of Mus81–Mms4 (Eme1) heterodimeric nuclease	[63]
Mms4	Eme1	EME1	Interacts with Mus81	[63]
Yen1	Not identified	GEN1	Symmetrical cleavage of HJs	[55]

Abbreviations: 53BP1, p53-binding protein 1; ATRIP, ATR-interacting protein; CHK2, checkpoint kinase 2; Rqh, RecQ homolog.

motifs present in Sae2 that could be mutated to determine whether Sae2 has a catalytic role in Spo11 removal.

In vegetative cells, DSBs made by the meganucleases HO or I-SceI can still be processed in *mre11*, *rad50* or *xrs2* null mutants, albeit more slowly [12,13]. The DNA fragments produced by HO cleavage are much more stable in *mre11* Δ mutants than in wild-type cells, but a sub-population of breaks initiates resection and is subsequently repaired by Rad51-dependent strand invasion. The *rad50S* and *sae2* Δ mutants exhibit a slight delay in processing HO-induced breaks, whereas no obvious defect in HO-induced DSB processing is seen in *mre11-nd* mutants [14,15]. The *rad50S*, *sae2* Δ and *mre11-nd* mutants show moderate sensitivity to IR, but the most striking phenotype in mitotic cells is a defect in the resolution of hairpin-capped DNA ends [16,17]. Thus, the Mre11 nuclease and Sae2 seem to be crucial for processing blocked ends, such as those produced by Spo11, hairpins and some IR-induced adducts. Recent studies show that the MRX complex and Sae2 initiate end resection by an endonuclease mechanism that removes the 5' strand in increments of around 50–100 nucleotides (Figure 2) [18,19]. This function for MRX and Sae2 in the initiation of end resection is consistent with previous studies showing an endonucleolytic mechanism for Spo11 removal and Mre11-dependent oligonucleotide formation in *Xenopus laevis* extracts [10,20]. Furthermore, cytological and chromatin immunoprecipitation studies show that Mre11 binds transiently to sequences immediately adjacent to the DSB and does not move away from the break site as might be expected for a processive nuclease activity [21,22]. The initial processing step by MRX and Sae2 is not essential for processing HO-induced DSBs in budding yeast, suggesting that other nucleases are able to resect the ends directly.

In fission yeast and mammalian cells the initial processing step seems to be more important for homology-dependent repair than in budding yeast. The equivalent mutants of fission yeast (Table 1), *ctp1* Δ (the apparent Sae2 ortholog) and *mre11-H134S*, are more sensitive to IR than the budding yeast mutants, and the phenotype is more similar to the *mre11* Δ mutant [23,24]. The formation of ssDNA after introduction of a site-specific DSB has not been examined directly in fission yeast, but the recruitment of RPA to sequences adjacent to an HO-induced DSB has been shown by chromatin immunoprecipitation and has been used as an indirect measure of resection [23]. RPA recruitment to DSBs is reduced in *ctp1* Δ mutants, similar to the low level observed in *mre11* Δ mutants, suggesting that resection is reduced. Similarly, CtIP (the apparent mammalian Sae2 ortholog) knockdown in human cells, or elimination of the Mre11 nuclease activity by use of an Mre11-H129N substitution mouse cell line, results in a dramatic reduction in the formation of IR-induced RPA foci [25,26].

Exo1 and Sgs1–Dna2 function in processive 5'–3' resection

The observation of 5'–3' resection in the absence of the MRX complex suggested that redundant activities are able to process HO-induced DSBs. Exo1 is a conserved member of the Rad2 (XPG [*Xeroderma pigmentosum* group G]) family of nucleases, which exhibit 5'–3' double-stranded DNA (dsDNA) exonuclease and 5' flap endonuclease activities *in vitro* [27]. Exo1 functions in mismatch repair, meiotic crossover control and the degradation of stalled replication forks and uncapped telomeres but has only a minor role in the 5'–3' resection of an HO-induced DSB [15,27–29]. Several studies suggest that Mre11 and Exo1

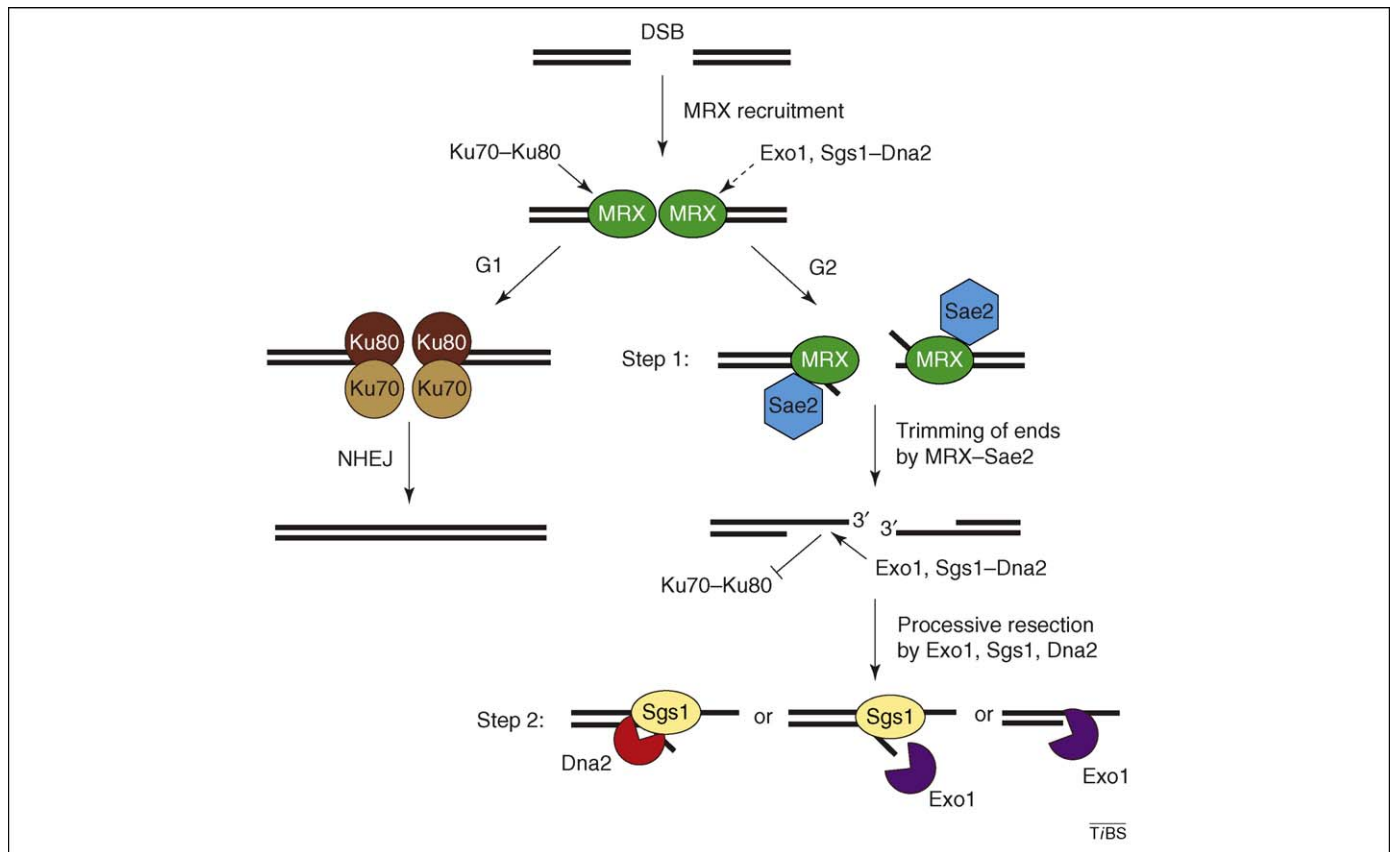


Figure 2. Model for the two-step mechanism of DSB processing. Immediately after a DSB is formed, the MRX complex (green) binds to the broken ends. If repaired by NHEJ, the ends should not be processed and are ligated back together with the help of the NHEJ pathway components (the Ku complex [brown], DNA ligase 4 and accessory factors). Repair of the break by HR requires 5'-3' resection, which takes place in two steps. In the first step, MRX and Sae2 (blue) trim the ends around the break to create short ssDNA overhangs. These are then substrates for further processing by the concerted action of a helicase, Sgs1 (orange), and two nucleases, Exo1 (purple) and Dna2 (red). In the absence of Exo1 and Sgs1-Dna2, further clipping by MRX and Sae2 can occur but seems to be inefficient. The initial processing step is required to remove terminal adducts and might also function to create a substrate that is less favorable for Ku binding and preferred by Sgs1 or Exo1. Sae2 is inactive in G1 cells, thus favoring repair by NHEJ.

have redundant roles in DSB processing in mitotic cells. Exo1 overexpression suppresses the DNA repair defect of *mre11-*, *rad50-* and *xrs2-* null mutants and can partially overcome the *mrx* defect in resection of an HO-induced DSB [2]. This activity is dependent on Exo1 nuclease activity, suggesting that Exo1 can perform some functions of the MRX complex in end resection [2]. Moreover, *exo1Δ mre11Δ* double mutants exhibit a synthetic growth defect, are more sensitive to MMS (methyl methanesulfonate) and IR and display an even longer delay in the processing of an HO-induced DSB than *mre11Δ* single mutants [2]. In *S. pombe*, radiation-induced Rad51 foci are not detected in the *exo1Δ rad50Δ* double mutant, which is consistent with the single-strand tails being very short [30]. However, residual resection and homology-dependent repair occur in the *exo1Δ mre11Δ* double mutant, suggesting further redundancy within the pathway.

One possible mechanism for DNA end resection would involve a helicase working in conjunction with a single-strand specific endo- or exonuclease. In *Escherichia coli*, the RecQ helicase and the RecJ 5'-3' exonuclease function in DSB resection in the absence of the dominant RecBCD activity [31], suggesting the possibility that the yeast RecQ-family helicase, Sgs1 [32], might also function in DSB resection. The *sgs1Δ* single mutant shows normal resection of sequences close to an HO endonuclease-

induced DSB, but resection is reduced for sequences >3 kb from the break site [18,19]. By contrast, only the products of MRX-Sae2-mediated cleavage are detected in the *exo1Δ sgs1Δ* double mutant, thereby resulting in very short ssDNA tails (Figure 2) [18,19]. Remarkably, Rad51-dependent repair still occurs, albeit with reduced efficiency [18,19,33]. It is likely that the extensive resection by Exo1 and Sgs1 after MRX-Sae2 clipping is fast, explaining why the initial processing in mitotic cells had previously escaped detection. The resection defect observed in the *sgs1Δ* mutant was also observed in mutants lacking the Sgs1-interacting proteins Top3 (DNA topoisomerase III, TopoIII α in mammals) and Rmi1 (RecQ-mediated genome instability 1), suggesting that the entire STR (Sgs1-Top3-Rmi1) complex is required for DSB processing [19]. A role for a RecQ family helicase in resection seems to be conserved in human. BLM (mutated in Bloom syndrome) is considered to be the Sgs1 ortholog based on protein-protein interactions and mutant phenotypes, and it is required for genome integrity and cancer prevention [34,35]. BLM can function in a parallel pathway with Exo1 to promote RPA recruitment to camptothecin-induced DSBs [33]. Because resection of HO-induced DSBs can occur in budding yeast *mre11Δ* and *sae2Δ* mutants, it is presumed that Exo1 or Sgs1 can still process 'clean' ends in the absence of the initial processing step. However, Exo1

and Sgs1 might not be able to process adducts produced by IR or Spo11, explaining the IR sensitivity and meiosis defects observed in *mre11-nd* and *sae2Δ* mutants.

Sgs1 must function with a nuclease to degrade the 5' strand. Zhu *et al.* [19] provided evidence that the nuclease acting with Sgs1 is Dna2, a conserved endonuclease/helicase implicated in Okazaki fragment processing. *DNA2* is an essential gene, but the lethality conferred by the null mutation is suppressed by deletion of *PIF1* (which encodes a helicase that functions in the nucleus and mitochondria) [36]. Like the *sgs1Δ* mutant, the *dna2Δ pif1-m2* mutant (defective for the nuclear function of Pif1) exhibits no defect in resection of sequences close to the DSB, but resection monitored 28 kb from the break site is reduced. The effect of the *dna2Δ* mutation seems to be more severe than *sgs1Δ*, possibly due to indirect effects on DNA replication during the long time course used to monitor resection. *DNA2* was also identified as the major activity responsible for 5'-3' degradation of linear DNA in *X. laevis* egg extracts [37]. Because the phenotype of the *exo1Δ dna2Δ* double mutant is similar to the *exo1Δ sgs1Δ* double mutant and the *dna2Δ sgs1Δ* resection defect is epistatic to *dna2Δ*, it seems that Sgs1-Dna2 functions in a parallel pathway to Exo1. However, it remains possible that Sgs1 provides a substrate that can be utilized by either Dna2 or Exo1. *In vitro* studies on the mammalian orthologs show that BLM can stimulate Exo1 nuclease activity [38]. The Dna2 end-processing function requires its nuclease activity, but not helicase activity, which is consistent with the view that Sgs1 unwinds DNA ends and Dna2 removes the 5' strand [19].

In vitro studies suggest that a two-step resection mechanism is also used in the Euryarchaea hyperthermophile *Pyrococcus furiosus* [39]. The genes encoding Mre11 and Rad50 are in the same operon as *HerA* and *NurA*, which encode an ATP-dependent bidirectional helicase and a 5'-3' exonuclease, respectively. The purified recombinant proteins interact functionally to promote 5' end resection of dsDNA. The MR complex is involved in the initial processing step by removing oligonucleotides of 15–55 nt from the 5' end, then the partially resected intermediate is rapidly processed by *HerA* and *NurA* to generate long 3' ssDNA tails.

Regulation of DSB resection

The repair of DSBs in vegetative cells can occur by HR or by non-homologous end joining (NHEJ). In NHEJ, sequence homology is not required for ligation of the two ends; repair can be accurate or associated with nucleotide deletion or addition at the junction [40]. HR is generally restricted to the S and G2 phases, when DNA has replicated and the sister chromatid is available as a repair template [41,42]. NHEJ, by contrast, operates throughout the cell cycle but seems to be more important in G1 [40,43]. The choice between the two pathways is governed by cyclin-dependent protein kinases (CDKs), and several lines of evidence suggest that DSB resection, an event necessary for HR and inhibitory for NHEJ, is a crucial regulator in this decision (Figure 2) [41,42]. Previous studies showed that Sae2 is phosphorylated periodically in cycling cells [44]. Huertas *et al.* [45] provided evidence that Sae2 is phos-

phorylated by Cdc28 (the CDK1 ortholog) on Ser267 within an evolutionarily conserved motif; the substitution of this site to a non-phosphorylatable residue, S267A, phenocopies *sae2Δ*, including hypersensitivity to camptothecin, defective sporulation, reduced hairpin-induced recombination and impaired DSB processing [45]. A mutant with a substitution of Ser267 to Glu to mimic phosphorylation has wild-type Sae2 function and also overcomes the requirement for CDK activity in DSB resection [45]. Mimicking or preventing phosphorylation by S267E and S267A substitutions, respectively, modulates the balance between HR and NHEJ during the cell cycle, suggesting that the commitment to DSB resection is highly regulated, thus ensuring that the cell engages in the most appropriate DNA repair pathway [45].

Although HR requires DNA end resection to generate ssDNA, extensive resection can be detrimental, giving rise to gross chromosome rearrangements [46]. Therefore, it is likely that the extent of DNA resection is regulated. Recent studies suggest that some checkpoint proteins not only recognize ssDNA but also affect the rate and extent at which ssDNA arises. The Tel1 (ATM) and Mec1 (mitosis entry checkpoint 1, the ATR [ataxia telangiectasia and Rad3 related] ortholog) protein kinases, which are members of the phosphoinositide-3-kinase-related protein kinase (PIKK) family, are required for DSB detection and downstream signaling (Table 1) [47]. The DNA damage signals that evoke these two kinases are distinct: Tel1 (ATM) has a primary role in the response to DSBs, whereas Mec1 (ATR) seems to respond to RPA-coated ssDNA. The clamp loader complex (Rad24 and replication factor C [RFC] subunits 2–5) binds at the ssDNA-dsDNA junction formed by resection and loads the 9-1-1 PCNA (proliferating cell nuclear antigen)-like DNA damage clamp (comprising Ddc1 [DNA damage checkpoint 1]-Rad17-Mec3 in *S. cerevisiae*). RPA-coated ssDNA also recruits the Mec1-Ddc2 heterodimer, which then activates the checkpoint cascade, leading to a Rad9-mediated Rad53 phosphorylation [48]. Rad9 acts first as an adaptor to mediate the interaction between Mec1 and Rad53 and then as a scaffold to allow Rad53 autophosphorylation and activation [48].

Rad9 is essential for damage-induced cell-cycle arrest and additionally contributes to DNA damage metabolism by inhibiting ssDNA accumulation at DSBs and uncapped telomeres via interaction of the Rad9 Tudor domain and the methylated K79 residue of histone H3 [49]. This mechanism regulates resection and seems to represent a strategy that coordinates cell-cycle arrest with nuclease progression, thus limiting the amount of ssDNA generated during the DNA damage response. In fact, loss of this inhibitory effect can partially bypass the CDK1 requirement for resection, suggesting that Rad9 could also be a target of the CDK1-dependent regulation of resection [49].

Recent studies have shown Exo1 activity to be regulated in a checkpoint-dependent manner [50,51]. Budding yeast Exo1 is phosphorylated in response to telomere uncapping, DNA damage induced by bleomycin or replication defects. This phosphorylation relies on Mec1, Rad9, Rad53, Rad24 and Rad17 [51]. Consistent with this finding, Exo1 was identified as a Rad53 target in a proteome-wide screen of

proteins phosphorylated in response to MMS treatment [52]. The phosphorylation sites were mapped to four serines in the C-terminal half of the protein [51]. The phenotypes of phospho-mimic and phosphorylation-defective *exo1* mutants are subtle, but they indicate that Exo1 phosphorylation inhibits its activity *in vivo* by limiting the accumulation of ssDNA at telomeres (Figure 3) [51]. Similarly, human Exo1 is phosphorylated by ATR and is degraded after treatment with the S-phase inhibitor hydroxyurea [50]. Thus, in both human and yeast, Exo1 participates in a negative-feedback loop to limit DSB resection and checkpoint activation. Sae2 also negatively regulates checkpoint signaling by modulating MRX complex association at damaged DNA (Figure 3). Sae2 loss delays Mre11 and Tel1 foci disassembly at DNA breaks, resulting in persistent Mec1 and Tel1 signaling to downstream components [21,53].

Resolution of recombination intermediates

In the DSB model, the dHJ intermediate formed by strand invasion and second end capture must be resolved to allow segregation of the recombinant duplexes (Figure 1). In *E. coli*, RuvC cleaves HJ-containing substrates to produce nicked duplex products that can be readily ligated [54]. GEN1 (XPG-like endonuclease 1), a member of a new sub-class of the Rad2 (XPG) family of nucleases with RuvC-like properties, was identified after extensive fractionation of HeLa cell nuclear extracts [55]. The same group, in an independent screen for yeast HJ resolvases, identified the yeast ortholog of GEN1, Yen1. Although the biochemical properties of GEN1 and Yen1 fit the paradigm for an HJ resolvase, there is currently no genetic evidence to suggest that they function in this capacity. The only published phenotype of the *yen1Δ* mutant is a synthetic growth defect when combined with a *dna2-2* conditional allele [56]. Genetic analysis of the function of Yen1 (GEN1) in recombination is likely to be complicated by the presence of other activities capable of HJ resolution. For example, the budding yeast Sgs1 helicase can reduce crossing over associated with DSB resection in mitotic and meiotic cells in addition to its role in DSB resection [57,58]. Human BLM forms a complex with TopoIII α , RMI1 and RMI2 that can branch migrate and dissolve dHJs *in vitro*, forming exclusively non-crossover products (Figure 1) [59–61]. To date, no yeast ortholog of RMI2 has been identified. The *in vitro* activity is consistent with cytologically and genetically observed BLM (Sgs1)-mediated crossover suppression [57,62].

The other activity known to have an important role in resolution of recombination intermediates is the Mus81 (MMS and UV sensitive 81)–Mms4 (MMS sensitive 4) heterodimer. Budding yeast Mus81 was identified in a two-hybrid screen for Rad54-interacting proteins and independently in a genetic screen for mutations that cause lethality in an *sgs1Δ* background [63]. Although budding yeast *mus81Δ* mutants are resistant to IR and exhibit only a subtle meiotic recombination defect, Mus81 is essential for meiosis in fission yeast [63]. Fission yeast *mus81Δ* mutants accumulate meiotic recombination intermediates, and this defect is partially suppressed by preventing the initiation of recombination

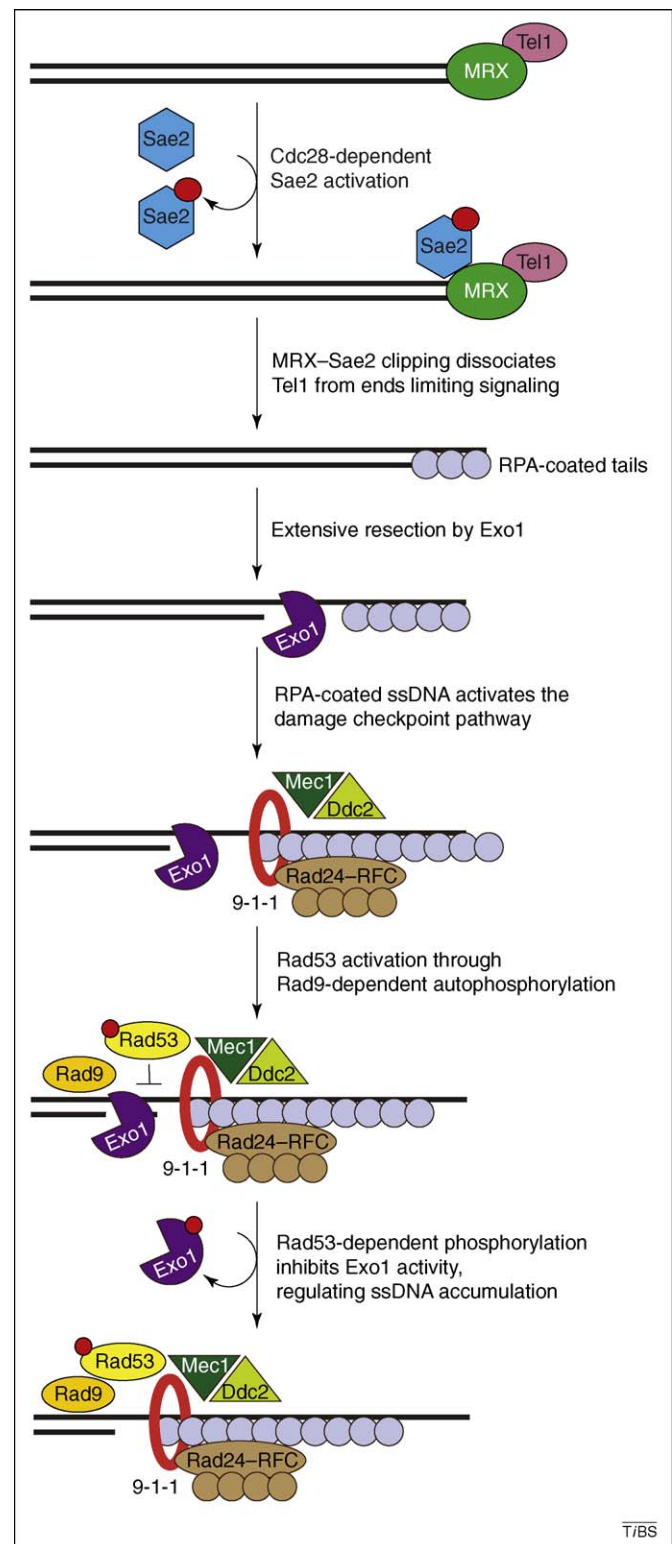


Figure 3. Regulation of DSB resection. The MRX complex (green) associated with the DSB ends rapidly recruits Tel1 (pink) via its association with Xrs2. Upon entry to S, phosphorylation/activation of Sae2 (blue) by Cdc28 initiates DSB resection, leading to the dissociation of Mre11 and Tel1 from the ends, thus limiting Tel1-dependent checkpoint signaling. Resection of the ends generates RPA (light blue)-coated ssDNA. The Rad24 complex (light brown) loads the 9-1-1 complex (red) onto the ssDNA–dsDNA junction. RPA-coated ssDNA activates the Mec1 (dark green)-dependent DNA damage pathway, leading to a Rad9 (orange)-mediated Rad53 (yellow) phosphorylation. In a negative-feedback loop, Exo1 (purple) is phosphorylated in a Rad53-dependent manner, which inhibits its activity, thus limiting the accumulation of ssDNA. For simplicity, only one end of the break and only the Exo1-dependent pathway of resection are depicted. Phosphorylation events are indicated by the addition of a red circle. Ddc2: light green.

or by expressing a bacterial HJ resolvase [64]. *In vitro*, Mus81–Mms4 (Eme1 [essential meiotic endonuclease 1] in fission yeast and human) cleaves a variety of branched DNA structures, including HJs. Based on the greater activity in cleavage of D-loops and nicked HJs, Osman *et al.* [65] proposed that Mus81–Eme1 processes an early strand exchange intermediate before ligation to form a dHJ intermediate, forming exclusively crossover products (Figure 1). Interestingly, *S. pombe* lacks a Yen1 ortholog; this might account for the strong requirement for Mus81–Eme1 in this organism. However, a Mus81–Eme1-independent crossover pathway does exist in *S. pombe* mitotic cells, suggesting that an activity other than Yen1 is responsible [66,67].

MutL homolog 1 (Mlh1) and Mlh3 regulate meiotic crossovers in budding yeast and mouse cells. Mutations in the *MLH1* and *MLH3* genes result in a synergistic defect in meiotic crossovers when combined with *mus81Δ* or *mms4Δ* [63,68]. Similarly, mice lacking *Mus81* are fertile, whereas the *Mus81^{-/-} Mlh1^{-/-}* double mutant exhibits a greater meiotic crossover defect than the *Mlh1^{-/-}* single mutant [69]. It remains unclear whether the residual crossovers that occur in the *mlh1Δ mus81Δ* double mutant are dependent on Yen1 or whether Yen1 functions in the context of the Mlh1-dependent crossover pathway. The observation that an endonuclease activity associates with the MutLα complex (Mlh1–Pms1 [post-meiotic segregation 1]), coupled with the functional importance of the Mlh3 endonuclease motif, raises the possibility that the Mlh1–Mlh3 complex could be directly involved in processing meiotic recombination intermediates [70,71].

The SDSA model for DSBR suggests that the invading end that is extended by DNA synthesis is displaced and anneals to a complementary sequence at the other side of the break exposed by resection. This displacement step could occur by unwinding of the D-loop intermediate by a helicase (Figure 1). The BLM helicase is able to unwind naked DNA D-loops *in vitro*, and this activity could contribute to the anti-crossover function [72]. The budding yeast Srs2 (suppressor of *rad6*), human RTEL1 (regulator of telomere length), human FANCM (Fanconi anemia group M) and fission yeast Fml1 (FANCM ortholog) DNA helicases also dissociate D-loops *in vitro* [67,73–75]. *srs2Δ* and *fml1Δ* mutants both show an increase in the proportion of crossover events during DSBR, which is consistent with a role in SDSA. In the absence of these factors, D-loop intermediates might be cleaved by Mus81 to create crossovers, or they might mature to a dHJ intermediate that can be dissolved by BLM (Sgs1) or cleaved by a HJ resolvase.

Concluding remarks

In the last year, enormous progress has been made in characterizing the mechanisms for DSB processing and resolution of recombination intermediates. A role for Sgs1 (BLM) in DNA end resection was unexpected because genetic studies had pointed to a role for it after Rad51 action, and biochemical studies indicated that the human BLM protein had a role in dissolving recombination intermediates. It remains unclear whether the activities of Exo1 and Sgs1–Dna2 are fully redundant during other processes

that involve end resection, such as meiosis and degradation of uncapped telomeres. New insight into how these activities are regulated during the cell cycle and in response to checkpoint signaling is also emerging. Several activities have been described that can resolve HJs or strand invasion intermediates. A near-term challenge is to determine whether *yen1Δ* mutants exhibit a defect in resolving HJ intermediates *in vivo* and, if so, how this activity intersects with other nucleases and helicases involved in the maturation of recombination intermediates.

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