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Current Opinion in Genetics & Development

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Structure-Specific Endonucleases (SSE) are specialized DNA endonucleases that recognize and process DNA secondary structures without any strict dependency on the nucleotide sequence context. This enables them to act virtually anywhere in the genome and to make key contributions to the maintenance of genome stability by removing DNA structures that may stall essential cellular processes such as DNA replication, transcription, repair and chromosome segregation. During repair of double strand breaks by homologous recombination mechanisms, DNA secondary structures are formed and processed in a timely manner. Their homeostasis relies on the combined action of helicases, SSE and topoisomerases. In this review, we focus on how SSE contribute to DNA end resection, single-strand annealing and double-strand break repair, with an emphasis on how their action is fine-tuned in those processes.

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Current Opinion in Genetics & Development 2021, **71**:195–205

This review comes from a themed issue on **Homologous recombination in meiosis and repair**

Edited by **Eric C Greene** and **Rodney J Rothstein**

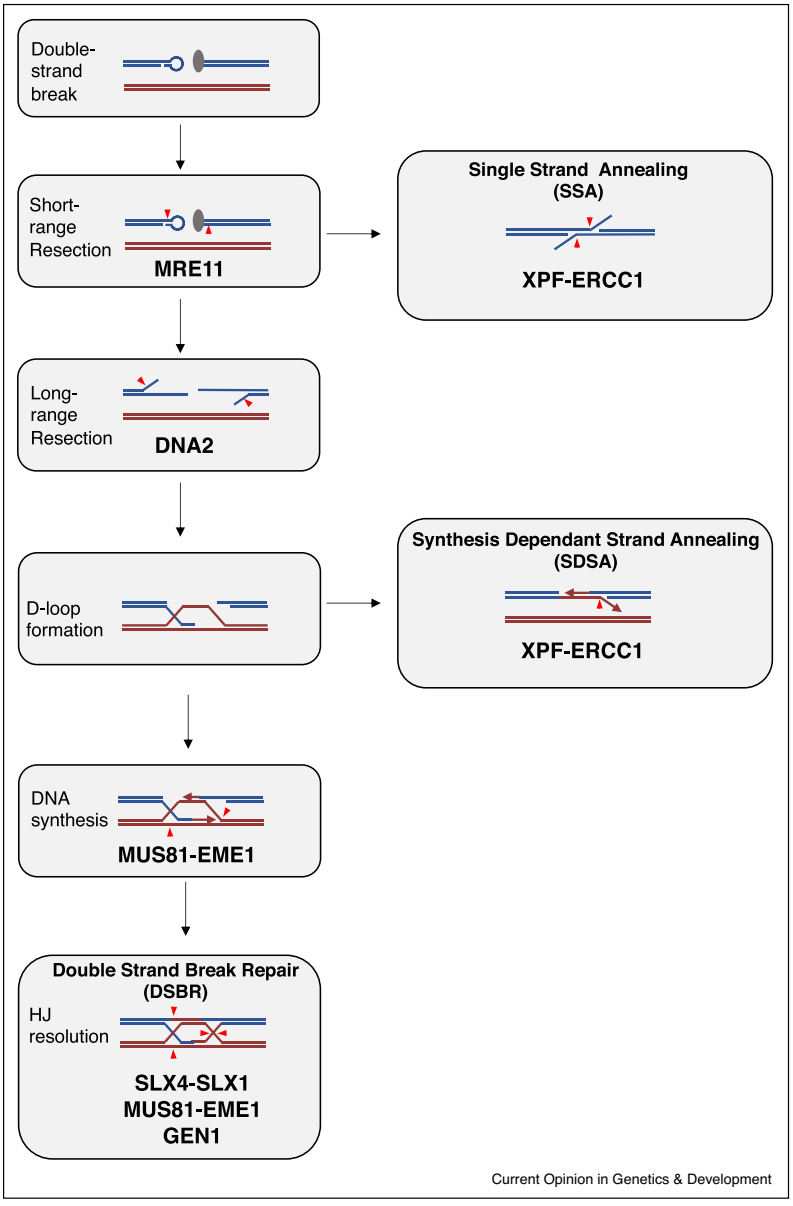
https://doi.org/10.1016/j.gde.2021.09.005

Introduction

Homology directed repair of DNA double-strand breaks (DSB) relies on elaborate DNA transactions that involve the formation of a variety of secondary DNA structures. These include double-stranded DNA ends, single-stranded DNA flaps as well as more complex structures such as displacement loops (D-loop) and Holliday junctions that are generated after the exchange of complementary strands between sister chromatids or homologous chromosomes (Figure 1). The homeostasis of these structures relies on the coordination of DNA processing enzymes including DNA helicases, topoisomerases and nucleases [1]. Here, we review the key contributions made by so-called structure-specific endonucleases, a specialized class of nucleases that recognize and process secondary DNA structures without any strict dependency on the DNA sequence context. This enables them to act virtually anywhere in the genome and to process secondary DNA structures at multiple steps of HR mechanisms (Figure 1).

The first step in DSB repair by HR that relies on SSEs is the so-called end resection mechanism that promotes the 5′ to 3′ resection of a DNA double-stranded end (Figures 1, 2). It is a key process that generates a 3′ single-strand overhang that will serve for homology search and annealing to the complementary homologous sequence in all HR pathways (Figures 1, 3). It is cell cycle regulated, occurring in S/G2 when a sister chromatid is available. Over the last two decades or so, tremendous efforts have been put into dissecting the ins and outs of this process with the latest developments reviewed in this issue [2]. We will focus here specifically on the tightly controlled action of MRE11 and DNA2 which are the two main SSEs that contribute to DNA end resection [3,4]. Following end resection, homology-directed repair of a double-ended DSB can be executed by single-strand annealing (SSA), synthesis-dependent strand annealing (SDSA) or double-strand break repair (DSBR) (Figure 1). SSA proceeds through the annealing of homologous sequences that flank the break site. This is a highly error prone mechanism that ends with the deletion of one of the repeats and the entire intervening sequence between the homologous repeats. It also results in the formation of two non-homologous single-strand 3′ tails that will need to be removed for completion of the repair process (Figures 1, 3b). Their removal is carried out by the XPF-ERCC1 SSE in mammals and its yeast counterparts $Rad1^{XPF}$-$Rad10^{ERCC1}$ and $Swi10^{XPF}$-$Rad16^{ERCC1}$ in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively [5]. We will review the important progress made on understanding how 3′ flap removal is controlled in yeast SSA. In contrast to SSA, SDSA and DSBR involve homology search and strand invasion of an intact sister chromatid or homolog chromosome. This leads to the formation of the D-Loop (Figures 1, 3a) where the 3′ end of the invading strand is used to prime DNA synthesis. In SDSA, the extended invading strand dissociates from the intact complementary donor strand and anneals to the 3′ overhang on the other side of the break following D-loop disassembly by helicases (Figures 1, 3c). This can result in the formation of a 3′ flap if DNA synthesis runs far enough before D-loop disassembly (Figures 1, 3c). As in

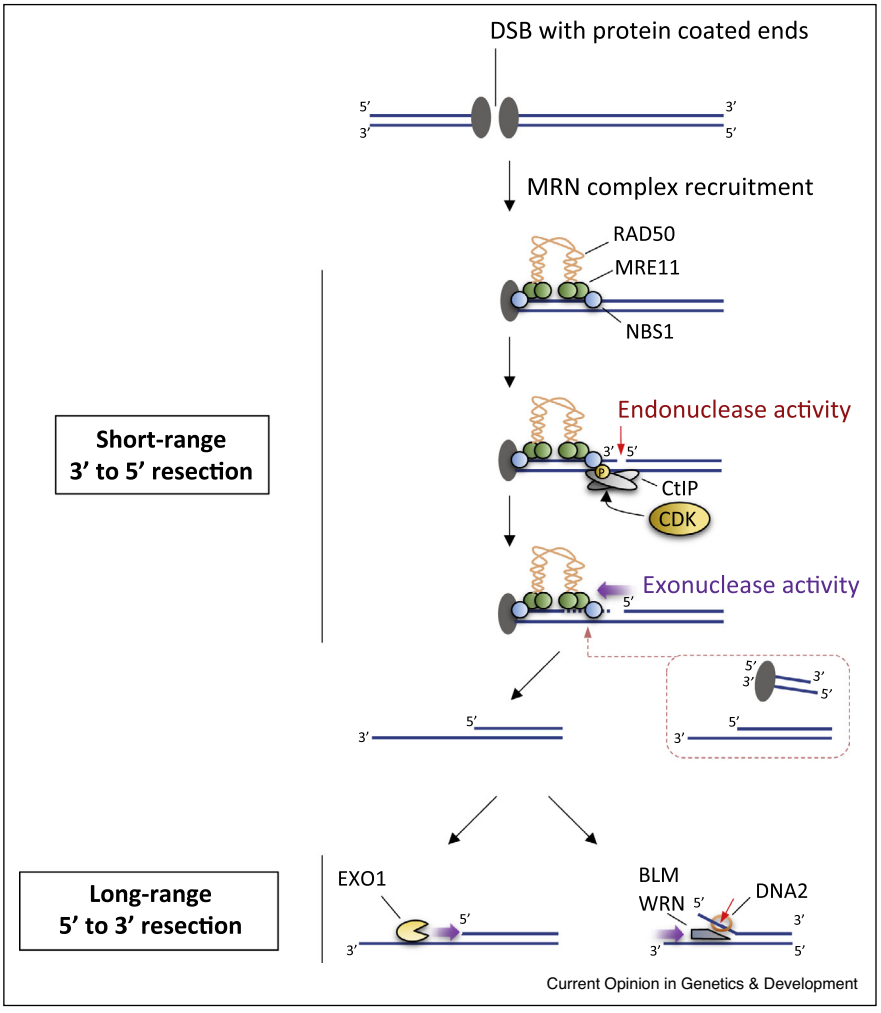
Figure 1



SSEs in HR-mediated repair of a two-ended DSB.
Simplified outline of homology directed DSB repair mechanisms showing the processing of secondary DNA structures by SSEs (preferential cut sites are indicated with red arrows). These range from protein-coated DNA ends (grey oval on the right end of the break) and/or ends with secondary DNA structures (shown on left end of the break) that block 5′–3′ resection, 3′ flaps to more complex branched DNA structures such as D-loops and Holliday junctions. As explained in the main text, it is noteworthy that there are important functional interactions at play between helicases and SSEs. Helicases can either generate secondary structures for SSEs to process, such as during end processing (see Figure 2 for details), or instead dismantle structures such as D-loops and dHJs that otherwise need to get processed by SSEs (see Figure 4 for details).

SSA, the 3′ flap will be endonucleolytically removed by XPF-ERCC1 in mammalian cells and $Rad1^{XPF}$-$Rad10^{ERCC1}$ in *S. cerevisiae* (for review Ref. [6]). However, compared to SSA, much less is currently known on how they are targeted and controlled in SDSA. Noteworthy, SDSA is a non-crossover (non-CO) process that avoids extensive exchange of genetic information, making it one of the least mutagenic HR mechanisms. If the D-loop persists, the non-invading 3′ overhang can anneal to the complementary intact donor strand that forms the single-stranded loop (Figure 1). This combined with extension of both 3′ ends of the break and ligation will ultimately lead to the formation of the canonical double-Holliday junction structure (dHJ) (Figure 1). HJs covalently link both sisters or homologs and need to be processed before chromosome segregation to avoid segregation defects and

Figure 2



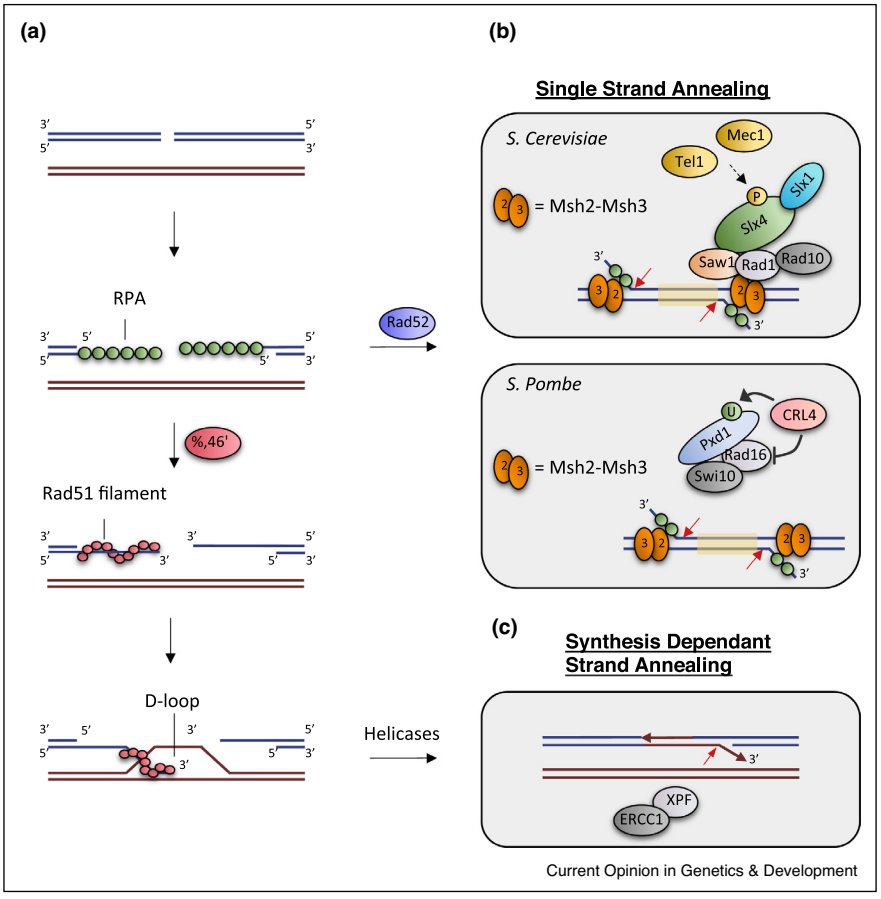
DNA end resection.
MRE11 initiates end-resection by nicking the strand that is 5′ terminated at the break (for simplicity end resection is shown for only one of the ends). It will then carry out 3′–5′ short-range resection with its 3′–5′ exonuclease activity while the gap it has generated constitutes an entry point for the EXO1 and DNA2 nucleases that will carry out long-range 5′–3′ resection. Whereas long-range resection carried out by Exo1 relies on a classical exonucleolytic 'chewing back' process of the DNA strand, it is carried out by a more elaborate mechanism when achieved by DNA2. Indeed, DNA2 is a single-strand DNA endonuclease that will drive 5′–3′ resection by nicking inside the single-strand 5′ flaps which is generated by 5′–3′ DNA unwinding catalyzed by BLM. The pink dotted arrow shows a second cut that can be made by MRE11 opposite its first cut or a gap. The cleavage products generated by that second cut are shown in the dotted lined box. These are a protein-coated short duplex DNA product and a protein free end that can undergo further resection. Note: cuts are indicated with red arrows.

aneuploidy. They can be removed by dHJ dissolution, which relies on the combined action of a DNA helicase and a type I topoisomerase, or by HJ resolution, which involves the nucleolytic processing of HJs by the MUS81-EME1, SLX1-SLX4 and GEN1 SSEs (Figure 4a) or by the MLH1-MLH3 nuclease complex. HJ resolution by MLH1-MLH3, which is not an SSE as such, is reviewed by Sanchez and colleagues in this issue and will not be further discussed here [7]. While HJ dissolution is exclusively a non-CO process like SDSA, HJ resolution can lead to both non-CO or CO. Controlling the balance between SDSA and DSBR is therefore critical in determining the genetic outcome of the recombination process. As reviewed in this issue, DNA helicases are key enzymes that weigh into that balance reversing D-loops and other intermediates to promote SDSA or eliminating dHJs in DSBR [8]. We will see how elaborate spatio-temporal control mechanisms of HJ-processing SSEs also contribute (Figure 4b).

MRE11 and DNA2 in DNA end resection

The first SSE to come into action in HR is MRE11, which displays both DNA endonuclease and 3′ to 5′ exonuclease activities. MRE11 initiates end resection by nicking the

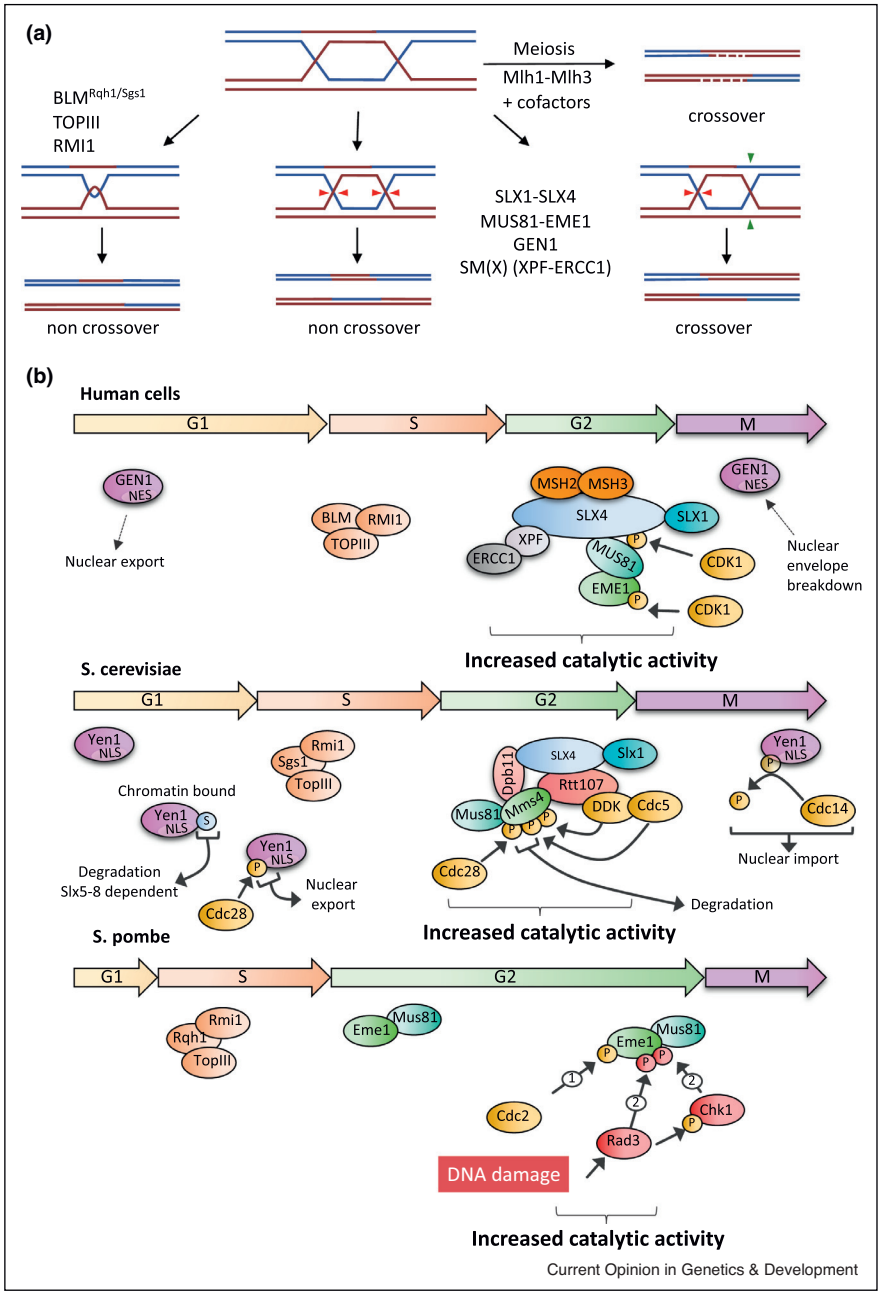
Figure 3



SDSA and SSA.
(a) A DSB with resected ends and RPA-coated 3′ overhangs can be channeled by RAD52 towards SSA if homologous repeats flank the break **(b)** or toward homology search and strand invasion of an intact double-stranded donor after replacement of RPA by RAD51 **(c)**.
(b) In SSA the 3′ overhangs anneal when complementary sequences (yellow hallow) corresponding to the homologous repeats flanking the break are uncovered by resection. This results in the formation of two 3′ single-stranded non-homologous tails that will be removed by Rad1-Rad10 in *S. cerevisiae* (ortholog of human XPF-ERCC1). As further detailed in the main text the recruitment, precise positioning and stimulation of Rad1-Rad10 relies on multiple protein-protein contacts established between Rad1-Rad10 and Msh2-Msh3, Saw1 and Rpa as well as Slx4 phosphorylated by Mec1/Tel1. In *S. pombe*, Pxd1 was identified as the Saw1 ortholog. It binds and stimulates Rad16-Swi10 (the ortholog of Rad1-Rad10 and XPF-ERCC1). Pxd1 can also interact with Dna2 and inhibit Rpa-mediated stimulation of Dna2 in S-phase. Pxd1 was recently shown to be degraded in S-phase after it is ubiquitinated by the CRL4-Cdt2 ubiquitin ligase. For simplicity only one SSA complex is shown. Note: cuts are indicated with red arrows.
(c) In SDSA, the invading strand is displaced after the D-loop gets dismantled by DNA helicases and annealed to the 3′ overhang on the other side of the break. If DNA synthesis and extension of the 3′ invading strand proceeds far enough, a 3′ flap will be generated after the invading strands anneals to the opposite 3′ overhang. The 3′ flap will be removed by XPF-ERCC1 and its counterparts in yeast. Note: cuts are indicated with red arrows.

strand that is 5′-terminated at the break. The endonucleolytic cut serves as an entry point for the EXO1 and DNA2 nucleases that carry out long-range 5′ to 3′ resection while short-range 3′ to 5′ resection towards the end is carried out by MRE11 exonuclease activity (Figure 2) [9•,10–12]. It operates in complex with RAD50 and NBS1, or Xrs2 in *S. cerevisiae*, as part of the MRN or MRX complex, respectively (Figure 2). Recruitment of MRE11 to the ends is controlled in multiple ways (reviewed in Ref. [3]), including the recently reported unsuspected contribution of GRB2 as a direct partner of MRE11 [13]. The MRE11-RAD50 (MR) complex represents the catalytic core where ATP binding and hydrolysis by RAD50 induces conformational changes that specifically stimulate the endonuclease activity of MRE11 while inhibiting its exonuclease activity [14–16]. The key regulatory cofactor of MRN/X is CtIP in mammals, $Ctp1^{CtIP}$ in *S. pombe* and $Sae2^{CtIP}$ in *S. cerevisiae* [17–20]. Intriguingly, both CtIP and $Sae2^{CtIP}$ have been reported to have endonuclease activity, but this remains controversial in the field [20–23]. Stimulation of MRE11 endonuclease activity by CtIP was recently shown to rely on a small conserved C-terminal motif [24••]. Phosphorylation of CtIP

Figure 4



dHJ processing in DSBR.
(a) During dHJ dissolution the two HJs are merged by the BLM (ortholog of the *S. pombe* and *S. cerevisiae* Rqh1 and Sgs1 helicases, respectively) helicase into a hemicatenaned structure where one strand of one chromosome is threaded between the two strands of the other chromosome. Processing of the hemicatenane by the type I topoisomerase that cuts and religates one of the intertwined strands completes the dissolution process without CO formation.
(b) Control mechanisms of HJ resolvases in human cells, *S. cerevisiae* and *S. pombe*. See main text for details. NLS = Nuclear Localization Signal. NES = Nuclear Export Signal. P = Phosphate. S = SUMO.

by various kinases including CDK1 in S/G2, ATM/ATR in response to DNA damage as well as CK2 in *S. pombe*, enhances its stimulatory effect by driving its interaction with the FHA and BRCT domains of NBS1 [24••,25–28], or with Rad50 in the case of $Sae2^{CtIP}$ [29•]. Importantly, CDK1-mediated phosphorylation is critical to restrict the initiation of end resection to S/G2 when a sister chromatid is available [17,30]. MRN/X endonuclease stimulation by CtIP is essential in the presence of protein-DNA complexes (DPC) at the ends of the DSB. This was first demonstrated *in*

vitro on dsDNA substrates with biotin-streptavidin coated ends [17,30–32]. Follow-up studies went on to show that KU forms a physiologically relevant DPC that stimulates MRN/X endonuclease activity [31,33–35]. DNA-PKcs further stimulates endonucleolytic cutting by MRE11 of KU-coated ends, enhancing the binding of CtIP to the MR core complex seven to eightfold [34••]. Remarkably, MRE11 endonuclease activity was shown to be stimulated by a nick or a gap on the opposite strand in the vicinity of protein-coated DNA ends [11,32,34••]. This ability of MRE11 to nick both strands was shown to drive the release of short dsDNA/KU and dsDNA/DNA-PKcs cleavage products detected both *in vitro* and *in vivo* (Figure 2) [11,34••]. Whether this is a general feature that applies to other naturally occurring protein blocks at DNA ends such as topoisomerase-DNA cleavage complexes and the meiotic topoisomerase-related Spo11 enzyme remains to be determined.

While all of the above relates to positive regulation of the MRE11 endonuclease activity, recent studies have identified new MRE11 binding partners that can negatively control DNA end resection. These factors influence MRE11 stability, DNA binding, DNA exonuclease activity and/or chromatin retention and it is not known whether some may directly modulate the endonuclease activity of MRE11 [36–38].

DNA2 is the next SSE to come into action during end-resection where it is recruited by MRN/X via direct interaction with MRE11 to promote long-range resection in coordination with the RecQ-like helicases BLM and WRN in mammals, Sgs1^{BLM} in *S. cerevisiae* and Rqh1^{BLM} in *S. pombe* [33,39–42,43•,44,45]. DNA2 is a bifunctional enzyme that carries both single-strand specific endonuclease and DNA helicase activities. It can introduce cuts within either 3′ or 5′ flaps [4]. The 3′ overhang generated by end resection is protected by RPA from DNA2 SSE activity, which is instead targeted to the 5′-flap generated by its RecQ helicase partner [40,46,47]. DNA2 helicase activity does not contribute to DNA unwinding but rather acts as a translocase that removes RPA from the 5′-flap so that it can get cleaved by DNA2 [46,47]. Remarkably, human phosphorylated CtIP (P-CtIP) is also a key co-activator of DNA2 [42,48••]. This is mediated by a central domain of CtIP that is not required for MRE11 stimulation and is missing in Sae2^{CtIP} [48••]. Accordingly, Sae2 ^{CtIP} is unable to stimulate Dna2 [48••]. Dna2 recruitment through direct binding to Mre11 is negatively regulated by the NHEJ factor Nej1, which competes with Dna2 for Mre11 binding and shifts the balance in favor of NHEJ [49]. In *S. cerevisiae*, SUMOylation of Dna2 in its N-terminal regulatory domain, that is not found in its human counterpart, was recently shown to impair its endonuclease activity *in vitro* [50], but to contribute to nuclear localization and recruitment of Dna2 to DNA damage induced foci, which is also stimulated by Cdk1 and Mec1^{ATR}-mediated phosphorylation of Dna2 [50,51]. In human cells, nuclear localization of DNA2, which lacks an NLS, instead strictly relies on K63 polyubiquitination mediated by the TRAF6 E3 ligase [52].

Considering the importance of P-CtIP in promoting end-resection in part through direct stimulation of both MRE11 and DNA2 SSEs, mechanisms that control its turnover are expected to be critical for controlling the timing and extent of resection. In line with this, protein phosphatase 1 coordinates with RIF1 to counteract DNA end resection by suppressing the accumulation of CtIP at DSBs immediately after damage and was proposed to achieve this by dephosphorylating P-CtIP [53]. Other possible avenues rely on the control of CtIP levels by proteasomal degradation [54,55]. Remarkably, ATM-mediated hyperphosphorylation of chromatin-bound CtIP stimulates its SUMOylation and subsequent degradation by the proteasome following polyubiquitination by the RNF4 SUMO targeted ubiquitin ligase (STUbL) [56,57]. Impeding on this process results in excessive resection and defective HR.

Control of 3′flap processing during SSA

SSA strongly relies on end resection which must proceed until complementary sequences are exposed in the resulting 3′ single-strand overhangs. Accordingly, SSA was found to depend on CtIP and to be low in G1 arrested human cells [58,59]. The pairing of the opposite overhangs driven by RAD52 results in the formation of two 3′ non-homologous single-stranded tails that will be removed by XPF-ERCC1 SSEs in mammals and yeast (Figure 3b).

A number of studies have unraveled the mechanisms that control the recruitment, DNA binding and catalytic activity of Rad1^{XPF}-Rad10^{ERCC1} during SSA in *S. cerevisiae*. Recruitment of Rad1^{XPF}-Rad10^{ERCC1} to recombination intermediates relies on both Saw1 and the Msh2-Msh3 mismatch repair complex [60,61]. Saw1 is a structure-specific DNA binding protein that also interacts directly with Rad1 [60]. Both properties are essential for targeting Rad1^{XPF}-Rad10^{ERCC1} to 3′ flaps that are over 30 nucleotides [60]. Msh2-Msh3 which is also a structure-specific DNA binding protein that binds ds/ss DNA junction at the base of a 3′ flap is required when repeat length is below 1 kb probably to stabilize the annealed 3′ overhangs [62,60]. Msh2-Msh3 also interacts directly with Rad1^{XPF}-Rad10^{ERCC1} [63••]. Biochemical studies show that Rad1^{XPF}-Rad10^{ERCC1} and Saw1 can form a stable complex in the absence of DNA and it is currently unclear whether Saw1 loads on the recombination intermediates before it recruits Rad1^{XPF}-Rad10^{ERCC1} or whether they arrive as a preformed ternary complex [60,63••]. Importantly, Saw1 stimulates Rad1^{XPF}-Rad10^{ERCC1} *in vitro* specifically on 3′ flap structures, not on other structures such as a model replication fork with no 3′ flap [60]. RPA also directly binds and stimulates Rad1^{XPF}-Rad10^{ERCC1} during SSA [63••,64••]. Intriguingly, earlier studies had shown that Slx4 stimulates Rad1^{XPF}-Rad10^{ERCC1} during SSA after its phosphorylation by Mec1^{ATR}/Tel1^{ATM} [65,66]. However, although Slx4 also

directly interacts with Rad1^{XPF}, it is not involved in the recruitment of Rad1^{XPF}-Rad10^{ERCC1} [60], which is in striking contrast with the key role fulfilled by mammalian SLX4 in the recruitment of XPF-ERCC1 to interstrand crosslinks or telomeres [67]. Separation of function *rad1* mutants that are unable to process 3′ flaps and are deficient in gene conversion and SSA but proficient for NER were generated by mutating conserved residues in the N-terminal domain of Rad1 [63••,64••]. Combined biochemical and genetic analyses of these mutants suggest that the timely and precise positioning of Rad1^{XPF}-Rad10^{ERCC1} at the base of the 3′ flap is mediated by its interaction with Msh2-Msh3, Saw1 and possibly RPA [63••,64••]. Interactions between Saw1 and DNA and Saw1 and Msh2-Msh3 also contribute to the process [63••]. However, further investigations will be necessary to better understand the catalytic stimulation of Rad1^{XPF}-Rad10^{ERCC1} by Saw1, RPA and/or Slx4 and get a complete picture of its spatio-temporal control during SSA.

Interestingly, Pxd1 was identified as the fission yeast Saw1 ortholog and a key regulator of SSA [68]. Remarkably though, Pxd1 interacts with Dna2 in addition to Rad16^{Rad1}-Swi10^{Rad10} [68] and while it promotes SSA by activating Rad16^{Rad1}-Swi10^{Rad10}, it inhibits RPA-mediated Dna2 activation [68]. Zhang *et al.* recently demonstrated that Pxd1 is degraded in S-phase after its ubiquitination by the CRL4-Cdt2 ubiquitin ligase [69•]. This ensures that S-phase related functions of Dna2 are not inhibited by Pxd1 while avoiding Pxd1-mediated stimulation of Rad16^{Rad1}-Swi10^{Rad10} and error prone SSA [69•].

Control of dHJ processing in DSBR

As previously mentioned, two different processes ensure removal of dHJs. One is the so-called dissolution mechanism that is achieved by the concerted action of a RecQ-like helicase (BLM in mammals, Sgs1^{BLM} or Rqh1^{BLM} in budding and fission yeasts, respectively) and a type 1A topoisomerase (TOPO IIIα in mammals, TopIII in both yeasts) in complex with an accessory factor Rmi1(for review Ref. [70]) (Figure 4a). dHJ dissolution is a conservative mechanism that leads exclusively to non-CO and is considered the pathway of choice in vegetative cells. HJ resolution is the alternative mechanism. It is carried out by SSEs that have acquired the capacity to introduce coordinated cuts on opposite strands at the junction. Unlike dHJ dissolution, HJ resolution by these so-called HJ resolvases can result in either non-CO or CO (Figure 4a) and nucleolytic processing of HJs and other HJ-related structures is a major source of genetic variability in meiosis. Importantly, HJ resolution is also the only mechanism for eliminating single HJs. Three conserved families of nuclear SSEs are capable of HJ resolution. These include MUS81-EME1 (Mus81-Mms4 in *S. cerevisiae*) that belongs to the XPF-family of endonucleases, Gen1 (Yen1 in *S. cerevisiae*) that is a member of the FEN1/XPG endonucleases and Slx1-Slx4 where Slx1 belongs to the GYI-YIG superfamily of nucleases ([71–77] and for review Ref. [78]). Remarkably, there is no Gen1 ortholog in fission yeast and an Slx4 ortholog has yet to be found in plants. Importantly, as reviewed in this issue, CO-biased resolution of HJs in meiosis relies primarily on the Mlh1-Mlh3 endonuclease and its co-factors in *S. cerevisiae* and mammals, while CO in *S. pombe* relies exclusively on Mus81-Eme1 [7].

Elaborate control mechanisms have been selected to ensure the timely hyperactivation and spatial control of HJ resolvases in late G2 and mitosis (Figure 4b). They guarantee efficient resolution of persisting HJs and other recombination intermediates before chromosome segregation. Importantly, they also provide time for conservative helicase-driven processing of those structures earlier in the cell cycle, thereby limiting CO in vegetative cells.

Control of MUS81-EME1

In *S. cerevisiae*, catalytic upregulation of Mus81-Mms4^{EME1} at the G2/M transition relies on the phosphorylation of Mms4 by Cdc28^{CDK1}, Cdc5^{PLK1} and DDK [79–82] (Figure 4b). This also triggers complex formation between Mus81-Mms4^{EME1} and the Rtt107, Slx4 and Dpb11 scaffolds, which is mediated by direct interactions between Mus81-Mms4^{EME1} and Rtt107 and Dpb11 [80]. Formation of this multifactorial complex seems to further contribute to Mus81-Mms4^{EME1} stimulation and is suspected to contribute to the timely recruitment of Mus81-Mms4^{EME1} but its exact functional relevance is uncertain. The importance of restricting hyper-activation of Mus81-Mms4^{EME1} to late G2 and mitosis is underscored by increased rates of aberrant CO and processing of replication intermediates in cells producing phosphomimetic *mms4* mutants [83]. Recently, an additional level of control was found to be imposed by the degradation of phosphorylated Mms4^{EME1} specifically [84•]. This is mediated by SUMOylation of phosphorylated Mms4^{EME1} by an as yet unknown process, and targeted ubiquitination by the Slx5-Slx8 STUbL and the Cul8-Mms21-Esc2 E3 ubiquitin ligase complex [84•]. It will be important to better understand why Mms4 needs to be ubiquitinated by two different and apparently independent ligases as well as whether Esc2 acts as a co-activator of Mus81-Mms4^{EME1}, as previously suggested [85], before promoting Mms4^{EME1} degradation [84•].

Human MUS81-EME1 also undergoes CDK1-mediated catalytic upregulation [79,86], but this relies instead on phosphorylation of the SLX4 nuclease scaffold which acts as a stimulatory cofactor of MUS81-EME1 [74–76] and shows enhanced interaction with MUS81 in G2/M following phosphorylation by CDK1 [86]. EME1 also appears to be phosphorylated by CDK1 but whether this also stimulates MUS81-EME1 remains to be established. Importantly, binding of SLX4 to the N-terminus of MUS81 relieves auto-inhibition of MUS81-EME1 imposed by MUS81's N-terminal Helix-hairpin-Helix domain [87]. *In vitro* studies showed that efficient HJ resolution by SLX4-MUS81-EME1 is strongly promoted by SLX1 within a SLX1-

SLX4-MUS81-EME1 (SLX-MUS) complex [88]. Productive HJ resolution by the SLX-MUS complex relies on a nick and counter mechanism that coordinates a first cut by SLX1 and a second cut on the opposite strand by MUS81-EME1. This reaction can be further stimulated *in vitro* when the N-terminus of SLX4 is in interaction with its XPF and MSH2 partners [87,89].

In *S. pombe*, stimulation of HJ resolution by Mus81-Eme1 is mediated by dual phosphorylation of Eme1 by $Cdc2^{CDK1}$ and $Rad3^{ATR}$ in response to DNA damage [90,91]. Importantly, hyper-phosphorylation of Eme1 by $Rad3^{ATR}$ requires that it is first phosphorylated by $Cdc2^{CDK1}$. This ensures rapid hyperactivation of Mus81-Eme1 in response to DNA damage in late G2/M. Preventing Mus81-Eme1 hyperactivation in cells lacking $Rqh1^{BLM}$ results in gross chromosomal rearrangements and impaired cell viability [90,91].

Control of GEN1

In *S. cerevisiae*, $Yen1^{GEN1}$ also undergoes $Cdc28^{CDK1}$-mediated phosphoregulation [79]. However, the outcomes are opposite to those described above for Mus81-$Mms4^{EME1}$, as phosphorylation of $Yen1^{GEN1}$ results in both its catalytic inhibition and nuclear exclusion [92,93] (Figure 4b). This inhibitory control of $Yen1^{GEN1}$ is relieved in anaphase after dephosphorylation by Cdc14 [92,93]. The importance of $Yen1^{GEN1}$ control is underscored by the detrimental impact of premature activation of Yen1 on genome stability [92–94]. Furthermore, $Cdc28^{CDK1}$-driven inhibition of $Yen1^{GEN1}$ was recently shown to be particularly important in meiosis where it prevents the premature resolution by $Yen1^{GEN1}$ of recombination intermediates that are needed to establish a controlled distribution of CO [95•]. Reminiscent of the previously mentioned Slx5-Slx8 mediated degradation of phosphorylated $Mms4^{EME1}$ [84•], $Yen1^{GEN1}$ turns out to be targeted for degradation by Slx5-Slx8 dependent ubiquitination at the G1/S transition [96•]. This additional layer of regulation is proposed to ensure that any chromatin associated active $Yen1^{GEN1}$ that remains at the end of G1 will be eliminated before the cells enter S phase [96•]. Preventing ubiquitination of $Yen1^{GEN1}$ by Slx5-Slx8 results in increased levels of CO [96•]. In human cells, control of GEN1 instead relies on a nuclear export signal that prevents GEN1 from accessing chromosomes until nuclear envelope breakdown occurs in mitosis [97].

Conclusion

Important progress has been made in recent years on dissecting the mechanisms underlying the control of SSEs in end-resection and on understanding how from a structural standpoint they modulate the enzymatic activity and/or DNA binding properties of the enzyme. This also stands true for the control of $Rad1^{XPF}$-$Rad10^{ERCC1}$ during SSA in budding yeast. However, as discussed throughout this review many questions still need to be answered before we can get a full picture of how SSEs are regulated. This relates for example to how XPF-ERCC1 is controlled in SSA in higher eukaryotes and to what extent the principles of $Rad1^{XPF}$-$Rad10^{ERCC1}$ control in budding yeast can be extrapolated to its human counterpart. Control of these SSEs in the SDSA pathway is also poorly understood. On another note, while remarkably elaborate regulatory networks that ensure the timely upregulation of MUS81-EME1 enzymes have been unraveled over this last decade, we still do not understand how catalytic stimulation mediated by phosphorylation of EME1 and $Mms4^{EME1}$ actually operates, not to mention whether phosphorylation of human EME1 has any relevance at all. Many more questions remain and there is no doubt that investigating how SSEs are controlled in HR and beyond will yield important new findings of potential therapeutic value.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

CRediT authorship contribution statement

Cédric Giaccherini: Writing - original draft, Visualization.
Pierre-Henri L Gaillard: Writing - review & editing.

Acknowledgements

We wish to thank Jean-Hugues Guervilly for critical reading of the manuscript and our colleagues of the CRCM 3R community for stimulating discussions with special thanks to Bertrand Llorente, Mauro Modesti, Valérie Garcia and Pierre-Marie Dehé.

We apologize to many of our colleagues whose work we were unable to reference due to the constraints imposed by the format of this short review.

This work was supported by grants from Institut National du Cancer (INCa-PLBio2019-152) awarded to PHLG. CG was a recipient of a fellowship awarded by the Fondation pour la Recherche Médicale (Equipe FRM grant number ECO20170637468) and the Fondation ARC (grant ARCDOC42020010001276).

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