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REVIEW

Coupling end resection with the checkpoint response at DNA double-strand breaks

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Abstract DNA double-strand breaks (DSBs) are a nasty form of damage that needs to be repaired to ensure genome stability. The DSB ends can undergo a strand-biased nucleolytic processing (resection) to generate 3′-ended single-stranded DNA (ssDNA) that channels DSB repair into homologous recombination. Generation of ssDNA also triggers the activation of the DNA damage checkpoint, which couples cell cycle progression with DSB repair. The checkpoint response is intimately linked to DSB resection, as some checkpoint proteins regulate the resection process. The present review will highlight recent works on the mechanism and regulation of DSB resection and its interplays with checkpoint activation/inactivation in budding yeast.

Keywords Double-strand break · Resection · MRX · Mec1 · Tel1 · Nucleases

Abbreviations

DSB	Double-strand break
ssDNA	Single-stranded DNA
dsDNA	Double-stranded DNA
MRX	Mre11-Rad50-Xrs2
RPA	Replication protein A
HR	Homologous recombination
NHEJ	Non-homologous end joining
Ku	Ku70–Ku80

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Introduction

DNA double-strand breaks (DSBs) are dangerous forms of DNA damage that need to be accurately repaired to preserve genome stability. Failure to repair them can result in genome instability that is a hallmark of cancer cells. DSBs can form accidentally as a result of failure in DNA replication, as well as of exposure to ionizing radiations or chemicals. Moreover, they can arise when telomeres undergo extensive erosion, which leads to the activation of a DNA damage response and to the onset of apoptosis and/or senescence. DSBs can also form in a programmed manner during physiological cellular processes, such as the prophase of the first meiotic division or the rearrangement of the immunoglobulin genes in lymphocytes.

DSB occurrence triggers the activation of a highly conserved pathway, called DNA damage checkpoint, which couples DSB repair with cell cycle progression [1]. Key checkpoint players include the protein kinases Mec1 and Tel1, whose mammalian orthologs are ATR and ATM, respectively [1]. Once Mec1/ATR and/or Tel1/ATM are activated, their checkpoint signals are propagated through the protein kinases Rad53 and Chk1 (Chk2 and Chk1 in mammals, respectively), whose activation requires the conserved protein Rad9 (53BP1 in mammals) [2–4].

DSBs are repaired by two major conserved pathways: non-homologous end-joining (NHEJ) and homologous recombination (HR). NHEJ directly rejoins together the two broken ends [5], whereas HR uses intact homologous duplex DNA sequences (sister chromatids or homologous chromosomes) as a template for accurate repair [6, 7]. The first step in HR is the degradation of the 5′ DNA strands on either side of the DSB to generate 3′ single-stranded DNA (ssDNA) tails through a process termed DNA end resection. The ssDNA tails are first coated by the ssDNA

binding complex replication protein A (RPA), which is then replaced by the evolutionarily conserved recombinase Rad51. This replacement leads to the formation of a right-handed helical filament that searches for homologous duplex DNA molecules and catalyzes their invasion. The invading 3′ end serves to prime DNA synthesis using the intact homologous DNA sequence as a template, followed by the resolution of the resulting DNA structures and DNA ligation [6, 7].

Much of our knowledge about the DNA end resection mechanism and its regulation has come from genetic and biochemical studies in the budding yeast *Saccharomyces cerevisiae*. Here, we will review the mechanism and regulation of end resection in this organism, as well as its interplays with the checkpoint response.

End resection by MRX, Exo1 and Dna2 nucleases

Genetic studies in the budding yeast *S. cerevisiae* identified the highly conserved MRX (Mre11-Rad50-Xrs2) complex, Exo1 and Dna2 as key nucleases for DSB resection. The MRX complex had long been known to be required for the processing of meiotic DSBs generated by the topoisomerase-like protein Spo11 [8–12]. MRX has a DNA binding activity with a preference toward DNA ends [13] and localizes very close to the DSB ends [14, 15]. The Rad50 subunit has an ATPase activity that induces conformational changes that regulate MRX functions in DNA damage signaling, resection and maintenance of the DSB ends tethered to each other [16–20]. Mre11 has a 3′–5′ dsDNA exonuclease activity, whose polarity is opposite to that needed to generate the 3′-ended ssDNA at the DSB ends [21, 22]. However, Mre11 has also a weak endonuclease activity on 5′-terminated DNA strands and on other DNA structures [23, 24]. This endonuclease activity is dependent on the ATPase activity of Rad50 and on the physical interaction between MRX and the Sae2 protein (CtIP in mammals), which strongly promotes the endonuclease activity of Mre11 within the context of the MRX complex [25]. The ability of MRX to cleave 5′-terminated DNA ends suggested that this complex initiates DNA resection via its endonuclease, rather than its exonuclease activity, by creating a nick that provides the access for nucleases capable of degrading DNA in a 5′–3′ direction [26].

In both yeast and mammals, the 5′–3′ exonuclease activity is provided by two partially overlapping pathways dependent on Dna2 and Exo1 nucleases [27, 28]. In yeast, inactivation of each single pathway results in only minor resection defects, whereas severe resection defects are observed when the two pathways are inactivated simultaneously [27, 28]. The MRX complex not only provides the access for Dna2 and Exo1 through its endonuclease activity, but it has also a structural role in promoting their stable association to DSBs [29]. This nuclease-independent role of MRX can explain why the lack of Mre11 causes a resection defect more severe than that caused by the lack of either Sae2 or Mre11 nuclease activity.

Although Dna2 is recruited on ssDNA ends, it has an endonuclease activity that can cleave both 5′ and 3′ ssDNA overhangs adjoining a duplex DNA, giving rise to degradation products of ~5 to 10 oligonucleotides in length [30, 31]. The separation of DNA strands in the Dna2-mediated nucleolytic processing is carried out by the RecQ helicase Sgs1 (BLM in humans), which unwinds double-stranded DNA (dsDNA) in a 3′–5′ polarity [27, 28, 32]. Unlike Dna2, Exo1 degrades the 5′-terminated strand within duplex DNA and therefore it does not require a helicase activity to unwind DNA [33–35]. Altogether, these data support a model in which MRX-Sae2 provides an initial endonucleolytic cleavage of the 5′ strand at both DSB ends. Then, the nick enables resection in a bidirectional manner, using Exo1 and Dna2-Sgs1 in the 5′–3′ direction away from the DSB end, and Mre11 in the 3′–5′ direction towards the DSB end (Fig. 1a).

Biochemical reconstitution studies have revealed that the RPA complex regulates DSB resection by preventing nonspecific binding of Exo1 to ssDNA, promoting the unwinding activity of Sgs1, stimulating the 5′–3′ resection polarity of Dna2 and attenuating the degradation of the 3′-terminated DNA strand [36, 37]. These in vitro findings have been supported in vivo, as RPA depletion in *S. cerevisiae* eliminates both the Sgs1-Dna2 and Exo1-dependent resection pathways [38].

The requirement for MRX-Sae2 nuclease activity in end resection is dependent on how well Exo1 and Sgs1-Dna2 can access the DSB ends. MRX and Sae2 are important to resect DSBs that possess either chemical modifications or proteins covalently bound at their 5′ ends that restrict the access of Exo1 and Sgs1-Dna2 (Fig. 1a). For example, *sae2Δ* and *mre11* nuclease defective mutants are defective in resecting meiotic DSBs, where Spo11 cleaves DNA by a topoisomerase-like transesterase mechanism, forming a covalent bond between a tyrosine residue of the enzyme and the 5′ ends of the DSB [8–12, 39–42]. Furthermore, the same mutants exhibit a marked sensitivity to ionizing radiations, which can generate double- and single-strand breaks and DNA–protein crosslinks [43, 44], as well as to camptothecin, which extends the half-life of DNA-topoisomerase cleavage complexes [45]. Consistent with this model, the endonuclease activity of MRX-Sae2 is strongly stimulated by the presence of dsDNA substrates in which one DNA end is blocked by a protein adduct [25].

Both Sae2 and Dna2 have been shown to be targets of cyclin-dependent kinase (Cdk1 in yeast)-Clb complexes

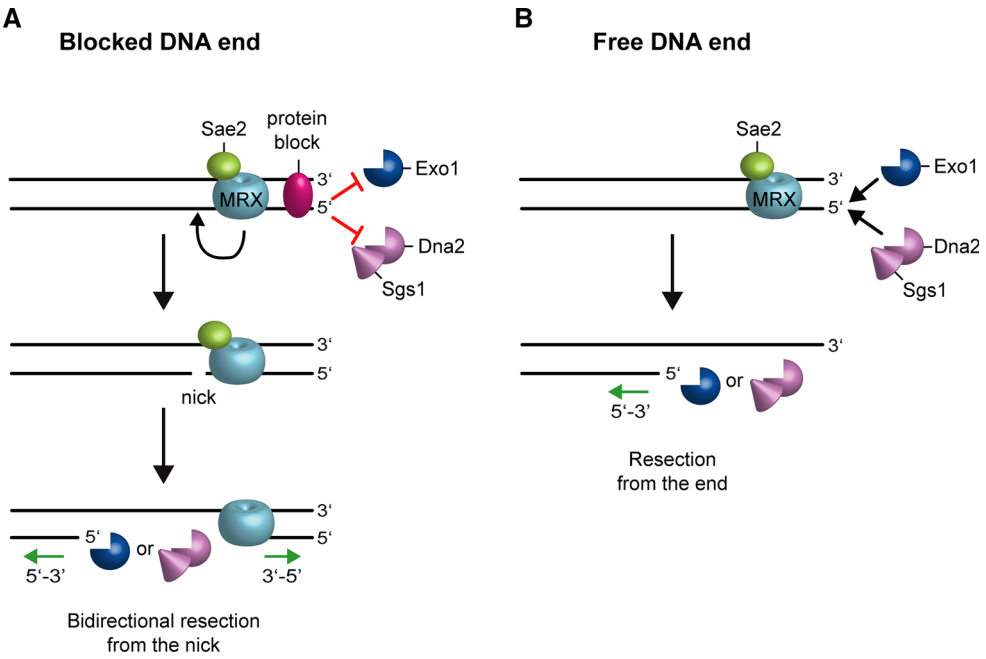

Fig. 1 Model for resection of blocked and free DNA ends. The MRX complex and Sae2 are recruited to DNA ends. **a** The 5′ strand of a DSB blocked by a covalent adduct or a tightly bound protein is not accessible to Exo1 and Sgs1-Dna2. MRX-Sae2-dependent incision of the 5′ strand allows bidirectional processing by Exo1/Sgs1-Dna2 in the 5′–3′ direction from the nick and by MRX in the 3′–5′ direction toward the DSB ends. **b** The processing of DNA ends with no covalent modifications can occur directly by Dna2-Sgs1 or Exo1. MRX is required to promote the association of Exo1 and Sgs1-Dna2 at both blocked and free DNA ends

[46, 47], which allows DSB resection to take place only during the S and G2 phases of the cell cycle, when sister chromatids or homologous chromosomes are present as repair templates [48, 49]. Substitution of Sae2 serine 267 with a nonphosphorylatable residue impairs DSB processing, whereas the same process takes place quite efficiently when Sae2 serine 267 is replaced by a glutamic residue mimicking constitutive phosphorylation [46]. Furthermore, substitution of three Cdk1 consensus sites of Dna2 with nonphosphorylatable residues reduces extensive resection [47].

Inhibition of end resection by Ku and Rad9

The initial endonucleolytic cleavage catalyzed by MRX and Sae2 is critical to resect DSBs whose DNA ends are not accessible to Exo1 and Sgs1-Dna2 due to the presence of chemical modifications or covalently bound-proteins [8–12, 39–42, 45] (Fig. 1a). By contrast, DNA ends (such as those generated by endonucleases) with free 3′ hydroxyl and 5′ phosphate groups are resected by Exo1 and Sgs1-Dna2 even in the absence of MRX-Sae2-mediated cleavage of the 5′ strand (Fig. 1b) [12]. However, initiation of resection of endonuclease-induced DSBs in *sae2Δ* cells occurs less efficiently than in wild type cells [50], suggesting that MRX-Sae2 can be important even at these DSBs to relieve possible inhibition of Dna2 and/or Sgs1-Dna2 activity.

The nucleolytic processing catalyzed by Exo1 is inhibited by the presence of the Ku complex bound at the DSB

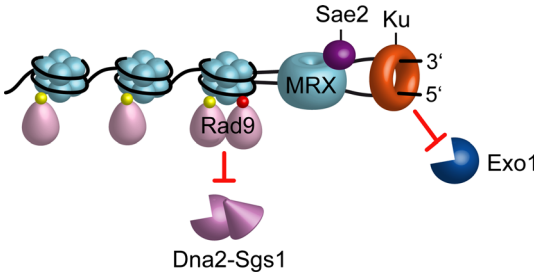

Fig. 2 Inhibition of DSB resection by Ku and Rad9. Ku is bound very close to the DSB end. Rad9 is bound to chromatin even in the absence of DSBs via interaction with methylated histone H3 (*yellow dots*). Rad9 association at DSBs is enhanced by γH2A generation (*red dots*). Ku and Rad9 inhibit DSB resection by limiting Exo1 and Sgs1-Dna2 access to DNA ends, respectively

ends (Fig. 2) [29, 51–54]. The absence of Ku suppresses the resection defect of *mre11Δ* and *sae2Δ* cells in an Exo1-dependent manner [29, 53, 54], indicating that Ku restricts Exo1-mediated resection. As Ku is bound very close to the DSB ends, the MRX-Sae2 clipping could allow Exo1 to initiate resection from a nick and this, in turn, would overcome the inhibition exerted by Ku on Exo1 activity.

By contrast, Sgs1-Dna2 is unable to initiate end resection without MRX even in the absence of Ku, suggesting the existence of another inhibitory pathway. The resection activity of Sgs1-Dna2 is inhibited mainly by the Rad9 protein (Fig. 2) [55, 56], which was originally identified as an adaptor in the DNA damage checkpoint pathway, linking the checkpoint kinases Mec1 and Tel1 to the activation of the effector kinases Rad53 and Chk1 [57]. Rad9 is already bound to chromatin even in the absence of DNA

damage by an interaction with methylated lysine 79 of histone H3 (H3-K79) [58–61]. Rad9 binding to the sites of damage is further strengthened by an interaction between its BRCT domain and histone H2A that has been phosphorylated on serine 129 (γH2A) by the checkpoint kinases Mec1 and Tel1 (Fig. 2) [62–64]. Inhibition of DSB resection by Rad9 requires its chromatin association, as the lack of the H3-K79 methyltransferase Dot1 or the presence of a H2A variant where serine 129 is substituted by a non-phosphorylatable alanine residue increases the resection efficiency [60, 65, 66]. Elimination of the ATP-dependent chromatin remodeler Fun30 increases Rad9 accumulation at DSBs, suggesting that Fun30 can overcome the barrier to resection imposed by Rad9-bound chromatin [65, 67, 68].

Several lines of evidence indicate that Rad9 acts as a barrier toward end processing enzymes by restricting the access of Sgs1-Dna2 to the DSB ends. The lack of Rad9 suppresses the resection defect of Sae2-deficient cells, which show an increased amount of Rad9 bound very close to the DSB ends [55, 56]. The lack of Rad9 increases the resection efficiency also in a wild type context [60, 69] and this rapid resection is mainly dependent on Sgs1, whose recruitment at DSBs is inhibited by Rad9 [55, 56]. Further support for a Rad9-mediated inhibition of Sgs1 comes from the recent identification of a hypermorphic allele of *SGS1* (*SGS1-SS*) that behaves like a *rad9Δ* phenocopy with respect to resection [55]. The Sgs1-ss variant, which suppresses both the hypersensitivity to DNA damaging agents and the resection defect of *sae2Δ* cells, is robustly associated to the DSB ends both in the presence and in the absence of Rad9 and resects the DSB more efficiently than wild type Sgs1 [55]. Altogether, these findings indicate that Rad9 inhibits the activity of Sgs1-Dna2 by limiting Sgs1 binding/persistence at DSB ends and that the Sgs1-ss mutant variant escapes this inhibition possibly because it is more tightly bound to DNA and exerts its helicase activity through Rad9-containing chromatin more efficiently than wild type Sgs1.

End resection and checkpoint activation

Generation of DSBs elicits the activation of the DNA damage checkpoint, whose key players include the *S. cerevisiae* protein kinases Mec1 and Tel1, as well as their mammalian orthologs ATR and ATM, respectively [1]. In both yeast and mammals, Mec1 physically interacts with Ddc2 (ATRIP in mammals), which helps the recruitment of Mec1 to the DSB ends [70–73]. By contrast, Tel1 activation depends on the MRX complex, which is required for Tel1 recruitment to the site of damage through direct interaction between Tel1 with Xrs2, as well as for Tel1 kinase activity [74–80]. Whereas Tel1 is recruited on blunt DSB ends or DNA ends with short ssDNA tails, Mec1 recognizes RPA-coated ssDNA that results from resection of the DSB ends [81–84].

Mec1 and Tel1 themselves regulate the generation of 3′-ended ssDNA at the DSB ends. Cells lacking Mec1 accelerate the generation of ssDNA at the DSBs, whereas the same process is impaired in *mec1-ad* cells that carry an hypermorphic *mec1* allele [66]. Mec1 inhibits DSB resection at least in two ways: (1) by inducing Rad53-dependent phosphorylation of Exo1 that leads to the inhibition of Exo1 activity [85, 86] and (2) by promoting the binding of the resection inhibitor Rad9 close to DNA lesions through phosphorylation of H2A on serine 129 (Fig. 3) [66]. In fact, while the lack of Mec1 reduces Rad9 recruitment at the DSB ends by impairing γH2A generation, the Mec1-ad variant enhances the association of Rad9 at DNA ends by increasing the efficiency of γH2A generation [66]. Furthermore, eliminating Rad9 or decreasing its binding to the DSB by preventing γH2A formation suppresses the resection defect of *mec1-ad* cells [66]. Consistent with a role of Mec1 in inhibiting DSB resection, the ring-shaped Ddc1-Mec3-Rad17 checkpoint complex, which is required for full Mec1 activation [87], inhibits resection by promoting the recruitment of Rad9 near DSBs [88]. In any case, the rapid resection in *mec1Δ* cells is not as efficient as in *rad9Δ* cells [66], suggesting that Mec1 also positively controls DSB resection. Consistent with this hypothesis, Mec1 is known to phosphorylate Sae2 and this phosphorylation is important for Sae2 function in DSB resection [89, 90].

In contrast to Mec1-deficient cells, cells lacking Tel1 slightly reduce the efficiency of resection [91] and concomitantly increases both precise and imprecise NHEJ events [92]. Interestingly, *tel1Δ* cells show an abnormally high persistence of Ku at the DSBs [93]. Since an increase of Ku level delays DSB processing [52], the high Ku association at DSBs in *tel1Δ* cells might explain the resection defect observed in the same cells.

Tel1, once loaded at DSBs by MRX, supports MRX function in a positive feedback loop (Fig. 3). In fact, the lack of Tel1 was shown to impair MRX association at DNA ends flanked by telomeric DNA repeats [94]. Furthermore, a synthetic phenotype screen has isolated a *rad50-V1269M* allele that sensitizes *tel1Δ* cells to genotoxic agents [95]. The *rad50-V1269M* mutation impairs MRX association at DSBs and the lack of Tel1 reduces further the amount of $MR^{V1269M}X$ bound at DSBs. As a consequence, *tel1Δ rad50-V1269M* cells are severely defective in keeping the DSB ends tethered to each other and in repairing a DSB by either HR or NHEJ [95]. Interestingly, MRX association to DNA has been shown to induce parallel orientation of the Rad50 coiled coils that favors intercomplex association needed for DNA tethering

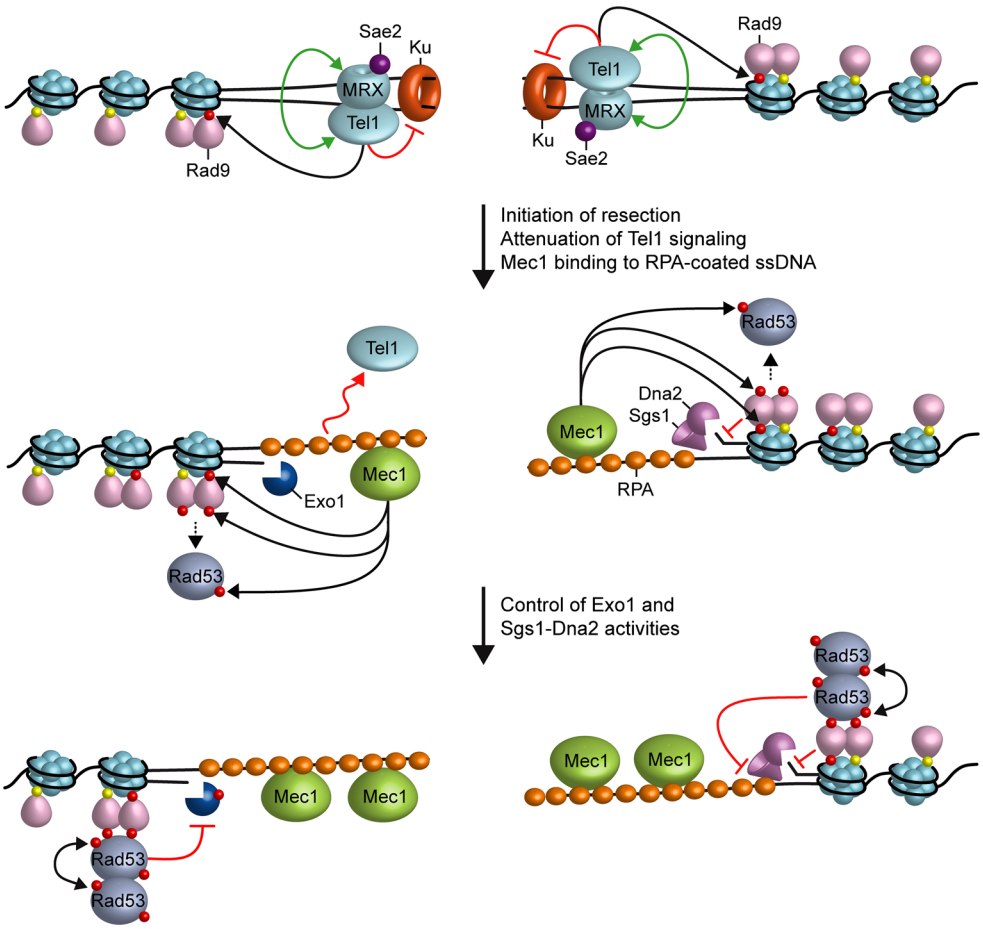

Fig. 3 Interplays between end resection and checkpoint. Rad9 is bound to methylated histone H3 (*yellow dots*) even in the absence of DSBs. When a DSB occurs, the MRX complex and Sae2 localize to the DSB ends. MRX is required for the recruitment at DSBs of Tel1, which in turn stabilizes MRX retention at DSBs in a positive feedback loop (*double green arrows*). Tel1 promotes the removal of Ku from the DSB and the initiation of resection. Furthermore, it contributes to the recruitment of Rad9 to the DSB ends through γH2A generation (*red dots*). When DSB resection takes place, the resulting 3′-ended ssDNA attenuates Tel1 signaling activity and, once coated by RPA, allows activation of Mec1. Activated Mec1 contributes to γH2A generation that leads to a further enrichment of Rad9 at DSBs, which provides a barrier to the resection activity of Sgs1-Dna2. Mec1 also phosphorylates Rad9 and these phosphorylation events create binding sites for Rad53 molecules, which then undergo in-trans autophosphorylation and activation (*double black arrows*). Mec1-dependent phosphorylation of Rad53 allows further autoactivation. Once activated by Mec1, Rad53 counteracts DSB resection by phosphorylating and inhibiting Exo1 and by restricting the access to the DSB of Sgs1-Dna2 possibly by reducing Sgs1 binding to RPA-coated DNA. Phosphorylation events are indicated by *black arrows* and *red dots*

[96]. These data suggest that Tel1, once loaded at DSBs by MRX, promotes a proper MRX-DNA association needed for the tethering of broken DNA ends and DSB repair. Tel1 exerts this function independent of its kinase activity [94, 95], suggesting that it plays a structural role in promoting/stabilizing MRX retention to DSBs.

Altogether these data support a model wherein the binding of MRX to DNA ends drives the recruitment of Tel1, which facilitates the removal of Ku from the DSB ends to prevent Ku-mediated end-joining and to facilitate resection of the DSB ends (Fig. 3). Tel1 also promotes proper MRX association at DSBs needed for end tethering. When DSB resection takes place, the resulting ssDNA-coated by RPA is recognized by Mec1 that phosphorylates H2A on serine 129. γH2A generation promotes the enrichment of Rad9 to the DSB ends, which inhibits DSB resection by counteracting Sgs1-Dna2 activity. Mec1 also phosphorylates Rad9 and these phosphorylation events create a binding site for Rad53, which then undergoes in-trans autophosphorylation events required for Rad53 activation as a kinase. Once activated, Rad53 in turn inhibits DSB resection by phosphorylating and inhibiting Exo1 and by promoting Rad9-mediated inhibition of Sgs1-Dna2 activity (see below). Because Mec1 is activated by RPA-loaded ssDNA, this Mec1-mediated inhibition of DSB resection contributes to keep under control Mec1 itself in a negative feedback loop, thus coupling resection with checkpoint activation.

End resection and checkpoint inactivation

In both yeast and mammals, Mec1 activation is coupled with ssDNA-dependent loss of Tel1 activation, suggesting that the increase in length of the single-stranded 3′ overhangs drives a switch from a Tel1- to a Mec1-dependent checkpoint [91, 97]. Defects in DSB resection caused by either dysfunctions of the resection machinery or Rad9 excess at DSBs causes unscheduled Tel1-mediated cell cycle arrest because it leads to a persistent MRX occupancy at DSBs [65, 66, 98, 99]. As the mammalian counterpart of MRX has been shown to bind ss/dsDNA junctions [100], one possibility is that the slowing down of DSB resection can generate stable ss/dsDNA junctions that are recognized by MRX and this, in turn, can lead to a persistent Tel1 signaling activity. Alternatively, the ssDNA at the dsDNA junction can be prone to breakage to generate a second DSB that activates MRX-Tel1, as suggested in [101]. Modulation of MRX-Tel1 activity by DSB resection can be important also at stalled replication forks, where Tel1 is involved in preventing rearrangements and accumulation of cruciform DNA structures [102, 103].

Persistent checkpoint activation caused by enhanced MRX signaling activity at DSBs contributes to the DNA damage hypersensitivity of Sae2-deficient cells. In fact, *mre11* mutant alleles that reduce MRX binding to DSBs restore DNA damage resistance in *sae2Δ* cells [104, 105]. Furthermore, impairment of Rad53 activity either by affecting its interaction with Rad9 or by abolishing its kinase activity suppresses the sensitivity to DNA damaging agents of *sae2Δ* cells [106]. Similarly, reduction in Tel1 binding to DNA ends or abrogation of its kinase activity restores DNA damage resistance in *sae2Δ* cells [106].

Defects in Rad53 or Tel1 signaling also suppress the resection defect of *sae2Δ* cells [106]. The bypass of Sae2 function in DSB resection by Rad53 and Tel1 impairment is due to decreased amount of Rad9 bound at the DSBs [106]. As Rad9 inhibits Sgs1-Dna2 [55, 56], reduced Rad9 association at DSBs likely bypasses Sae2 function in DNA damage resistance and DSB resection by relieving the inhibition of the Sgs1-Dna2 resection machinery. Altogether, these findings suggest that the primary cause of the resection defect caused by the lack of Sae2 is an enhanced Rad9 binding to DSBs that is promoted by the persistent MRX-dependent Tel1 and Rad53 signaling activities. While Tel1 can control Rad9 association through γH2A generation [106], how Rad53 can facilitate Rad9-mediated inhibition of Sgs1-Dna2 activity remains to be determined. Because Rad53 and RPA compete for binding to Sgs1 [107], it is tempting to propose that activation of Rad53 signaling activity may shift Sgs1 binding preference from RPA to Rad53, leading to decreased Sgs1 association to RPA-coated ssDNA that in turn can potentiate the barrier to resection imposed by Rad9-bound chromatin (Fig. 3).

In any case, the bypass of Sae2 function in DSB resection by Rad53 or Tel1 dysfunction needs a sufficient amount of MRX to be present at DSBs to promote stable association of Sgs1-Dna2 to DNA ends. In fact, attenuation of Rad53 signaling by *mre11* mutant alleles that reduce MRX binding to DSBs is not capable to restore wild type levels of resection in *sae2Δ* cells [104, 105]. Furthermore, *TEL1* deletion, which reduces the association of MRX to DSBs [94, 95], is not capable to suppress the resection defect of *sae2Δ* cells, whereas the same process is restored by the lack of Tel1 kinase activity via generation of a kinase-dead allele [104–106].

Conclusion

It is clear that the nucleolytic processing of the DSB ends generates stretches of 3′ ended ssDNA that are necessary to initiate HR and to elicit a checkpoint response. On the other hand, this process has to be strictly regulated. In fact, long ssDNA formed at DSBs, at dysfunctional replication forks or after break-induced DNA replication can be the source of clustered mutations (kataegis) frequently occurring during carcinogenesis [108–112]. Furthermore, extensive DNA end resection may induce error-prone repair events that can cause DNA deletions and translocations. Given the importance of DSB repair mechanisms in tumor biology, further understanding of their positive and negative regulators as well as of their connections with the checkpoint mechanisms is strongly relevant to human disease.

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