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DNA end resection during homologous recombination

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Abstract

Exposure to environmental mutagens but also cell-endogenous processes can create DNA double-strand breaks (DSBs) in a cell's genome. DSBs need to be repaired accurately and timely to ensure genomic integrity and cell survival. One major DSB repair mechanism, called homologous recombination, relies on the nucleolytic degradation of the 5'-terminated strands in a process termed end resection. Here, we review new insights into end resection with a focus on the mechanistic interplay of the nucleases, helicases, and accessory factors involved.

Keywords

homologous recombination; nuclease; MRX/N; Sae2

Introduction

DNA double-strand breaks (DSBs) can form in a cell's genome due to exposure to irradiation or chemicals [1], but also as a consequence of cell-endogenous processes, such as DNA replication, transcription, and the generation of reactive oxygen species. Moreover, DSBs are necessary intermediates during certain cell developmental processes, such as meiosis [2]. DSBs are highly cytotoxic and need to be repaired timely and efficiently to ensure genomic integrity and cell survival.

The two major classes of DSB repair pathways are non-homologous end-joining (NHEJ) and homologous recombination (HR) [3] (Figure 1). During NHEJ, DSB ends are bound by the Ku70-Ku80 (Ku) complex, which recruits DNA ligase 4 and accessory factors for mostly accurate re-ligation of the DNA ends. HR is more complex and initiates with the nucleolytic degradation of the DSB 5' strands in a process called end resection [4]. The 3' single-stranded DNA (ssDNA) overhangs generated are bound by the ssDNAbinding protein complex RPA, which is then replaced with the Rad51 recombinase in a process catalyzed by mediator proteins. The ssDNA-Rad51 nucleoprotein filament executes a homology search and invades into an intact copy of the broken sequence, which is usually the sister chromatid. The invading 3' end is then extended by DNA synthesis. Reannealing with the other DSB end, fill-in synthesis, and ligation complete the repair process. End resection plays a crucial role in DSB repair pathway choice, as the ssDNA generated is a poor substrate for Ku binding and, thus, channels repair towards the HR pathway. Moreover,

RPA-coated ssDNA plays an important role in a DSB signaling cascade that orchestrates the DNA damage response. In this review we discuss new insights into the mechanism of end resection with a focus on the interplay of the involved nucleases, helicases, and accessory factors. We will not cover resection at replication forks, and 53BP1-dependent resection suppression and fill-in synthesis in mammalian cells. We refer the interested reader to excellent reviews discussing these topics [5,6].

Initiation of end resection

The Mre11-Rad50-Xrs2/Nbs1 (MRX in *Saccharomyces cerevisiae*/MRN in mammals) complex is one of the first repair factors to be recruited to DSBs. MRN can slide along DNA and bypass protein obstacles, such as nucleosomes, and this facilitated diffusion might support fast recruitment to DSBs [7]. Once localized at the DSB, MRX initiates end resection by nicking the 5' strands internal to the ends and resecting in 3'−5' direction back towards the DSB [8] (Figure 1). MRX nicking is particularly important if the DSB ends contain protein blocks, such as the meiotic DSB-forming Spo11 endonuclease (see section "Meiotic resection"), or hairpin-capped ends (see next section). *In vitro* data revealed that MRX nicking is in fact stimulated by artificial and physiological protein blocks, such as streptavidin, nuclease-dead restriction enzymes, Ku, and RPA [9–11]. A recent study implies that MRX bound to DSB ends can itself stimulate nicking by adjacent MRX complexes [12]. Upon MRX nicking, the long-range resection machineries further degrade the 5' strand in a 5'−3' direction (see next section and Figure 1).

The MRX/N complex consists of two copies of each subunit [13] (Figure 2). The catalytic head region contains the Mre11 nuclease and the Rad50 ATPase domains. Two long Rad50 coiled coils protrude from the head region and interact at the apex via a zinc hook. The Xrs2/Nbs1 subunit is specific for eukaryotic organisms and supports nuclear localization, Mre11 dimerization, interaction with other proteins, and – in the case of higher eukaryotes – Mre11 nuclease activity [14,15]. MRX can adopt at least two conformations called "open" or "resting state" and "closed" or "cutting state", and the transition between these involves ATP hydrolysis by Rad50 [16]. Previous structural studies used complexes with truncated Rad50 coiled coils to investigate these states. A recent cryo-electron microscopy study presented the two conformations for the full-length *Escherichia coli* MR homolog SbcCD [17]. In the *E. coli* MR resting state, the Mre11 nuclease domain is positioned below the Rad50 ATPase domain, which contains two ATP molecules, and the Rad50 coiled coils adopt a ring-shaped conformation (Figure 2a). Upon addition of DNA and ATP hydrolysis, the complex adopted the cutting state, where the Mre11 nuclease domains are repositioned to the site of the Rad50 ATPase domains (Figure 2b). DNA is bound by the Rad50 subunits and one of the Mre11 nuclease domains and positioned for nucleolytic attack. Interestingly, the Rad50 coiled coils are zipped up, adopt a rod-like conformation, and participate in DNA binding at their base. Only a single DNA duplex end can fit into the cutting state complex and this might explain how Mre11 nuclease activity is restricted to DSB ends and does not attack internal DNA.

A crucial Mre11-Rad50 interaction in the cutting state of the *E. coli* MR complex relies on a specific Mre11 peptide loop. Eukaryotic Mre11 lacks this loop, but the

corresponding Rad50 region interacts with the regulatory cofactor Sae2 (in *S. cerevisiae*, Ctp1 in *Schizosaccharomyces pombe*, CtIP in mammals). Sae2 is the target of multiple kinases and its phosphorylation status is a crucial determinant for MRX nuclease activity. Sae2 phosphorylation by cyclin-dependent kinase (CDK) is necessary to stimulate MRX nicking and restricts end resection initiation to S and G2 cell cycle stages, when a sister chromatid is available to template repair. Additional Sae2 phosphorylation at multiple sites by DNA damage signaling kinases (see section "Resection and the DNA damage response") is necessary for full MRX nicking stimulation, likely by regulating the transition from an inactive multimeric to an active tetrameric state [18]. Tetramer formation depends on the N-terminal region of Ctp1 [19]. Besides nicking, the 3'−5' exonuclease activity of MRX has also been shown to be stimulated by phosphorylated Sae2 [12]. Sae2 phosphorylation is important for binding to the MRX complex [14,18,20]. Once bound to the MRX complex, an evolutionarily conserved C-terminal fifteen-amino-acid peptide is necessary and sufficient for nuclease stimulation [20]. Altogether, these studies suggest that phosphorylated Sae2 stimulates MRX nuclease activities by stabilizing its cutting state conformation. Recent work has shown that the telomeric protein Rif2 has the opposite effect on MRX activity [21–23]. A Rif2 N-terminal motif destabilizes the MRX cutting state at telomeres to prevent detrimental telomere processing and DNA damage signaling. Interestingly, both, Rif2 and Sae2 bind to the same Rad50 surface. Further studies are needed to elucidate the molecular and structural details of MRX nicking regulation and to clarify how nicking is restricted to the 5' strand of DSB ends.

Long-range end resection

After MRX nicking, long-range resection machineries extend the resection tracts [4] (Figure 1). One of the two partially redundant machineries is the 5'−3' exonuclease Exo1. The other machinery consists of the endonuclease Dna2, which works in concert with Sgs1 (in *S. cerevisiae*, Rqh1 in *S. pombe*, and BLM or WRN in mammals) and accessory factors, such as Top3, Rmi1 (and RMI2 in mammals), and RPA. The entry site for long-range resection can be 5' resected or gapped DNA substrates. Exo1 has also been shown to resect from nicks and recent work established that the same is true for the Sgs1-Top3-Rmi1-Dna2 (STR-Dna2) complex [24].

RPA plays multiple important roles during DNA end resection. *In vitro* experiments have shown that interaction with RPA stimulates Dna2 degradation of the 5'-terminated strands, while protecting 3'-terminated strands [25,26], and the Dna2 crystal structure has elucidated the underlying molecular mechanism [27]. RPA supports recruitment of Dna2 and Sgs1 [27–29] and *in vitro* studies have demonstrated that RPA stimulates the Sgs1 helicase activity [24,29] as well as resection by BLM in combination with EXO1 or DNA2 [30]. These data confirm previous *in vivo* findings showing that RPA depletion inhibits resection by both Exo1 and STR-Dna2 [28]. The regulatory role of RPA in end resection suggests a mechanism ensuring that the generated ssDNA is immediately coated with RPA. In fact, RPA coating of resected DNA has been shown to play important safeguarding roles. It prevents formation of DNA secondary structures, which are subject to nucleolytic attack, for example by the MRX complex [28], and prevents promiscuous microhomology annealing, which can give rise to genomic rearrangements [31,32]. Moreover, RPA-coated ssDNA

plays important functions downstream of resection during DNA damage signaling (see next section) as well as Rad51 filament formation. Interestingly, another downstream HR factor has recently been shown to regulate long-range resection. Rad52 is known to help RPA replacement with Rad51, but also restricts loading and activity of Sgs1 in *S. cerevisiae* and Rqh1 in *S. pombe* [33].

The MRX complex not only initiates resection, but also stimulates long-range resection. As discussed above, MRX nuclease activities generate ssDNA, which is a suitable substrate for both long-range resection machineries and stimulates STR-Dna2 recruitment and activity via RPA binding. Moreover, MRX physically interacts with Sgs1 and MRN has recently been shown to physically interact with EXO1 [7]. Correspondingly, MRX directly recruits the long-range resection machineries to DSBs and stimulates resection [24–26]. Recent single-molecule microscopy experiments suggest that MRN stays in close proximity to resecting EXO1 [7]. Although MRN did not influence resection kinetics, it prevented an inhibitory effect of RPA previously seen under these conditions [34]. More work is needed to clarify if MRX stays in direct contact with resection factors during long-range resection. A potential role of MRX in this context could be to nick behind resection-stalling lesions or protein-adducts to restart long-range resection.

Another example for crosstalk between resection initiation and long-range resection has recently been discovered. Besides its long-known role in activating MRN nicking, CtIP also stimulates long-range resection via DNA2. CtIP helps to recruit DNA2 to DSBs [35] and recent work shows that it also activates DNA2-mediated resection [36]. DNA2 possesses a helicase domain in addition to its nuclease activity, and the former is stimulated by phosphorylated CtIP. Moreover, phosphorylated CtIP stimulates BLM helicase activity [36,37]. Interestingly, the *S. cerevisiae* CtIP homolog Sae2 does not stimulate yeast Dna2 but supports long-range resection by regulating DNA damage signaling (see next section).

Resection and the DNA damage response

DSBs trigger a complicated signaling response [38] (Figure 3). The apical kinases Tel1 (in *S. cerevisiae*, ATM in mammals) and Mec1 (in *S. cerevisiae*, ATR in mammals) initiate the DNA damage response by phosphorylating common target proteins. Tel1 is recruited to DSBs via interaction with the MRX complex, while Mec1 is recruited via Ddc2 (in *S. cerevisiae*, ATRIP in mammals) to RPA-coated ssDNA resulting from end resection. Thus, DNA damage signaling transitions from Tel1 to Mec1 when end resection initiates. DNA damage signaling has both positive and negative effects on resection. As mentioned above, Tel1 and Mec1 phosphorylation of Sae2 stimulates resection initiation by MRX [18]. Tel1 and Mec1 also phosphorylate the histone subunit H2A and the 9–1-1 complex, which support recruitment and Tel1 or Mec1-mediated phosphorylation of the adapter protein Rad9 (53BP1 in mammals) [38]. Chromatin-bound Rad9 next to DSBs inhibits long-range resection via both Exo1 and STR-Dna2 [39,40]. Rad9 also helps recruitment and activation of the effector kinase Rad53 (CHK2 in mammals), which phosphorylates and inhibits Exo1 [41].

Recent work has elucidated new functional interactions between components of the DNA damage response and resection factors. Sae2 phosphorylation by Tel1 and Mec1 not only

activates MRX nicking, but also dampens phosphorylation of other Tel1 and Mec1 targets, such as Rad9, to counteract resection suppression [42]. Besides recruiting Rad9, the 9–1-1 complex also recruits Exo1 and STR-Dna2 and stimulates long-range resection in regions of low Rad9 abundance, such as at uncapped telomeres [40]. In contrast, initial resection by MRX is suppressed by the 9–1-1 complex independently of its role in DNA damage signaling [43]. Finally, the stimulatory role of RPA on BLM-EXO1 and BLM-DNA2-mediated resection has recently been found to be suppressed upon RPA phosphorylation by DNA damage kinases [30]. It will be interesting to see if these functional interactions are conserved between different organisms.

Resection in the chromatin context

Resection of naked DNA *in vitro* is much faster than resection of chromatinized DNA *in vivo* [44]. In a reconstituted system, nucleosomes were shown to impede STR-Dna2 and especially Exo1-mediated resection [45]. Recent *in vitro* studies showed that the long-range resection machineries can mobilize, but not evict nucleosomes, and are stalled upon encountering a dense nucleosome array [30,46]. Accordingly, several chromatin remodelers have been implicated in supporting resection in the chromatin context. Both the RSC and SWI/SNF complexes support MRX recruitment to DSBs and early resection with redundant roles of the INO80 complex [47–50]. Further, a recent study implicated the RSC and SWI/SNF remodeler complexes in long-range resection [51]. Another chromatin remodeler supporting long-range resection is Fun30, which counteracts the inhibitory effect of Rad9 [49,52,53]. Interestingly, Fun30 recruitment to DSBs is regulated by CDK phosphorylation-dependent binding to the 9–1-1 interactor Dbp11, establishing another layer of cell cycle-regulated resection control [54]. While an *in vitro* study suggested that histones are not evicted upon resection [55], a recent *in vivo* study showed that nucleosomes are not present on resected DNA [51]. More work is needed to fully understand the redundancy and synergism of chromatin remodelers in resection and to clarify if nucleosome eviction precedes or is coupled to end resection.

Meiotic Resection

Meiosis generates haploid gametes from diploid precursor cells and relies on HR for proper homologous chromosome pairing and segregation. Meiotic HR initiates with Spo11-mediated DSB formation and resection of these breaks [2]. A deep sequencing-based approach termed S1-seq was developed to monitor meiotic resection genome-wide in *S. cerevisiae* [56]. Meiotic resection initiates by MRX nicking and 3’−5’ resection to remove the covalently linked topoisomerase-like protein Spo11 from DSB ends. Limited long-range resection in meiosis depends on Exo1, while STR-Dna2 is dispensable in yeast. The S1-seq method and an alternative deep sequencing-based method (END-seq) have recently been applied to study resection during mouse meiosis [57,58]. Surprisingly, mouse meiotic resection depends only mildly on EXO1. It will be interesting to see if DNA2 or another nuclease mediates long-range resection in mouse meiosis.

Interestingly, meiotic resection tracts seem to reach their final length quickly (ca. 0.8 kb and 1.1 kb in yeast and mouse, respectively), after which resection ceases [56–58]. Meiotic resection is not considerably increased upon depletion of strand invasion activities. In

contrast, resection of a single DSB in mitotically dividing, recombination-deficient yeast cells continues without cessation at a constant speed of ca. 4 kb/h, generating tracts of ssDNA extending to tens of kb [44]. These differences might be due to the large number of DSBs, specialized chromatin context, and the activities and regulation of resection factors and chromatin remodelers during meiosis. It is possible that limited meiotic resection could prevent exhaustion of recombination factors. More work is needed to clarify the common and unique features of mitotic and meiotic resection.

Conclusion

End resection serves important functions in HR, repair pathway choice, and DNA damage signaling. However, unscheduled or extensive resection can pose risks to genomic integrity, as ssDNA is more vulnerable to mutagenesis than dsDNA and long ssDNA tracts can trigger aberrant recombination. Thus, end resection is tightly controlled, and recent studies have added to our understanding of the complex interaction networks that integrate end resection, DNA damage signaling, and downstream HR steps. Many mechanistic details of these interactions are still unknown, but we anticipate new and exciting discoveries in the coming years.

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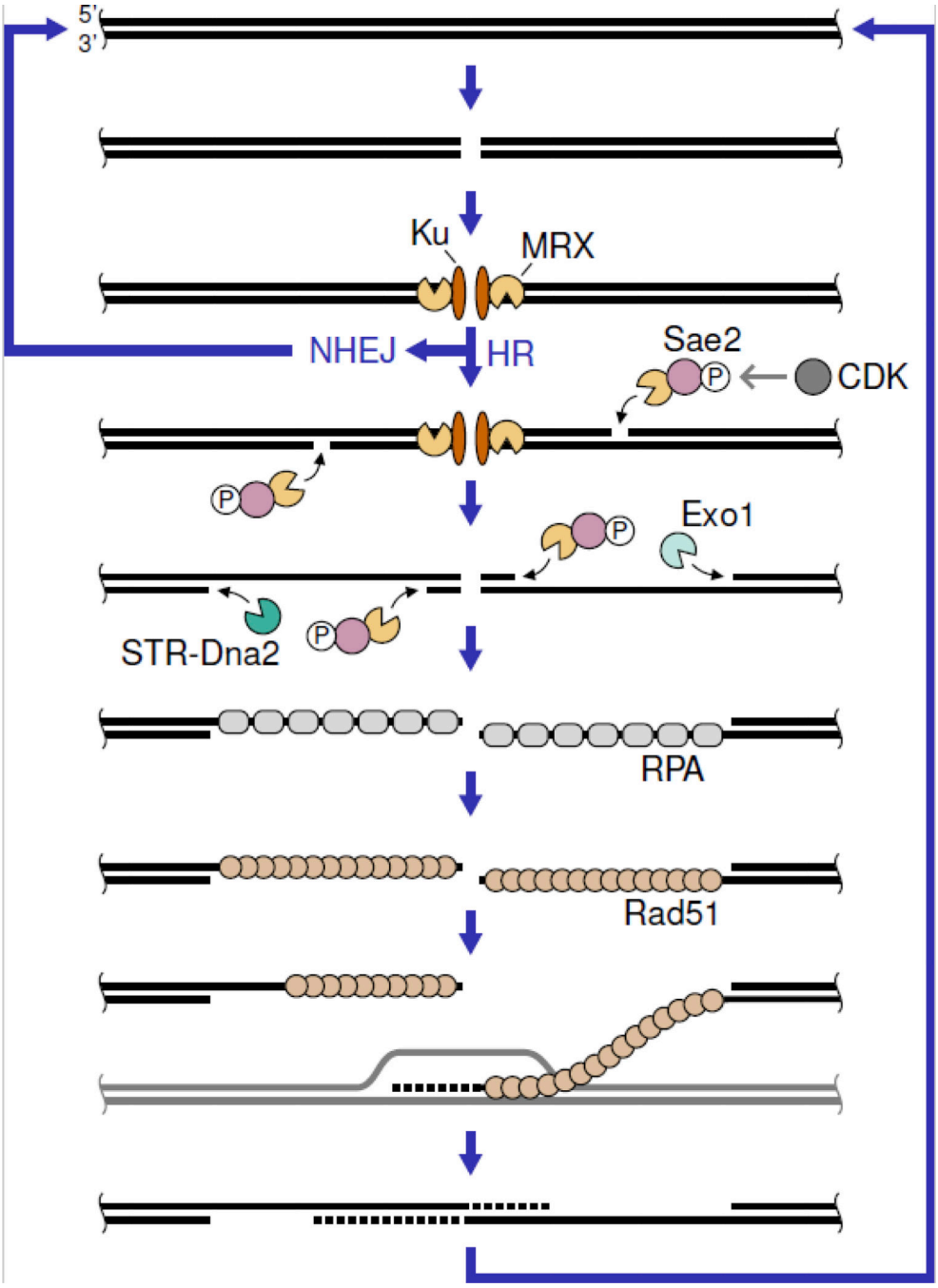

Figure 1: DSB are repaired by NHEJ or HR.

While NHEJ directly re-ligates DSBs, HR requires nucleolytic processing of the DSB ends by MRX-mediated initial and Exo1 or STR (Sgs1-Top3-Rmi1)-Dna2-mediated long-range resection. The ssDNA generated is coated by RPA and subsequently bound by the Rad51 recombinase for strand invasion and repair synthesis. The *S. cerevisiae* protein names are shown. See text for homologs in other organisms.

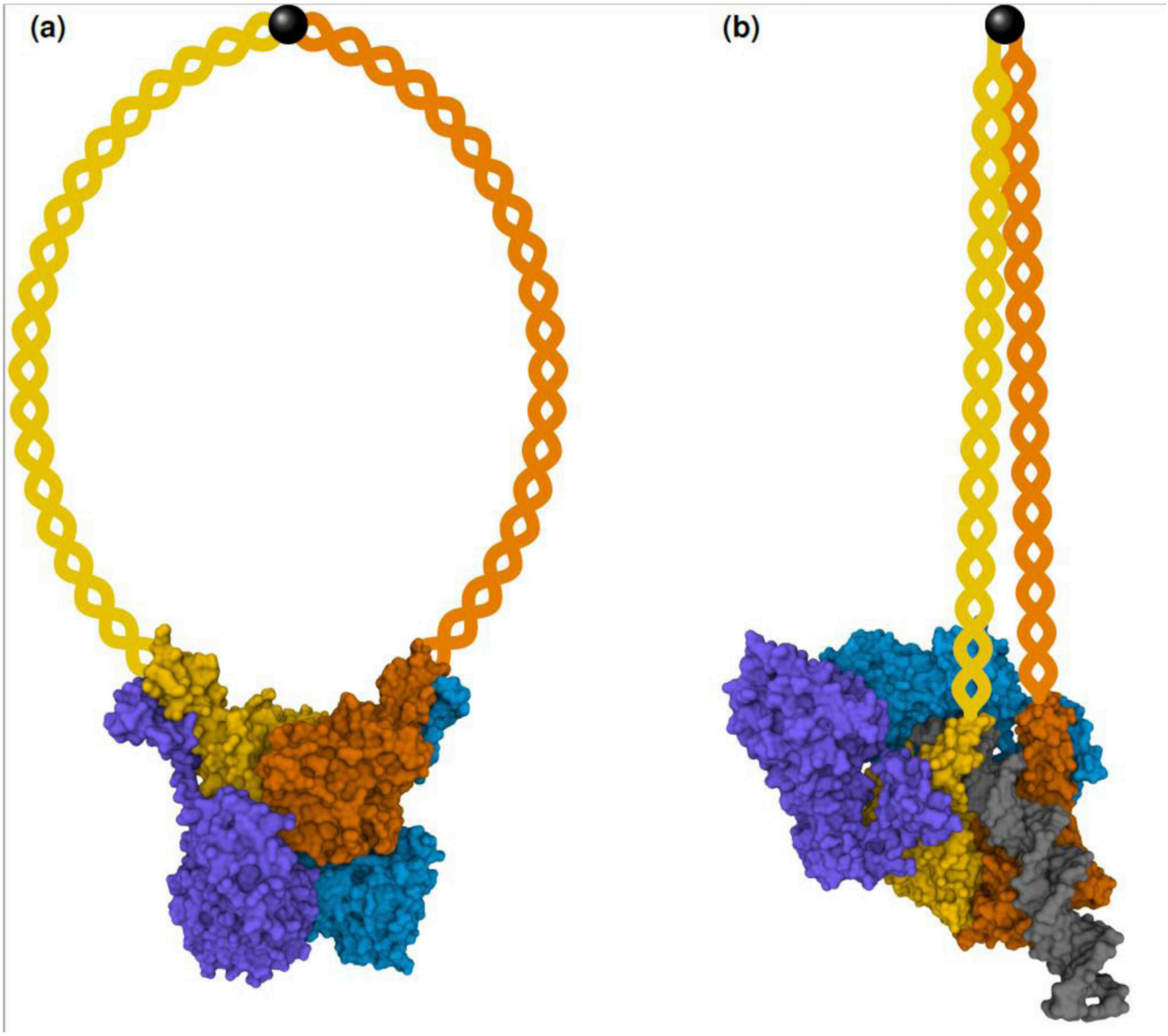

Figure 2: "Resting state" (a) and "cutting state" (b) of the *E. coli* MR (SbcCD) complex.
The Mre11 subunits are in blue and purple and the Rad50 subunits are in yellow and orange. The DNA duplex in the "cutting state" is in gray. The protruding Rad50 coiled coils and the zinc hook, whose structures could not be determined due to their flexibility, are schematically depicted. Molecular surface structures are from [17] (PDB IDs 6S6V and 6S85 [59]) and were rendered with Mol* [60].

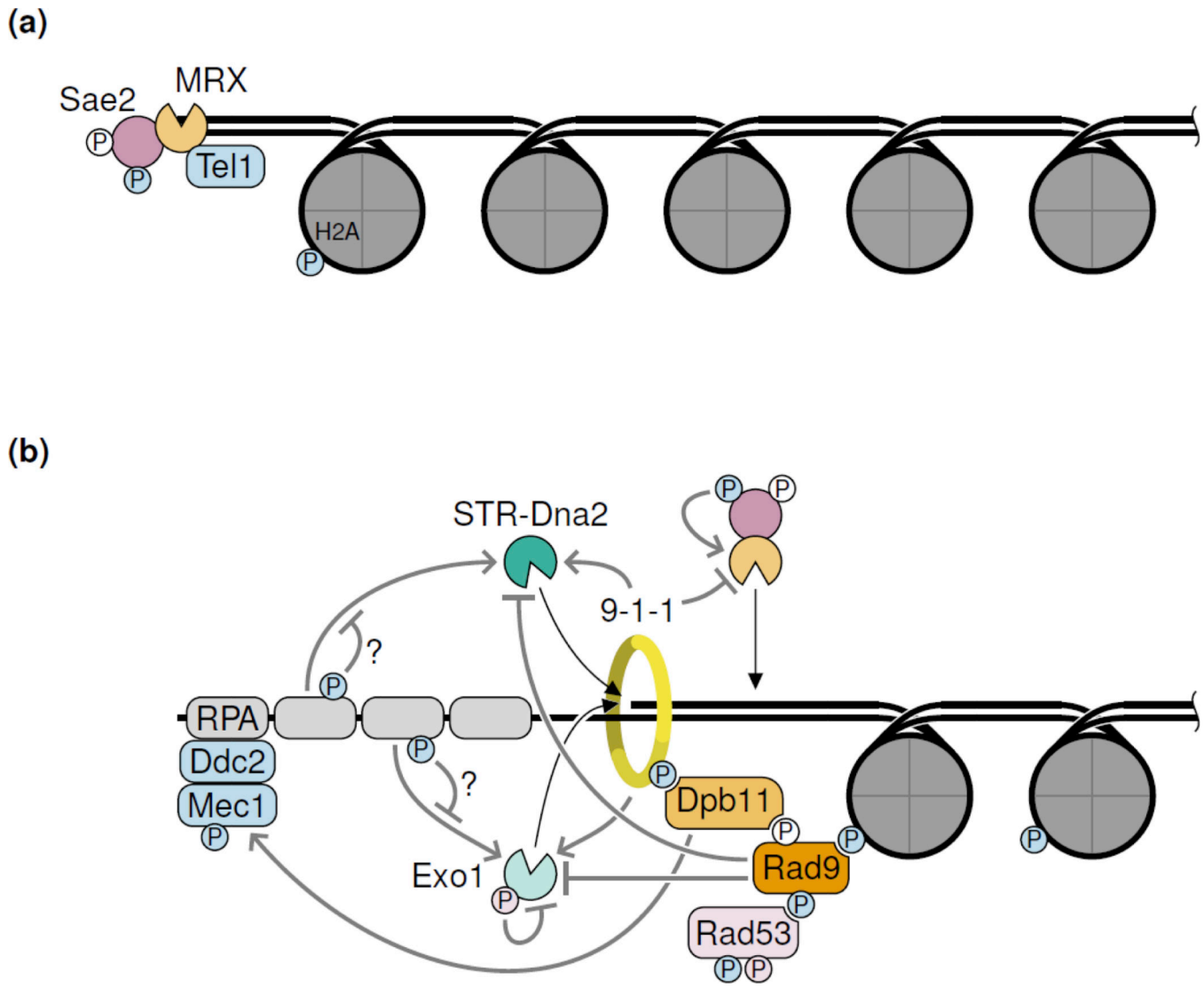

Figure 3: Resection and the DNA damage response (DDR).

(a) Tel1 is recruited to DSBs by MRX. (b) Upon resection and RPA binding, Mec1 is recruited. Multiple DDR proteins as well as DDR-induced phosphorylation events regulate the resection nucleases both in a stimulatory and inhibitory fashion, giving rise to a complex regulatory network. Phosphorylation events (P) are colored according to the responsible kinase. White Ps denote CDK-mediated phosphorylation. Question marks denote regulations that were described for mammals but have not yet been established in other organisms. The *S. cerevisiae* protein names are shown. See text for homologs in other organisms.