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Mini-review

Making the best of the loose ends: Mre11/Rad50 complexes and Sae2 promote DNA double-strand break resection

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ABSTRACT

Double-strand breaks in chromosomal DNA are repaired efficiently in eukaryotic cells through pathways that involve direct religation of broken ends, or through pathways that utilize an unbroken, homologous DNA molecule as a template for replication. Pathways of repair that require homology initiate with the resection of the 5′ strand at the break site, to uncover the 3′ single-stranded DNA that becomes a critical intermediate in single-strand annealing and in homologous strand exchange. Resection of the 5′ strand is regulated to occur most efficiently in S and G_2 phases of the cell cycle when sister chromatids are present as recombination templates. The mechanisms governing resection in eukaryotes have been elusive for many years, but recent work has identified the major players in short-range processing of DNA ends as well as the extensive resection of breaks that has been observed in vivo. This review focuses on the Mre11/Rad50/Xrs2(Nbs1) complex and the Sae2(CtIP) protein and their roles in initiating both short-range and long-range resection, the effects of topoisomerase-DNA conjugates on resection in vivo, and the relationship between these factors and NHEJ proteins in regulating 5′ strand resection in eukaryotic cells.

Contents

1. Introduction

The repair of DNA double-strand breaks (DSBs) through mechanisms of homologous recombination initiates with the processing of the broken DNA strands, primarily removal of the 5′ strand [1,2]. This process of 5′ strand resection is essential for the eventual establishment of a Rad51 filament on the 3′ strand, which performs the homology search for a target to use as a template for replication [3]. In eukaryotes, the 3′ single-stranded DNA (ssDNA) generated during resection is first coated by the RPA complex before exchange for Rad51 by mediator proteins [4], and plays an important biological role in recruitment and activation of Mec1(ATR) in the replication checkpoint [5]. In budding yeast, both resection and checkpoint activation occur much more efficiently in S and

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G_2 phases of the cell cycle when sister chromatids are present [6,7]. 5′ strand resection is thus a critical event in the initiation of homologous recombination as well as in S phase cell cycle checkpoint control in eukaryotic cells. Resection enzymes and their roles in vivo have recently been reviewed in DNA Repair [8], thus this review focuses primarily on the mechanisms by which the Mre11/Rad50/Xrs2(Nbs1) (MRX(N)) complex and Sae2(CtIP) facilitate DNA end processing.

2. DSB resection in budding yeast

DSB resection was observed decades ago [1] but only in the last few years have the molecular details of this process become more apparent [8–10]. Most of these details have been elucidated in *S. cerevisiae*, where resection of DSBs has primarily been measured during meiotic recombination, or at sites of inducible endonuclease-generated breaks in vegetatively growing cells. The extent of resection can vary depending on the presence of functional strand invasion machinery, as well as the availability and location of a homologous template, which also determines the kinetics of repair [11,12]. Under circumstances where strand invasion is blocked by the absence of recombination factors or a homologous template, tens of thousands of nucleotides can be removed from the 5′ strand at the break site [11,13]. When a homologous template is available and strand invasion can occur, the extent of resection is significantly less (∼2 to 4 kb in the case of allelic recombination and ∼3 to 6 kb in the case of ectopic recombination), which correlates well with the faster kinetics of gene conversion between homologs relative to recombination between different chromosomes [11]. The rate of resection has been reported to be approximately 4 kb/h in vivo at different chromosomal locations [11–14], although the initiation of resection is not necessarily concerted [2].

3. Mechanisms of MRX/Sae2 stimulation of resection

3.1. MRX and Sae2 introduction

Mre11/Rad50 complexes are found in all organisms and are important for efficient DSB repair, as well as for signaling of DSBs that occurs through the Tel1(ATM) protein kinase in eukaryotes [15,16]. The Mre11 protein is in the lambda phosphatase family of phosphoesterases, and recombinant Mre11 proteins from several divergent species exhibit exo- and endonuclease activity in vitro [17–24]. Exonuclease activity in manganese occurs in the 3′ to 5′ direction, while endonuclease activity is observed in manganese on single-strand/double-strand transitions and on hairpin loops. In magnesium, Mre11 does not act as an exonuclease but rather shows weak endonucleolytic activity on the 5′ strand of linear DNA ends [25–27]. Mre11 binds to the Rad50 protein, which is in the Structural Maintenance of Chromosomes (SMC) family of large ATPases containing long coiled-coils that separate Walker A and Walker B ATP-binding domains. Rad50 proteins exhibit ATPase and adenylate kinase activities in vitro [28–30], and the human MRN complex was also shown to catalyze opening of duplex DNA ends in a manner that is stimulated by ATP [31]. In eukaryotic cells, Mre11 and Rad50 also associate with a third protein, Nbs1(NBN, Nibrin, Xrs2 in budding yeast), which regulates the activities of the other components and is essential for the functional interactions between the MRX(N) complex and Tel1(ATM) [31–35].

In organisms that undergo sexual reproduction, MRX(N) complexes are essential for the processing of DSBs that are created by Spo11, a topoisomerase-like enzyme related to TOP6A from archaea [36] that forms a covalent attachment with DNA at a catalytic tyrosine residue [37,38]. In many organisms, MRN also regulates telomere maintenance through telomerase activity and also through telomerase-independent recombination [8], and in some organisms MRN is important for efficient non-homologous end joining (NHEJ) [39]. All of these scenarios involve DNA ends, which are bound by MRN and in some cases also processed through the activity of Mre11 [25–27]. MRX complexes are required for the association of DSB ends in vivo in budding yeast [40,41], and have been observed tethering DNA ends together in vitro [42]. Mre11 dimers from *P. furiosus* were crystallized with two molecules of DNA associating end to end [43], suggesting that Mre11 may hold the ends of DNA molecules together in the context of Mre11/Rad50 complexes.

The Sae2 protein in budding yeast does not associate physically with MRX but deletion strains share many phenotypes with strains expressing hypomorphic alleles of the MRX complex, most notably Mre11 nuclease-deficient alleles and *rad50S* alleles that block meiotic DSB processing [44–46]. Sae2-deficient yeast strains were also found to exhibit defects in mitotic DSB repair, specifically those involving hairpin structures as intermediates [47–49]. Rattray et al. observed that large palindromic duplications resulted from misrepair of a DSB within an inverted repeat in *sae2Δ* cells, which was postulated to result from fold-back and replication of 3′ single-stranded DNA intermediates [47,48]. Lobachev et al. also found that the processing of spontaneous DNA breaks at sites of inverted repeats was altered in *sae2Δ*, MRX-deficient, Mre11 nuclease-deficient, and *rad50S* strains, which was also suggested to arise because of a failure to process hairpin DNA structures [49]. Recombinant Sae2 binds linear DNA independently of MRX and exhibits endonuclease activity on 5′ flap structures as well as on hairpin DNA in vitro [50]. Hairpin structures were cleaved within single-stranded regions adjacent to the hairpin rather than at the hairpin tip, thus it was suggested that Mre11 nuclease activity might help to create single-stranded DNA which could then be cleaved by Sae2.

The MRX complex and the Sae2/Com1 protein have been associated with DSB resection for many years because mutations in the genes encoding these factors generate a complete block to 5′ strand removal in meiosis. *rad50S* and *mre11S* hypomorphic mutations, Mre11 nuclease-deficient alleles, and null mutations in Sae2 yield covalent complexes of Spo11 on the 5′ strand of DNA at the break site [24,44–46,51,52]. The strict requirement for Mre11 nuclease activity and for the Sae2 protein is not as pronounced in vegetatively growing yeast cells, however, where resection of endonuclease-induced breaks is only delayed in the absence of MRX [53], and nearly unaffected by mutations that inactivate Mre11 nuclease activity [52]. Deletion of Sae2 also causes a delay of resection, similar to that seen with *rad50S* strains [54]. The primary difference between meiotic and mitotic DSBs is the presence of covalent Spo11-DNA intermediates in meiosis, which presumably are removed by a combination of MRX nuclease activity and the activity of Sae2.

3.2. Resection of DSBs in yeast

Long-range resection of endonuclease-induced breaks in budding yeast was shown to be dependent on two redundant pathways: the 5′ to 3′ exo- and endonuclease Exo1, or an alternative pathway consisting of the 5′ flap endonuclease Dna2 in combination with the helicase Sgs1 and its partners RmiI and Top3 [13,55–57]. Although inactivation of either of these pathways alone has little effect on DSB resection, removal of both blocks all long-range (0.5–30 kb) resection. In cells lacking Exo1 and either Sgs1 or Dna2, the broken DNA ends were processed very slowly on the 5′ strand but only 50–300 nt was removed [13,55]. This short-range processing was found to be dependent on the MRX complex and the Sae2 protein.

MRX-deficient yeast cells with functional Exo1 and Dna2/Sgs1/Rmi1/Top3 pathways show deficiencies in resec-