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Mini review

Interplays between ATM/Tel1 and ATR/Mec1 in sensing and signaling DNA double-strand breaks

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ABSTRACT

DNA double-strand breaks (DSBs) are highly hazardous for genome integrity because they have the potential to cause mutations, chromosomal rearrangements and genomic instability. The cellular response to DSBs is orchestrated by signal transduction pathways, known as DNA damage checkpoints, which are conserved from yeasts to humans. These pathways can sense DNA damage and transduce this information to specific cellular targets, which in turn regulate cell cycle transitions and DNA repair. The mammalian protein kinases ATM and ATR, as well as their budding yeast corresponding orthologs Tel1 and Mec1, act as master regulators of the checkpoint response to DSBs. Here, we review the early steps of DSB processing and the role of DNA-end structures in activating ATM/Tel1 and ATR/Mec1 in an orderly and reciprocal manner.

Contents

1. Introduction

DNA double-strand breaks (DSBs) are among the most cytotoxic forms of DNA damage because failure to repair them can lead to loss of genetic information and chromosome rearrangements, which are hallmarks of cancer cells. DSBs can occur either accidentally during normal cell metabolism or can be caused by exposure to exogenous agents, such as certain types of chemotherapeutic drugs or ionizing radiation (IR). Nevertheless, they are also obligate intermediates during physiological cellular processes, such as meiotic recombination and lymphoid differentiation. Moreover, the ends of eukaryotic chromosomes, i.e. the telomeres, are structurally related to DSBs.

Two major pathways take care of repairing DSBs: non-homologous end-joining (NHEJ) and homologous recombination (HR). NHEJ directly ligates together the two broken ends with little or no processing [1] and is highly efficient, but it can lead to mutations at the joining sites, as well as inversions and translocations. HR is more accurate, because it uses undamaged homologous DNA sequences (sister chromatids or homologous chromosomes) as a template for repair in an error-free manner [2]. Making the right choice between NHEJ and HR is important to ensure genome stability.

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Generation of DNA DSBs elicits the activation of sophisticated surveillance mechanisms, the DNA damage checkpoints, which initiate a coordinated cellular response [3]. Activation of the DNA damage checkpoint results in cell cycle arrest and DNA repair or programmed cell death. Key players in the checkpoint response are phosphatidylinositol 3-kinase related protein kinases, such as mammalian ATM (ataxia-telangiectasia-mutated) and ATR (ATM- and Rad3-related), *Saccharomyces cerevisiae* Tel1 and Mec1, and *Schizosaccharomyces pombe* Tel1 and Rad3. In humans, ATM congenital deficiency results in ataxia-telangiectasia [4], which is a rare, autosomal recessive disorder characterized by progressive cerebellar ataxia, neuro-degeneration, radiosensitivity, checkpoint defects, genome instability and predisposition to cancer. Similarly, mutations in ATR are associated with Seckel syndrome, a clinically distinct disorder characterized by proportionate growth retardation and severe microcephaly [5]. Here we will focus on the work done in *S. cerevisiae* and mammals to review the early steps in DSB processing and signaling, as well as the regulation of ATM/Tel1 and ATR/Mec1 signaling activities in responding to DNA DSBs.

2. Resection of DNA ends

The highly conserved MRN/MRX complex (Mre11-Rad50-Nbs1 in metazoan; Mre11-Rad50-Xrs2 in yeast) and the Ku70/Ku80 heterodimer (hereafter referred to as Ku) are the first protein complexes to be recruited at DSBs [6]. The presence of Ku and MRN/MRX mediates the recruitment of proteins that religate the broken DNA ends by NHEJ [7–9]. NHEJ is active only on blunt or minimally processed DNA ends, and therefore is inhibited by the nucleolytic degradation of the 5′ strands. The latter process, referred to as 5′–3′ resection, generates 3′ single-stranded DNA (ssDNA) tails at the DSB ends and commits DSB repair to HR [10]. The Replication Protein A (RPA) complex binds to the ssDNA tails and recruits the ATR/Mec1 checkpoint kinase. Thus the decision to resect a DSB is fundamental not only to initiate DSB repair by HR, but also to activate the ATR/Mec1-mediated checkpoint response.

2.1. Positive regulators of DSB resection

In *S. cerevisiae*, the MRX complex initiates DSB resection together with the Sae2 protein [11,12]. The Mre11 component of MRX exhibits 3′–5′ double-strand DNA (dsDNA) DNA exonuclease activity and ssDNA endonuclease activity [13–15]. It has been proposed that MRX together with Sae2 can remove oligonucleotides from the 5′ ends of the break, giving rise to short 3′-ended ssDNA tails that are then subjected to extensive resection [16,17] (Fig. 1). Sae2 shows endonuclease activity in vitro that is stimulated by the MRX complex [18]. Whether Sae2 promotes DSB resection by regulating MRX nuclease activity or by acting as a nuclease itself remains to be determined. Sae2 involvement in DSB processing is conserved among eukaryotes, as also its putative orthologs in humans and *S. pombe* (CtIP and Ctp1, respectively) have critical functions in DSB resection [19,20].

The function of *S. cerevisiae* Sae2 in end resection requires its phosphorylation on Ser267 by cyclin-dependent kinases (CDK) [21]. In fact, a *sae2-S267A* mutant exhibits defective generation of 3′-ended ssDNA and reduced HR-mediated DSB repair. On the contrary, the phospho-mimicking mutant *sae2-S267E* does not cause these phenotypes and partially bypasses the requirement for CDK activity in DSB ends processing. This CDK-dependent control of Sae2 function in DSB resection is conserved also in the Sae2 vertebrate ortholog CtIP, two sites of which are phosphorylated by CDK and control the efficiency of resection [22–24]. How CDK phosphorylation promotes Sae2/CtIP activity in DSB resection is still unknown, but the use of CDK activity to promote Sae2 function in resection is one of the mechanisms that cells use to suppress HR during the G1 phase of the cell cycle (when sister chromatids are not yet present for HR and CDK activity is low) to avoid genomic rearrangements [25,26].

The requirement for MRX and Sae2 in end resection depends upon the nature of DNA ends. The initial endonucleolytic cleavage of the 5′ strands catalyzed by MRX and Sae2 is crucial for the processing of “dirty” DNA ends such as those created after exposure to IR, camptothecin, bleomycin and methylating agents, where protein-DNA adducts or altered DSB ends structures must be removed to allow further processing [27–32]. Conversely, resection of “clean” DSB ends, such as those generated by endonucleases, can occur also in the absence of MRX and Sae2. In fact, initiation of resection at an endonuclease-induced DSB is impaired in cells lacking MRX or Sae2, but once resection is initiated its rate is similar to that of wild-type cells [11,12,28,29,31]. It is worth noting that the defect in initiating resection is more severe in *mre11*Δ cells than in *sae2*Δ cells or *mre11* nuclease defective mutants, and this difference is likely due to reduced recruitment at DSBs of other proteins involved in resection (Sgs1, Dna2 and Exo1) rather than to a specific requirement for MRX to initiate resection.

More extensive DSB resection is catalyzed by the 5′-3′ exonuclease Exo1 and the 3′-5′ RecQ helicase Sgs1, which control two partially redundant pathways [16,17] (Fig. 1). The ssDNA formed by Sgs1-mediated DNA unwinding is degraded by the bipolar 5′ flap endonuclease Dna2, which is a CDK target in DSB resection [33]. Sgs1 interacts with the type I topoisomerase Top3 and the oligonucleotide/oligosaccharide-binding (OB)-fold containing protein Rmi1 to form the STR complex [34,35]. Recruitment of Sgs1, Dna2 and Exo1 to DSBs requires the MRX complex [36], and this can explain why *mre11*Δ cells have more severe resection defects than *sae2*Δ and *mre11* nuclease defective mutants. By contrast, Sgs1 and Dna2 are still recruited in *sae2*Δ and *mre11* nuclease defective mutants, indicating that these proteins can compensate for MRX-Sae2 nuclease function in initiation of resection.

In vitro data indicate that resection in humans occurs via two pathways, which are similar to those described for *S. cerevisiae*. In one of them, the human counterpart of Sgs1, BLM and DNA2 physically interact and collaborate in 5′–3′ resection of DNA ends [37], while MRN promotes resection by recruiting BLM to DNA ends [37]. In the second pathway, MRN, RPA and BLM stimulate resection by promoting the action of human EXO1 to DNA ends, with BLM enhancing EXO1 affinity for DSB ends and MRN increasing EXO1 processivity [38].

Interestingly, these reconstitution in vitro experiments of the resection machinery has revealed an essential role for the RPA complex in promoting the unwinding activity of Sgs1/BLM and enforcing the 5′–3′ resection polarity of Dna2 [34,35,38,39]. These in vitro findings have been recently confirmed in vivo, as depletion of *S. cerevisiae* RPA eliminates both the Sgs1-Dna2 and Exo1-dependent resection pathways [40]. Furthermore, RPA shields the 3′-ended ssDNA overhangs from nucleolytic attack and inappropriate annealing that could lead to genetic rearrangements [40].

DSB resection is also influenced by histone modifications and ATP-dependent chromatin remodeling reactions [41]. Interestingly, recent data indicate that Exo1- and Sgs1/Dna2-mediated DSB processing require distinct chromatin remodeling events [42]. In fact, either removal of H2A-H2B dimers or incorporation of the histone variant H2A.Z markedly enhances Exo1 activity, suggesting that ATP-dependent chromatin-remodeling enzymes regulate Exo1-mediated resection. By contrast, resection by the Sgs1-Dna2 machinery remains efficient when chromatin fibers are subsaturated with nucleosomes, suggesting that initiation of resection by