A game of musical chairs: Pro- and anti-resection factors compete for TOPBP1 binding after DNA damage

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DNA double strand breaks (DSBs) are generally repaired through nonhomologous end joining or homologous recombination. In this issue, Liu et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201607031) report that the conserved scaffold protein TOPBP1^{Dpb11} provides binding sites for both pro- and anti-resection factors at DSBs, providing insights into repair pathway regulation.

Double strand breaks (DSBs) are the most deleterious type of DNA damage because they can cause the loss or rearrangement of genomic sequences. Eukaryotic cells have two major repair pathways to tackle this threat: nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ involves the DNA end-binding protein Ku and ligase LIG4, which directly ligates the two DSB ends together. However, sometimes, a short DNA sequence is inserted or deleted in the process or, worse, wrong ends are joined, leading to translocations. In contrast, HR is largely error-free, as it uses an undamaged homologous chromatid as template for repair. The search for this homologous template requires a 3′ single stranded DNA (ssDNA) overhang at the site of damage, which is initiated by the MRN complex (MRX in *Saccharomyces cerevisiae*) with the help of CtIP/Sae2, and extended by conserved exonucleases and helicases EXO1, BLM/Sgs1, and DNA2 (Hustedt and Durocher, 2016). This DSB processing, known as "end resection," is the critical step that commits the repair pathway to HR because NHEJ is only active on minimally or non-resected DNA ends. NHEJ is active throughout the cell cycle and is prevalent in G1, whereas HR is the preferred pathway of repair in S/G2 phase, particularly when an intact sister chromatid is available to serve as the template for repair. Indeed, preference for repair by HR correlates with cyclin dependent kinase (CDK) activity, which is low in G1 and elevated in S/G2 phase. CDK targets multiple end processing factors including CtIP and EXO1 and stimulates end resection activity (Hustedt and Durocher, 2016). There are, however, additional layers of control that regulate repair pathway choice at DSBs. Examples are the DNA end-protection activity of Ku, which impedes nuclease-mediated resection in G1 (Barlow et al., 2008; Clerici et al., 2008), or the TONSL–MMS22L complex, which recognizes newly loaded histone H4 during replication to mark post-replicative chromatin and favor HR (Saredi et al., 2016). In addition, the anti-resection factor 53BP1 accumulates at DSBs through interaction with H4K20 methylation and H2AK16 ubiquitination to block end resection. This end protection activity requires recruitment of RIF1 and PTIP through ATM-mediated phosphorylation of the 53BP1 N terminus (Hustedt and Durocher, 2016). The pro-resection factor BRCA1 is thought to counteract this effect of 53BP1 by inhibiting recruitment of RIF1 in S phase (Hustedt and Durocher, 2016). However, the molecular mechanism by which 53BP1 and BRCA1 antagonize each other is not fully understood. In this issue, Liu et al. sheds light on the struggle between pro- and anti-resection factors, which regulate repair pathway at DSBs and the lesions associated with stalled replication fork.

TOPBP1 (in human)/Dpb11 (in yeast *S. cerevisiae*) is a conserved scaffold protein that functions in initiation of DNA replication, DNA damage checkpoint signaling, and DNA repair (Wardlaw et al., 2014). TOPBP1/Dpb11 contains multiple BRCT domains that recognize phosphorylated motifs, and it is rapidly recruited to the DNA damage site through its interaction with the phosphorylated 9-1-1 DNA damage clamp (Ohashi et al., 2014). The 53BP1 homologue in *S. cerevisiae*, Rad9, has a similar role as 53BP1 in blocking resection and protecting DNA ends (Lazzaro et al., 2008), but it is better known as a mediator of the DNA damage checkpoint effector kinases, Rad53 and Chk1 (Pfander and Diffley, 2011). The Smolka group had previously discovered that the Slx4–Rtt107 complex in *S. cerevisiae* negatively regulates the activation of the DNA damage checkpoint by counteracting the interaction of 53BP1/Rad9 with TOPBP1/Dpb11 (Ohouo et al., 2013). Slx4-Rtt107 was also suggested to promote resection (Dibitetto et al., 2016), so Liu et al. (2017) hypothesized that the regulation of end resection in yeast depends on competitive binding between the anti-resection protein Rad9 and the pro-resection scaffold Slx4-Rtt107. To test this hypothesis, Liu et al. (2017) examined whether stably targeting Rad9 to the 9-1-1 complex blocks resection, using *S. cerevisiae* as a model. The researchers generated a fusion protein made of the yeast Dpb11-BRCT(3/4) domains and Rad9 (BRCT(3/4)-Rad9), and found that BRCT(3/4)–Rad9 expression strongly reduced resection at the HO endonuclease-induced DSB, especially at sequences distal from the break. This was not the case when they expressed either wild-type Rad9 or a BRCT–point mutant Rad9 fusion that fails to interact with 9-1-1. Liu et al. (2017) also showed that

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the BRCT(3/4)–Rad9 fusion protein abolishes methyl methanesulfonate–induced ssDNA and HR focus formation, as monitored by Rfa1 and Rad52 foci, respectively. This suggested that stable Rad9 association to 9-1-1 diminishes end resection at the site of damage associated with stalled replication fork in S phase. Remarkably, coexpressing a BRCT(3/4)–Rtt107 fusion reversed all the phenotypes induced by the BRCT(3/4)–Rad9 fusion, arguing that Slx4–Rtt107 antagonizes the resection blockade set up by Rad9. Because the Slx4–Rtt107 interaction with Dpb11 was greatly enhanced after Mec1 checkpoint kinase-mediated phosphorylation of Slx4 (Ohouo et al., 2010; Liu et al., 2017), Liu et al. (2017) next tested whether Mec1 phosphosite mutations in *SLX4* leads to resection defects. Indeed, *slx4* mutant cells carrying seven phospho-acceptor site mutations showed a marked reduction in end resection at the HO endonuclease-induced DSB, as did yeast cells deleted for *SLX4*. This data suggested that Mec1 phosphorylation of Slx4 is crucial for the pro-resection function of Slx4–Rtt107 and likely important for its ability to compete with Rad9 in yeast (Dibitetto et al., 2016; Liu et al., 2017).

Liu et al. (2017) further explored whether or not this competition mechanism exists in human cells. They pulled down TOPBP1 in the presence or absence of the replication fork–stalling drug hydroxyurea (HU) and asked whether TOPBP1 interactions as assessed by mass spectrometry are altered on HU. This unbiased TOPBP1 interaction analysis revealed that BRCA1 and its binding factors were the most enriched TOP BP1 interactors on HU, whereas 53BP1 was the most reduced in abundance. The researchers further demonstrated that pharmacological inhibition of ATR (the mammalian homologue of Mec1) greatly reduces the TOPBP1–BRCA1 interaction, suggesting that ATR-dependent phosphorylation enforces this interaction. These findings suggest that the TOPBP1–BRCA1 interaction in human cells mirrors that of Dpb11–Slx4–Rtt107 in yeast.

To confirm the relevance of their findings to repair outcome, Liu et al. (2017) tested if the stable targeting of 53BP1 to TOPBP1 impacts HR repair. They expressed a fusion of TOPBP1 binding module and 53BP1 (CTR–53BP1) in human cells, similar to the BRCT(3/4)–Rad9 fusion in yeast. The expression of CTR–53BP1 increased its focus formation on HU. The foci overlapped with the end-protection repair factor RIF1 but excluded the HR markers RPA and RAD51; the cells also displayed reduced ssDNA formation and HR efficiency as monitored in the direct repeat–GFP system, an assay routinely used to assess HR in human cells. The expression of CTR–53BP1, but not wild-type 53BP1, also induced mitotic chromosome aberrations upon poly(ADP-ribose) polymerase inhibition, which is reminiscent of the phenotype caused by uncontrolled 53BP1 in BRCA1-deficient cells.

Overall, the data presented by Liu et al. (2017) support a model in which TOPBP1/Dpb11 serves as a conserved platform at the DSB and the replication fork–associated damage, where the anti- and pro-resection factors 53BP1/Rad9 and BRCA1 in human cells or Slx4–Rtt107 in yeast engage and compete against each other to regulate repair pathway choice. What is the importance of fine-tuning this competitive mechanism? A recent study suggested that 53BP1 binding limits end resection capacity in S/G2 to avoid the hyper-resection that could result in mutagenic RAD52-dependent single strand annealing (Ochs et al., 2016). Therefore, balanced activity of NHEJ and HR might be important to ensure the fidelity of HR.

An interesting and unresolved question is how 53BP1 and BRCA1 engagement at the DSB is controlled. TOPBP1/Dpb11 also functions as ATR/Mec1 checkpoint kinase activator. Liu et al. (2017) speculate that TOPBP1 together with ATR/Mec1 might shift a swift gear from the end protective mode to the fast resection mode at the site of damage. A new study demonstrated that ATR phosphorylates PALB2, which associates with the HR factor BRCA2, and this phosphorylation enhances the BRCA1–PALB2 interaction after resection, thereby promoting HR (Buisson et al., 2017). These results suggest that ATR/Mec1 is a key HR enhancer. Another study demonstrated that BRCA1 promotes 53BP1 dephosphorylation by the PP4 phosphatase, which in turn suppresses NHEJ by releasing RIF1 (Isono et al., 2017). A model for DSB repair regulation summarized from recent findings is shown in Fig. 1. Interestingly, it has been shown that the phosphatase PP4 complex (Pph3–Psy2) in yeast physically interacts with ATR/Mec1 through its regulatory subunit DDC2 and that it counteracts Mec1 targets (Hustedt et al., 2015). How this signaling circuitry regulates the DSB repair choice is an important question. The findings from Liu et al. (2017) open new avenues of research toward understanding the role of BRCT-mediated protein interactions and their regulation in modulating the early engagement of pro-HR and pro-NHEJ factors at sites of damage. TOP BP1/Dpb11 appears to interact with numerous factors not limited to 53BP1/Rad9, BRCA1 in human, and Slx4 in yeast (Wardlaw et al., 2014) and, given its multiple BRCT domains, which can potentially accommodate many factors at a time, one could imagine that the competition dictating repair pathway choice is even more complex than we know.

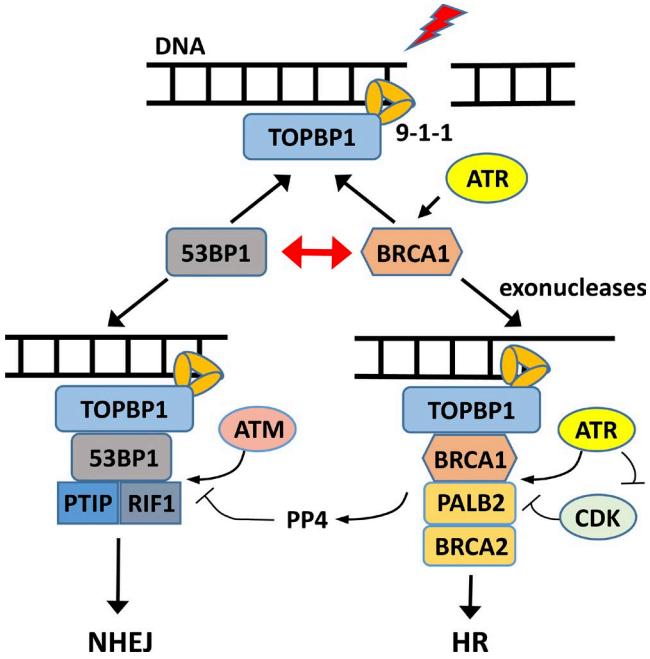

Figure 1. **A model for DSB repair regulation.** TOPBP1 is rapidly recruited to the DNA damage site through its interaction with the 9-1-1 complex. 53BP1 (Rad9 in yeast) and BRCA1 compete for binding the TOPBP1 scaffold protein at DSB. ATR (Mec1 in yeast) enhances BRCA1 interaction with TOPBP1 (Liu et al., 2017). 53BP1 phosphorylation by ATM promotes the recruitment of RIF1 and PTIP, which blocks end resection, thereby promoting NHEJ. CDK phosphorylates PALB2 and inhibits the BRCA1–PALB2 interaction. At the resected end, ATR promotes the BRCA1–PALB2 interaction by phosphorylating PALB2 in conjunction with CDK inhibition, which facilitates BRCA2-dependent HR (Buisson et al., 2017). BRCA1 counteracts NHEJ by promoting PP4-dependent dephosphorylation of 53BP1, which results in RIF1 release (Isono et al., 2017).

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