THE JOURNAL OF CELL BIOLOGY

TOPBP1 takes RADical command in recombinational DNA repair

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TOPBP1 is a key player in DNA replication and DNA damage signaling. In this issue, Moudry et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201507042) uncover a crucial role for TOPBP1 in DNA repair by revealing its requirement for RAD51 loading during repair of double strand breaks by homologous recombination.

Proper replication and maintenance of the eukaryotic genome requires the involvement of the scaffolding protein TOPBP1. Over the last 20 years, studies in yeast, frog, and mammals have revealed conserved roles for TOPBP1 in initiation of DNA replication and activation of DNA damage signaling. TOPBP1 has been shown to assemble ternary protein complexes necessary to jump-start DNA replication or to initiate DNA damage signaling events by recognizing distinct phosphoproteins via its multiple BRCA1 C terminus (BRCT) domains (Fig. 1 D; Wardlaw et al., 2014). In this issue, Moudry et al. add to the list of crucial TOPBP1 roles in genome biology and reveal that TOPBP1 is also required for proper repair of double strand breaks (DSBs) by homologous recombination (HR).

Moudry et al. (2016) report that depletion of TOPBP1 makes cells highly sensitive to the poly (ADP-ribose) polymerase inhibitor olaparib, a drug known to sensitize cells with an already dysfunctional HR machinery. In particular, olaparib hypersensitizes cells that carry mutations in the bona fide HR factors and tumor suppressors *BRCA1* or *BRCA2*. In this work, the authors first identified TOPBP1 as a hit in a high-content RNAi screen for proteins whose depletion resulted in higher toxicity after olaparib treatment in osteosarcoma cells, which suggests that loss or inactivation of TOPBP1 predicts the response of cancer cells to this drug. Moudry et al. (2016) observed that RNAi-mediated knockdown of TOPBP1 in cancer cells treated with olaparib increased the level of DNA damage and induced DNA DSB markers. The researchers subsequently examined whether olaparib sensitivity reflected defective HR in TOPBP1-depleted cells by measuring HR activity through several parameters and confirmed that TOPBP1-depleted cells showed reduced HR activity.

The HR process encompasses several phases, including end resection and chromatin loading of RPA and RAD51, which can be visualized by formation of microscopically detectable foci. Moudry et al. (2016) searched for which step of HR was compromised in cells depleted for TOPBP1 and found that DNA end resection, i.e., the processing of the 5′ recessed end that exposes a 3′ overhang used for homology search, seemed not to be affected, as evaluated by the amounts of single stranded DNA detected by BrdU incorporation under nondenaturing conditions. Interestingly, they found that the next key stage in HR, in which the RAD51 recombinase protein is loaded at these 3′ overhangs (Fig. 1 A), was greatly impaired, based on the assessment of the formation of RAD51 foci by microscopy and of the biochemical analysis of RAD51 accumulation on chromatin. Although the mechanism by which TOPBP1 promotes the loading of RAD51 remains unclear, the authors propose an interesting model in which TOPBP1 plays a scaffolding role to direct Polo-like Kinase 1 (PLK1), which phosphorylates RAD51 and facilitates its loading to DNA damage sites (Fig. 1 A; Yata et al., 2012). Consistent with this model, they show that TOPBP1 physically interacts with PLK1 and that depletion of TOPBP1 impairs PLK1-dependent RAD51 phosphorylation. Although more work is needed to prove that the TOPBP1–PLK1 interaction is required for this phosphorylation event, the results are exciting as they suggest another important functional link between TOPBP1 and a kinase. During DNA damage signaling, TOPBP1 plays an established role in activating the ATR kinase (Kumagai et al., 2006) and is believed to direct ATR's action toward specific substrates. This latter function is best understood in yeast, in which TOPBP1/Dpb11 forms a ternary complex to direct ATR/Mec1 action to phosphorylate the downstream kinase Rad53. Interestingly, recent data from fission yeast also suggest that TOPBP1 interacts with yet another kinase, CDK, and directs its kinase action (Qu et al., 2013). The emerging scenario is that TOPBP1 may function as a scaffolding hub for controlling the action of distinct kinases to ensure genome integrity (Fig. 1 B).

Although the work of Moudry et al. (2016) is the first to show a clear role for TOPBP1 in RAD51 loading, studies in budding yeast have proposed links between the TOPBP1 orthologue Dpb11 and HR-mediated repair. It was shown that the temperature-sensitive *dpb11-1* mutant displays a sensitivity to DNA damage that is not further increased by deletion of *RAD51*, suggesting that Dpb11 functions in HR repair (Ogiwara et al., 2006). In addition, other groups showed that TOPBP1/Dpb11 is required for DSB-induced mating-type switching and also reached the conclusion that TOPBP1/Dpb11 is required for HR-mediated repair of a DSB (Germann et al., 2011; Hicks et al., 2011). These studies provided compelling evidence that the role for TOPBP1/Dpb11 in DSB repair is independent of

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 212 No. 3 263–266
www.jcb.org/cgi/doi/10.1083/jcb.201601028

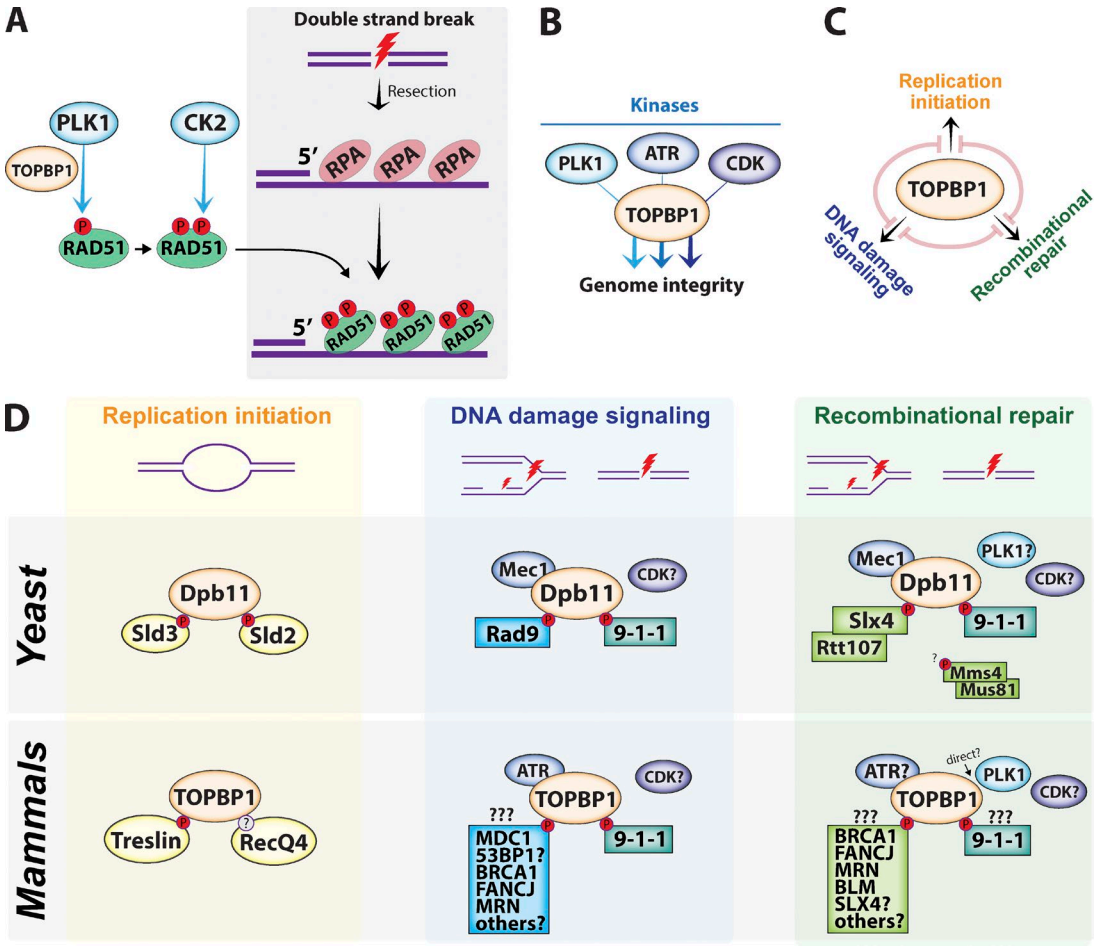

Figure 1. **Involvement of TOPBP1 in HR-mediated repair.** (A) The work by Moudry et al. (2016) supports a model in which TOPBP1 directs the action of PLK1 kinase toward RAD51, mediating a phosphorylation event that licenses a second phosphorylation event mediated by the casein kinase 2 (CK2) kinase. These phosphorylation events are believed to facilitate the loading of RAD51, which replaces RPA, at damage sites and favor HR. (B) TOPBP1 as a hub for coordinating the action of multiple kinases toward genome maintenance. (C) Speculative model for the mutually exclusive engagement of TOPBP1 in distinct cellular processes as a strategy for coordinating genome replication and DNA damage responses (see text for details). (D) Current understanding of how TOPBP1 and its yeast orthologue Dpb11 mediate the formation of ternary complexes for replication initiation, DNA damage signaling, and recombinational repair. In mammals, the indicated proteins have been shown to interact with TOPBP1, but the roles of most of those interactions remain unclear. For simplicity, some TOPBP1 interactions and their cofactors are not depicted.

its roles in replication initiation and DNA damage signaling. In humans, there also is evidence pointing to potential roles for TOPBP1 in DNA repair, as depletion of TOPBP1 was found to increase sensitivity to ionizing radiation and lead to defective DSB repair by HR (Morishima et al., 2007).

The new set of results provided by Moudry et al. (2016) clearly place TOPBP1 at the center stage of HR-mediated repair in what seems to be yet another key and evolutionarily conserved role for TOPBP1, in addition to replication initiation and DNA damage signaling. An intriguing and unanswered question relates to defining the evolutionary benefit conferred by maintaining these crucial roles in the same protein. It is tempting to speculate that having a single protein module in command of key licensing events helps ensure the ordered and mutually exclusive execution of distinct cellular processes (Fig. 1 C). This is a particularly attractive and well-suited idea for the established role of DNA damage signaling in inhibiting origin firing during DNA replication. Sequestration of TOPBP1 into a complex involved in DNA damage signaling would help ensure that replication initiation is inhibited. Consistent with this hypothesis, it is established in yeast that the same BRCT domains involved in replication initiation are also required for DNA damage signaling. In addition, it was recently shown that competition between DNA damage signaling proteins and DNA repair factors for binding to the BRCT domains of TOPBP1/Dpb11 is a mechanism to remove TOPBP1/Dpb11 from a pro-DNA damage signaling complex, resulting in dampening of DNA damage signaling (Ohouo et al., 2013; Cussiol et al., 2015). It will be exciting to further explore this competition-based regulatory mechanism in human cells, as well as in the coordination of DNA damage signaling with DNA repair. In this direction, it is crucial that the precise molecular mechanism by which TOPBP1 promotes HR repair is elucidated, including defining which TOPBP1 BRCT domains are required and which factors they are binding to favor RAD51 loading or other pro-HR functions. Through truncation mutation analyses, Moudry et al. (2016) show that the specific BRCT domains 7/8 of TOPBP1 are essential for TOPBP1's role in promoting HR. However, it remains unclear how this is accomplished mechanistically.

To make the scenario even more complicated, TOPBP1 is known to physically interact with an extensive network of repair factors, including, but not limited to, BRCA1, 53BP1,

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MRN, FANCJ, and BLM (Greenberg et al., 2006; Wardlaw et al., 2014). This points to an extremely complex system by which TOPBP1 could be coordinating the action of a range of repair factors and repair pathways (Fig. 1 D). It would not be surprising if TOPBP1 was found to be key for the regulation of other steps in HR-mediated repair as well as other repair pathways in response to varied types of genotoxic insults, including DNA replication stress. In yeast, the interaction between TOPBP1/Dpb11 and the repair scaffold Slx4 provides an additional example of the rich range of possibilities by which TOPBP1/Dpb11 functions in DNA repair. In addition to sequestering TOPBP1/Dpb11 and dampening DNA damage signaling (Ohouo et al., 2013; Cussiol et al., 2015), the Slx4–TOPBP1/Dpb11 interaction was recently found to control DNA end resection (Dibitetto et al., 2015) and was proposed to affect the late step of resolution of repair intermediates (Gritenaite et al., 2014). The TOPBP1–SLX4 interaction is conserved in humans; however, it remains unclear how this interaction impacts DNA repair in higher eukaryotes. Moreover, whereas in yeast it is possible to clearly define a pro-DNA damage signaling complex and a pro-recombinational repair complex (Fig. 1 D), in mammals the scenario is more complex and it is currently unclear what the precise contributions of different TOPBP1 interactions are in DNA damage signaling and/or recombinational repair. Finally, because the ATR kinase is expected to regulate several DNA repair factors, it is likely that the ATR-activating function of TOPBP1 plays important roles in some aspect of DNA repair. A major experimental avenue to explore this possibility and improve our understanding of the other roles for TOPBP1 in DNA repair will be the generation of separation-of-function mutants that do not interfere with DNA replication or DNA damage signaling.

Following the findings reported by Moudry et al. (2016), it is interesting to speculate on the implications of understanding TOPBP1's role in HR-mediated repair for cancer research and treatment. Little is known about the role of TOPBP1 in carcinogenesis. It was found that TOPBP1 expression and subcellular localization are altered in a subset of breast cancer samples (Going et al., 2007; Liu et al., 2009; Forma et al., 2012) and Moudry et al. (2016) also report altered TOPBP1 protein expression in ovarian cancers, although at modest frequencies. Nonetheless, as we learn more about TOPBP1 mechanisms of action in HR, it is possible that it may become an important target for manipulating the HR response by using small molecules such as Calcein AM, which targets BRCT domains 7/8 of TOPBP1 (Chowdhury et al., 2014) and was shown by Moudry et al. (2016) to impair HR. Concerning the finding that TOPBP1 plays a pro-HR function very much like BRCA1 and BRCA2, whose genes are most frequently mutated in ovarian and breast cancers, it is intriguing that although *TOPBP1* mutations have been found in cancers (Rebbeck et al., 2009; Forma et al., 2013), they are relatively infrequent and are likely not driver mutations. If TOPBP1 plays a key role in RAD51 loading, which is the step severely perturbed in *BRCA1*- or *BRCA2*-mutated cancer cells, it is not clear how more cancer-driving mutations have not been identified in *TOPBP1*. One possibility is that *TOPBP1* mutations affecting TOPBP1's pro-HR function also affect DNA replication and DNA damage signaling and impair the replicative capacity of cancer cells. Disentangling potential antagonistic roles for TOPBP1 in both suppressing and supporting tumorigenesis could lead to exciting new directions to study this complex multifunctional protein and to potentially develop new therapeutic strategies.

Acknowledgments

We thank Dr. Robert Weiss, Jennie Sims, and Dr. José Cussiol for productive discussions.

This work was supported by a National Institutes of Health M.B.S. grant (R01-GM097272).

The authors declare no competing financial interests.

Submitted: 8 January 2016
Accepted: 8 January 2016

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