COMMENTARY

Enhanced Tel1^{ATM} Checkpoint Signaling at Protein-Bound Double-Strand Breaks[▽]

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DNA double-strand breaks (DSBs) pose particularly grave threats to genome integrity and cell survival, as they not only can cause genetic mutations but also can lead to loss of chromosome fragments or genome rearrangements. Just like any structural damage, no two DSBs formed by ionizing radiation or clastogenic agents are exactly alike, and thus the cellular responses that are required to head off a potential catastrophe must be highly adaptable. The ataxia-telangiectasia mutated (ATM) protein kinase controls many of the cellular responses to DSBs in humans (7). In this issue, Fukunaga et al. (4) report that Tel1, the *Saccharomyces cerevisiae* ortholog of ATM, is hyperactivated when DNA ends are covalently bound to proteins. These unexpected results suggest that the strength of the checkpoint response can be increased by “dirty” DNA ends, which may be more difficult to repair.

ATM phosphorylates a plethora of substrates that are involved in control of the cell cycle checkpoint, DNA repair, and apoptosis in higher eukaryotes (7). In humans, defects in the ATM gene can cause neurodegeneration, immunodeficiencies, and severe radiosensitivity. While some of the downstream functions of ATM have been characterized, key aspects of the ATM activation mechanism remain unsolved. ATM exists as a homodimer in its inactive state *in vivo* (1). Inactive ATM recognizes and interacts with the heterotrimeric Mre11-Rad50-Nbs1 (MRN) complex, which binds the DNA ends generated at a DSB. This process, as well as *trans* autophosphorylation between each partner in the ATM-ATM dimer, generates active ATM monomers.

Fukunaga et al. initiated their study by establishing a sensitive assay that measures the activity of immunoprecipitated Tel1. This analysis confirmed that stimulation of Tel1 activity by DSBs relies on its association with the Mre11-Rad50-Xrs2^{Nbs1} (MRX) protein complex. Furthermore, this interaction requires the C terminus of Xrs2. These findings parallel studies done with human and *Xenopus* ATM (3, 5, 6), although interestingly, Tel1 recruitment to DSBs does not depend on its kinase activity, representing a significant difference from ATM (2).

The MRX protein complex processes DNA ends in association with Sae2 nuclease, which is homologous to human CtIP

and fission yeast Ctp1. Together with other enzymes that can catalyze the 5′-to-3′ resection of DNA ends, MRX and Sae2 generate the 3′ single-strand DNA overhangs that are essential for homologous recombination repair of DSBs. Interestingly, deletion of *SAE2* enhances Tel1 function *in vivo* (10), but the mechanism underlying this effect is unknown. Fukunaga and colleagues show that Sae2 is particularly important for processing DNA ends that are covalently bound to proteins. This was previously shown for removal of Spo11, a topoisomerase-related protein that becomes covalently bound to the 5′ end of DSBs formed during meiosis (8). Fukunaga et al. report that Sae2 is also required for the efficient removal of histones that become covalently bound to DNA in cells treated with phleomycin, a chemical clastogen. They detected a similar requirement for Sae2 and Mre11 nuclease activity in the efficient removal of topoisomerase I (Top1), which can be trapped on 3′ DNA ends by camptothecin (CPT), a Top1 poison. The defect in Top1 removal in *sae2Δ* cells correlated with an enhancement of Tel1 activation. To test whether DNA ends that are covalently bound to proteins might hyperactivate Tel1, *in vitro* kinase assays were performed with purified Tel1 and MRX complex in the presence of double-stranded DNAs whose ends were prebound with Fab antibody fragments (4). Remarkably, these DNA-Fab fragment complexes stimulated Tel1 activity to a level 2-fold greater than that produced by naked DNA duplexes alone (Fig. 1). Importantly, Fab fragments bound to internal regions of the DNA duplexes had no such effect.

As tethering of Fab fragments to DNA ends slows the rate of MRX-mediated nucleolytic DNA end processing, an obvious prediction from the data by Fukunaga et al. was that any method of inhibiting DNA end processing might increase Tel1 activation. They tested this model by repeating the assays using a nuclease-defective form of Mre11 (encoded by *mre11-3*), which lingers at DSBs due to a defect in DNA end processing. However, this nuclease-defective Mre11 mutant did not enhance Tel1 activation in the presence of protein-free DNA ends. Interestingly, Tel1 kinase activity gradually diminished over time when wild-type MRX complex was incubated with duplex DNA molecules that were blocked at their ends with Fab fragments. This reduction in Tel1 activity was not observed when the experiment was performed with the nuclease-dead Mre11 mutant. These results suggest that Fab fragments do not fully protect DNA ends from MRX-mediated end processing, which eventually converts the DNA molecules into forms that cannot activate Tel1, a process that has also been observed for ATM (9).

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[▽] Published ahead of print on 28 March 2011.

FIG. 1. Enhanced activation of Tel1^{ATM} at protein-bound DNA ends. Ionizing radiation, phleomycin, and other clastogenic agents can lead to the formation of DSBs with “clean” DNA ends, which may be relatively easy to repair (left), or “dirty” DNA ends (right), which may be bound to proteins (represented by the yellow ovoid). These modified DNA ends may require extra or more difficult nucleolytic processing as a prerequisite for DSB repair by homologous recombination. The MRX (Mre11-Rad50-Nbs1) protein complex, Sae2, and Tel1 localize to the DNA ends, with the recruitment of Tel1 mediated through an interaction with the C terminus of Xrs2. Tel1 is activated at both types of DNA ends, but its activity is enhanced at protein-bound DSBs.

The studies by Fukunaga et al. also make some intriguing observations regarding the roles of MRX and Sae2 in removal of proteins that become covalently bound to DNA after genotoxic stress. Fukunaga et al. found that nuclease activity of both Sae2 and Mre11 is required to remove Top1 from DNA following CPT treatment, whereas only Sae2 was required to remove cross-linked histones from DNA after treatment with the radiomimetic agent phleomycin. These results suggest separable roles for the MRX complex and Sae2 in the removal of covalent protein-DNA complexes. One possibility is that there are distinct mechanisms for the removal of terminally bound proteins (e.g., topoisomerases) versus internally bound proteins (e.g., histones) from DNA. An alternative possibility is that the chemical natures of histone-DNA cross-links and Top1-DNA complexes are significantly different, thus requiring separate mechanisms for their removal.

The studies by Fukunaga et al. also raise some interesting new questions. Perhaps the most prominent question concerns the mechanism by which protein-bound DNA ends enhance Tel1 activation. As noted above, slowing down DNA end processing appears to be insufficient to hyperactivate Tel1. Fukunaga et al. speculate that MRX is able to detect protein-modified DNA ends and enhance Tel1 activation accordingly. Might this involve a conformational change in the MRX complex? At present it is unclear how this might occur and how it could modulate Tel1 activity. On the other hand, the data by Fukunaga et al. do not appear to rule out the possibility that direct interactions between Tel1 and proteins bound to DNA ends might be responsible for the enhanced activation of Tel1. Like its mammalian ortholog, ATM, Tel1 is a huge protein (2,787 amino acids) with a large amino-terminal HEAT (huntingtin, elongation factor 3, subunit A of protein phosphatase 2A, and TOR1) repeat domain. As the name implies, HEAT

repeat domains are found in a diverse set of protein families, which includes other phosphatidylinositol-3'-OH kinase-like kinases (PIKKs), such as ATR and DNA-dependent protein kinases (DNA-PKs), as well as karyopherins, to name only some. The tandem-arranged HEAT repeats are typically linked by a flexible interunit loop. Structural studies of HEAT repeat domains have shown that they form superhelical scaffolding matrices that can engage other molecules with a high degree of flexibility. It is possible that the HEAT repeat domain of Tel1 might directly engage proteins and other bulky adducts at DNA ends and thereby mediate enhanced activation of Tel1.

Another question concerns the functional significance of the enhanced activation of Tel1 at modified DNA ends. In contrast to ATM in mammals, Tel1 is not critical for checkpoint signaling or regulating DNA repair pathways in yeast, but its function can be revealed by inactivating other DNA damage response proteins, in particular the ATR ortholog Mec1. The studies by Fukunaga et al. also underscore the fact that little is known about how the processing of DNA ends leads to a decrease in Tel1 activity. This might involve conformational changes in the MRX complex that do not favor Tel1 activation or that decrease the affinity of Tel1 for the MRX complex. Alternatively, the outright removal of MRX from processed DNA ends may be the critical step leading to Tel1 inactivation. These questions remain for future studies.

ACKNOWLEDGMENTS

G.E.D. was supported by a National Institutes of Health National Research Service Award. DNA damage response studies in our laboratory are supported by National Institutes of Health grants CA77325, CA117638, and GM59447.

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