Fixin' to Divide

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Fixin’ to Divide

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In this issue of Molecular Cell, Yata et al. (2012) show that the mitotic kinase and cell-cycle regulator Plk1 can directly stimulate the DNA repair process, providing a potential mechanism of crosstalk between DNA repair and cell-cycle signaling.

The mitotic kinase Plk1 acts as a central choreographer for every substage of mitosis as well as subsequent events during cytokinesis. In addition, Plk1 is required for DNA-damaged cells to re-enter mitosis after repair is concluded and cell-cycle checkpoint signaling is silenced. What no one would have expected, however, is a paradoxical role for this mitotic kinase in stimulating the repair process itself.

DNA damage activates checkpoint pathways—ATM-Chk2, ATR-Chk1, and p38MAPK-MK2—that disable cyclin/Cdkks, resulting in cell-cycle arrest in G1, S, or G2. In addition, Plk1 activity is inhibited by the DNA damage response. After DNA repair, Plk1 drives mitotic entry by inhibiting the activity of these checkpoint kinase pathways (van Vugt and Medema, 2005). Simply put, the G2 checkpoint pathways directly oppose cyclin/CDK activity, and the checkpoint kinases that do this are shut down by the activity of Plk1. DNA repair is also modulated by the cell-cycle response. Repair differs depending on the cell-cycle stage; double-strand DNA breaks (DSBs) in G1, for example, are repaired by error-prone nonhomologous end-joining (NHEJ). In contrast, during S and G2, repair by error-free homologous recombination (HR) can occur because of the availability of the sister chromatid (Moynahan and Jasin, 2010). During HR, 5’ to 3’ resection of the DSB results in activation of the ATR arm of the DNA damage response as well as the generation of an HR-competent DNA template. For HR to occur in an S- and G2-dependent manner, the cell-cycle machinery and the DNA damage response must intersect. Mre11 and CtIP are two such molecules that “read” both cell-cycle state and DNA damage signaling. Mre11, an exo- and endonuclease component of the Mre11/Rad50/Nbs1 (MRN) complex that is involved in both damage signaling and repair, specifically associates with cyclin A/Cdk2 during late S and G2, the HR competent points in the cell cycle. Similarly, CtIP, which associates with DSBs exclusively at S and G2, is phosphorylated by Cdk2 to generate a BRCA1 phospho-docking site and stabilize CtIP itself (Buis et al., 2012; Sartori et al., 2007). The resulting Mre11/CtIP/BRCA1 complex resects the break, creating a DNA molecule with 3′ single-stranded overhangs competent for RPA loading. BRCA2 then loads Rad51 onto the RPA-coated molecule (Figure 1) and inhibits Rad51’s ATPase activity to stabilize the Rad51-ssDNA complex. This complex, in turn, invades the sister chromatid, ultimately repairing the damage through homologous recombination (Moynahan and Jasin, 2010; Shin et al., 2003). In this issue, a fascinating new paper by Yata and colleagues now implicates Plk1 phosphorylation of Rad51 in the same process (Yata et al., 2012).

The findings of Yata et al. are surprising, given that the major accepted role of Plk1 is driving cells from G2 into M phase, a stage of the cell cycle where DNA damage signals thought to be required for recruitment of repair machinery are suppressed. Furthermore, Plk1 is known to phosphorylate 53BP1, a molecule important for NHEJ, blocking its ability to form foci after DNA damage (van Vugt et al., 2010). Using a series of in vitro kinase assays, Yata et al. now demonstrate that Rad51 is also a substrate of Plk1. Plk1 phosphorylation of Rad51 at S14 primes Rad51 for subsequent CK2 phosphorylation at T13. This CK2-phosphorylated form of Rad51 can then bind directly to the FHA- and tandem

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BRCT domain-containing N terminus of Nbs1. These findings led the authors to infer that Rad51 phosphorylation by CK2 might recruit both Rad51 and Nbs1 proteins to foci in a manner similar to that previously reported for CK2-dependent interactions between Nbs1 and MDC1 (Lloyd et al., 2009; Williams et al., 2009). In agreement with this, phosphorylation of endogenous Rad51 at the Plk1 and CK2 sites could be observed in cells following nocodazole arrest, and briefly following 4 Gy IR. Evidence for a direct Rad51-Nbs1 interaction in vivo, however, still awaits conformation, something that could be difficult given the relatively high K_d of the complex and its potentially transient nature.

In agreement with the model proposed by Yata et al., Rad51 mutagenesis studies confirmed roles for the Plk1 phosphorylation site in both Rad51 foci formation and in DNA repair. Cells expressing nonphosphorylatable T13A and S14A mutants of Rad51 had reduced Rad51 foci formation; conversely, the phosphomimetic Rad51 S14D mutant displayed increased foci formation. Intriguingly, the S14A Rad51 mutant fared the worst in a clonogenic survival assay after 4 Gy IR, even in the background of WT Rad51, while the S14D mutants had enhanced survival. Together, these data indicate that phosphorylation of Rad51 at an in vitro Plk1 site enhances Rad51 foci formation and survival after IR and that nonphosphorylatable S14A mutants of Rad51 have dominant-negative effects.

The authors further postulated that Plk1 and CK2 phosphorylation may result in BRCA2-independent recruitment of Rad51 to chromatin (Figure 1). In the background of WT endogenous Rad51, BRCA2 knockdown resulted in a reduction of all forms of Rad51 foci formation, with the S14A variant most dependent on BRCA2 expression and the S14D mutant least dependent. BRCA2-deficient cells are known to be exquisitely sensitive to PARP inhibitors, and resistance to PARP inhibitors is shown to develop through the reactivation of HR pathways. Yata and colleagues therefore examined the effects of PARP inhibitors on BRCA2.

**Figure 1. A Model for DSB Repair by HR Using BRCA2-Dependent and Plk1/CK2-Dependent Pathways**

DSBs generated in S and G2 undergo 5' → 3' resection by the activity of cyclin A/Cdk2, Mre11, and CtIP. The 3' single-strand overhang is then coated with RPA. RPA is displaced by Rad51 through BRCA2- or Plk1/CK2-dependent events involving destabilization of Rad51 oligomers, loading of Rad51 monomers onto the ssDNA, and inhibition of Rad51 ATPase activity. Plk1 and CK2 phosphorylation of Rad51 enhances Rad51 recruitment to sites of DNA damage through a putative interaction with NBS1.
knockdown cells expressing the various Rad51 mutants. The S14D phospho-mimetic mutant of Rad51 led to better survival than WT Rad51, while the S14A and T13A mutant cells survived worse, implying that Plk1 and CK2 phosphorylation of Rad51 could be a mechanism by which BRCA2-deficient cells acquire resistance to PARP inhibitors. Intriguingly, in a recent issue of Molecular Cell, D’Andrea and colleagues described a novel antirecombinase protein, PARI, that stimulates Rad51 ATPase activity to unload Rad51 from ssDNA (Moldovan et al., 2012). Perhaps Plk1- and CK2-dependent phosphorylation of Rad51 interferes with PARI binding, stabilizing the Rad51-ssDNA filaments independently of BRCA2.

On the basis of these observations, one would expect phosphorylated forms of Rad51 to promote HR. Paradoxically, however, using two different HR reporter assays, Yata and colleagues found that all the different mutations in Rad51—both phospho-mimicking and nonphosphorylatable analogs—showed reduced HR relative to cells expressing WT Rad51. The authors interpret this as evidence that dynamic phosphorylation and dephosphorylation of Rad51 is likely important for its HR-promoting function.

Exactly when Plk1 and Rad51 would function in concert to facilitate HR is unclear. Perhaps Plk1 phosphorylation of Rad51 marks specific times in the cell cycle when HR activity can be enhanced—when damage occurs very late in G2 prior to Plk1 inactivation by checkpoint kinases, for example, or upon Plk1 reactivation and silencing of the checkpoint during the late stages of recovery from damage. Alternatively, Plk3, a Plk1 paralog known to be specifically activated by DNA damage (Xie et al., 2001), may be the relevant S14 kinase under some conditions. In either case, it seems clear that the potential roles for Plk1 as a master regulator of the cell cycle after DNA damage have expanded to include disruption of 53BP1 foci formation, enhancement of Rad51 foci formation, inactivation of Chk1 and Chk2, and reactivation of cyclin/CDK activity. Yata et al.’s findings of a Plk1-Rad51 connection show that the roles of Plk1 may be more than just modulating the machinery of mitosis itself.

REFERENCES
