A Novel Function for BRCA1 In Crosslink Repair

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In this issue of *Molecular Cell*, Bunting et al. (2012) provide new evidence that BRCA1 plays an important role in DNA interstrand crosslink repair that is distinct from its established function in promoting DNA end resection during homologous recombination.

DNA interstrand crosslinks (ICLs) are extremely cytotoxic DNA lesions that create a covalent bond between the Watson and Crick strands of DNA, thereby blocking DNA replication and transcription. DNA crosslinking agents are widely used in chemotherapy, but their efficacy is limited because tumors frequently upregulate repair mechanisms and acquire resistance. In addition, failure to repair endogenous ICLs may underlie the human genetic disorder Fanconi anemia (FA), which is caused by mutations in any one of 15 "FANC" proteins. FA is characterized by developmental abnormalities, bone marrow failure, and a high incidence of cancer. Cellular resistance to ICLs is dependent on both FANC and BRCA proteins, which act in a common DNA repair pathway that involves homologous recombination (HR). However, the exact role these proteins play in ICL repair, as well as their functional interplay, remains murky. In this issue of *Molecular Cell*, new data from Andre Nussenzweig and colleagues indicate that BRCA1 promotes ICL repair independent of homologous recombination, by helping to activate the Fanconi anemia pathway.

ICL repair occurs in S phase when one or two DNA replication forks collide with the lesion (Figure 1A). Fork stalling at the ICL triggers a DNA damage response that includes activation of checkpoint proteins, translesion DNA polymerases, structure-specific endonucleases, recombinases, and the FANC and BRCA proteins. A key step in ICL repair involves monoubiquitylation of the FANCI-FANCD2 complex, which stimulates its recruitment to chromatin. Ubiquitylated FANCI-FANCD2 then promotes DNA incisions that support "unhooking" of the ICL and lead to formation of a DNA double strand break (DSB) (Figure 1B) that is subsequently repaired by HR (Figures 1C and 1D) (Knipscheer et al., 2009; Long et al., 2011).

The tumor suppressor protein BRCA1 is mutated in familial breast and ovarian cancers and appears to play multiple roles in HR. BRCA1 promotes resection of the double stranded DNA ends at a break to generate a 3′ ssDNA tail that is bound by the recombinase Rad51. In addition, BRCA1 has been shown to associate with BRCA2, which stimulates Rad51 loading onto the 3′ ssDNA tail. Importantly, BRCA1 mutants are sensitive to various DNA damaging agents, such as PARP inhibitors that induce DSBs, as well as ICL-inducing agents. In both cases, cellular sensitivity was thought to result from defects in the canonical HR functions of BRCA1.

Nussenzweig and colleagues have made remarkable progress understanding the biology of BRCA1 by crossing *Brca1*-deficient mice with mutations in components of the nonhomologous end joining (NHEJ) pathway. Unlike HR, NHEJ involves direct ligation of broken DNA ends in an error-prone process. 53BP1, an important regulator of NHEJ, modulates chromatin structure surrounding the break site. Remarkably, mutations in *53BP1* almost completely reverse defects associated with the loss of BRCA1. Thus, unlike *Brca1* mutants, *Brca1/53BP1* double mutants are resistant to PARP inhibitor, are proficient for HR, and assemble RPA foci after DNA damage. These results argued that the primary function of BRCA1 in DSB repair is to promote resection by antagonizing 53BP1 (Bunting et al., 2010).

In their new paper, Bunting et al. (2012) made the unexpected observation that while *53BP1* mutation reversed the sensitivity of *Brca1*-deficient cells to PARP inhibitor, it did not alleviate their sensitivity to ICL-inducing agents, even though Rad51 foci formation was largely restored (Bunting et al., 2012). One interpretation of these results is that ICL repair is more sensitive than DSB repair to subtle problems in HR. However, the authors also showed that in the absence of BRCA1, FANCD2 foci formation was suppressed (even though FANCD2 ubiquitylation was normal), and that this defect was not rescued by 53BP1 loss. These results support a model in which BRCA1 is required for ICL repair independent of homologous recombination by promoting FANCD2 recruitment to sites of DNA damage.

Additional insight came from combining BRCA1 deficiency with depletion of another NHEJ protein called Ku (Ku70/Ku80 heterodimer), which binds to DNA ends and coordinates end joining. Unlike *53BP1* mutation, loss of Ku70 rescued FANCD2 foci formation and ICL sensitivity in *Brca1*-deficient cells. Therefore, during ICL repair, BRCA1 not only promotes HR, but also antagonizes an inhibitory effect of Ku on FANCD2 foci formation. The mechanism whereby Ku inhibits FANCD2 foci formation is mysterious, because ubiquitylated FANCD2 likely binds to ICLs before any DNA ends have been created (Knipscheer et al., 2009). Another interesting question is why the presence of 53BP1 does not inhibit ICL repair in the BRCA1/Ku mutants, even though this reaction requires HR. Perhaps ICL repair does not require the same level of resection as DSB repair because at least one of the incised ends already contains a 3′ ssDNA overhang (Figure 1B).

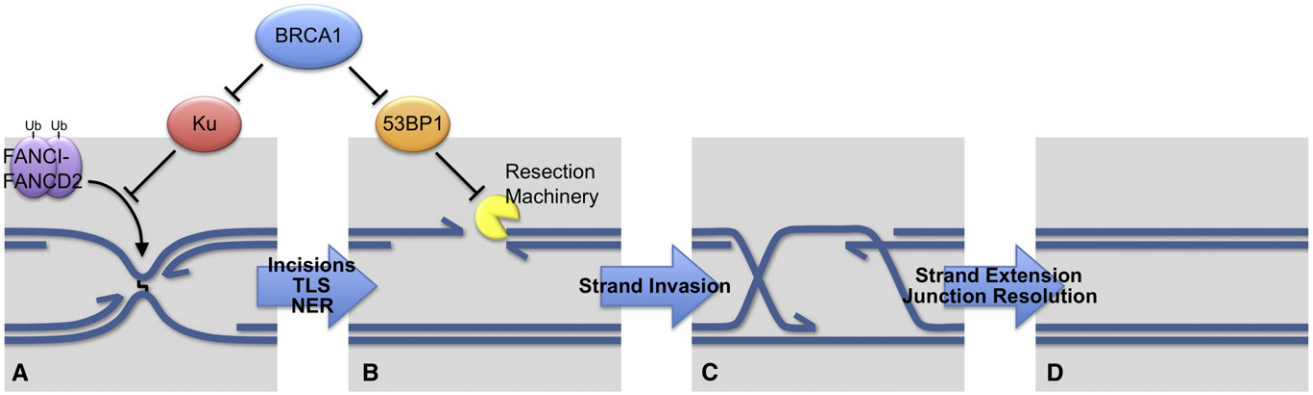

Figure 1. Possible Roles for BRCA1 in ICL Repair

(A) Replication fork stalling at an ICL leads to FANCI-FANCD2 ubiquitylation, which promotes DNA incisions that unhook the crosslink and create a DSB in one sister chromatid. In the absence of BRCA1, FANCD2 localization is suppressed by Ku.

(B) After DNA incisions, the bottom sister chromatid is restored by translesion DNA synthesis (TLS) and nucleotide excision repair (NER), and the ends of the DSB are resected to produce 3′ ssDNA overhangs. In the absence of BRCA1, 53BP1 inhibits resection of DNA ends, thereby favoring error-prone repair by NHEJ that can promote genomic instability.

(C and D) Rad51 promotes strand exchange between the sister chromatids (C), resulting in two fully intact DNA duplexes (D).

Although FANCD2 foci formation was previously shown to be defective in BRCA1-deficient cells (Garcia-Higuera et al., 2001; Vandenberg et al., 2003), this observation has not gained much attention. The clear correlation between ICL sensitivity and defective FANCD2 foci formation in various BRCA1 single and double mutants now places the spotlight back on BRCA1 as an important regulator of FANCD2 localization. Notably, this function of BRCA1 is most important in the presence of Ku. Together, the data suggest that BRCA1 modifies stalled replication forks directly or indirectly to antagonize Ku and prepare the structure for binding by ubiquitylated FANCD2.

Bunting et al. (2012) report another provocative observation regarding the interplay of the FA/BRCA and NHEJ pathways. Two recent papers showed that ICL sensitivity in FANC-deficient worms and chicken cells can be alleviated by simultaneous mutation of the NHEJ factors Ku or DNA ligase IV (Adamo et al., 2010; Pace et al., 2010). Moreover, siRNA-based experiments suggested that a similar phenomenon occurs in human cells. These observations generated considerable excitement because they implied that there was no intrinsic requirement for the FA pathway in ICL repair and that, in principle, ICL repair could be restored in FA patients. However, Bunting et al. (2012) report that genetic knockout of 53BP1 or Ku in *FANCD2*$^{-/-}$ mouse cells actually exacerbates their sensitivity to DNA crosslinks. Based on this data, FANCD2 appears to perform an intrinsic function in ICL repair that cannot be circumvented. More work will be required to understand the basis of these different results.

In summary, Bunting et al. (2012) provide evidence that FANCD2 plays an essential role in ICL repair that depends on an HR-independent function of BRCA1. These data provide the basis for more in-depth mechanistic studies of the relationships and functional interplay between the FA, NHEJ, and HR repair pathways.

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