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When the RAP (80) fades out, you can hear BRCA1 RING

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The tumor suppressor protein BRCA1 plays an important role in DNA repair by homologous recombination. Despite being encoded by the first familial breast and ovarian cancer gene identified, how BRCA1 is recruited to sites of DNA damage to execute its repair functions has remained poorly understood. Several recent studies highlight the role of its constitutive interaction partner BARD1 in this process. In this issue, parallel work by Sherker *et al* (2021) focused on a second route of BRCA1 recruitment, connected to the BRCA1-A complex protein RAP80. Studying BRCA1 recruitment in RAP80-deficient cells exposed a critical role for the BRCA1 RING domain and its associated ubiquitin ligase activity. Given that tumors expressing RING-less BRCA1 isoforms can become resistant to therapy, targeting the RAP80 recruitment axis in such tumors might restore effective treatment.

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See also: **A Sherker *et al*** (December 2021)

Our genome continually experiences a myriad of DNA lesions, of which DNA double-strand breaks (DSBs) are among the most toxic and pose the greatest threat to genome integrity. Dedicated DNA damage response pathways, including non-homologous end joining (NHEJ) and homologous recombination (HR), have evolved to repair DSBs. BRCA1 is needed for several steps in the HR pathway, and mutations in the *BRCA1* gene lead to HR defects and promote the development of familial breast and ovarian cancers (Tarsounas & Sung, 2020). How exactly BRCA1 functions in HR repair and how its recruitment to DSBs is regulated are difficult to elucidate, in part due to its involvement in multiple protein complexes with both distinct and overlapping functions.

An important clue came from recent findings that the obligatory interaction partner of BRCA1, the tumor suppressor protein BARD1, binds lysine 20 of histone H4 in its unmethylated form (H4K20me0) through an ankyrin repeat domain (Nakamura *et al*, 2019). H4K20me0 specifically marks replicated chromatin and thereby targets BARD1-BRCA1 to those areas of the genome, where a replicated template is available for HR repair. Replicated chromatin not only serves as a binding platform for BARD1-BRCA1, but is also refractory to the binding of the resection antagonist 53BP1 (Michelena *et al*, 2021), which through its tandem Tudor domain interacts with H4K20me2 but not with H4K20me0.

Although H4K20me0- versus H4K20me2-dependent recruitment of BARD1-BRCA1 and 53BP1, respectively, can explain the preferential use of HR in replicated chromatin, H4K20 methylation status does not discriminate well between damaged and undamaged chromatin. This task is achieved by the histone mark H2AK15ub, which is deposited by the DNA damage-induced ubiquitin E3 ligase RNF168 and which is then recognized by a ubiquitination-dependent recruitment (UDR) motif in 53BP1. The dual recognition of H4K20me2 and H2AK15ub thus targets 53BP1 preferentially to unreplicated damaged chromatin to constrain mutagenic DNA end resection there.

But how does the BARD1-BRCA1 complex discriminate damaged from undamaged chromatin? Analogous to 53BP1, which as multivalent chromatin reader integrates information about damage and replication status, several recent studies demonstrate that BARD1, in addition to its H4K20me0-binding ankyrin repeat domain, contains a tandem BRCT domain-associated ubiquitin-dependent recruitment (BUDR) motif, which interacts with H2AK15ub and positions the BARD1-BRCA1 complex so that it can ubiquitylate the C-terminal tail of H2A/H2AX (Becker *et al*, 2021; Dai *et al*, 2021; Hu *et al*, 2021; Krais *et al*, 2021; Witus *et al*, 2021). 53BP1 and BARD1-BRCA1 thus compete for the same damage-induced histone mark, and the bivalent recognition of H2AK15ub and H4K20me0 allows the BARD1-BRCA1 complex to suppress 53BP1 binding specifically in replicated areas of the genome.

In parallel work by Sherker *et al* (2021), a distinct route of BRCA1 recruitment was investigated. BRCA1 recruitment to sites of DNA damage not only depends on H2AK15ub recognition by BARD1, but is also linked to the polyubiquitin sensor RAP80 and its interaction partner Abraxas, two components of the BRCA1-A complex. However, due to BARD1-mediated recruitment of BRCA1, RAP80-deficient cells have hardly any defect in BRCA1 foci formation around DSBs. Conversely, residual BRCA1 foci in cells in which the BARD1-dependent recruitment of BRCA1 is disrupted depend on RNF8, RAP80, Abraxas, and the BRCA1 BRCT domains (Becker *et al*, 2021; Dai *et al*, 2021; Krais *et al*, 2021; Sherker *et al*, 2021). Consistent with the new structural, biochemical and functional data on BARD1-dependent recruitment of the BARD1-BRCA1 complex to damaged chromatin (Becker *et al*, 2021; Dai *et al*, 2021; Hu *et al*, 2021; Krais *et al*, 2021), Sherker *et al* show that BRCA1 recruitment in RAP80-deficient cells depends on the BRCA1 RING domain. Interestingly, however, the data also reveal a role

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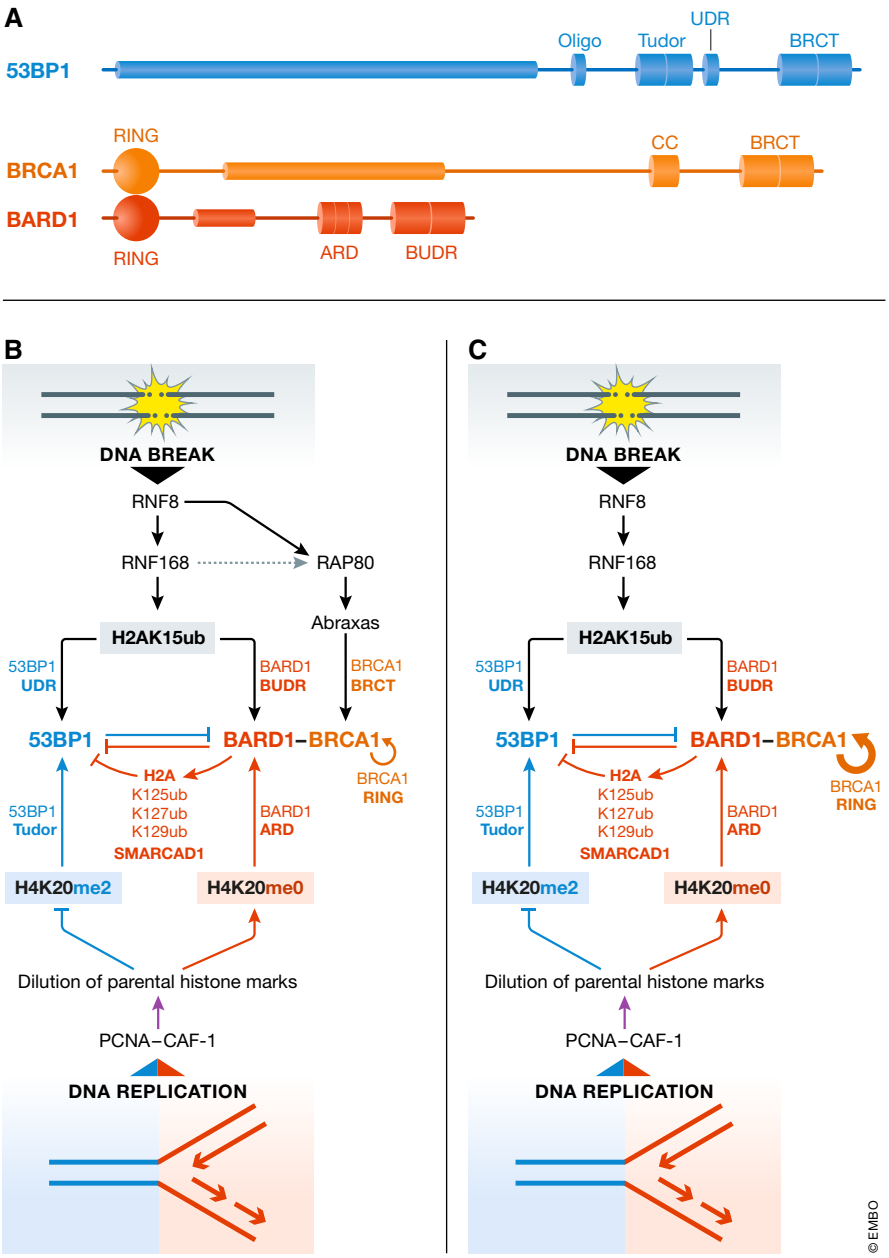

Figure 1. Regulatory switches, reinforcement, and redundancy in DSB repair pathway choice.

(A) Protein domain overview for the antagonistic players in DSB repair, 53BP1 and BARD1-BRCA1. ARD, ankyrin repeat domain; BRCT, BRCA1 C-terminal domain; BUDR, BRCT domain-associated ubiquitin-dependent recruitment motif; CC, coiled-coil domain; Oligo, oligomerization domain; RING, really interesting new gene (RING) ubiquitin E3 ligase domain; Tudor, tandem Tudor domain; UDR, ubiquitination-dependent recruitment motif. (B) 53BP1 and BARD1-BRCA1 are both multivalent chromatin readers, whose reader domains decode information about the occurrence of DNA damage (H2AK15ub) and replication status of damaged chromatin (H4K20me0 versus H4K20me2). Once recruited to damaged replicated chromatin through the BARD1 ARD and BUDR motif, BARD1-BRCA1 reinforces a switch to HR repair through direct competition for 53BP1's binding mark H2AK15ub, and through ubiquitylation of the C-terminal tail of H2A at K125/127/129, which attracts the chromatin remodeler SMARCAD1 to relocate 53BP1 away from the break site. In a parallel pathway downstream of RNF8, the polyubiquitin sensor RAP80 promotes BRCA1 recruitment as part of the BRCA1-A complex. (C) Whereas the RAP80-mediated BRCA1 recruitment depends on the BRCA1 BRCT domain, in the absence of RAP80, the accumulation of BRCA1 around DSBs becomes critically dependent on its RING domain-associated ubiquitin ligase activity. Combined deficiency in BRCT and RING domain abolishes BRCA1 recruitment as well as RAD51 loading and sensitizes cells to PARP inhibitors.

for the BRCA1 RING domain that is independent of BARD1-mediated BRCA1 recruitment: Sherker *et al* demonstrate that BRCA1 RING mutants, which still interact with BARD1 but show impaired nucleosome (K70A/R71A) or E2 (I26A) interaction and have impaired ubiquitylation activity, lose BRCA1 recruitment in a RAP80-deficient background. Also, the I26A mutation sensitizes BRCA1-ΔBRCT cells, in which RAP80-mediated recruitment is blocked, to PARP inhibitors. Based on these findings, Sherker

et al propose that tumors expressing RING-less BRCA1 isoforms with acquired resistance to therapy might be re-sensitized to treatments by targeting the RAP80-Abraxas-BRCT recruitment axis.

The findings by Sherker *et al,* together with the other studies (Becker *et al*, 2021; Dai *et al*, 2021; Hu *et al*, 2021; Krais *et al*, 2021; Witus *et al*, 2021), shed new light on the role and regulation of BRCA1 in DSB repair and on how a robust switch to HR repair in replicated chromatin is achieved (Fig 1). In addition, the findings by Sherker *et al* also reemphasize the complexity in BRCA1 recruitment by its different interaction partners and the complexity in their respective roles in DNA end resection, RAD51 loading, and HR repair. And they open interesting new questions about the mechanism of BRCA1 RING activity-dependent reinforcement of its own recruitment to DSBs. Previous work had demonstrated that BARD1-BRCA1-dependent ubiquitylation of the H2A C-terminal tail recruits the chromatin remodeler SMARCAD1, which repositions 53BP1 away from the break site and promotes DNA end resection (Densham *et al*, 2016). Given the antagonistic relationship between 53BP1 and BARD1-BRCA1 and their competition for H2AK15ub binding, self-reinforced BARD1-BRCA1 recruitment through H2AK125/127/129 ubiquitylation and downstream eviction of 53BP1 might be particularly important, and hence more easily revealed, when redundant pathways, such as the RAP80 recruitment axis, are silenced (Fig 1).

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