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More end resection is not merrier

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Abstract

Unrestrained 53BP1 activity causes fusions of dysfunctional telomeres and embryonic lethality associated with misrepair of DNA double-strand breaks in BRCA1-deficient mice. However, the physiological role of 53BP1 remains unclear, because it presumably did not evolve to carry out these pathological functions. A new report proposes that 53BP1 activity prevents hyper-resection and thereby promotes error-free DNA repair while suppressing alternative mutagenic pathways.

DNA double-strand breaks (DSBs) are among the most dangerous insults to genetic material. If not repaired rapidly and faithfully, DSBs cause a variety of pathological conditions including embryonic lethality, premature aging and cancer. Cells have evolved elaborate mechanisms to repair DSBs and these activities are primarily cell-cycle regulated^{1,2}. During the S and G2 phases of the cell cycle, DSBs are preferentially repaired by Rad51-mediated gene conversion (GC). GC is an error-free pathway of homology-directed repair (HDR) in which a processed DNA end invades the intact sister chromatid and uses it as a template for repair-associated DNA synthesis. In addition to GC, a different type of HDR relying on Rad52 can potentially occur between two repeat sequences that flank a DSB, thereby resulting in excision of the intervening sequences. This form of deletional recombination, known as single-strand annealing (SSA), is always mutagenic. Both GC and SSA are initiated by nuclease-mediated 5'–3' resection of DNA ends, thus generating recombinase-coated single-stranded overhangs that can anneal to homologous sequences within their corresponding templates. Cells can also repair DSBs through a pathway called nonhomologous end joining (NHEJ)³, which involves nontemplated end ligation and is active throughout the cell cycle. Although recent studies have shed light on how cells choose between NHEJ and HDR pathways⁴, the mechanism by which cells enforce GC over SSA is poorly understood. In this issue, Ochs *et al.*⁵ provide evidence that the choice to engage GC or SSA is determined largely by the extent of DNA end resection, which is in turn controlled by 53BP1 (Fig. 1).

The physiological function of 53BP1 during DSB repair has long been an enigma, given that it lacks enzymatic activity. Originally, 53BP1 was shown to promote long-range NHEJ during antigen-receptor rearrangements and in response to dysfunctional telomeres^{6–9}. Beyond these NHEJ functions in G1, 53BP1 has been shown to antagonize the activity of the tumor suppressor and HDR protein BRCA1 in the S and G2 phases of the cell cycle.

COMPETING FINANCIAL INTERESTS

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Dramatically, genetic ablation of 53BP1 rescues the embryonic lethal phenotype associated with BRCA1 deficiency¹⁰. Thus, in addition to its NHEJ-promoting role, unbridled 53BP1 activity causes lethality by blocking BRCA1-mediated HDR^{11,12}. Further work has revealed that 53BP1 interacts with downstream effectors, RIF1 and PTIP, through phosphorylation-dependent protein interactions, and recruits them to damaged chromatin, where they block DSBs from resection by the nuclease CtIP^{13,14}. This barrier to resection must be actively relieved by BRCA1 in S and G2 to enable repair by HDR; otherwise, unprocessed DSBs become potential substrates for nontemplate-driven ligation by error-prone NHEJ, thus resulting in the formation of highly aberrant toxic intermediates¹². These studies have collectively contributed to the notion that 53BP1 is a general suppressor of end resection and HDR.

Ochs *et al.*⁵ now report that the function of 53BP1 in HDR regulation may be more nuanced than previously thought (Fig. 2). Recently, it has emerged that the ability of meta-zoan cells to mobilize 53BP1 is restricted by the relatively narrow dynamic range of RNF168, an inherently unstable E3 ubiquitin ligase that ubiquitinates a critical histone H2A lysine residue that is required for 53BP1 binding at sites of damaged chromatin^{15–17}. In humans, the self-limiting nature of RNF168 activity can support 53BP1 assembly at approximately 20–40 breaks^{5,17}. Above this threshold, 53BP1 binding no longer increases with increasing DSB load. Given the critical role of 53BP1 in shielding DNA ends against resection, HDR might be expected to be progressively enhanced in cells that incur higher levels of DSBs as more ends devoid of 53BP1 become resected. Yet Ochs *et al.*⁵ have found that, despite massive formation of RPA-coated single-stranded DNA indicative of hyper-resection, Rad51 recruitment and GC efficiency diminish with increasing DSB load. This decline in Rad51 response occurs even when the protein is in excess, thus suggesting that extensively resected ends may have lower affinity for Rad51 binding. Augmentation of RNF168-mediated 53BP1 mobilization to, or partial reduction of CtIP-mediated resection at, unprotected ends is sufficient to enhance Rad51-dependent GC under these conditions. The most surprising consequence of attenuated Rad51 binding at 53BP1-devoid hyper-resected ends is the channeling of these structures toward Rad52 binding, which initiates mutagenic SSA (Fig. 2). Therefore, a major function of 53BP1 in DSB repair is to limit the extent of DSB resection within a range that allows for Rad51-dependent GC while preventing alternative mutagenic repair mechanisms that might jeopardize genome integrity.

At first, it may seem counterintuitive that 53BP1 blocks DSBs from nucleases yet at the same time is required for limited end resection. Recent studies have shown that although 53BP1 initially accumulates at DSB sites, it is subsequently deposited at regions more distal to the break in a manner requiring the ubiquitin ligase activity of BRCA1 (refs. 18–20). It is possible that BRCA1-mediated repositioning of 53BP1 away from DSB termini to the periphery effectively delineates an insulated chromatin domain within which end resection and error-free HDR is permitted. When BRCA1 is absent, a substantial amount of 53BP1 may remain near the break site, blocking nuclease access and thereby suppressing end resection and HDR. When both BRCA1 and 53BP1 are absent, those DNA ends that exhibit limited resection in BRCA1-deficient cells are capable of supporting Rad51-dependent GC because they are no longer blocked by 53BP1.

Depletion of 53BP1 leads not only to increased levels of GC but also to increased SSA in BRCA1-deficient cells^{5,21} (Fig. 2). Indeed, gene targeting efficiency, a functional indicator of successful GC, is impaired in BRCA1-deficient cells and is only partially restored after loss of 53BP1 (ref. 11). Moreover, in agreement with their increased reliance on SSA-mediated mutagenic HDR, cells doubly deficient in BRCA1 and 53BP1, compared with their wild-type counterparts, display increased genomic instability^{10,12} and are hypersensitive to Rad52 inhibition⁵. Together, these data strengthen the emerging view that organismal viability requires certain levels of HDR competency, though not necessarily genome stability. Most spontaneous DSBs are thought to be caused by perturbations during DNA replication². In wild-type cells, protecting stalled DNA replication forks from nuclease-mediated degradation may prevent the formation of DSBs under physiological conditions^{22,23}. However, in a scenario in which forks cannot be adequately protected, such as in cells doubly deficient in BRCA1 and 53BP1²², replication-associated DSBs would ensue, and some of these breaks might be aberrantly repaired by SSA (Fig. 2). The finding that Rad52 deletion partially suppresses the development of T-cell lymphoma in ATM-deficient mice supports the oncogenic potential of SSA²⁴. Although mice doubly deficient in BRCA1 and 53BP1 do not appear to develop spontaneous tumors¹⁰, it would nevertheless be interesting to test whether they are tumor prone when challenged with carcinogens or agents that induce replication stress. Finally, because BRCA1-deficient tumor cells can become resistant to chemotherapeutic agents through loss of 53BP1, Rad52 inhibitors may prove to be useful in a clinical setting. In any case, the work by Ochs *et al.*⁵ underscores that HDR is complex and consists of both error-free and error-prone subpathways whose activities must be tightly regulated and fine-tuned to ensure genome integrity. Future studies aimed at enhancing understanding of the causes of synthetic viability and chemotherapy resistance may enable rational design of new treatment regimens and more effective cancer therapies.

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Figure 1.

53BP1 restrains end resection, thereby promoting gene conversion and preventing single-strand annealing. 53BP1 is recruited to sites of DSBs, where it binds histone H2A ubiquitinated by RNF168. RNF168 activity has a narrow dynamic range capable of supporting maximal 53BP1 assembly at approximately 20–40 DSBs. When cells incur relatively few DSBs, each break is able to accrue a sufficient number of 53BP1 molecules, thus limiting the extent of end resection to a range that allows for Rad51-dependent GC. As the DSB load increases, progressively more breaks fail to accrue an adequate amount of 53BP1. Such insufficiently protected ends are vulnerable to hyper-resection, which reduces their affinity for Rad51, and they instead recruit Rad52 to catalyze repair through mutagenic SSA.

Figure 2.

A model of pathway choice during DSB repair. In mammalian cells, DSBs can be produced directly, by genotoxic insult on DNA, as well as indirectly, after failure of a BRCA1-mediated mechanism that protects stalled replication forks from nuclease-mediated degradation. In wild-type cells, 53BP1 initially accumulates at DSB sites but is subsequently repositioned distal to the break, thus enabling limited resection followed by Rad51 loading onto the resected single-stranded ends. Rad51 then mediates strand invasion into the homologous template on the sister chromatid and promotes error-free repair through GC events. In the absence of 53BP1, DSBs are extensively resected, thus exposing long stretches of single-stranded DNA and biasing repair toward Rad52-dependent, mutagenic SSA. In the absence of BRCA1, 53BP1 repositioning is inhibited, thereby resulting in blocked resection. These insufficiently resected DSBs do not become substrates for HDR but are instead repaired by NHEJ, thus leading to gross chromosomal abnormalities and loss of cellular viability. Finally, resection is restored, and the ability to carry out HDR is regained, in BRCA1-deficient cells after loss of 53BP1. Depending on the extent of resection at individual breaks, HDR in BRCA1- and 53BP1-deficient cells is probably the result of a combination of GC and SSA and may contribute to the restoration of viability in these cells.