Acta Biochim Biophys Sin, 2016, 48(7), 641–646
doi: 10.1093/abbs/gmw045
Advance Access Publication Date: 23 May 2016
Review

Review

DNA double-strand break repair: a tale of pathway choices

Jing Li* and Xingzhi Xu*

Beijing Key Laboratory of DNA Damage Response and College of Life Sciences, Capital Normal University, Beijing 100048, China

*Correspondence address. Tel: +86-10-68909575; Fax: +86-10-68906307; E-mail: jing_li@cnu.edu.cn (J.L.)/
Tel: +86-10-68902440; Fax: +86-10-68906307; xingzhi_xu@cnu.edu.cn (X.X.)

Received 12 January 2016; Accepted 15 April 2016

Abstract

Deoxyribonucleic acid double-strand breaks (DSBs) are cytotoxic lesions that must be repaired either through homologous recombination (HR) or non-homologous end-joining (NHEJ) pathways. DSB repair is critical for genome integrity, cellular homeostasis and also constitutes the biological foundation for radiotherapy and the majority of chemotherapy. The choice between HR and NHEJ is a complex yet not completely understood process that will entail more future efforts. Herein we review our current understandings about how the choice is made over an antagonizing balance between p53-binding protein 1 and breast cancer 1 in the context of cell cycle stages, downstream effects, and distinct chromosomal histone marks. These exciting areas of research will surely bring more mechanistic insights about DSB repair and be utilized in the clinical settings.

Key words: DSB, 53BP1, RIF1, PTIP, BRCA1

Introduction

Deoxyribonucleic acid (DNA) double-strand breaks (DSBs), the most harmful type of DNA lesions, are generated in response to extrinsic ionizing radiation (IR) or intrinsically to free radicals during cellular metabolism or DNA abducts/abnormal DNA structures during DNA replication [1,2]. They also occur inherently during many physiological processes such as meiosis I [3], mating-type switching in yeasts [4], V(D)J recombination, and immunoglobulin class-switching recombination (CSR) in B-lymphocytes [5]. We will focus on DSB repair in mammalian cells.

The repair of DSBs is channeled into two pathways, non-homologous end joining (NHEJ) and homologous recombination (HR). During NHEJ, the Ku70/80 heterodimers bind to DNA ends and recruit the DNA protein kinase (DNA-PK) [6]. Once bound, DNA-PK activates its own catalytic subunit (DNA-PKcs) and further enlists the endonuclease Artemis (also known as SNM1c). At a subset of DSBs, Artemis removes excess single-strand DNA (ssDNA), and generates a substrate that will be ligated by DNA ligase IV. NHEJ involves blunt-end ligation independent of sequence homology by the canonical DNA-PKcs/Ku70/80 complex. During the cell cycle, NHEJ occurs predominantly in G_0/G_1 and G_2 [7,8]. In fact, NHEJ is the only DSB repair pathway in the G_0 and G_1 phases. In the review, we will focus on the resection-mediated pathway of NHEJ which represents the slow component of NHEJ.

HR functions in the S and G_2 phases and plays a major role in the repair of replication-associated DSBs. The DSB ends are resected to expose 3′ ssDNA tails, primarily by the MRE11-RAD50-NBS1 (MRN) complex [9]. Then, the adjacent sister chromatid will be used as a template, and the ssDNA will invade the template mediated by the recombinase Rad51, displacing an intact strand to form a D-loop. D-loop extension is followed by branch migration to produce double-Holliday junctions, the resolvation of which completes the repair cycle. During HR, the ability of the Ku70/80 complex to bind DNA ends is restricted by the MRN complex. Moreover, it could also be displaced by the E3 ligase RNF138, also called Nemo-like kinase (NLK)-associated ring finger protein (NARF) [10].

In comparison, NHEJ is fast [11], yet mutagenic, often accompanied by short deletions and base changes. HR often requires

error-prone polymerases, yet is typically viewed as error-free. In reality, the fidelity of HR is not as high as DNA replication, and may result in broadly distributed base substitutions [12].

The early divergent step between the two pathways is end resection, and is regulated by many factors. It is first modulated by cell cycle phases: the cyclin-dependent kinase (CDK) phosphorylates the exonuclease Exo1 and promotes end resection, while attenuation of Exo1 phosphorylation increases NHEJ [13]. Then, it is mediated by post-translational modifications. Breast cancer 1 (BRCA1), in complex with MRN, plays an instrumental role in promoting end resection, and is poly adenosine diphosphate (ADP)-ribosylated by PARP1 [14,15]. Third, it is regulated by accessory factors, where the balance between BRCA1 and p53-binding protein 1 (53BP1) comes into play.

53BP1 Dictates the NHEJ Pathway

53BP1 is a large protein with 28 N-terminal Ser-Gln or Thr-Gln (SQ/TQ) sites, middle tandem Tudor domains, a ubiquitination-dependent recruitment (UDR) motif and the C-terminal BRCA1 carboxyl-terminal (BRCT) repeats [16] (**Fig. 1A**). As its name suggests, it was first discovered as a binding protein of the tumor suppressor protein p53 [17]. Two decades later, although it is still enigmatic why 53BP1 interacts with p53, its central role in DSB repair has become clear. 53BP1 promotes accurate NHEJ and restricts resection. Nevertheless, NHEJ occurs well in cells lacking 53BP1.

The SQ/TQ sites are partially phosphorylated by the ataxia-telangiectasia mutated (ATM) kinase, the AT and Rad3-related kinase (ATR), and/or DNA-PKcs [18]. ATM-dependent phosphorylation of 53BP1 is essential for the interaction of 53BP1 with downstream targets, including RAP1-interacting factor 1 (RIF1) [19] and PAX transactivation domain-interacting protein (PTIP or PAXIP1) [20] (**Fig. 2**), although 53BP1-PTIP interaction is not critical for PTIP recruitment to DSB sites in mammalian cells [20].

The antagonist relationship between 53BP1 and BRCA1 is manifested by the following evidence: first, 53BP1 and BRCA1 occupy distinct DSB sites [21]; second, BRCA1 attenuates RIF1 accumulation in DSBs during the S phase [22]. The chromatin removal of 53BP1 is not dependent on the E3 ligase activity of BRCA1 [23], but rather, dependent on the interaction of BRCA1 with CtBP-interacting protein (CtIP) [23,24]. The BRCT domain of BRCA1 recruits the E3 ligase ubiquitin-like, with plant homeodomain (PHD) and RING finger domain 1 (UHRF1), which mediates K63-polyubiquitination of RIF1 and subsequent dissociation of Rif1 from 53BP1 [25]. Thus, BRCA1-CtIP and BRCA1-UHRF1 pathways antagonize 53BP1-Rif1 in S–G_2 in favor of HR, and 53BP1-Rif1 attenuates BRCA1 in G_1. We will expand the 53BP1-dictated NHEJ pathway below.

The 53BP1-Rif1-Rev7/Mad2L2 axis

The Rif1 protein does not contain any known protein motifs, and was first identified in yeasts to modulate telomere homeostasis [26]. The human Rif1 is not involved in telomeres, but rather interacts with 53BP1 to block DSB resection in G_1 [19]. ATM-dependent phosphorylation of 53BP1 at its N-terminal SQ/TQ sites recruits RIF1 to damaged chromatin foci [22–24,27,28]. In a reporter cell line, knockdown of 53BP1, Rif1, or both results in NHEJ defects at comparable levels, suggesting that 53BP1 and Rif1 act in the same pathway [22]. In the adaptive immune response, 53BP1 is essential for CSR. During CSR, knockdown of Rif1 leads to hyper-resection

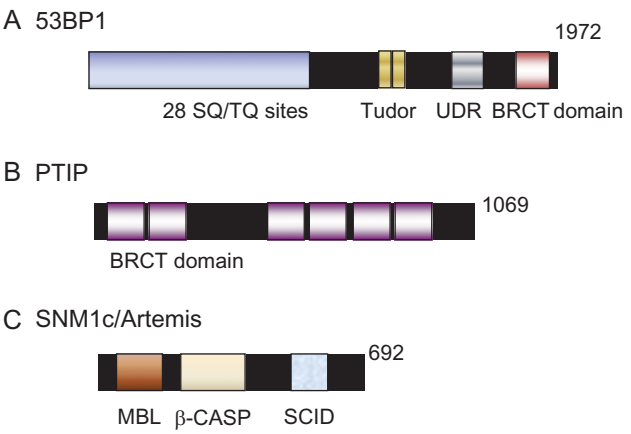

Figure 1. Diagrams showing domain structures of 53BP1, PTIP, and SNM1c/Artemis (A) Schema of the 53BP1 protein structure, showing the 28 SQ/TQ sites at its N-terminus, middle tandem Tudor domains and a UDR motif and the C-terminal BRCT repeats. (B) Schema of the PTIP protein structure, showing the multiple BRCT domains. (C) Schema of the SNM1c/Artemis protein structure, showing the N-terminal metallo-β-lactamase (MBL) domain, middle β-CASP domain and the C-terminal domain involved in a radiosensitive severe combined immunodeficiency disorder (SCID).

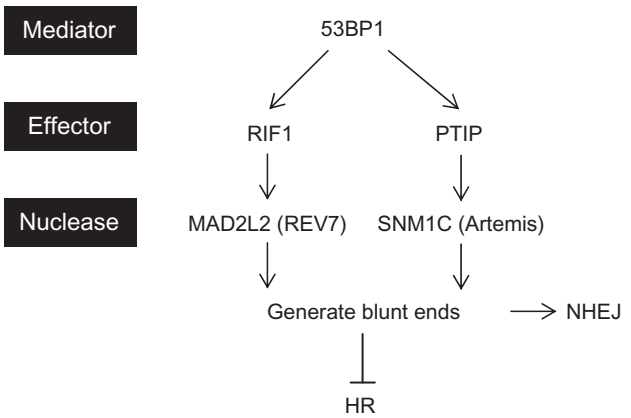

Figure 2. DSB repair pathway choice involves a balance between HR and NHEJ Under both physiological (CSR) and pathological (telomere fusion) conditions, the 53BP1-Rif1-Rev7/Mad2L2 axis operates to generate blunt ends, thus promoting NHEJ and inhibiting HR by BRCA1. The 53BP1-PTIP-SNM1c/Artemis axis only functions under pathological conditions to promote NHEJ.

of DSBs within the immunoglobulin heavy chain locus [22,28]. In Rif1-deficient mouse cells, IR sensitivity is comparable to cells deficient in both 53BP1 and Rif1 [22,23]. These data suggest that Rif1 and 53BP1 act concertedly in a common pathway.

Proteins downstream of Rif1 have been under hot pursuit, until earlier in 2015 two groups independently identified Rev7/Mad2L2 [29–31], a protein familiar to the field of DSB repair. Rev7 was first identified to be involved in ultraviolet sensitivity in yeasts [32]. It is a component of DNA polymerase ζ (zeta), regulating DNA translesion synthesis [33]. It re-emerged as a homolog of yeast mitotic arrest-deficient (Mad2) protein, and was named Mad2L2/Mad2B [34]. As Mad2 is a sensor of the mitotic spindle assembly checkpoint (SAC), Mad2L2 modulates the ubiquitin E3 ligase activity of anaphase-promoting complex/cyclosome (APC/C) by sequestering its co-activator, Cdh1, thus preventing premature APC/C activation [35].

Mad2L2 is also involved in the formation of a functional spindle and ensures correct chromosome segregation [36]. During DSB repair pathway choice, Rev7/Mad2L2 inhibits 5′ DNA end resection and accumulates at IR-induced DSBs under the control of 53BP1 and Rif1, thus promoting NHEJ-dependent events, such as telomere fusion and CSR [29–31]. It remains to be elucidated why Rev7/Mad2L2 does not complex with 53BP1 or Rif1 directly, although evidence suggests that chromatin contexts might play a role [29].

It has also been noted that the function of 53BP1 is not totally dependent on Rif1. While loss of 53BP1 rescued the lethal phenotype of BRCA1 null, loss of Rif1 only partially rescued HR defects in BRCA1 defective cells [23,24,27].

The 53BP1-PTIP-SNM1c/Artemis axis

PTIP contains BRCT repeats (**Fig. 1B**) that interact directly with ATM-dependent phosphorylation of 53BP1 at S25 [20,37]. Independent of its role in DNA repair, PTIP also mediates transcription together with the mixed-lineage leukemia 3 (MLL3)-MLL4 H3K4 methyltransferase [37]. Similar to 53BP1 and RIF1, PTIP blocks DSB end resection, and is involved in telomere fusion, but is not used for CSR. Therefore, PTIP could be considered as a context-specific effector [16].

Downstream of PTIP is the nuclease, Artemis/SNM1c [38]. Artemis belongs to the MBL family of DNA nuclease [39] (**Fig. 1C**). It possesses both endonuclease activities at DNA hairpins and at 5′ and 3′-DNA overhangs of duplex DNA in a DNA-PKcs-dependent manner, and also 5′-exonuclease activities on ssDNA and 5′-overhangs that is DNA-PKcs-independent [39]. A hotly debated question has been whether Artemis contains one single active site for both its exonucleolytic and endonucleolytic activities, which was answered recently by Li *et al.* [40], showing that not only the 5′-exonuclease and the endonuclease activities were co-purified but also a putative active site mutation-H115A markedly reduced both nuclease activities. A unified view of Artemis nuclease activity has also been proposed when it was discovered that Artemis resects into blunt DNA ends, the effectiveness of which depends on the AT content of the DNA ends and the Ku proteins [41].

PTIP interacts with Artemis through its second BRCT domain, while Artemis interacts with PTIP through damage-dependent phosphorylation of six S/T sites at the very C-terminus of Artemis, with T656 as the most critical residue [38]. Artemis localizes to DNA-damage sites, and loss of Artemis increased Rad51 and RPA foci in BRCA1-deficient cells [38]. In breast or ovarian cancer patients with *BRCA1/2* mutations, PARP inhibitors (PARPis) are widely used to induce synthetic lethality of tumor cells in the absence of BRCA1-mediated HR [42,43]. Loss of Artemis or using the nuclease-inactive mutant, H35AD37N, of Artemis leads to PARPi resistance [38]. Taken together, Artemis interacts with PTIP in a damage-dependent manner, and trims DNA ends to promote NHEJ depending on its nuclease activity.

DSB Repair in the Context of Chromatin Environment

DSB repair occurs in the context of chromatins, which are DNA structures surrounded by histones. Therefore, the chromatin association of 53BP1 antagonizes against BRCA1, leading to distinct subsequent pathway choice. The importance of histone modifications is exemplified by the very first step of DNA-damage signaling cascade, in which ATM phosphorylates the histone H2A variant H2A.X (or γH2AX), ensued by subsequent recruitment of DNA-damage effectors. The structure of 53BP1 also signifies the histone code: the Tudor domain binds histone H4K20me2, and the UDR motif interacts with histone H2AK15ub [16]. Several histone marks and other chromatin factors will be discussed below.

H4K20me vs. H4K16ac

The Tudor domain of 53BP1 binds to H4K20me and H4K20me2, which is essential for the recruitment of 53BP1 to DSB foci [44]. H4K20me2 is directly affected by acetylation of adjoining H4K16. The lysine acetyltransferase KAT5 (or TIP60) induces DNA damage–dependent H4K16ac, disrupting binding of the 53BP1 Tudor domain with the H4 tail [45]. The H4K16 acetyltransferase males absent on the first protein is phosphorylated by ATM at T392 and modulates 53BP1 function (MOF) [46]. However, the histone deacetylases HDAC1 and HDAC2 deacetylate H4K16 following DSB induction, thus promoting the association of 53BP1 with H4K20me (1/2) [47]. Therefore, the crosstalk of histone acetylation and methylation determines the association of 53BP1 with methylated chromatins.

In addition, the Polycomb protein L3MBTL1 and the demethylase Jumonji domain-containing protein 2A (JMJD2A/KDM4A) compete against 53BP1 for methylated chromatin binding with their H4K20me2-binding domains [48,49]. In the absence of DNA damages, they localize to H4K20me2. Upon DNA damages, RING finger 8 (RNF8) and RNF168 ubiquitinate both proteins, resulting in dissociation of L3MBTL1 from the chromatin and proteasome degradation of JMJD2A [50,51].

Moreover, a local enhancement of H4K20 methylation by the histone methyltransferases SET domain-containing protein 8 (SETD8/PRSET7) and multiple myeloma SET domain-containing protein (MMSET/WHSC1) has also been proposed [52–54]. However, it remains elusive whether H4K20 methylation formed in the pathway pertains to DNA damage.

H2AK15ub

The UDR motif in the vicinity of the Tudor domain recognizes DNA damage-induced ubiquitination of H2A by the E3 ligase RNF168 [55,56], which is necessary for the DNA damage-induced focus formation of 53BP1. Indeed, point mutations in the UDR motif abolish 53BP1 focus formation upon DNA damage and consequently the association of H2AK15ub with 53BP1 [23], but not that of H4K20me2. The DNA damage-specific combination of histone marks–H4K20me2 plus H2AK15ub–ensures the focus formation of 53BP1 at DSBs, and 53BP1 only binds to mononucleosomes that contain both histone marks. The dual specificity is sufficient and necessary for chromatin recruitment of 53BP1.

A negative regulator of H2AK15ub binding with 53BP1 is RNF169, another E3 ligase [57,58]. It has been proposed that RNF169 accumulates on damaged chromatins and competes against 53BP1 for H2AK15ub, but it remains enigmatic as how the balance is tipped upon DNA damage.

H3K36me3

H3K36me3 associates with transcription elongation and accumulates on actively transcribed genes [59]. H3K36me3 channels DSBs into the HR pathway at both heterochromatins (details discussed in the next section) and actively transcribing regions. Contrary to the

histone marks aforementioned, H3K36me3 is not induced by DNA damages, but rather, pre-established [60]. Using three experimental systems, I-SceI-, radiation-, and AsiSI-induced DSBs, three groups independently identified that active transcription marked by H3K36me3 and the main H3K36me3 methyltransferase SETD2 are necessary for HR repair [60–62].

H3K36me2, which is present on around 40% of nucleosomes, promotes NHEJ [63]. Besides the core histones, the linker histone 1 (H1) was identified to be the key target of RNF8 and UBC13 (or UBE2N), and K63-ubiquitinated H1 is read by RNF168, the recruitment of which induces H2A ubiquitination [64]. Therefore, more efforts are needed to generate a more unified view of the histone code that governs repair pathway choice.

Other chromatin cues: nuclear positions, nucleolar DNA and heterochromatins

DNA is distributed to distinct nuclear compartments: the vicinity of nuclear membranes, nuclear pores, lamina-associated, or nuclear interior. Although DNA damages occur throughout the nucleus, the fate of the damages is far from alike. Recent evidence suggests that DSBs at the nuclear pores or nuclear interior are more permissive for HR, while DSBs at the nuclear membranes are repaired by alternative end joining [65]. Therefore, nuclear compartmentalization also contributes to pathway choice.

Another specialized nuclear structure is the nucleolus, which harbors ribosomal DNA (rDNA) arrays and is the site of ribosome biogenesis. The nucleolar interior contains actively transcribing rDNA repeats, while the nucleolar periphery is packed with heterochromatins. DSBs introduced specifically to rDNA result in ATM-dependent inhibition of rDNA transcription, coupled with outward movement of rDNA to the periphery, resulting in easier access to the repair factors [66].

DSBs occurring in heterochromatin regions are repaired specifically by an HR pathway involving ATM, Artemis, RNF8, 53BP1, and RNF168 [67,68]. It has been proposed that NHEJ failures in these regions might be caused by the chromatin compaction in the heterochromatins, thus resulting in the subsequent heterochromatin de-condensation and HR factor recruitment [69]. Mechanistically, DSB leads to dissociation of heterochromatin protein 1 (HP1) from the heterochromatin mark H3K9me3, which enlists KAT5/TIP60 binding via its chromodomain, resulting in nucleosome resection and HR repair [70].

Open Questions and Translational Perspective

Many questions remain enigmatic in the field. To begin with, how is 53BP1 fine-tuned? Recently, ubiquitin-conjugating enzyme H7 (UbcH7/Ube2L3) was identified to regulate proteasome-dependent degradation of 53BP1 during both the steady state and replication stress [71]. UbcH7 depletion stabilizes 53BP1, and sensitizes the cells to DNA damages due to the error-prone NHEJ pathway [71]. Thus, UbcH7 could be utilized to enhance radiotherapy or chemotherapy by stabilizing 53BP1. It also makes one wonder: what is the E3 ligase for 53BP1? Next, can we biochemically reconstitute damaged chromatins? Third, are there other factors acting in concert with BRCA1 to antagonize 53BP1?

The vast majority (around 85%) of IR- or drug-induced DSBs are repaired by NHEJ, even in G_2 [8,72]. Thus, the DSB repair pathway choice may entail translational perspectives. For instance, when chemotherapies are conducted using PARPis in breast or ovarian cancer patients with *BRCA1/2* mutations, one needs to take into consideration that *BRCA1*$^{-/-}$ *53BP1*$^{-/-}$, *BRCA1*$^{-/-}$*Rev7*$^{-/-}$, *BRCA1*$^{-/-}$ *Artemis*$^{-/-}$ cells will render PARPis inefficient [29,38].

Another devastating human hereditary disease, Cockayne syndrome (CS), is mainly caused by the *ERCC6* gene which encodes Cockayne syndrome group B protein (CSB). CSB was discovered to localize to DSBs. Loss of CSB or a CS-associated CSB mutation impairs BRCA1 recruitment, but promotes 53BP1-Rif1 foci [73]. Therefore, targeting 53BP1 might raise a new clinical venue for the CS patients.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 31461143012 and 31530016), the National Basic Research Program of China (Nos. 2013CB911002 and 2015CB910601) to X.X., and from Young Talent Development Plan in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (No. CIT&TCD201404158) and Beijing Nova Program Interdisciplinary Cooperation Project to J.L.

References

1. Aparicio T, Baer R, Gautier J. DNA double-strand break repair pathway choice and cancer. *DNA Repair* 2014, 19: 169–175.
2. Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol* 2016, 26: 52–64.
3. de Massy B. Initiation of meiotic recombination: how and where? Conservation and specificities among eukaryotes. *Annu Rev Genet* 2013, 47: 563–599.
4. Haber JE. Mating-type genes and MAT switching in Saccharomyces cerevisiae. *Genetics* 2012, 191: 33–64.
5. Soulas-Sprauel P, Rivera-Munoz P, Malivert L, Le Guyader G, Abramowski V, Revy P, de Villartay JP. V(D)J and immunoglobulin class switch recombinations: a paradigm to study the regulation of DNA end-joining. *Oncogene* 2007, 26: 7780–7791.
6. Cannan WJ, Pederson DS. Mechanisms and consequences of double-strand DNA break formation in chromatin. *J Cell Physiol* 2015, 231: 3–14.
7. Chiruvella KK, Liang Z, Wilson TE. Repair of double-strand breaks by end joining. *Cold Spring Harb Perspect Biol* 2013, 5: a012757.
8. Karanam K, Kafri R, Loewer A, Lahav G. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. *Mol Cell* 2012, 47: 320–329.
9. Heyer WD, Ehmsen KT, Liu J. Regulation of homologous recombination in eukaryotes. *Annu Rev Genet* 2010, 44: 113–139.
10. Ismail IH, Gagne JP, Genois MM, Strickfaden H, McDonald D, Xu Z, Poirier GG, *et al*. The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. *Nat Cell Biol* 2015, 17: 1446–1457.
11. Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, Max EE, Ried T, *et al*. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 2000, 404: 510–514.
12. Malkova A, Haber JE. Mutations arising during repair of chromosome breaks. *Annu Rev Genet* 2012, 46: 455–473.
13. Tomimatsu N, Mukherjee B, Catherine Hardebeck M, Ilcheva M, Vanessa Camacho C, Louise Harris J, Porteus M, *et al*. Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. *Nat Commun* 2014, 5: 3561.
14. Hu Y, Petit SA, Ficarro SB, Toomire KJ, Xie A, Lim E, Cao SA, *et al*. PARP1-driven poly-ADP-ribosylation regulates BRCA1 function in homologous recombination-mediated DNA repair. *Cancer Discov* 2014, 4: 1430–1447.

15. Badie S, Carlos AR, Folio C, Okamoto K, Bouwman P, Jonkers J, Tarsounas M. BRCA1 and CtIP promote alternative non-homologous end-joining at uncapped telomeres. *EMBO J* 2015, 34: 828.
16. Panier S, Boulton SJ. Double-strand break repair: 53BP1 comes into focus. *Nat Rev Mol Cell Biol* 2014, 15: 7–18.
17. Iwabuchi K, Bartel PL, Li B, Marraccino R, Fields S. Two cellular proteins that bind to wild-type but not mutant p53. *Proc Natl Acad Sci USA* 1994, 91: 6098–6102.
18. Adams MM, Carpenter PB. Tying the loose ends together in DNA double strand break repair with 53BP1. *Cell Div* 2006, 1: 19.
19. Silverman J, Takai H, Buonomo SB, Eisenhaber F, de Lange T. Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. *Genes Dev* 2004, 18: 2108–2119.
20. Munoz IM, Jowsey PA, Toth R, Rouse J. Phospho-epitope binding by the BRCT domains of hPTIP controls multiple aspects of the cellular response to DNA damage. *Nucleic Acids Res* 2007, 35: 5312–5322.
21. Chapman JR, Sossick AJ, Boulton SJ, Jackson SP. BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. *J Cell Sci* 2012, 125: 3529–3534.
22. Chapman JR, Barral P, Vannier JB, Borel V, Steger M, Tomas-Loba A, Sartori AA, *et al*. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol Cell* 2013, 49: 858–871.
23. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkac J, Cook MA, *et al*. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 2013, 49: 872–883.
24. Feng L, Fong KW, Wang J, Wang W, Chen J. RIF1 counteracts BRCA1-mediated end resection during DNA repair. *J Biol Chem* 2013, 288: 11135–11143.
25. Zhang H, Liu H, Chen Y, Yang X, Wang P, Liu T, Deng M, *et al*. A cell cycle-dependent BRCA1-UHRF1 cascade regulates DNA double-strand break repair pathway choice. *Nat Commun* 2016, 7: 10201.
26. Hardy CF, Sussel L, Shore D. A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. *Genes Dev* 1992, 6: 801–814.
27. Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5′ end resection. *Science* 2013, 339: 700–704.
28. Di Virgilio M, Callen E, Yamane A, Zhang W, Jankovic M, Gitlin AD, Feldhahn N, *et al*. Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 2013, 339: 711–715.
29. Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, Bouwman P, Bartkova J, *et al*. REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 2015, 521: 541–544.
30. Boersma V, Moatti N, Segura-Bayona S, Peuscher MH, van der Torre J, Wevers BA, Orthwein A, *et al*. MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5′ end resection. *Nature* 2015, 521: 537–540.
31. Sale JE. REV7/MAD2L2: the multitasking maestro emerges as a barrier to recombination. *EMBO J* 2015, 34: 1609–1611.
32. Lawrence CW, Das G, Christensen RB. REV7, a new gene concerned with UV mutagenesis in yeast. *Mol Gen Genet* 1985, 200: 80–85.
33. Tomida J, Takata K, Lange SS, Schibler AC, Yousefzadeh MJ, Bhetawal S, Dent SY, *et al*. REV7 is essential for DNA damage tolerance via two REV3L binding sites in mammalian DNA polymerase zeta. *Nucleic Acids Res* 2015, 43: 1000–1011.
34. Cahill DP, da Costa LT, Carson-Walter EB, Kinzler KW, Vogelstein B, Lengauer C. Characterization of MAD2B and other mitotic spindle checkpoint genes. *Genomics* 1999, 58: 181–187.
35. Listovsky T, Sale JE. Sequestration of CDH1 by MAD2L2 prevents premature APC/C activation prior to anaphase onset. *J Cell Biol* 2013, 203: 87–100.
36. Bhat A, Wu Z, Maher VM, McCormick JJ, Xiao W. Rev7/Mad2B plays a critical role in the assembly of a functional mitotic spindle. *Cell Cycle* 2015, 14: 3929–3938.
37. Callen E, Di Virgilio M, Kruhlak MJ, Nieto-Soler M, Wong N, Chen HT, Faryabi RB, *et al*. 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell* 2013, 153: 1266–1280.
38. Wang J, Aroumougame A, Lobrich M, Li Y, Chen D, Chen J, Gong Z. PTIP associates with Artemis to dictate DNA repair pathway choice. *Genes Dev* 2014, 28: 2693–2698.
39. Fernandez FJ, Lopez-Estepa M, Vega MC. Nucleases of metallo-beta-lactamase and protein phophatase families in DNA repair. In: Storici F ed. DNA repair-on the pathways to fixing DNA damage and errors. InTech, ISBN 978-953-307-649-2, Published: September 9, 2011.
40. Li S, Chang HH, Niewolik D, Hedrick MP, Pinkerton AB, Hassig CA, Schwarz K, *et al*. Evidence that the DNA endonuclease ARTEMIS also has intrinsic 5′-exonuclease activity. *J Biol Chem* 2014, 289: 7825–7834.
41. Chang HH, Watanabe G, Lieber MR. Unifying the DNA end-processing roles of the artemis nuclease: Ku-dependent artemis resection at blunt DNA ends. *J Biol Chem* 2015, 290: 24036–24050.
42. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, *et al*. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 2010, 376: 235–244.
43. Chan SL, Mok T. PARP inhibition in BRCA-mutated breast and ovarian cancers. *Lancet* 2010, 376: 211–213.
44. Huyen Y, Zgheib O, Ditullio RA Jr, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, *et al*. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 2004, 432: 406–411.
45. Tang J, Cho NW, Cui G, Manion EM, Shanbhag NM, Botuyan MV, Mer G, *et al*. Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. *Nat Struct Mol Biol* 2013, 20: 317–325.
46. Gupta A, Hunt CR, Hegde ML, Chakraborty S, Chakraborty S, Udayakumar D, Horikoshi N, *et al*. MOF phosphorylation by ATM regulates 53BP1-mediated double-strand break repair pathway choice. *Cell Rep* 2014, 8: 177–189.
47. Miller KM, Tjeertes JV, Coates J, Legube G, Polo SE, Britton S, Jackson SP. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. *Nat Struct Mol Biol* 2010, 17: 1144–1151.
48. Min J, Allali-Hassani A, Nady N, Qi C, Ouyang H, Liu Y, MacKenzie F, *et al*. L3MBTL1 recognition of mono- and dimethylated histones. *Nat Struct Mol Biol* 2007, 14: 1229–1230.
49. Lee J, Thompson JR, Botuyan MV, Mer G. Distinct binding modes specify the recognition of methylated histones H3K4 and H4K20 by JMJD2A-tudor. *Nat Struct Mol Biol* 2008, 15: 109–111.
50. Acs K, Luijsterburg MS, Ackermann L, Salomons FA, Hoppe T, Dantuma NP. The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat Struct Mol Biol* 2011, 18: 1345–1350.
51. Mallette FA, Mattiroli F, Cui G, Young LC, Hendzel MJ, Mer G, Sixma TK, *et al*. RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J* 2012, 31: 1865–1878.
52. Oda H, Hubner MR, Beck DB, Vermeulen M, Hurwitz J, Spector DL, Reinberg D. Regulation of the histone H4 monomethylase PR-Set7 by CRL4(Cdt2)-mediated PCNA-dependent degradation during DNA damage. *Mol Cell* 2010, 40: 364–376.
53. Pei H, Zhang L, Luo K, Qin Y, Chesi M, Fei F, Bergsagel PL, *et al*. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. *Nature* 2011, 470: 124–128.
54. Tuzon CT, Spektor T, Kong X, Congdon LM, Wu S, Schotta G, Yokomori K, *et al*. Concerted activities of distinct H4K20 methyltransferases at DNA double-strand breaks regulate 53BP1 nucleation and NHEJ-directed repair. *Cell Rep* 2014, 8: 430–438.
55. Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, Ellenberg J, *et al*. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 2009, 136: 435–446.
56. Stewart GS, Panier S, Townsend K, Al-Hakim AK, Kolas NK, Miller ES, Nakada S, *et al*. The RIDDLE syndrome protein mediates a ubiquitin-

dependent signaling cascade at sites of DNA damage. *Cell* 2009, 136: 420–434.
57. Panier S, Ichijima Y, Fradet-Turcotte A, Leung CC, Kaustov L, Arrowsmith CH, Durocher D. Tandem protein interaction modules organize the ubiquitin-dependent response to DNA double-strand breaks. *Mol Cell* 2012, 47: 383–395.
58. Poulsen M, Lukas C, Lukas J, Bekker-Jensen S, Mailand N. Human RNF169 is a negative regulator of the ubiquitin-dependent response to DNA double-strand breaks. *J Cell Biol* 2012, 197: 189–199.
59. Edmunds JW, Mahadevan LC, Clayton AL. Dynamic histone H3 methylation during gene induction: HYPB/Setd2 mediates all H3K36 trimethylation. *EMBO J* 2008, 27: 406–420.
60. Aymard F, Bugler B, Schmidt CK, Guillou E, Caron P, Briois S, Iacovoni JS, *et al*. Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat Struct Mol Biol* 2014, 21: 366–374.
61. Carvalho S, Vitor AC, Sridhara SC, Martins FB, Raposo AC, Desterro JM, Ferreira J, *et al*. SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint. *Elife* 2014, 3: e02482.
62. Pfister SX, Ahrabi S, Zalmas LP, Sarkar S, Aymard F, Bachrati CZ, Helleday T, *et al*. SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability. *Cell Rep* 2014, 7: 2006–2018.
63. Fnu S, Williamson EA, De Haro LP, Brenneman M, Wray J, Shaheen M, Radhakrishnan K, *et al*. Methylation of histone H3 lysine 36 enhances DNA repair by nonhomologous end-joining. *Proc Natl Acad Sci USA* 2011, 108: 540–545.
64. Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, Narita T, *et al*. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 2015, 527: 389–393.
65. Lemaitre C, Grabarz A, Tsouroula K, Andronov L, Furst A, Pankotai T, Heyer V, *et al*. Nuclear position dictates DNA repair pathway choice. *Genes Dev* 2014, 28: 2450–2463.
66. van Sluis M, McStay B. A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. *Genes Dev* 2015, 29: 1151–1163.
67. Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Lobrich M, Jeggo PA. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol Cell* 2008, 31: 167–177.
68. Noon AT, Shibata A, Rief N, Lobrich M, Stewart GS, Jeggo PA, Goodarzi AA. 53BP1-dependent robust localized KAP-1 phosphorylation is essential for heterochromatic DNA double-strand break repair. *Nat Cell Biol* 2010, 12: 177–184.
69. Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A, Kakarougkas A, *et al*. Factors determining DNA double-strand break repair pathway choice in G2 phase. *EMBO J* 2011, 30: 1079–1092.
70. Sun Y, Jiang X, Xu Y, Ayrapetov MK, Moreau LA, Whetstine JR, Price BD. Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nat Cell Biol* 2009, 11: 1376–1382.
71. Han X, Zhang L, Chung J, Mayca Pozo F, Tran A, Seachrist DD, Jacobberger JW, *et al*. UbcH7 regulates 53BP1 stability and DSB repair. *Proc Natl Acad Sci USA* 2014, 111: 17456–17461.
72. Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, Goodarzi AA, *et al*. ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *EMBO J* 2009, 28: 3413–3427.
73. Batenburg NL, Thompson EL, Hendrickson EA, Zhu XD. Cockayne syndrome group B protein regulates DNA double-strand break repair and checkpoint activation. *EMBO J* 2015, 34: 1399–1416.