‘A mover and a shaker’: 53BP1 allows DNA doublestrand breaks a chance to dance and unite

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F1000 Biology Reports 2009, **1**:21 (doi: 10.3410/B1-21)

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Abstract

The DNA damage response mediator protein, p53-binding protein 1 (53BP1), is dispensable for the repair of most DNA doublestrand breaks (DSBs) induced by ionising radiation. However, two recent studies have shown that 53BP1 is required for rejoining of distant DSB ends and that it promotes the movement of uncapped telomeres. These results are discussed in the light of recent findings, and a model for the role of 53BP1 in DSB rejoining is presented.

Introduction and context

Ataxia telangiectasia (A-T) mutated protein (ATM) lies at the heart of the DNA doublestrand break (DSB) signal transduction response [1]. Following DSB detection, in a step dependent upon the Mre11-Rad50-Nbs1 (MRN) complex, a range of so-called ‘mediator’ proteins, including MDC1 (mediator of DNA damage checkpoint 1), BRCA1 (breast cancer 1, early onset), RNF8 (ring finger protein 8), and 53BP1 (p53-binding protein 1), are recruited to the DSB. Although significant insight into the orchestrated assembly of the complex of mediator proteins has been gained, their precise role in the damage response has remained elusive [2–4]. The ‘mediators’ accumulate over a large distance surrounding the DSB and, perhaps critically, serve to recruit and maintain ATM at the DSB site. ATM phosphorylates multiple substrates, including p53, the Chk2 transducer kinase, and the mediator proteins themselves. Either directly or indirectly, these phosphorylation events regulate end points such as cell cycle checkpoint arrest or apoptosis. Importantly, they also influence DSB repair.

Although cells lacking individual mediator proteins display genomic instability, defects in the response to DSBs are subtle. ATM-dependent substrate phosphorylation is only marginally impaired, cell cycle checkpoint arrest is normal at all but very low DSB numbers, and

most DSB repair occurs efficiently [5,6]. However, two studies recently have provided significant insight into the function of 53BP1, a mediator protein that is the closest homologue of the *Saccharomyces cerevisiae* and *S. pombe* proteins, Rad9 and Crb2, respectively [7,8]. Both studies demonstrate roles for 53BP1 in the rejoining of distant DSB ends. Here, we consider the significance of these findings, which provide insight into the elusive role of 53BP1.

Major recent advances

53BP1 promotes non-homologous end-joining between distant DNA breaks

The major process that repairs radiation-induced DSBs in mammalian cells is DNA non-homologous end-joining (NHEJ). Although the majority of NHEJ occurs independently of ATM signalling, recent studies have shown that ATM and 53BP1 are required for the repair of approximately 15% of ionising radiation (IR)-induced DSBs [6]. NHEJ has also been shown to rejoin DSBs that arise at deprotected telomeres [9]. Dimitrova and colleagues [7] examined the role of 53BP1 in the rejoining of deprotected telomeres, obtained using a conditional deletion of TRF2, a component of the shelterin complex that protects telomere ends. Mouse telomeres lacking TRF2 are recognised as DSBs, inducing the recruitment of ATM signalling proteins and NHEJ-dependent rejoining

Figure 1. DSB-induced / 53BP1-mediated increased chromatin mobility

Higher order chromatin structure serves as a barrier limiting DNA accessibility, mobility, and some repair processes. Mounting evidence suggests that a function of ataxia telangiectasia mutated protein (ATM) is to affect localised chromatin changes required for some, but not all, aspects of double-strand break (DSB) repair. ATM is an inactive multimer in undamaged cells. In the presence of DSBs, the Mre11-Rad50-Nbs1 (MRN) complex recruits ATM to the DSB site, resulting in its autophosphorylation, monomerisation, and activation. ATM signalling causes phosphorylation of histone H2AX, followed by MDC1 recruitment, recruitment of ubiquitin ligases (for example, RNF8), histone mono-ubiquitination (mono-ub), and finally 53BP1 recruitment. 53BP1 recruitment is required for ATM retention at the DSB site. This facilitates ATM-mediated localised chromatin modifications, which enhance chromatin relaxation. This is required for some, but not all, DSB-processing events. It appears to be dispensable for most non-homologous end-joining (NHEJ) but is required for long-range NHEJ events, for telomere fusions, and for rejoining DSBs located within heterochromatin. The precise nature of the chromatin modifications required for these events is still unclear.

to generate telomere end-to-end fusions. Such unprotected ends, therefore, are processed like radiation-induced DSBs by NHEJ. However, in contrast to most NHEJ rejoining, telomere fusion events caused by elimination of TRF2 require ATM and 53BP1. To assess the role of 53BP1, Dimitrova and colleagues created a fluorescently tagged fragment of 53BP1, which was able to localise to telomere ends tagged with GFP-TRF1, another shelterin component. Remarkably, time-lapse microscopy performed after loss of TRF2 revealed that, in the presence of 53BP1, the deprotected telomeres were more mobile, moving through larger territories within the nucleus compared with cells lacking 53BP1. The authors interpreted their findings to suggest that 53BP1 may cause localised changes to chromatin that promotes movement of the DNA ends, a necessity for NHEJ-dependent rejoining of distant DSBs.

NHEJ also functions during V(D)J – variable (diversity) joining – recombination and class switch recombination (CSR), two critical intrachromosomal joining events during immune development. Since A-T patients do not manifest severe combined immunodeficiency and since cells lacking ATM and 53BP1 are largely proficient for

V(D)J recombination, it has been assumed that, like most radiation-induced DSBs, the rejoining of DSBs induced during V(D)J recombination is ATM- and 53BP1-independent. However, Difilippantonio and colleagues [8] focused on the observation that, although 53BP1^{-/-} mice are largely proficient for V(D)J recombination, they have reduced T and B cell numbers and decreased thymus cellularity. More detailed analysis revealed distinct abnormalities in T-cell antigen receptor (TCR) coding junctions, including TCR-β, TCR-α, and TCR-δ, that were reminiscent of the aberrant short-range intra-switch recombination products observed in CSR junctions in 53BP1^{-/-} mice. Specific analysis of joining of distal gene segments in 53BP1^{-/-} thymocytes revealed that, whilst short-range rearrangements were similar or even more numerous, long-range rearrangements in 53BP1 knockout mice were significantly and specifically diminished. This unexpected and exciting finding exposes a specific role for 53BP1 in long-range V(D)J rejoining events.

53BP1s impact on chromatin organisation

Although 53BP1 is not a core NHEJ component, it has now been shown to function in several NHEJ-dependent rejoining events: (a) a subcomponent of IR-induced DSB

repair, (b) telomere fusions at deprotected telomeres, and (c) long-range V(D)J recombination. In addition to these processes, 53BP1 is required for CSR, another DSB rejoining process that, at least to some extent, is also NHEJ-dependent [10–12]. Curiously, although long-range rejoining of DSBs is beneficial to help create diversity during immune development and is perhaps preferable to the persistence of unprotected telomeres, long-range translocations following IR-induced DNA doublestrand breakage will likely promote genomic instability and therefore be undesirable. Notwithstanding the different outcomes and benefits, it is tempting to anticipate that all events arise via a common role of 53BP1.

Recent studies have shown that those radiation-induced DSBs requiring ATM for repair are located within heterochromatin and that ATM phosphorylates KAP-1, a heterochromatic-building factor [13]. Since the need for 53BP1 is epistatic to ATM in DSB repair, it is possible that 53BP1 also functions to promote KAP-1 phosphorylation, enhancing the phosphorylation of KAP-1 at the DSB site to facilitate chromatin relaxation. It is currently unclear whether KAP-1 phosphorylation is required for telomere fusions. Although KAP-1 may not be the critical ATM substrate for unprotected telomere repair, one potential model is that ATM-dependent phosphorylation events arising in a 53BP1-dependent manner confer chromatin relaxation (Figure 1). MDC1 is dispensable for ATM activation but is required for ATM retention at the DSB site, and indirect evidence suggests that 53BP1 may also serve to localise ATM to the site of damage [14]. Thus, a potential model is that 53BP1 is specifically required for localised chromatin modification in the vicinity of the DSB. Whether the 'movement' observed at the uncapped telomeres is the critical event promoting rejoining or a consequence of chromatin relaxation remains to be determined. Strikingly, loss of MDC1 and phosphorylated histone γ H2AX imparts a milder impact on long-range V(D)J recombination rejoining in stark contrast to radiation-induced DSBs, in which loss of MDC1, γ H2AX, and 53BP1 exerts similar and epistatic phenotypes. Thus, 53BP1 may have an additional role, as proposed by Difilippantonio and colleagues, reflecting a function in enhanced DSB synapsis via 'basal' chromatin binding (not highlighted in Figure 1).

Future directions

The finding that 53BP1 promotes movement of telomere ends is exciting and novel and it will be important to establish whether this is a phenotype directly underlying telomere fusion events or an indirect consequence of chromatin changes. An exciting question to address now is what chromatin changes underlie these different

53BP1 rejoining events and whether or not they are mechanistically related.

Abbreviations

53BP1, p53-binding protein 1; A-T, ataxia telangiectasia; ATM, ataxia telangiectasia mutated protein; CSR, class switch recombination; DSB, doublestrand break; IR, ionising radiation; MDC1, mediator of DNA damage checkpoint 1; NHEJ, non-homologous end-joining; TCR, T-cell antigen receptor; V(D)J, variable (diversity) joining.

Competing interests

The authors declare that they have no competing interests.

References

- Lavin MF: **Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer.** *Nat Rev Mol Cell Biol* 2008, **9**:759-69.
 - Mailand N, Bekker-Jensen S, Fastrup H, Melander F, Bartek J, Lukas C, Lukas J: **RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins.** *Cell* 2007, **131**:887-900.
- F1000 Factor 4.8 *Must Read*
 Evaluated by Tim Humphrey 6 Dec 2007, Stephen Jackson 20 Dec 2007
- Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J: **RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly.** *Cell* 2007, **131**:901-14.
- F1000 Factor 6.0 *Must Read*
 Evaluated by Stephen Jackson 20 Dec 2007
- Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, Pelletier L, Jackson SP, Durocher D: **Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase.** *Science* 2007, **318**:1637-40.
 - Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, Naka K, Xia Z, Camerini-Otero RD, Motoyama N, Carpenter PB, Bonner WM, Chen J, Nussenzweig A: **DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1.** *Nat Cell Biol* 2002, **4**:993-7.
 - Riballo E, Kuhne M, Rief N, Doherty A, Smith GC, Recio MJ, Reis C, Dahm K, Fricke A, Krempler A, Parker AR, Jackson SP, Gennery A, Jeggo PA, Lobrich M: **A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci.** *Mol Cell* 2004, **16**:715-24.
 - Dimitrova N, Chen YC, Spector DL, de Lange T: **53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility.** *Nature* 2008, **456**:524-8.
- F1000 Factor 6.0 *Must Read*
 Evaluated by Jiri Lukas 7 Nov 2008
- Difilippantonio S, Gapud E, Wong N, Huang CY, Mahowald G, Chen HT, Kruhlak MJ, Callen E, Livak F, Nussenzweig MC, Sleckman BP, Nussenzweig A: **53BP1 facilitates long-range DNA end-joining during V(D)J recombination.** *Nature* 2008, **456**:529-33.
- F1000 Factor 3.0 *Recommended*
 Evaluated by Pierre Ferrier 30 Oct 2008
- Celli GB, Denchi EL, de Lange T: **Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination.** *Nat Cell Biol* 2006, **8**:885-90.

10. Ward IM, Reina-San-Martin B, Olaru A, Minn K, Tamada K, Lau JS, Cascalho M, Chen L, Nussenzweig A, Livak F, Nussenzweig MC, Chen J: **53BP1 is required for class switch recombination.** *J Cell Biol* 2004, **165**:459-64.
11. Manis JP, Morales JC, Xia Z, Kutok JL, Alt FW, Carpenter PB: **53BP1 links DNA damage-response pathways to immunoglobulin heavy chain class-switch recombination.** *Nat Immunol* 2004, **5**:481-7.
12. Franco S, Murphy MM, Li G, Borjeson T, Boboila C, Alt FW: **DNA-PKcs and Artemis function in the end-joining phase of immunoglobulin heavy chain class switch recombination.** *J Exp Med* 2008, **205**:557-64.
13. 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**:167-77.
14. Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, Alt FW, Chen J: **MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals.** *Mol Cell* 2006, **21**:187-200.

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