Editorial Commentary

An emerging regulatory network of NHEJ via DYNLL1-mediated 53BP1 redistribution

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Our bodies are continuously exposed to a wide range of carcinogens and other DNA-damaging agents, together with endogenous forms of DNA damage, resulting in mutations and complex chromosomal aberrations that can affect oncogenes or tumour suppressor genes. These errors, which can be inherited by daughter cells if they are not resolved, can contribute to malignant transformation (1). In tumours, the gradual acquisition of genetic aberrations can also contribute to the development of drug resistance. Various mechanisms that mediate the repair of DNA lesions are critical in suppressing genome instability, thus elucidating these processes is crucial to our understanding of tumour biology and cancer evolution.

Whether as a result of an aberrant event or a programmed cellular process, a double-strand break (DSB) is one of the most dangerous forms of DNA damage. DSBs are predominantly repaired by homologous recombination (HR) or non-homologous end-joining (NHEJ), which are highly regulated cellular processes that involve numerous protein networks. TP53-binding protein 1 (53BP1) is a key mediator of DSB resolution that contains a variety of interaction protein domains, which mediate its functions as a scaffold for DSB-responsive factors, and is required for the decision of whether a cell will undergo HR or NHEJ (2).

In a new article in *Nature Communications*, Becker *et al.* suggest that the multifunctional protein DYNLL1 acts as a protein hub for the oligomerisation of 53BP1 and its recruitment to DSBs (3). The molecular interaction between 53BP1 and DYNLL1 was identified and investigated in detail, by the elegant use of a variety of 53BP1 mutant constructs. These two proteins were found to co-localise to ionizing radiation (IR)-induced nuclear foci (IRIF), where DYNLL1 is essential for the oligomerisation domain (OD)-independent recruitment of 53BP1 to DSB sites. Functionally, this interaction was shown to mediate the regulation of the p53 response to nutlin, the synthetic relationship between BRCA1 and PARP inhibition (PARPi), and adaptive immunity in mice, which collectively support the emerging role of DYNLL1 as a universal regulator of NHEJ.

Building on previous findings that 53BP1 can be recruited to IRIFs independently of its oligomerisation domain, the authors defined that the OD-independent 53BP1 complex formation is reliant on its interaction with DYNLL1, which regulates the efficiency of NHEJ. The findings of this report are consistent with previous studies, which have shown that deficiency of ASCIZ/ATMIN, the transcriptional regulator of DYNLL1 (4), is associated with defective 53BP1 foci formation (5). Interestingly, recent work from the Chowdhury lab described only a moderate reduction of 53BP1 foci upon DYNLL1 ablation. Specifically, although the median number of 53BP1 foci did not change significantly, the distribution of foci was profoundly affected, as the number of cells with a high

number of foci was markedly reduced (6). These apparently opposing observations could be reconcilable, considering that they may reflect differential responses of two distinct cell populations, as categorised by the number of 53BP1 foci they display in response to IR.

Becker *et al.* also demonstrated that 53BP1 foci were formed throughout interphase and were ubiquitously regulated by DYNLL1 and the OD of 53BP1. However, localisation of 53BP1 has been shown to have specific functions in different phases of the cell cycle as, for example, 53BP1 nuclear bodies found exclusively in G1 are thought to be a result of unrepaired replication stress (RS)-related aberrations (7). Interestingly, ASCIZ/ATMIN has been reported to be required for the formation of RAD51 foci in response to alkylating agents (8) or 53BP1 foci (5,9) and in some studies for ATM signalling in response to various stimuli (5,9,10). Its requirement in response to RS is most notable in late-passage primary mouse embryonic fibroblasts when ATMIN deficiency is often associated with premature senescence, a process thought to be driven by RS-induced damage in cultured cells (11). The role of ASCIZ/ATMIN and ATM signalling in response to RS has been a point of debate in the field (9,12) and a closer investigation into the effects of ASCIZ/ATMIN and DYNLL1 on 53BP1 might help clarify their functions.

In addition, although IR seems not to affect the interaction between DYNLL1 and 53BP1, Becker *et al.* provide evidence to suggest that upstream activation of the full-length protein is important. The latter is particularly obvious when focusing on a 53BP1 construct with 28 serine to alanine substitutions, which still contains the DYNLL1 interaction domains but cannot override the IRIF localisation defects caused by the mutation of the OD domains. This phenotype suggests the interesting possibility that some of these S/T-Q ATM/R phosphorylation sites could be essential in 53BP1 recruitment to DSBs. In addition, DYNLL1 phosphorylation at Ser88 has been reported to be necessary for the modulation of its protein function (6). Given that DYNLL1 serves as a scaffold for several proteins (13), it is possible that different partners bind to DYNLL1 depending on its phosphorylation status and subsequently alter its functions, and in particular regulation of 53BP1. Taken together, these observations suggest that cellular signalling may be contributing to additional layers of regulation of 53BP1 by DYNLL1.

Particularly relevant to cancer research, this study also revealed that the requirement of DYNLL1 in regulating 53BP1 recruitment to the DSB extends to a functional outcome, as DYNLL1 affects BRCA1-deficient tumour sensitivity to PARPi. Specifically, the authors demonstrated that loss of either DYNLL1 or ASCIZ/ATMIN conferred growth advantages to BRCA1-deficient cells and organoids exposed to olaparib. Despite initially showing promising clinical data, drug resistance against PARPi has started to manifest in the clinic, driven by diverse mechanisms, including through perturbation of key components of NHEJ: 53BP1, RIF1 and REV7 (14). The recent identification of shieldin, a protein complex that acts alongside these proteins to promote NHEJ, offers additional insight into PARPi resistance mechanisms. Loss of shieldin renders BRCA-mutant cells resistant to olaparib but sensitive to ionizing radiation and platinum therapy, making these treatment strategies particularly attractive for overcoming PARPi resistance (15). Interestingly, in a genome-wide screen in BRCA1-deficient cells, both ASCIZ/ATMIN and DYNLL1 were identified as strong drivers of resistance to not only PARPi but also platinum agents (6). The differential response to platinum resistance between these recently identified NHEJ complexes and the ASCIZ/ATMIN–DYNLL1 axis is intriguing, and can be utilised to understand the mechanism of NHEJ and PARPi resistance regulation by DYNLL1. Moreover, considering that DYNLL1 regulates 53BP1 accumulation at the IRIF, it will also be of great clinical interest to investigate its role in IR-sensitivity.

Looking beyond the repair of IR-mediated DSBs, Becker *et al.* implicated DYNLL1 in the regulation of class switch recombination (CSR) and p53 pathway activation. Specifically, overcoming the requirement for DYNLL1 in normal B cell development by using a mature B cell system, the authors showed that DYNLL1-loss, as well as loss of its upstream regulator ASCIZ/ATMIN, leads to defective CSR. These findings are reminiscent of previous work that identified a role for ASCIZ/ATMIN in the maintenance of genomic stability and tumour suppression in B cells, where ASCIZ/ATMIN deletion was associated with defective peripheral V(D)J rearrangement and CSR resulting from inefficient repair of DSBs generated during somatic recombination (16). In addition, Becker *et al.* identified that DYNLL1-dependent 53BP1 oligomerisation has a role in the canonical response to inhibition of MDM2 and subsequent regulation of its target p53, as resistance to nutlin-mediated apoptosis was only partially suppressed upon addition of the 53BP1 mutation that lacks the ability to bind DYNLL1.

The authors propose a bivalent model of 53BP1 activation, primarily resulting from its ability to oligomerise.

Their findings from diverse experimental systems lead to the conclusion that 53BP1 functions are mediated by the combinatorial effects of the OD and DYNLL1 interaction domains. Investigating IRIF-localisation of 53BP1, the authors discovered that mutation or loss of either of these domains contributes to partial mis-localisation of 53BP1 foci, with the disruption of both domains resulting in the most profound phenotypes. They identified that individual contributions of each domain was apparent in the stability of the 53BP1 association with chromatin around the DSB, in which the DYNLL1-mediated interaction alone was not sufficient to ensure localisation to the DSB and the defect was predominantly driven by the disruption of the OD. Similarly, in mouse B cells, loss of DYNLL1 interaction caused a substantial decrease in CSR deficiency; however, it was the loss of 53BP1 oligomerisation via disruption of its OD that seemed to predominantly manifest the defect. This effect was also evident in the ability of DYNLL1 to rescue drug-induced phenotypes, as nutlin sensitivity was also driven predominantly by the OD-mediated interaction. Although detailed investigation of these domains suggests an important role for DYNLL1 interactions, the OD-mediated interaction might be the primary driver of 53BP1 oligomerisation and function. It will be very interesting to establish whether this relationship applies to other 53BP1-mediated cellular responses, including its role in sensitivity of BRCA1-deficient cells to PARP inhibition.

An important addition to the model proposed by Becker *et al.* might come from a concordant recent report, which showed that DYNLL1 limits DNA end-resection at the break site (6). The loss of DYNLL1 increased both the end-resection rate and MRE11, RPA32, and RAD51 foci formation in response to damage, postulating that DYNLL1 limits DNA end-resection through its interaction with the MRE11, RAD50, NBS1 (MRN) complex. In addition, the authors have shown an epistatic relationship between ASCIZ/ATMIN and DYNLL1, potentially placing DYNLL1 downstream of ATM. This finding is consistent with previous reports that established ASCIZ/ATMIN as the transcriptional regulator of DYNLL1 (4) and the findings by Becker *et al.* that ASCIZ/ATMIN-loss-associated phenotypes are rescued by the addition of DYNLL1 (3). However, the nature of this relationship might be more complex, as DYNLL1 itself has been shown to bind ASCIZ/ATMIN (13).

As DYNLL1 regulates 53BP1 localisation and function, and may be directly and/or indirectly regulated by ATM, it is conceivable that DYNLL1 could be involved in telomere end protection, where ATM and 53BP1 both have significant functions (17). At telomeres, chromosome ends are masked by the functions of components of the shelterin complex, which suppress ATM signalling and canonical NHEJ (c-NHEJ). In addition to c-NHEJ and considering that DYNLL1 regulates 53BP1 foci formation in S and G2 phases, affects PARP signalling and interacts with the MRN complex (3,6), DYNLL1 could also be relevant in regulating alternative NHEJ (alt-NHEJ), a repair process that depends on DNA microhomology and the functions of ATM, MRN and 53BP1. The roles of ASCIZ/ATMIN and DYNLL1 in these processes have been underexplored and there is exciting work ahead as these pathways are further deciphered.

In summary, work discussed here demonstrates a role for DYNLL1 in regulating 53BP1 complexes and raises important biological questions about the mechanisms of the cellular response to DSBs. Is the ASCIZ/ATMIN-DYNLL1 axis a master regulator of 53BP1 or is it particularly important in specific NHEJ-regulated processes? Driven by its ability to bind 53BP1, can DYNLL1 act as a spatial and temporal regulator that fine-tunes the recruitment of DNA repair proteins at the DSB? And finally, does the ASCIZ/ATMIN-DYNLL1 axis contribute to mechanisms that regulate the exact timing of the interplay between the 53BP1–RIF and the BRCA1-CTIP complexes and, therefore, the selection between error-free HR and error-prone NHEJ? Understanding the roles and interactions of these highly complex DNA repair cascades is a crucial step in implementing improvements in cancer therapy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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