The demise of a TUDOR under stress opens a chromatin link to 53BP1

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The mechanisms that localise 53BP1 to sites of DNA double-strand breaks (DSBs) have remained elusive, despite this protein's key roles in DNA damage response signalling and repair processes. Recent studies, including the work by Mallette *et al* (2012) in this issue of *The EMBO Journal*, now provide crucial insights into the roles of ubiquitin-dependent signalling cascades at DNA damage sites required for chromatin-mediated 53BP1 recruitment.

Ever since its identification over 15 years ago, 53BP1—originally suggested to regulate p53-dependent transcription—has remained a rather enigmatic protein. The realisation that 53BP1 played a role in the cellular response to DNA damage redirected attention towards its involvement in coordinating DNA DSB repair, but this aspect of 53BP1 has never been far from controversy. It has been implicated in many different reactions to genotoxic insults, such as localising repair proteins to break sites, activation of the intra-S- and G2/M-phase DNA damage checkpoints, ATM-dependent signal transduction and DNA DSB repair (reviewed by Noon and Goodarzi, 2011). Recent studies of knockout mouse models suggest that 53BP1 promotes nonhomologous DNA end-joining (NHEJ) by limiting DNA end-resection and counteracting homologous recombination repair. This role of 53BP1 becomes most apparent in lymphoid development, where it stimulates NHEJ repair of distally located DSBs induced during V(D)J recombination and immunoglobulin class switch recombination. As a consequence, mice lacking 53BP1 exhibit severe immunodeficiency characterised by lymphopenia and agammaglobulinemia (Noon and Goodarzi, 2011).

The controversy surrounding 53BP1 is not just limited to its function. Like many DSB mediator/adaptor proteins, 53BP1 is rapidly recruited to regions of chromatin located proximal to the DNA break. In contrast to its evolutionary relatives in lower eukaryotes, such as *S. cerevisiae* Rad9 and *S. pombe* Crb2 (Hammet *et al*, 2007; Sofueva *et al*, 2010), this recruitment is not mediated via its C-terminal tandem BRCT domains binding to phosphorylated histone H2A. Rather, relocalisation of 53BP1 to DSBs requires its TUDOR domain, which recognises dimethylated histones residing locally at the damaged DNA region. Similar to budding yeast Rad9, the TUDOR domain of 53BP1 was originally proposed to bind lysine-79 of histone H3 following dimethylation by the Dot1 histone methyltransferase (Huyen *et al*, 2004; Wysocki *et al*, 2005). However, the ability of 53BP1 to form DNA

damage-induced foci was later found to require its TUDOR domain preferentially associating instead with histone H4 dimethylated on lysine-20 (H4K20Me₂), a mechanism also found for fission yeast Crb2 (Botuyan *et al*, 2006; Kim *et al*, 2006). Interestingly, several candidate histone H4K20 methyltransferases, such as MMSET, PR-Set7 and SUV20-4h1/2, have been implicated in potentiating the focal recruitment of 53BP1, yet no real consensus has been reached over this issue (Botuyan *et al*, 2006; Schotta *et al*, 2008; Pei *et al*, 2011). While it is conceivable that more than one methyltransferase, with each being specific to a particular type of chromatin structure, may regulate 53BP1 relocalisation, a more likely scenario is that loss of each of these enzymes disrupts the chromatin configuration in such a way that it is no longer permissive for 53BP1 to recognise its methylated targeting motif. Irrespective of this, a perplexing aspect is that global levels of H4K20Me₂ do not increase following the induction of DNA damage (Botuyan *et al*, 2006). One model posits that the dimethylated H4K20 moiety remains buried within the nucleosome, and that local chromatin remodelling around the break site makes it visible to the TUDOR domain of 53BP1. The demonstration that ionising radiation-induced 53BP1 foci are mildly disrupted in cells lacking components of the p400–TIP60 chromatin remodelling complex, lending some credence to this hypothesis (Murr *et al*, 2006; Xu *et al*, 2010).

Over the last few years, the ubiquitin–proteasome system has emerged as another major factor in controlling the recruitment of repair factors to the sites of DNA damage and the surrounding chromatin. The ubiquitin-dependent DNA damage response (Ub-DDR) is coordinated by a cascade of E3 ubiquitin ligases, including RNF8 and RNF168, which direct the relocalisation of both 53BP1 and the BRCA1–A complex to DSBs by mediating the formation of K63-linked ubiquitin chains on H2A-type histones (reviewed by Stewart, 2009). These chains recruit the BRCA1–A repair complex through the paired ubiquitin-interacting motifs (UIMs) of the complex subunit RAP80. On the other hand, the role of the RNF8–RNF168-dependent Ub-DDR in promoting 53BP1 recruitment has remained less clear, although it was suggested that histone ubiquitylation too might trigger chromatin relaxation, allowing the di-methylated histone H4 modification to be recognised by 53BP1.

Local increases in H4K20Me₂ levels associated with artificially induced DSBs have been documented, requiring

Figure 1 Following the induction of DNA double-strand breaks (DSBs) and their detection by the MRN–ATM–MDC1 pathway, the ubiquitin ligases RNF8 and RNF168 are recruited to the chromatin proximal to the break, where they ubiquitylate both H2A-type histones and the H4K20-Me2-binding TUDOR domain proteins JMJD2A/B and L3MBTL1. Proteasome-dependent JMJD2A/B degradation, p97/VCP-mediated L3MBTL1 removal, and localised nucleosome remodelling by the p400–TIP60 complex all contribute to revealing di-methylated lysine-20 of histone H4 to the TUDOR domain of 53BP1. In parallel, RNF8/RNF168-dependent poly-ubiquitylation of H2A/H2AX in the nucleosomes either side of the DSB promotes recruitment of the BRCA1-A complex via interaction with the UIM domains of RAP80.

activity of the methyltransferase MMSET (Pei *et al*, 2011); however, this increase in H4K20 dimethylation, as measured by chromatin-immunoprecipitation, could likewise be explained by an increase in the visibility of this histone mark, rather than a net change in its local levels. This raises the interesting possibility that the ability of this histone mark to recruit 53BP1 may in fact be actively masked by the cell, with the block only being removed upon DNA damage. In support of this, the new work by Stephane Richard and colleagues (Mallette *et al*, 2012) identifies two TUDOR domain-containing lysine demethylase enzymes, JMJD2A and JMJD2B, as a critical link between the RNF8–RNF168-dependent Ub–DDR recruitment cascade and the chromatin-mediated 53BP1 relocalisation to DNA breaks. Mallette *et al* (2012) found that JMJD2A/B, but not other members of the JMJD2 family, become polyubiquitylated and degraded in response to DNA damage, and that this requires the E3 ligase activity of both RNF8 and RNF168. Earlier work had shown that the TUDOR domain of these closely related lysine demethylases can bind *in vitro* with high affinity to a K20-dimethylated H4 peptide (Kim *et al*, 2006), in a manner

similar to 53BP1. Therefore, 53BP1 and JMJD2A/B might compete for the same histone mark. Based on this, the ubiquitin-dependent loss of JMJD2A/B upon the induction of DNA damage would consequently allow binding of 53BP1. Consistent with this hypothesis, Richard and colleagues demonstrated that overexpression of JMJD2A/B could selectively inhibit the formation of 53BP1 foci, but not RAP80 foci, in cells exposed to ionising radiation. This suppression depended on the integrity of the methyl-histone-binding TUDOR domain, since mutants lacking demethylase activity inhibited 53BP1 foci formation in a manner similar to that of wild-type JMJD2A/B demonstrating a nonenzymatic role of JMJD2A/B. As a consequence, forced overexpression of wild-type JMJD2A or JMJD2B but not the TUDOR domain mutant also hypersensitised cells to DSB-inducing agents. Taken together, these data would predict that the defective 53BP1 relocalisation to DSBs in cells lacking RNF8 and RNF168 results from an inability to degrade JMJD2A/B following exposure to ionising radiation. Furthermore, another prediction from this would be that loss of JMJD2A/B in

cells lacking either RNF8 or RNF168 should restore 53BP1 recruitment, which was indeed confirmed to be the case.

While this elegant model (Figure 1) nicely ties up several loose ends, true to form, it is unlikely to be the whole story. Interestingly, other recent publications have implicated the AAA-ATPase p97/VCP in controlling the Ub-DDR (Acs *et al*, 2011; Meerang *et al*, 2011). Meerang *et al* (2011) demonstrated that RNF8 can catalyse the formation of K48-linked as well as K63-linked ubiquitin chains at the sites of DNA damage, and that this mediates the recruitment of p97/VCP and its cofactors. Furthermore, they went on to show that p97/VCP may play a role in the removal and/or disassembly of K48-linked ubiquitin chains formed during the DDR, as its depletion increased the abundance of these chains at sites of damage. Moreover, Meerang *et al* (2011) demonstrated that loss of p97/VCP also reduced, albeit mildly, the extent of 53BP1 relocalisation—which could suggest that efficient unmasking of the H4K20Me₂ mark after DNA damage, as observed in the new work by Malette *et al*, may not only require RNF8-dependent JMJD2A/B polyubiquitylation, but also the polyubiquitin-coupled ‘removase’ activity of p97/VCP for the active extraction of modified JMJD2A/B from chromatin prior to its degradation.

The possibility that in addition to JMJD2A/B, other proteins capable of binding and masking the H4K20Me₂ mark may also require removal and/or degradation upon DNA damage is supported by the recent findings of Acs *et al* (2011), who demonstrated that p97/VCP is required for the RNF8- (and to a lesser extent RNF168-) dependent removal of the H4K20Me₂-binding protein L3MBTL1 from damage sites (Figure 1). However, whether L3MBTL1 can also mask the H4 methylation mark and as a consequence block its recognition by 53BP1 was not yet demonstrated. If this were the case, then overexpression of L3MBTL1 would be expected

to inhibit radiation-induced 53BP1 foci formation, while L3MBTL1 depletion should be capable of rescuing 53BP1 recruitment defects in cells lacking RNF8 or RNF168; it will certainly be interesting to see these predictions are substantiated by future work. It also remains to be determined if loss of L3MBTL1 from damage-associated chromatin requires ubiquitin-dependent degradation or ubiquitin-dependent chromatin remodelling. Therefore, it is at this stage difficult to definitively conclude whether L3MBTL1 and JMJD2A/B function similarly to mask sites of histone methylation from 53BP1 in the absence of DNA damage. One obvious question arising from these studies is, why would cells utilise multiple methyl-lysine-binding proteins to negatively regulate 53BP1 relocalisation? It is tempting to speculate that 53BP1 recruitment to sites proximal to a DSB may be differentially regulated, depending on the chromatin context where the break is located, that is, heterochromatic versus euchromatic regions. In this way, the existence of non-redundant methyl-mark masking proteins could be explained by them functioning separately in distinct chromatin compartments.

Clearly, we still have much to learn about the involvement of 53BP1 in the DNA damage response and the mechanisms underpinning its recruitment to sites of DNA damage; despite the exciting advances from the discussed recent studies, if we have learned anything from 53BP1 over the years it is likely that this is still just the tip of the iceberg.

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Conflict of interest

The author declares that he has no conflict of interest.

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