Mixed ubiquitin chains regulate DNA repair

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Diverse linkage in polyubiquitin chain structure gives cells an unparalleled complexity to virtually modulate all aspects of cell biology. Substrates can be covalently modified by ubiquitin chains of different topology. Proper DNA damage response takes advantage of this regulatory system and heavily relies on ubiquitin-based signaling. Moreover, increasing evidence suggests that chain specificity dictates DNA repair outcome. In this issue of *Genes & Development*, Wu and colleagues (pp. 1702–1717) show that Cezanne and Cezanne2, two paralogous deubiquitinating enzymes that are recruited to sites of DNA damage, ensure proper local polyubiquitin chain composition for downstream DNA repair protein assembly. Their study offers a key insight into the mechanism of crosstalk between linkage-specific ubiquitylation at DNA damage sites, while simultaneously raising important questions for future research.

The ubiquitin landscape is key for propagating and fine-tuning the DNA damage response (DDR) as shown by ever-growing evidence over the last decade (Schwertman et al. 2016). Target proteins can be polyubiquitylated by ubiquitin chains in which ubiquitin moieties are linked through their seven lysine residues (Lys6, 11, 27, 29, 33, 48, and 63) or through the N-terminal methionine, resulting in chains with distinct topologies that orchestrate different biological outputs (Komander and Rape 2012; Swatek and Komander 2016; Haakonsen and Rape 2019). Homotypic chains are connected through the same ubiquitin residue, whereas heterotypic chains are built up by ubiquitin monomers linked through different residues. However, chain complexity does not stop here since these polymers could branch out when a subunit is modified on two or more residues at the same time (Haakonsen and Rape 2019). Proteasomal degradation through Lys48-linked ubiquitin chains, as well as signaling events driven by Lys63-linked ubiquitin chains, have been attributed to proper DDR signaling. Although other linkage-specific ubiquitylation events have been documented during DDR (Elia et al. 2015), their functional roles remain largely unclear.

In response to double stranded breaks (DSBs), one of the earliest enzymes involved is the RING Finger ubiquitin ligase 8 (RNF8), which decorates histone H1 and L3MBTL2 with Lys63-linked ubiquitin chains, generating a platform for the recruitment of downstream repair proteins (e.g., 53BP1 and BRCA1) (Uckelmann and Sixma 2017; Nowsheen et al. 2018). Subsequently, through the combined action of RNF8 and RNF168, Lys63-linked, Lys48-linked, and Lys27-linked ubiquitin chains can arise at DSBs (Lok et al. 2012; Gatti et al. 2015). Recently, RNF8 was also shown to propagate Lys11 chains at DSBs with the help of the ubiquitin-conjugating enzyme, UBE2S, in an ATM-dependent fashion. Interestingly, this activity of RNF8 is antagonized by the deubiquitinating enzyme (DUB) Cezanne, which has a high specificity for Lys11 polyubiquitin chains (Mevissen et al. 2013; Paul and Wang 2017). Local enrichment of these chains has been proven to be necessary for proper DNA damage-induced transcriptional silencing, complementing H2A monoubiquitylation by the cPRC1 and ncPRC1.1 complexes (Wang et al. 2004; Shanbhag et al. 2010; Rona et al. 2018). Just by looking at RNF8, RNF168, and the different types of polyubiquitin chains they build, one could argue for the existence of a potential crosstalk between linkage-specific ubiquitination at the site of DNA damage. Therefore, it is important to gain a better understanding of how the polyubiquitin chain profile at DNA damage sites is regulated.

Wu et al. (2019) showed how local Lys11 and Lys63 polyubiquitin chain composition at DSBs dictate downstream DNA repair protein recruitment. The polyubiquitin chain profile generated by the ligase activity of RNF8 (either through utilizing UBCH8 for Lys48-linked chains, UBC13 for Lys63-linked chains, or UBE2S for Lys11-linked chains) is fine-tuned by the Cezanne and Cezanne2, novel "readers" and "erasers" of the polyubiquitin signal at DSBs.

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The nucleating role of Lys63-linked chains is demonstrated by the fact that there is no proper DSB repair without UBC13 (Zhao et al., 2007). Therefore, identification of novel responders to these emerging chains might give a better understanding of DNA repair processes. To this end, Wu et al. (2019), using a protein array-based screening, identified the ubiquitin-associated (UBA) domains of Cezanne and Cezanne2 as novel and specific binders of Lys63-linked polyubiquitin chains. Accordingly, Cezanne is recruited to laser-induced damage sites in a UBA domain-dependent manner and is necessary for proper DNA damage repair. The authors had previously shown that UBE2S depletion does not impair the recruitment of 53BP1 and components of the BRCA1-A complex (BRCA1, Rap80, and Ambraxas) to ionizing radiation induced foci (IRIF). In fact, the recruitment of these proteins relies on the Lys63-linked chains, not Lys11-linked chains. However, depletion of Cezanne led to fewer number of cells with Rad18 and BRCA1-A IRIF, while not affecting the formation of γH2A.X, 53BP1, and RNF168 foci. Rescue experiments proved that this function of Cezanne relies on its UBA domain and its Lys11-specific DUB activity. In favor of this hypothesis, codepletion of UBE2S rescued the phenotypes of Cezanne knockdown cells: (1) the formation of BRCA1-A foci, (2) the cell's ability to repair DNA lesions (assayed by the disappearance of γH2A.X foci over time), and (3) IR sensitivity. In vitro binding assays showed that the ubiquitin interacting motif of Rap80 is inhibited by Lys11-linked ubiquitin moieties present in a Lys63-linked chain. Therefore, Cezanne seems to facilitate the recruitment of downstream repair proteins by limiting mixed Lys11/Lys63 branched polyubiquitin chains in favor of chains with Lys63 linkages. The authors also looked at the contribution of Cezanne2, a paralog of Cezanne with similar Lys11-specific activity and a similar UBA motif capable of binding Lys63-linked chains. Cezanne2 does not seem to be redundant with Cezanne, but facilitates the function of the latter. On its own it is unable to affect the formation of BRCA1-A IRIF, as well as other phenotypes associated with Cezanne depletion. However, when the authors codepleted Cezanne and Cezanne2, they consistently observed more severe phenotypes.

The work by Wu et al. (2019) raises several questions for the field. What does regulate the interaction of RNF8 with at least three different ubiquitin-conjugating enzymes? How does the ATM-dependent, RNF8-mediated Lys11-linked ubiquitin chains act together with the PARP1-dependent, cPRC1- and ncPRC1.1-mediated transcriptional repression at DNA lesions? Do Cezanne and Cezanne2 activity antagonize local transcriptional repression? What other ubiquitin chains contribute to DNA repair and in what way? And, finally and more generally, what are the other readers and writers of this "ubiquitin-code" at DSBs?

The work described here shows that the ubiquitin signal at DNA damage sites is even more complex than assumed before and reminds us that polyubiquitin-linkage specificity dictates signaling events that need to be investigated in detail.

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