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RESEARCH HIGHLIGHT

H1 provides the missing link

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Adding to the Histone Code at DNA double-strand breaks, Mailand and colleagues now uncover non-degradative ubiquitin marks on linker histone H1 as key signaling intermediates in the DNA damage signal transduction cascade.

Ever since the original observation that histone H2AX mobilizes DNA repair factors into nuclear foci [1], the inception of a "Histone Code" in orchestrating DNA damage signal transduction and repair processes has sparked immense interests to define histone modifications at DNA damage sites.

Cells utilize a growing collection of histone modifications to coordinate chromatin responses at DNA double-strand breaks (DSBs), one of the most cytotoxic forms of DNA lesions. In particular, ubiquitin adducts deposited at the vicinity of DSBs provide docking sites for a cohort of DNA repair factors. Key to the formation of these DSB-associated ubiquitin adducts are the E3 ubiquitin ligases RNF8 and RNF168 [2-7]. RNF8 and RNF168 docking at DSBs follow a hierarchical sequence, and while the pair of E3 enzymes have been envisaged to, in concerted efforts, assemble DNA repair factors by ubiquitylating core histone proteins, the molecular basis that underlies their strict order of appearance at DSBs has remained elusive.

RNF168, also known as the RIDDLE syndrome protein, was first described in a patient with radiosensitivity and immunodeficiency, clinical manifestations that are characteristic of defective responses to DSBs [6, 8]. In addition to possessing a signature RING domain, the E3 ubiquitin ligase RNF168 also harbors two clusters of protein-interacting motifs (the authors referred to these as UDM1 and UDM2; see Figure 1) that supported its accrual at DNA damage sites [6, 7, 9, 10]. Notably, although both UDMs were each endowed with ubiquitin-binding properties, they were unique in having evolved to target specialized ubiquitin structures at DSBs. Indeed, while UDM2 specifically interacted with ubiquitylated H2A histones, division of labor has it that UDM1 was important in bringing RNF168 to DSBs via an hitherto unknown factor [9].

Notwithstanding the formidable nature in singling out this factor amongst the multiplicity of ubiquitin structures at DSBs, the Mailand team embarked on the mission to tease out exactly how RNF168 migrates to DSBs [11]. Because RNF168 is recruited to DSBs via an RNF8-UBC13-dependent ubiquitylation process [6, 7], and the E3-E2 pair encoded the major K63-based ubiquitylating activity at DSBs, the authors took an unbiased proteomics approach and quantitatively compared the abundance of K63-ub conjugates in control cells with those that have been exposed to ionizing radiation (IR). To do so, they utilized an ubiquitin-binding cassette that bears remarkable specificity for K63-based ubiquitin polymers, and fished out chromatin factor(s) that showed enrichment after IR treatment. Much to anyone's surprise, linker histones, and not core histone proteins, were major *bona fide* targets of K63-linked ubiquitylation reactions!

To examine whether K63-ubiquitylated H1 was duly responsible for anchoring RNF168 onto DSBs, the authors depleted H1 using an RNA interference approach, and found that H1 downregulation phenocopied RNF8 and UBC13 deficiencies. The observations that silencing H1 impaired assembly of RNF168 and its downstream DNA repair factors at DSBs argue favorably that K63-based ubiquitin-modified linker histones were the major chromatin components that targeted RNF168 to DSBs. The authors went on to show that RNF168 UDM1, previously predicted to bind to certain RNF8-dependent ubiquitylated structure [9], specifically associated with ubiquitylated species of histone H1, highlighting the preposition that linker histones represent major RNF8-UBC13-ubiquitylated chromatin substrates. Together, these results provide unequivocal evidence in support of a key role of linker histones as signaling intermediates in the ubiquitin-driven DSB signal transduction cascade, and implicate ubiquitylated H1 as new histone marks at DSBs.

What remains to be understood of the RNF8-RNF168 axis in DSB responses? From a structural perspective, it remains largely speculative how the RNF168 UDMs impart such high-level specificity for their respective histone targets. In particular, how exactly do the tandem protein-interacting motifs within UDM1 orientate when bound to ubiquitylated linker histones? Since deletion of either UMI or MIU within the UDM1 cluster did not noticeably affect RNF168 damage foci [9], do they play redundant roles in RNF168 targeting onto DSBs?

Moreover, the biochemistry that pertains to the RNF168-ubiquitylating activity *in vivo* remains obscure. Does RNF168 pair up with a specific E2 ubiquitin conjugating enzyme to

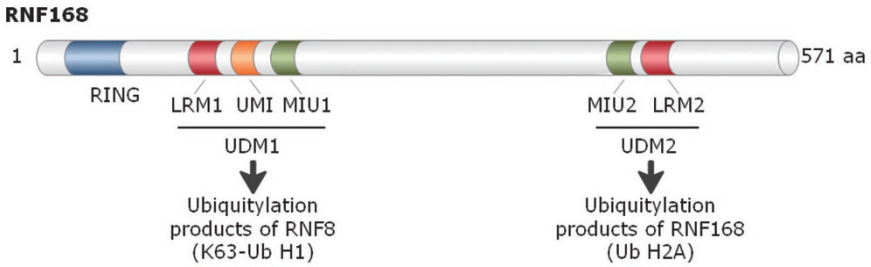

Figure 1 The RING finger protein 168 gene (RNF168) encodes a protein of 571 amino acids. RNF168 has six conserved motifs organized in three major regions: the really interesting new gene (RING) domain at N-terminus; cluster of protein-interacting motif 1 (UDM1) at N-terminal region and cluster of protein-interacting motif 2 (UDM2) at C-terminal region. UDM1, which interacts with ubiquitylation products of RNF8 especially histone H1 ubiquitylated at Lysine 63 (K63-Ub H1), contains LR motif 1 (LRM1), UIM- and MIU-related ubiquitin binding domain (UMI) and motif interacting with ubiquitin 1 (MIU1). UDM2, which interacts with ubiquitylation products of RNF168 especially ubiquitylated H2A-type histone (Ub H2A), contains MIU2 and LRM2 motifs. UIM, ubiquitin-interacting motif; aa, amino acids.

promote chromatin ubiquitylation at DSBs [12]? Given the multiplicity of ubiquitin structures at DSBs, it is also formally possible that RNF168 interacts with different E2s and targets different substrates to orchestrate the furcated DSB signal transduction sub-pathways. Addressing these outstanding issues will likely require cross-disciplinary approaches, but what seems clear is that linker histones are entering center stage in contributing to the Histone Code that dictates all aspects of biological processes.

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