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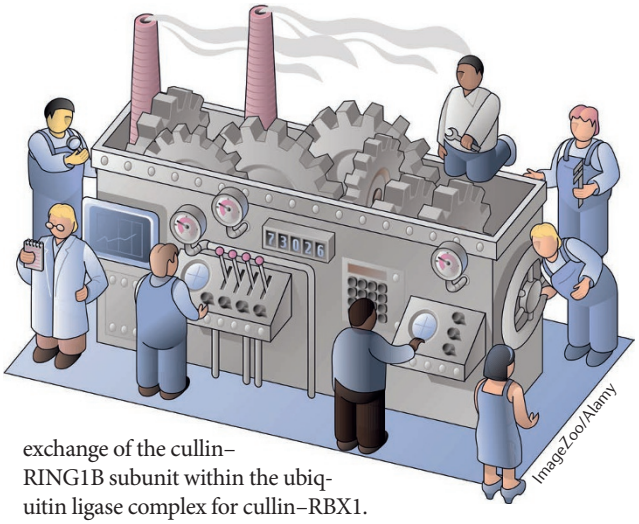

DNA DAMAGE RESPONSE

Controlling ubiquitylation at DNA lesions

To counteract various genotoxic insults and prevent genomic instability, cells have evolved intricate DNA damage response (DDR) pathways. Ubiquitylation was shown to have important roles in the repair of DNA damage; however, the mechanisms of ubiquitin signalling during the DDR are only partially understood. Recent work from two independent laboratories sheds new light on the regulation of ubiquitylation in different DNA damage repair pathways.

Wang *et al.* studied the DDR of double-strand breaks (DSBs), which relies on histone H2A ubiquitylation at Lys13 and Lys15 (H2AK13,15ub) for the recruitment of the repair machinery to the lesion. The authors performed a short hairpin RNA (shRNA) screen and uncovered a new deubiquitylase, ubiquitin-specific peptidase 51 (USP51), whose depletion was associated with an increase in spontaneous DDR. Notably, both silencing and overexpression experiments in cultured cells revealed that USP51 negatively regulates the levels of ubiquitylated H2A, and *in vitro* experiments validated that USP51 functions as a deubiquitylase of H2AK13,15ub.

Analysis of cells that were exposed to ionizing radiation to induce DSBs revealed that irradiation first caused a decrease in the chromatin occupancy of USP51, followed by a robust increase in USP51 recruitment to chromatin. Concomitantly, chromatin-bound H2AK13,15ub was prominent following irradiation but nearly undetectable 4 hours later. Notably, USP51 depletion accelerated the formation of H2AK13,15ub immunopositive foci following irradiation, and these foci were not efficiently resolved. This increase and persistence of H2AK13,15ub was associated with elevated recruitment of DSB repair factors to the DNA, enhanced DDR and increased cellular sensitivity to radiation. Collectively, these results indicate that USP51 is a novel regulator of DDR, which, through controlling H2A ubiquitylation levels, ensures timely and efficient repair of DSBs.

Gracheva *et al.* investigated ubiquitylation-mediated regulation of nucleotide excision repair (NER). NER removes ultraviolet (UV) radiation-induced DNA lesions through a mechanism involving H2A ubiquitylation mediated by the ubiquitin ligase complex composed of damage-specific DNA-binding protein 1 (DDB1), DDB2, RING-box 1 E3 ubiquitin protein ligase (RBX1) and cullin 4A (CUL4A) or CUL4B. The authors revealed that yet another ubiquitin ligase, RING finger protein 1B (RING1B), is involved in H2A ubiquitylation following UV irradiation, and biochemical analyses demonstrated that in this context, RING1B forms a previously unrecognized complex with DDB1, DDB2 and CUL4B.

As previous studies identified zuotin-related factor 1 (ZRF1) as a negative regulator of RING1B-chromatin interactions, its role in NER was next investigated. Interestingly, ZRF1 depletion enhanced RING1B occupancy at the chromatin following UV treatment but impaired the recruitment of CUL4A to the chromatin. This, together with *in vitro* experiments, indicated that ZRF1, through displacing RING1B from chromatin, acts at UV-induced lesions to mediate the exchange of the cullin–RING1B subunit within the ubiquitin ligase complex for cullin–RBX1. This suggested that ZRF1 functions as a molecular switch, which orchestrates ubiquitylating activity during NER. In line with this, ZRF1 depletion impaired ubiquitylation of xeroderma pigmentosum, complementation group C (XPC), which is a main initiator of NER and a known substrate of RBX1 ubiquitylation. The authors propose that the RING1B-containing ubiquitin ligase complex is recruited to UV-mediated lesions early during NER and ubiquitylates H2A, thereby providing an initial platform for the recruitment of NER factors. However, for proper execution of NER, this ubiquitin ligase complex needs to be remodelled through the ZRF1-mediated exchange of RING1B for RBX1, which can then ubiquitylate further targets.

In sum, both studies highlight that the regulation of ubiquitylation at DNA damage sites is highly complex and dynamic. It will now be important to integrate results obtained by studying different types of DNA damage in diverse models to gain a more holistic understanding of how ubiquitin-mediated signalling contributes to genome integrity.

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> “ubiquitylation at DNA damage sites is highly complex and dynamic”

ORIGINAL ARTICLES Wang, Z. *et al.* USP51 deubiquitylates H2AK13,15ub and regulates DNA damage response. *Genes Dev.* **30**, 946–959 (2016) | Gracheva, E. *et al.* ZRF1 mediates remodeling of E3 ligases at DNA lesion sites during nucleotide excision repair. *J. Cell Biol.* **213**, 185–200 (2016)
FURTHER READING Schwertman, P., Bekker-Jensen, S. & Mailand, N. Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat. Rev. Mol. Cell Biol.* http://dx.doi.org/10.1038/nrm.2016.58 (2016) | Marteijn, J. A. *et al.* Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat. Rev. Mol. Cell Biol.* **15**, 465–481 (2014)