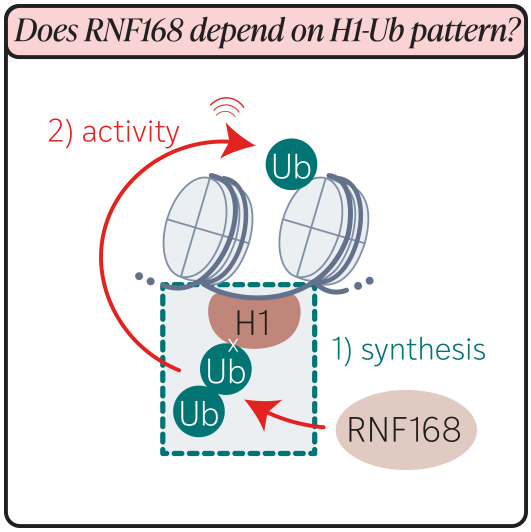
A chemical biology approach to decipher chromatin ubiquitylation by RNF168

P. Franz[1], C. Delvaux de Fenffe[2], B. Fierz[1]*

[1]EPFL, [2]Utrecht University

Recognition, integration and propagation of post-translational modifications (PTMs) on histone proteins play a crucial role in the DNA damage response. In particular, the ubiquitylation cascade mediated by the E3 ubiquitin ligase RNF168 is central in promoting homologous recombination (HR) and nonhomologous end-joining (NHEJ) following DNA double-strand breaks. RNF168 is recruited to DNA damage sites by binding to ubiquitylated linker histone H1 [1], where it further ubiquitylates H2AK13-15 [2], thereby acting as reader and writer of chromatin PTMs. However, the exact mechanisms underlying RNF168 regulation remain elusive, as the limited availability of specifically poly-ubiquitylated H1 restricts mechanistic research on a molecular level.

Therefore, we develop an approach employing expressed proteins, chemical derivatization, and in vitro reconstitution strategies to poly-ubiquitylate H1, with site- and chain-length specificity. Thus, we tightly control and systematically vary the chromatin fiber modification state. Using such ubiquitylated 'designer chromatin', we found that the ubiquitylation activity of RNF168 correlates with the chromatin ubiquitylation state. Currently, we are working on incorporating synthetic ubiquitylated H1 in cells via bead loading [3] to investigate the dependence of RNF168 activity in a physiological environment. This will reveal the chromatin-state-dependent activity of RNF168 in DNA damage repair pathways on a molecular level.



[1] T. Thorslund, A. Ripplinger, S. Hoffmann, T. Wild, M. Uckelmann, B. Villumsen, T. Narita, T. K. Sixma, C. Choudhary, S. Bekker-Jensen, N. Mailand, *Nature* **2015**, *527*, 389–393.
[2] F. Mattiroli, J. H. A. Vissers, W. J. van Dijk, P. Ikpa, E. Citterio, W. Vermeulen, J. A. Marteijn, T. K. Sixma, *Cell* **2012**, *150*, 1182–1195.
[3] Mann, G., Sadhu P., Brik A. *ChemBioChem* **2022**, 23, e2022001.