Structural characterisation and protein tool generation for investigating SLF1 complexes in replication-coupled DNA repair.

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Understanding how cells repair damaged DNA is highly relevant to cancer biology. DNA damage, such as interstrand crosslinks, can stall the replication fork, prompting the engagement of DNA damage response (DDR) proteins, such as SMC5/6 Localisation Factor (SLF) 1. SLF1 interacts with RAD18 (a ubiquitin ligase required for post-replication repair of damaged DNA); a specific histone marker present in replicated DNA; and SLF2, to regulate recruitment of the Structural Maintenance of Chromatin (SMC) complex, SMC5/6. However, the structural mechanism of the SLF1-SLF2 interaction network remains unclear, with a lack of protein tools to modulate their function within the DDR. Our study reveals that SLF1's tandem BRCA1 C Terminal (tBRCT) domain interacts directly with phosphorylated RAD18 (S442, S444) via a conserved tBRCT phospho-recognition mechanism. Additionally, we determined the crystal structure of the Ankyrin Repeat Domain (ARD) of SLF1 bound to histone H4 peptide unmethylated at lysine 20 (H4K20me0). Isolation of high-affinity Affimers, small non-antibody binding proteins, against the tBRCT and ARD of SLF1 also provides tools to probe the significance of SLF1 complexes *in vivo*. This research enhances our understanding of SLF1 function during replication-dependent repair and offers potential for developing DNA repair inhibitors for sensitising cancer cells to existing chemotherapeutics.