UBIQUITIN SIGNALLING

Post-translational modification with ubiquitin (Ub) initiated by sequential actions of Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3) regulates diverse cellular processes, including signal transduction, cell cycle progression, apoptosis and gene transcription. Deregulation in the Ub pathway is often associated with human pathogenesis, including cancer. Our group uses structural biology and biochemical approaches to study the enzymes in the Ub pathway to understand their regulation, mechanistic function and mutation-induced deregulation. We anticipate that the knowledge gained from our structural studies will assist in the development of selective therapeutic targets within the Ub pathway.

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes, namely E1, E2 and E3 (Figure 1). E1 adenylates Ub’s C-terminus in the presence of Mg²⁺ and ATP, forming a E2–Ub thiolester intermediate (– indicates the thioester bond). E3 generally consists of an E2–binding module (HECT, RING, RBR or U-box domain) and a protein–protein interaction domain that can recruit the substrate directly or indirectly.

Deregulation in CBL ubiquitin ligase

CBL proteins (CBLs) are RING E3s that negatively regulate RTKs, tyrosine kinases and other proteins by promoting their ubiquitination and degradation by the proteasome or lysosome. Mutations in CBL have been observed in human patients with myeloproliferative diseases. Investigating the mechanism by which CBL mutants exert oncoesis, we showed that CBL mutants inactivate E3 activity, thereby functioning as an adaptor to recruit other proteins such as CIN85 to elicit oncogenic signaling. Mechanistically, CBL mutants bound to receptor tyrosine kinases such as EGFR, which led to phosphorylation of CBL mutants’ C-terminal tyrosines. Phosphorylated tyrosines induced conformational changes that enabled CBL mutant–CIN85 interaction, whereas the CBL mutants could not ubiquitinate CIN85, leading to deregulated CBL-CIN85 signalling which altered transcriptome landscape, that in tumour cells, impaired K48-linked polyUb chain formation. To visualise this reaction, we chemically trapped UBE2K covalently linked to donor Ub and acceptor K48-linked di-Ub, where the C-terminus of donor Ub was linked to UBE2K’s active site cysteine and K48 of the acceptor di-Ub was linked to an UBE2K active site residue. We then determined the crystal structure of this cross-linked UBE2K complex and a RING E3 (Figure 3). We performed various NMR analyses and mutagenesis coupled with biochemical assays to validate our structure and demonstrated that our structure approximated the transition state of the K48-linked Ub chain synthesis. Our structure revealed that UBE2K’s active site residues and the C-terminal Ub associated (UBA) domain bound the acceptor di-Ub and oriented its K48 toward the UBE2K–Ub allosteric site for catalysis. Importantly, the UBE2K active site was not conserved in other E2s, indicating K48-linked specificity whereas the UBA domain functioned to stabilise the conformational flexibility of acceptor Ub. Unexpectedly, our structure unveiled multiple Ub acceptor di-Ub chains linked to the UBE2K active site, suggesting that inhibitors that target MDM2 E3 activity could overcome to accelerate Ub chain extension. Moreover, we showed that UBA domain exhibited a preference for K63-linked polyUb chain as the acceptor and thereby promoted branched K48–K63 polyUb chain formation. Our work explains the molecular basis for K48-linked Ub chain synthesis and how UBA domain promotes polyUb chain formation (Nakason et al., 2022, Nature Chemical Biology).