Structural insight on ubiquitination of NEMO for canonical activation of NF-κB

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Activation of canonical NF-κB signaling is mediated through the ubiquitination of the NF-κB essential modulator (NEMO). LUBAC is a E3 ubiquitin ligase that catalyzes the linear ubiquitination of NEMO. LUBAC is a multisubunit enzyme which consists of HOIP, HOIL-1L and SHARPIN. HOIP is the catalytic subunit of LUBAC which consists of a catalytic RING-in between-RING domain followed by the linear chain determining domain (RBR-LDD). RBR-LDD is known to synthesize linear ubiquitin chains. HOIP also consists of a NZF1 domain which has been identified to recognize NEMO as a substrate for ubiquitination. To get an insight on the LUBAC-mediated ubiquitination of NEMO, we crystallized NEMO in complex with linear diubiquitin and HOIP-NZF1. To obtain the crystal structure, the proteins were individually expressed as GST-fusion proteins in E.coli and purified using chromatography techniques. The three proteins were mixed in equimolar ratio for crystallization. The crystals were obtained after six weeks in sitting drops containing 0.1 M Tris-HCl (pH 8.5), and 22 % v/v PEG 11 Smear Broad. In this heterotrimeric structure, HOIP NZF1 binds to NEMO and ubiquitin, simultaneously. The C-terminal tail of the ubiquitin is oriented towards the ubiquitination site on NEMO. This suggests that the LUBAC recognizes the monoubiquitinated NEMO and recruits the catalytic domain, thereby facilitating linear ubiquitin chain elongation. We also determined the binding affinities of HOIP NZF1 with NEMO and ubiquitin using Surface Plasmon Resonance. HOIP-NZF1 binds to NEMO and ubiquitin with a low binding affinity KD of 86.7 μM and 5.2 μM respectively, suggesting that the binding is preferential to the ubiquitinated NEMO. HOIP overall does not initiate the ubiquitination process, but catalyzes the chain elongation on the mono ubiquitinated substrate. HOIL-1L could be responsible for priming of the first ubiquitin on the substrate, but the exact mechanism is yet to be explored

Crystal structure of NEMO CoZi in complex with HOIP NZF1 and linear di-ubiquitin

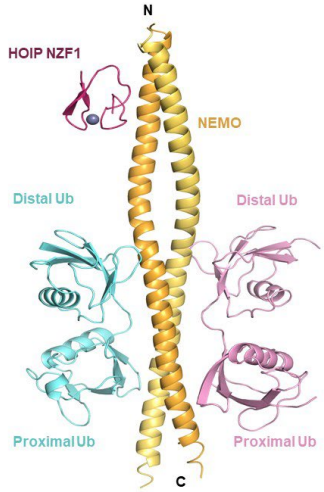

Figure 1