Specific Recognition of Linear Polyubiquitin Chains by NF-κB Essential Modulator (NEMO)

NEMO (NF-κB essential modulator) is a regulatory subunit of the IKK (IκB kinase) complex which phosphorylates IκB (inhibitor of NF-κB), and activates the transcription factor NF-κB (nuclear factor kappa B). Linear polyubiquitin chains play an important role in the activation of NF-κB through their binding to NEMO. The crystal structure of NEMO in complex with linear diubiquitin chains reveals that two diubiquitins symmetrically bind to either side of the coiled-coil NEMO homodimer, making extensive interaction with the linkage of the linear ubiquitin chains, and enabling linkage-specific recognition.

NF-κB is a transcription factor which activates the transcription of various genes involved in processes such as inflammation, cell growth, and apoptosis. Canonical NF-κB signalling requires phosphorylation of the inhibitor of NF-κB (IκB) by the IκB kinase (IKK) complex, which consists of three subunits – IKKα, IKKβ and NEMO (also known as IKKγ). Recently, NEMO was found to be modified by a novel ubiquitin chain, a linear polyubiquitin chain which is formed by normal polypeptide linkage between the carboxy terminal glycine and amino terminal methionine. This modification was found to be important for NF-κB signaling [1].

The CoZi (coil zipper) domain centrally located in the amino acid sequence of NEMO is responsible for the interaction of NEMO with linear polyubiquitin. To gain insight into the mechanism of IKK activation, we determined the structure of mouse NEMO CoZi domain comprising residues 250-339 at 2.8 Å resolution. NEMO CoZi forms a parallel coiled-coil homo-dimer with an overall length of 120 Å [Fig. 1(a, b)] [2]. Pro292 induces a kink in the overall helical structure, which gives rise to a deviation from perfect two-fold symmetry. However, when dividing the structure into N-terminal (260 to 291) and C-terminal (293 to 330) fragments, each subdomain displays an approximate dyad symmetry around the coiled-coil axes so that the Cα atoms of the two protomer subdomains can be superimposed with RMS deviations of 0.88 Å and 0.92 Å, respectively [Fig. 1(c)].

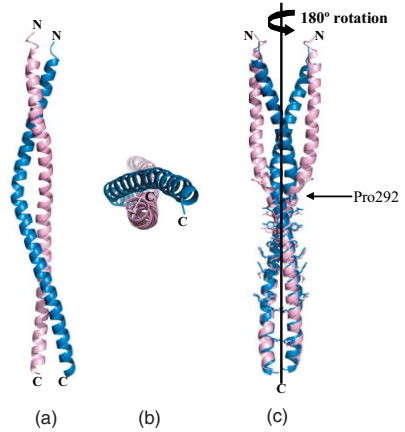

Figure 1
Crystal structure of the coiled-coil dimer of the NEMO CoZi domain(a)Side view (b) Top view (c) Superimposition of the structures rotated by 180 degrees.

For co-crystallization, linear diubiquitin, consisting of two tandem ubiquitins (where the N-terminal and C-terminal ubiquitins are respectively called distal and proximal ubiquitins), was used as a minimum unit of the linear polyubiquitin chains. The structure of NEMO CoZi in complex with diubiquitin was determined at 2.7 Å resolution [2]. Each asymmetric unit of the *C*2 crystal lattice contains one complex in which NEMO protomer and diubiquitin are present with a 2:2 stoichiometry. As expected from the pseudo two-fold symmetry of the homodimeric structure of NEMO CoZi alone, the NEMO dimer accommodates two diubiquitins, one on either side of the coiled-coil [Fig. 2(a, b)]. Both NEMO protomers contribute to each of the diubiquitin binding surfaces, which are comprised of residues Glu289 to Glu320.

Diubiquitin binding induces a slight unwinding of the coiled-coil of NEMO CoZi, making the kink in NEMO caused by Pro292 rather straight by the interaction with distal ubiquitin [Fig. 2(a)]. These structural adjustments are likely to change the overall shape of NEMO, which may alter the interaction of the upstream region of NEMO with the catalytic subunits of the IKK complex.

The two diubiquitins in the complex adopt very similar conformations (with a RMS deviation of 1.17 Å for the Cα positions of residues 1-146). However, the distal and proximal ubiquitins bind NEMO in a remarkably divergent fashion [Fig. 2(a, c)]. The distal ubiquitin contacts NEMO using its C-terminal tail and the canonical hydrophobic Ile44 surface, whereas the proximal ubiquitin employs residues located adjacent to, but not overlapping with, the Ile44 patch, which engage mainly in polar interactions with a single NEMO protomer [Fig. 2(c)].

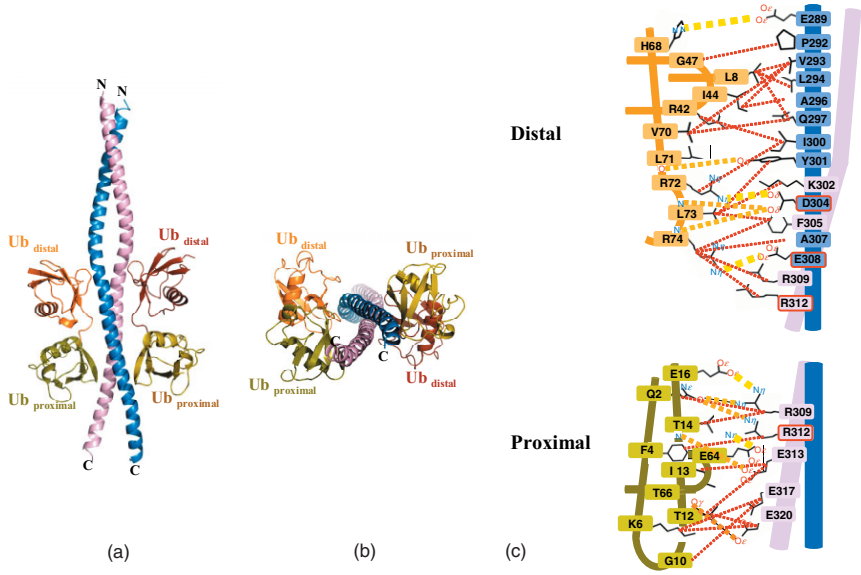

Figure 2
Crystal structure of the NEMO dimer in complex with two linear polyubiquitin chains. Each chain consists of distal and proximal ubiquitins (a) Side view (b) Top view. (c) Schematic drawing of the interface between NEMO and the linear polyubiquitin chains. Different ubiquitin surfaces are used for the distal and proximal interactions with NEMO. These interactions are possible only with linear polyubiquitin chains, and not with other linkages.

Notably, the linkage between distal and proximal ubiquitins, comprising Arg72, Leu73 and Arg74 of distal ubiquitin and Gln2 of proximal ubiquitin, is extensively recognized by NEMO [Fig. 2(c)]. Therefore polyubiquitins with other linkages are not able to bind in the same way as linear polyubiquitin, thus enabling the selectivity of NEMO to the linkage type of polyubiquitin chains.

Mutational analyses based on the complex structure confirmed that both distal and proximal ubiquitin bindings are necessary for full NF-κB activation. Importantly, mutations found in anhidrotic ectodermal dysplasia patients with immunodeficiency occur at the NEMO residues which recognize the linkage of linear polyubiquitin chains. These findings reveal that the recognition of linear polyubiquitin chains by NEMO is important for the activation cascade of NF-κB. The structure provides us with an understanding of polyubiquitin chain selectivity and useful insights for the development of therapies targeting NF-κB.

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