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Structural studies of NEMO and TRAF6: implications in NF-κB activation

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NF-κB proteins are evolutionarily conserved master regulators of immune and inflammatory responses (Gilmore, 2006). They play critical roles in a wide array of biological processes including innate and adaptive immunity, oncogenesis and development. They are activated in response to ligation of many receptors including T-cell receptors, B-cell receptors, members of the tumor necrosis factor (TNF) receptor superfamily and the Toll-like receptor/interleukin-1 receptor (TLR/IL-1R) superfamily.

The Ser/Thr-specific IκB kinase (IKK) signalosome is at the bottleneck for NF-κB activation because activated IKK phosphorylates IκB, leading to Lys48 (K48)-linked polyubiquitination and subsequent degradation of IκB by the proteasome (Scheidereit, 2006). The freed NF-κB dimers translocate to the nucleus to mediate specific target gene transcription. The IKK signalosome contains the kinase, IKKα and/or IKKβ, and the highly conserved regulatory protein NEMO (also known as IKKγ or FIP-3) (Scheidereit, 2006). IKK activity relies on the interaction between the kinase and NEMO. In cells lacking NEMO, IKKα and IKKβ cannot be activated by any of the classical NF-κB inducers. Sequence analysis of NEMO indicates a high helical content with an N-terminal kinase-binding domain (KBD), three coiled coil regions (CC1, CC2 and LZ) and a zinc finger (ZF) domain (Scheidereit, 2006).

Recent studies have shown that the ability of the CC2-LZ region of NEMO to interact with both linear and Lys63-linked polyubiquitin chains is crucial for IKK recruitment to receptor signaling complexes and for IKK activation (Ea et al., 2006; Tokunaga et al., 2009; Wu et al., 2006). In an effort to understand how NEMO interacts with both linear and Lys63-linked polyubiquitin chains, we performed and reported both biochemical and structural studies on the CC2-LZ region of NEMO (Lo et al., 2009). We showed that CC2 and LZ together form a continuous coiled coil structure with a prominent bent at the junction between CC2 and LZ. Affinity measurement by isothermal titration calorimetry showed that CC2-LZ interacts with linear diubiquitin and Lys63-linked diubiquitin at dissociation constants of 1.4 μM and 131 μM, respectively. No interaction could be detected between NEMO and monoubiquitin.

We used structure-based mutagenesis and nuclear magnetic resonance (NMR) to map the interaction between CC2-LZ and linear or Lys63-linked diubiquitin. These experiments revealed that the binding sites for diubiquitins at LZ are composites of both chains and that the proximal and distal ubiquitins interact with CC2-LZ differently. For tandem diubiquitin, the distal ubiquitin uses the conserved hydrophobic patch and the C-terminal tail while the proximal ubiquitin uses an adjacent surface patch. For Lys63-linked diubiquitin, the proximal ubiquitin uses its conserved hydrophobic patch while the distal ubiquitin mostly employs the C-terminal arm including the K63-linkage residue. These studies uncover the energetics for mutual recognition of NEMO and diubiquitins.

In a separate study, we elucidate how TRAF6 acts as an E3 in mediating Lys63-linked polyubiquitination.

Tumor necrosis factor (TNF) receptor associated factors (TRAFs) act upstream of IKK in the same receptor-mediated NF-κB signaling pathways. TRAF6, in particular, has been shown to mediate Lys63 (K63)-linked polyubiquitination via its N-terminal RING and zinc finger domains. To elucidate the ubiquitin ligase (E3) function of TRAF6, we reported the crystal structures of TRAF6, alone and in complex with the ubiquitin conjugating enzyme (E2) Ubc13 (Yin et al., 2009). The RING and zinc fingers of TRAF6 assume a rigid, strikingly elongated structure. Interaction of TRAF6 with Ubc13 involves direct contacts of the RING and the preceding residues while the first zinc finger plays a structural role. Surprisingly, this region of TRAF6 is dimeric both in the crystal and in solution, different from the trimeric C-terminal TRAF domain. Structure-based mutagenesis reveals that TRAF6 dimerization is critical for polyubiquitin synthesis and auto-ubiquitination. Fluorescence energy transfer analysis shows that TRAF6 dimerization induces higher order oligomerization of full-length TRAF6. The mismatch of dimeric and trimeric symmetry may provide a mode of infinite oligomerization that facilitates ligand-dependent signal transduction of these receptors.

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