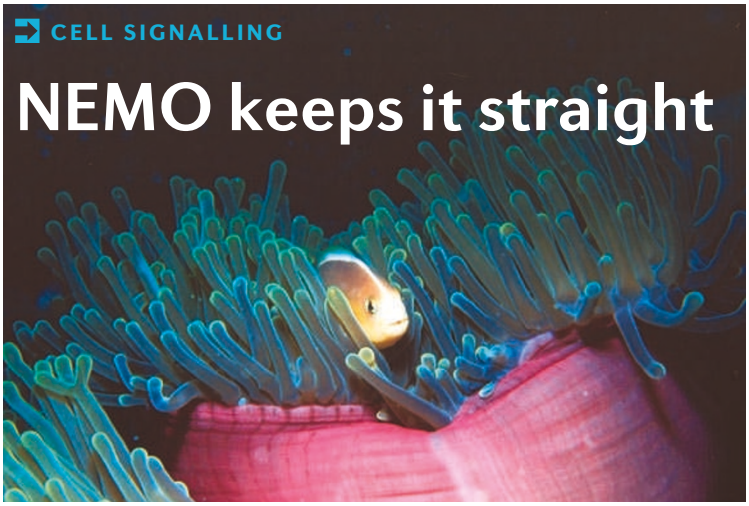
Nature Reviews Molecular Cell Biology | AOP, published online 4 February 2009; doi:10.1038/nrm2638

CELL SIGNALLING

NEMO keeps it straight



Giles Abrey

> “…LUBAC conjugates linear polyubiquitin chains to NEMO.”

The ubiquitin system regulates the function of proteins by conjugating different types of polyubiquitin chains. Non-linear Lys48-linked polyubiquitin chains (in which the carboxyl group of ubiquitin is linked to a Lys side chain of another ubiquitin) serve as signals for degradation by the 26S proteasome, whereas non-linear Lys63-linked polyubiquitin chains function in DNA damage and cellular signalling. Now, Kazuhiro Iwai and co-workers show that a new type of *in vivo* ubiquitin chain, head-to-tail-linked linear polyubiquitin (in which the C terminus of ubiquitin is conjugated to the amino terminus of another ubiquitin), activates the canonical nuclear factor-κB (NF-κB) pathway.

The team had previously found that the ubiquitin ligase complex LUBAC conjugates head-to-tail-linked linear polyubiquitin chains to substrates *in vitro*, but whether this occurs *in vivo* has been unclear. Simultaneous expression of HOIL-1L (also known as RBCK1) and HOIP (also known as RNF31), the two RING-finger proteins that form LUBAC, induces NF-κB signalling in various mammalian cells, and knockdown of these factors reduces NF-κB activity. Mutations in the domains of HOIP and HOIL-1L that are involved in the formation of LUBAC and in its linear polyubiquitylation activity block NF-κB activation. These results link LUBAC specifically to NF-κB signalling.

IκB kinase (IKK) is a canonical activator of NF-κB that contains two catalytic subunits (IKKα and IKKβ) and NF-κB-essential modulator (NEMO), which is the regulatory subunit. LUBAC interacts with NEMO but not IKKα or IKKβ in immunoprecipitation assays *in vivo*, and mutations in the zinc-finger domains of HOIP and HOIL-1L or the coiled-coil-2 (CC2) and Leu zipper (LZ) motifs of NEMO prevent the interaction. So, does LUBAC ubiquitylate NEMO?

Indeed, LUBAC ubiquitylates NEMO, but not IKKα and IKKβ, *in vitro*. Furthermore, an antibody that specifically recognizes head-to-tail-linked ubiquitin moieties reacts with NEMO that has been ubiquitylated by LUBAC *in vitro* and in cultured cells. So, LUBAC conjugates linear polyubiquitin chains to NEMO.

Using mass spectrometry, the authors identified Lys285 and Lys309 in the CC2 and LZ domains of NEMO as the acceptor sites of LUBAC-mediated ubiquitylation. Mutations in these residues block NF-κB signalling in cells, and deletion of *Ubc13*, which encodes a ubiquitin-conjugating enzyme that is crucial for generating Lys63-linked ubiquitin chains, does not affect LUBAC-induced NF-κB signalling.

To investigate the physiological role of LUBAC, Iwai and co-workers genetically ablated *Hoil1* in mice and found that the canonical NF-κB signalling pathway is not induced by pro-inflammation cytokines. Instead, increased JUN N-terminal kinase (JNK) signalling leads to apoptosis in hepatocytes of mutant mice.

These important data show that LUBAC activates the canonical NF-κB signalling pathway by binding to NEMO and conjugating linear polyubiquitin chains. Other physiological functions of linear polyubiquitylation remain to be investigated.

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ORIGINAL RESEARCH PAPER Tokunaga, F. *et al.* Involvement of linear polyubiquitylation of NEMO in NF-κB activation. *Nature Cell Biol.* 11 Jan 2009 (doi:10.1038/ncb1821)
FURTHER READING Perkins, N. D. Integrating cell-signalling pathways with NF-κB and IKK function. *Nature Rev. Mol. Cell Biol.* **8**, 49–62 (2007)