CHAPTER 8

FUNCTIONS OF LINEAR UBIQUITIN CHAINS IN THE NF-κB PATHWAY

Linear Polyubiquitin in NF-κB Signaling

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Abstract: The ubiquitin conjugation system regulates a wide variety of biological phenomena, in most cases, by modulating protein function via polyubiquitin conjugation. Several types of polyubiquitin chains exist in cells and the type of chain conjugated to a protein seems to determine how the protein is regulated. The polyubiquitin chains that have been reported thus far are generated by conjugation via Lys residues of ubiquitin. We have identified a novel linear polyubiquitin chain, in which the C-terminal Gly of one ubiquitin is conjugated to the α-amino group of the N-terminal Met of another ubiquitin and the ubiquitin ligase complex mediating these reactions specifically generates linear chains. We have shown that linear polyubiquitination is involved in activation of the canonical NF-κB pathway. The regulatory roles of Lys63-linked ubiquitin chains in the NF-κB pathway have been extensively studied. In this chapter, we will discuss the distinct roles of linear and K63-linked ubiquitin chains in TNF-α mediated NF-κB activation and the future directions for linear ubiquitin chain research.

Ubiquitin is a highly conserved small globular protein in eukaryotic organisms that is conjugated to proteins by a cascade of reactions catalyzed by three enzymes, E1, E2 and E3. Although the ubiquitin conjugation system was first identified as part of an energy dependent protein degradation system, the system is now recognized to be involved in a vast array of biological phenomena and to regulate protein function in various ways. Conjugation of polyubiquitin chains, which are polymers of ubiquitin, is crucial for regulating protein function, although mono-ubiquitination has also been shown to have signaling functions in the endocytic pathway. Recent reports now indicate that there exist several kinds of polyubiquitin chains in cells and that the mode

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of regulation of substrate proteins may depend on the type of conjugated polyubiquitin chain. For example, polyubiquitin chains linked via the ubiquitin K48 residue function as signals for degradation of the conjugated protein by the proteasome. In contrast, polyubiquitin chains linking via the ubiquitin K63 residue mediate DNA repair and signal transduction without functioning as a degradation signal. Polyubiquitin chains are thought to be generated by conjugation of the C-terminal Gly of one ubiquitin to a Lys residue in the next ubiquitin. The presence of isopeptide linkages via all seven Lys residues of ubiquitin has been revealed by mass spectrometric analyses and further broadens the scope of ubiquitin mediated regulation of cellular function. Indeed, a recent report by Jin et al has indicated that K11-linked polyubiquitin chains are specifically generated by UbcH10 (E2) and APC/C (anaphase promoting complex/cyclosome, an E3) and target the conjugated substrate for degradation by the proteasome.

IDENTIFICATION OF A UBIQUITIN LIGASE COMPLEX THAT SPECIFICALLY GENERATES HEAD-TO-TAIL LINEAR POLYUBIQUITIN CHAINS

We have identified a new, linear type of polyubiquitin chain, in which the C-terminal Gly of one ubiquitin is conjugated to the α-amino group of another ubiquitin. The linear polyubiquitin chain is generated by a unique ubiquitin ligase complex that we have named LUBAC (linear ubiquitin chain assembly complex). LUBAC is composed of two RING-IBR-RING proteins, HOIL-1L and HOIP, which have molecular masses of 58 kD and 120 kD, respectively (Fig. 1). We have hypothesized that the complex is composed of two or three molecules of each protein since the molecular mass of the complex, estimated by gel filtration, is approximately 600 kD. Among the domains that are present in HOIL-1L and HOIP, the UBA domain of HOIP and the UBL domain of HOIL-1L are involved in LUBAC formation. LUBAC can generate linear chains in concert with several E2s, including UbcH5s, E2-25K and UbcH7. Furthermore, LUBAC cannot generate polyubiquitin from N-terminally tagged wild type ubiquitin, indicating that it only generates linear (and not Lys-linked) chains. Notably, E2-25K exclusively generates K48-linked chains in the absence of an E3 in vitro, but generates linear chains in the presence of LUBAC in vitro. Therefore, LUBAC, not the E2 enzyme, appears to be predominantly responsible for determining the linkage specificity. This situation differs from the generation of K63- and K11-linked chains, since the specificity of both these types of chains is determined by E2s (K63: a Ubc13-containing E2 complex, K11: UbcH10).

Figure 1. Schematic structure of the LUBAC subunits HOIL-1L and HOIP. The NZF motifs of LUBAC possess both ubiquitin binding activity and NF-κB activation, although they are dispensable for linear polyubiquitin chain formation.