New Tools for Ubiquitin Signaling

Protein ubiquitination is rapidly emerging as a key regulatory mechanism of many cellular processes. Important roles in proteasomal degradation are contrasted by various non-degradative functions of ubiquitin in intracellular trafficking, signal transduction and the DNA damage response. This versatility arises from eight structurally and functionally distinct polyubiquitin chains that co-exist in cells. Biochemical characterization identified proteins and enzymes that specifically assemble, recognize and hydrolyze polyubiquitin in a linkage-specific fashion, and structural analysis starts to reveal mechanisms of linkage recognition.

What has been much more challenging is to understand the cellular roles of individual chain types and the interplay of the different forms of ubiquitination. A particularly fascinating area of ubiquitin research involves around cytokine signaling pathways, for example, the response to tumor necrosis factor (TNF)-α. In addition to canonical roles of Lys48-linked chains as a degradation signal, the first described instances of non-degradative Lys63-linked ubiquitination in nuclear factor κB (NFκB) signaling more than 10 years ago opened the door for a vast number of discoveries. Recently, the picture became even more complex with reports implicating Lys11 chains and linear (or Met1-linked) chains in TNFα signaling. The idea that linear chains cooperate with Lys63-linkages in activating cytokine-triggered kinases including inhibitor of κB kinase has resulted in some debate. However, the recent identification of SHARPIN as a component of the linear ubiquitin chain assembly complex, LUBAC, and its important roles in inflammation further supported that linear chains are key players in NFκB signaling. Despite this biochemical and genetic evidence, reagents that directly interrogate the roles of linear versus other polyubiquitin chains in vivo have been lacking.

In this issue of Journal of Molecular Biology, Matsumoto et al. report the engineering of a linkage-specific antibody against linear ubiquitin chains and show its application in Western blotting, immunoprecipitation and immunofluorescence studies. A strategy that combined phage display and affinity maturation as well as structure-guided engineering identified antibodies that were able to distinguish linear from structurally similar Lys63-linkages and other chain types in Western blotting applications. A co-crystal structure of the Fab fragment in complex with a linear diubiquitin revealed that the antibody recognizes both ubiquitin moieties in a compact chain conformation not previously observed. This binding mode is reminiscent to, but not identical with, that of the Lys63-specific antibody.

The new antibody displayed significant cross-reactivity with Lys63-linkages in conventional immunoprecipitation analysis, which is perhaps not surprising since linear and Lys63-linked chains can adopt similar conformations. The authors established an improved protocol and found that addition of 7 M urea recovered the remarkable linkage specificity of the antibody for linear chains, as probed by mass spectrometry.

Finally, the authors performed a variety of cellular studies, showing that overexpression of LUBAC results in accumulation of linear chains, which can be detected by Western blotting and also by immunofluorescence. A key result is that TNFα stimulation of HEK293 cells resulted in rapid up-regulation of linear chains. Interestingly, in this immunoprecipitation experiment using the linear-specific protocol, the precipitated substrates of linear chains appeared to harbor additional chain types, as mass spectrometry reveals significant amounts of Lys48- and Lys63-linkages.

The unambiguous confirmation of linear chain production upon TNFα stimulation adds to the overwhelming evidence that this chain type may have independent roles in NFκB activation. Nevertheless, key questions remain. What is the timing of linear chain assembly with respect to other chain types? What are the targets of linear chains in the TNF–receptor complex? Are there further receptors for linear chains? What are the deubiquitinases that reverse linear ubiquitination events? The described antibody will be a key tool to shed light on these lingering mysteries of ubiquitin signaling.
References


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