

P014 Probing the ubiquitinome linkage profile
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Protein ubiquitination through lysine residues on targets regulates many crucial cellular processes. The ubiquitin molecule itself contains seven lysines, any of which can also serve as a receptor for another ubiquitin. It has been shown that ubiquitin chains containing all seven possible linkages exist *in vivo*. Furthermore, target proteins can be modified by a single ubiquitin (monoubiquitination) or by multiple ubiquitin molecules on separate lysine residues (multiubiquitination). Thus, the global composition of the ubiquitin pool in cells is complex. In order to distinguish between the different modifications and to estimate their relative abundance under various conditions, we used wt ubiquitin and ubiquitin mutants in which one or more lysine residues were replaced by arginine, and expressed them in yeast cells. Using novel quantitative proteomics methods we examined the effects of these mutants on the forms of conjugated ubiquitin present within cells. Our study suggests that mono and multi-ubiquitin modifications comprise a large portion of the conjugated ubiquitin landscape. Among the polyubiquitinated species, Lysine48- and Lysine63-linked chains are the two most abundant. We have found conditions that emphasize a critical role for cellular homeostasis of lysine48 chains.