Characterization of ubiquitin metabolism in mammalian cells by fluorescence tracking

P-02.3-19

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Despite almost 40 years having passed from the initial discovery of ubiquitin (Ub), fundamental questions related to its intracellular metabolism are still enigmatic. Here we utilized fluorescent tracking for monitoring ubiquitin turnover in mammalian cells, resulting in obtaining qualitatively new data. We report (1) short Ub half-life estimated as 4 h; (2) for a median of six Ub molecules per substrate as a dynamic equilibrium between Ub ligases and deubiquitinated enzymes (DUBs); (3) loss on average of one Ub molecule per four acts of engagement of polyubiquitinated substrate by the proteasome; (4) direct correlation between incorporation of Ub into the distinct type of chains and Ub half-life; and (5) critical influence of the single lysine residue K27 on the stability of the whole Ub molecule. Concluding, our data provide a comprehensive understanding of ubiquitin-proteasome system dynamics on the previously unreachable state of the art. The reported study was supported by Russian Scientific Foundation project # 19-14-00262.