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Exploring neighborhoods of ubiquitinated proteins with proximity-utilizing biotinylation

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Post-translational modification of proteins by ubiquitin and similar molecules (SUMO, ATG12, NEDD8 etc) termed summarily UBLs is involved in a plethora of biological processes including protein turnover, DNA repair and replication, signal transduction, cell cycle control, metabolism, stress response, homeostasis, mRNA processing etc. Among the proteins involved in ubiquitin-mediated signal transduction, there are two E1, around 40 E2 and around 600 E3-ligases responsible for activation and covalent attachment of ubiquitin to protein targets (designated as “writers”) around 90 DUB (Deubiquitinating enzymes) that catalyze its removal (“erasers”) and many proteins that recognize ubiquitinated proteins via so-called UBD (Ubiquitin-Binding Domains) of various subtypes (“readers”). We have developed a systematic proteomics-based approach to study ubiquitin-dependent signaling and regulation based on identification of the UBL-modified proteins associated to a particular protein of interest which uses Proximity Utilizing Biotinylation (PUB). The co-expression of a protein of interest, fused to BirA ligase with the fusion of a ubiquitin with BAP (Biotin-Acceptor Peptide, specifically biotinylated by BirA) leads to biotinylation of ubiquitin-modified proteins interacting with (or else in proximity to) the BirA-fusion. We validated our approach using a well characterized interaction between the translesion DNA polymerase POLH and ubiquitinated PCNA. We showed that the preferential biotinylation of BAP-Ubi-PCNA by the BirA-POLH fusion depends on the integrity of the UBZ and PCNA-binding domains of POLH protein, i.e., on the binding between the two proteins. Further, we demonstrated that the approach can be used for mass-spectrometry identification of the ubiquitinated proteins interacting with the BirA fusion of interest.

Biography

Arman Kulyyassov graduated from the Novosibirsk State University (Russia) in 1992. He has completed his PhD on specialty of bioorganic chemistry. He has published more than 40 papers in international journals related to chemistry of natural compounds. During Post-doctoral studies in the Institut de Cancérologie Gustave Roussy in 2006-2010 (Villejuif, France) he specialized in proteomics, molecular and cell biology. At present he is the leading researcher in the stem cell lab in National Center for biotechnology (Astana, Kazakhstan) and has 5 papers published in peer-reviewed journals on proteomics.

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