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Study of the compartmentalization of cell using proximity utilizing enzymatic ligation

Arman Kulyyassov¹, Gulsamal Zhubanova¹ and Vasily Ogryzko²

¹National Center for Biotechnology, Kazakhstan

²University of Paris-Sud, France

Human cells consist of many membrane-bound structures called organelles. The membranes of these organelles serve as walls that separate the cell into compartments which helps cells to perform specific functions in their district regions. This compartmentalization allows eukaryotic cells to do otherwise incompatible chemical reactions simultaneously and also increase the surface area of the cell membranes, which helps for obtaining nutrients and excreting waste. Standard proteomic approaches to study spatial organization and compartmentalization of cell include fluorescence resonance energy transfer (FRET), protein complementation assays (PCA), and various two-hybrid systems. But these methods have some limitations such as restricted sensitivity, dynamic range and detection of short-range proximities. One of the perspective approaches to overcome some of these limitations is method based on using of enzyme/substrate pair. One protein of interest is fused to biotin ligase (BirA) or lipoic acid ligase (LplA), while another protein is fused to enzyme's "acceptor peptide" (AP) substrate. In case of interaction or proximity of the two proteins A and B, enzyme will catalyze site-specific covalent attachment of biotin or lipoic acid to corresponding AP, and we can detect modified AP by streptavidin or lipoic acid antibody staining or western blotting. Using this approach, we demonstrate that sharing the same intracellular compartment can also give a signal above the background. As model proteins, we used GFP (which is found in both nucleus and cytoplasm) and nuclear proteins. Thus, with appropriate controls, our approach can provide a new kind of information, complementary to data obtained by other methodologies.

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.

akulyyassov@gmail.com

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