Dissecting interacting molecular populations by FRET

Miklós Petrás[1], Zsolt Fazekas[1], Fábián Ákos[1], Gábor Horváth[1], Péter Nagy[1], György Vereb[1], János Szöllosi[1,2]

[1]Department of Biophysics and Cell Biology, Medical and Health Science Center and [2]Cell Biology and Signaling Research Group of the Hungarian Academy of Sciences, Research Center for Molecular Medicine, University of Debrecen, Nagyerdei krt 98, 4012 Debrecen, Hungary. E-mail: szollo@dote.hu

The role of the expression patterns of proteins involved in oncogenesis can be understood after characterizing their multimolecular interactions. Conventional FRET methods permit the analysis of interaction between two molecular species at the most, which necessitates the introduction of new approaches for studying multicomponent signaling complexes. Flow cytometric as well as microscopic donor (dbFRET) and acceptor (abFRET) photobleaching FRET measurements were performed to determine the association states of ErbB2, [beta]1-integrin, and CD44 receptors. Based on consecutively applied abFRET and dbFRET methods (two-sided FRET), the relationship of [beta]1-integrin-ErbB2 heteroassociation to ErbB2 homoassociation and of [beta]1-integrin-ErbB2 heteroassociation to ErbB2-CD44 heteroassociation was studied by correlating pixel-by-pixel FRET values of the corresponding abFRET and dbFRET images in contour plots. Anticorrelation was observed between [beta]1-integrin-ErbB2 heteroassociation and ErbB2 homoassociation on trastuzumab sensitive N87 and SK-BR-3 cells, while modest positive correlation was found between [beta]1-integrin-ErbB2 and ErbB2-CD44 heteroassociation on trastuzumab resistant MKN-7 cells. The FRET efficiency values of [beta]1-integrin-ErbB2 heteroassociation were markedly higher at the focal adhesion regions on attached cells than those measured by flow cytometry on detached cells. To complement our two-sided FRET method, we have developed a novel technique, triple-FRET, that allows for the measurement of energy transfer efficiency between three fluorescent dyes and the characterization of association pattern of three proteins simultaneously. Another advantage of this new method is that it can be used in confocal microscopy or flow cytometry, allowing us to exploit the capabilities of both instrumentations and to make the results obtained with different instruments more comparable. The triple FRET method was applied to cells where ErbB2-[beta]1-integrin-CD44 molecules were labeled with fluorescently tagged antibodies. On the basis of our results, we assume that the homoassociation state of ErbB2 is dynamically modulated by its interaction with [beta]1-integrins.
Key Words: Two-sided FRET, triple FRET, ErbB2