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Single-molecule tracking of signaling molecules undergoing anomalous diffusion in the plasma membrane

Kuniko Sakamoto, Takahiro Fujiwara, Hideji Murakoshi, and Akihiro Kusumi

Membrane Mechanisms Project, ICORP-JST, The Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
Tel: +81-75-751-4112, Fax: +81-75-751-4113
E-mail: kunikos@frontier.kyoto-u.ac.jp

Abstract:

Techniques that allow researchers to track single molecules in living cells are becoming important tools for investigating cells. I will show their unique capabilities using two examples in this presentation.

First, this ability of tracking single molecules has fostered a new fundamental understanding of molecular diffusion in the cell membrane: the plasma membranes of practically all of the mammalian cells in culture are partitioned into many small compartments of 30-200 nm, and that virtually all of the molecules incorporated in the membrane undergo short-term confined diffusion within a compartment and long-term hop diffusion between these compartments in the cell membrane. This entails a paradigm shift of the structure of the plasma membrane: the plasma membrane in the space scales greater than several 10s of nanometers should not be considered as a 2-dimensional continuum fluid, but that partitioned into closely apposed compartments.

Second, a single-molecule fluorescence resonance energy transfer (FRET) method has been developed to observe the activation of the small G protein Ras at the level of individual molecules. KB cells expressing H- or K-Ras fused with YFP (donor) were microinjected with the fluorescent GTP analogue BodipyTR-GTP (acceptor), and the EGF-induced binding of BodipyTR-GTP to YFP-(H or K)-Ras was monitored by single-molecule FRET. Upon activation, Ras diffusion rates were decreased by a factor of ~5, suggesting the formation of large activated-Ras signaling complexes. These may work as platforms for transducing the Ras signal to effector molecules, further suggesting that Ras signal transduction requires more than simple collisions with effector molecules. GAP334-GFP recruited to the membrane was also stationary, suggesting its binding to the signaling complex. The single-molecule FRET method developed here provides a new, powerful technique to study the signal transduction mechanisms of various G proteins, as well as anomalous diffusion of these molecules upon activation.