Triple-Color Single-Molecule Imaging of Signaling Molecules on the Plasma Membrane in Living Cells to Reveal Signal Regulation Mechanisms

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Living cells maintain their lives through sensing variety of extracellular stimuli and making appropriate responses to adopt to the environment. Considering the low concentration of some stimulants with substantial fluctuation, the receptors and signal transduction system have to possess high sensitivity and noise resistance mechanisms. To understand the mechanism, analysis of behavior of each receptor and downstream molecule in living cells is desired. In particular, receptor oligomerization, which occurs generally on various receptors upon ligand stimulation, might be a key factor to realize the receptor functions. The detail dynamics and roles of the oligomerization in receptor functions, however, has not been fully understood.

In our present study, we constructed a triple-color single-molecule imaging microscope. Using this microscope system, we tried to analyze oligomerization, ligand binding, and signaling efficacy of each formyl peptide receptor 1 (FPR1), a G protein-coupled receptor that is involved in chemotactic response of immune cells, through simultaneous single-molecule monitoring of a G protein-coupled receptor of FPR1, its agonists, and G protein in living cells. The single-molecule imaging data showed that FPR1 monomers and oligomers coexisted even before agonist stimulation. Upon agonist administration, the FPR1 monomer/oligomer equilibrium shifted to increase oligomers, and agonist-bound oligomers exhibited a longer interaction with G proteins compared to agonist-free oligomers and agonist-bound monomers did. Important finding is that inhibitor administration also resulted in an increase in the oligomer fraction, but both inhibitor-free and inhibitor-bound oligomers did not exhibit elongation of G protein interaction duration. Based on these results, we proposed a synergistic activation model in which ligand binding and successive oligomerization of FPR1 synergistically controls G protein activation.

The finding on GPCR function mentioned above was achieved through use of triple-color single-molecule imaging of ligand, GPCR, and G protein in living cells. This approach provides methodological advances that will potentially open up innovative avenues of research on signal transduction regulation mechanisms on receptor molecules.