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It is widely accepted that G protein alpha subunits (G α) are precoupled to G protein coupled receptors (GPCRs) in inactive form and are released from its receptor after activation by ligand. However, single molecule observation of G α 2 in Dictyostelium cells has revealed that at the plasma membrane, diffusion coefficient of G α 2 as well as G α 2 active mutant decreases to that of the cAMP receptor, one of GPCRs after cAMP stimulation. This finding implies that active G α 2 couples with the receptor after ligand binding.

In order to clarify the interaction between cAMP receptor and G α 2 after activation, we studied a C-terminal truncation mutant of cAMP receptor. Activation of G proteins by the mutant receptor was confirmed by PH domain translocation after addition of cAMP. Although cells expressing mutant receptor showed normal activation of G proteins, the diffusion of G α 2 did not change after cAMP stimulation. This result indicates the interaction of active G α 2 and cAMP receptor in the wildtype cell, requiring the receptor C-terminus. Interestingly, cells expressing mutant receptor had lower chemotaxis efficiency in cAMP gradients suggesting that slowed G α 2 diffusion, representing active G α 2 interactions with the receptor, may influence gradient sensing of Dictyostelium cells.

2K1424 G 蛋白質共役受容体との複合体形成による活性化 G 蛋白質 α サブユニットの局在制御

G protein coupled receptors modulate the localization of activated G protein alpha subunits through complex formation

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G-protein coupled receptors (GPCRs) and their associated heterotrimeric G-proteins constitute an important family of signaling partners that detect numerous physiological stimuli in eukaryotic cells. It is generally thought that G protein subunits, G α and G $\beta\gamma$, and receptors dissociate following ligand-dependent G protein activation. It is debated whether the receptor and G proteins are constitutively assembled as inactive complexes prior to ligand stimulation, or form complexes only after stimulation. We applied single molecule imaging analysis to a chemoattractant responsive GPCR and G protein signaling system. Before ligand stimulation, the lateral diffusion of GPCRs and G-proteins differed substantially demonstrating an absence of inactive complexes. After ligand stimulation, a substantial fraction of G α subunits showed diffusion coefficient matching the receptor. Furthermore, when we constrained receptor diffusion by tethering to a substrate, the G α subunit showed a corresponding reduction in diffusion. These data indicate that G-proteins form stable complexes with the receptor after ligand binding, and therefore potentially while in the activated state. Our analyses of constitutively active G α subunits support this hypothesis. Formation of GPCR- G α complexes increases the membrane-binding time of individual G α subunits and therefore results in the local accumulation of G α at the stimulated membrane on focal chemoattractant stimulation. This mechanism may positively reinforce spatially localized signaling from the receptor.

2K1436 GPCR (β 2 アドレナリン受容体) のダイマー・モノマー平衡: 1 分子イメージング法による研究

Dynamic monomer-dimer equilibrium of a prototypical GPCR, beta2 adrenergic receptor: a single molecule imaging study

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Beta2 adrenergic receptor (BAR) is a well-studied prototypical GPCR. However, whether it exists as monomers or dimers is controversial. Generally, for class A GPCRs, which BAR belongs to, the existence and function of dimers remain unresolved. Here, we fully determined the dynamic equilibrium between BAR monomers and dimers at 37°C, using a single-molecule imaging method. This is the second molecule, for which dissociation-association equilibrium is fully characterized in the membrane. The determined constants for BAR (for comparison with another class A GPCR, the values for formyl peptide receptor, FPR, are given, which was the first molecule for such determination; Kasai et al. 2011 J. Cell Biol.) are the following: the two-dimensional dimer dissociation constant = 1.6 (3.6) copies/square microns; a dimer lifetime of 80 (90) ms [a dissociation rate constant of 12.6 (11.0) /s]; and a 2D association rate constant of 7.9 (3.1) /[copies/square microns]/s. Rough agreement of these values between BAR and FPR suggests that class A GPCRs tend to form transient dimers with

their lifetimes of ~85 ms. These values were determined for non-engaged receptor, but were unchanged by liganding. Under a BAR (FPR) physiological expression level of ~260 (2.1) copies/square microns [740,000 (6,000) copies/cell] in dog heart cells (neutrophils), 95% (42%) of the molecules exist as dimers. Note that the dimer lifetimes are the same irrespective of the receptor density, whereas the monomer lifetimes become shorter with an increase of the receptor density.

2K1448 側方拡散運動の状態遷移解析が示す EGF 受容体分子間の動的相互作用

State transition of lateral diffusion movements shows dynamic interactions between epidermal growth factor receptors on the cell membrane

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Epidermal growth factor (EGF) induces activation (trans-phosphorylation) of its receptor (EGFR) via formation of EGFR homodimer. Although recent studies addressed the detail of the formation of EGFR dimers or clusters, dynamic aspects of the interactions between EGFR molecules remain unclear especially in correlation with the activation process of EGFR.

We carried out a single-molecule imaging of EGFR fused with green fluorescent protein (EGFR-GFP) diffusing along the plasma membrane of living cells. The variational Bayesian algorithm applied to the hidden Markov model of the motional modes suggested rapid three-state transitions of the EGFR-GFP motions and assigned each diffusion step in the trajectories to one of the three states with different diffusion coefficients. EGFR molecules in the slowest state diffused only within a confinement region and those in the faster states moved between the regions. A decrease of the slowest fraction by cellular-cholesterol depletion suggested that the region was a cholesterol-enriched micro-domain. The size of EGFR clusters calculated from the fluorescence intensities changed systematically during the diffusion within the confinement region. This change was observed in the absence of EGF and became more evident in the presence of EGF. Therefore, the confinement region was suggested as a platform for dynamic molecular interactions related to the activation of EGFR. Moreover, activation of EGFR could occur even in the ligand-free conditions.

2K1512 EGF 受容体活性化による細胞膜コンパートメント内への閉じ込め 昂進: 超高速 1 蛍光分子追跡による解明

Enhanced confinement of activated EGF receptor in the plasma membrane compartments revealed by ultra high-speed single-molecule tracking

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Upon the binding of EGF to its receptor (EGFR) on the plasma membrane, localized/polarized actin response was induced, which would require the regulated propagation of the cellular signal along the 2D plasma membrane. Although the 2D signal propagation was considered mediated by the bimolecular collision of engaged and non-engaged EGFR, the exact mechanism for spatiotemporally regulating signal propagation has remained unknown. To address this problem, we observed the movement of single fluorescently-tagged EGFR molecules in living T24 cells at 37 °C, at an imaging frame rate as fast as 6 kHz (every 0.167 ms), combined with a new quantitative method to detect hops (if they occur) in a single-molecule trajectory. Before stimulation, virtually all of the EGFR molecules were found to undergo hop diffusion in a 2D-space partitioned into 110-nm (median) compartments, with a residency time (exponential lifetime) within a compartment of 38 ms. This behavior is virtually the same as that found for transferrin receptor. At 2.5 min after stimulation with 10 nM EGF, ~70% of the cells exhibited slowed hop diffusion of EGFR (the remaining ~30% failed to respond in this period), within the same median compartment size of 110 nm, but with an elongated exponential residency time of 57 ms. This result shows that the engaged EGFR molecules undergo slowed diffusion before they finally become assembled, which is considered advantageous for localized signaling.

2K1524 細胞膜に沿ったシグナル伝播の制御機構: 1 分子追跡法による研究

Regulation mechanism for signal propagation along the plasma membrane: a single-molecule tracking study