(565KKKRRK570) immediately following helix 8 in the c-terminus of M3R was found to be necessary for the decrease in Gq-V mobility. We tested the hypothesis that an electrostatic interaction was involved in the interaction between M3Rs and Gq by repeating our FRAP experiments in permeabilized cells exposed to buffer solutions with high and low ionic strength. High ionic strength solutions inhibited the decrease in Gq mobility, whereas low ionic strength solutions enhanced this effect. These results suggest that an electrostatic interaction mediates an interaction between the c-terminus of M3Rs and Gq heterotrimers. The functional significance of this interaction is currently under study.

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1519-Pos

The Human Muscarinic Receptor Couples to GαI3 Via Catalytic Collision

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Hundreds of G-protein coupled receptors (GPCR) are encoded in the human genome. All GPCRs react to a vast variety of ligands and initiate the G-protein activation cycle, by catalyzing the exchange of GDP by GTP on the Gα subunit. Classically, this mode of activation has been proposed to be of catalytic collision coupling nature, where a single receptor sequentially activates several G-proteins. However, recent biophysical and imaging studies challenged this concept and suggested that some GPCR and G-proteins form stable non-dissociating complexes prior to and after activation. We were interested in determining the mode of coupling between the human muscarinic 2 receptor (m2R) and Gαi3βγ. We used the G-protein activated K+ channel (GIRK) as a reporter for receptor activation and systematically quantified receptor's and channel's plasma membrane concentrations using fluorescent methods and radioligand assays. We found a decrease in activation time at high receptor density, with no change in channel concentration. However, maximal amplitude was attained at lower receptor density, suggesting an amplification process. No change in Gβγ concentration was observed, as judged by the unchanged Gβγ-dependent basal activity of GIRK. Additionally, increasing amounts of m2R did not increase Gαi concentration. Together, these results suggest a catalytic collision coupling mechanism. We constructed a model describing m2R's activation scheme and predicted that excessive Gα subunits should slow the activation process by occupying the activated receptor in "dead-end" interactions, not leading to channel activation. Increasing amounts of two fluorescent Gαi3 subunits were used to test the prediction. Indeed, both subunits slowed the evoked-current, without change in current amplitude. These results, together with our previous observations, suggest that the m2R activates Gαi3 via a catalytic collision coupling mechanism, where one receptor diffuses and activates several Gαβγ subunits, leading to the activation of GIRK.

1520-Pos

Structural Characterization of the N-terminal Region of the Saccharomyces Cerevisiae G-Protein Coupled Receptor, Ste2P

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Binding of α-factor pheromone to the G-protein coupled receptor, Ste2p, initiates signal transduction events that lead to mating of the yeast *Saccharomyces cerevisiae*. Recent indirect evidence also implicates the N-terminal region of Ste2p in modulating cell wall degradation and membrane fusion during later steps of mating. Toward deciphering mechanisms, structural studies have been initiated on the N-terminus of Ste2p. Initially, residues 1-71 of Ste2p were expressed as a fusion protein with HIS and KSI tags and affinity purified from *E.coli* in mg quantities. Subsequent cyanogens bromide cleavage at methionines yielded a hydrophobic peptide (Ste2p 2-54) that consistently disappeared upon HPLC enrichment. Similarly, a chemically synthesized fragment corresponding to Ste2p residues 14-43 could not be purified by HPLC. However, the addition of three lysines to both termini was found to decrease hydrophobicity sufficiently to enable HPLC purification. Circular dichroism studies of a chemically synthesized K^3-Ste2p-14-43-K^3 peptide indicated mostly random structure, with ~45% β-strand and a small percentage of α-helix in buffered water. The structure was found to be stable at temperatures up to 40°C. These results correlate with predicted 2o structure for the Ste2p N-terminal domain including: random chain with a β-strand-loop-β-strand fold followed by a C-terminal α-helix (Shi et al., J. Cell. Biochem. 107:630-38) and recent NMR evidence suggesting α-helix in a C-terminal overlapping region (residues 39-47; Neumoin *et al.,* 2009 Biophysical Journal 96: 3187-96). Preliminary ^{1}H-^{1}H NOESY and TOCSY NMR data for the K^3-Ste2p 14-43-K^3 peptide have been collected. As well, a recombinant version of K^3-Ste2p-2-43-K^3 is being produced to extend the N-terminal region to be analyzed and facilitate isotopic labeling for complete structural elucidation.

1521-Pos

Arrestin can Bind to a Single G-Protein Coupled Receptor

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Termination of G protein-coupled receptor (GPCR) signaling typically involves phosphorylation of the receptor, followed by binding of a protein called arrestin. Here we tested the minimal stoichiometry required for this interaction, by determining if a single rhodopsin molecule can bind arrestin. To do this, we prepared nanoscale phospholipids particles, so-called nanodiscs, which contain only monomeric rhodopsin and measured their ability to bind visual arrestin. Our data clearly show that visual arrestin can bind to monomeric phosphorylated rhodopsin to stabilize its active form, called metarhodopsin II. Interestingly, we find beta-arrestin can also bind to monomeric rhodopsin in nanodiscs and stabilize metarhodopsin II. Together, these results suggest that in general, the minimal unit for arrestin binding is a monomeric GPCR.

1522-Pos

Solid-State NMR Demonstrates that Active Signaling Complexes of Bacterial Chemoreceptors Do Not Adopt the Proposed Trimer-Of-Dimers Structure

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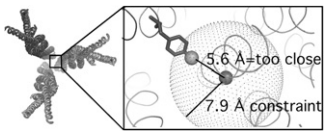
The receptor dimers that mediate bacterial chemotaxis form signaling complexes with CheW and the kinase CheA. Based on the packing arrangement observed in two different crystal structures of two different receptor cytoplasmic fragments, two different models have been proposed for receptor signaling arrays: the trimers-of-dimers and hedgerow models. We have identified an inter-dimer distance predicted to be substantially different by the two models, labeled the atoms defining this distance through isotopic enrichment, and measured it with ^{19}F-^{13}C REDOR. This was done in two types of receptor samples: isolated bacterial membranes containing overexpressed, intact receptor, and soluble receptor fragments reconstituted into kinase-active signaling complexes. In both cases, the distance found was incompatible with *both* the trimers-of-dimers and the hedgerow models. Comparisons of simulated and observed REDOR dephasing were used to deduce a closest-approach distance at this interface, which provides a constraint for the possible arrangements of receptor assemblies in the kinase-active signaling state.



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1523-Pos

Site-Specific Fluorescent Labeling of Purified G-Protein-Coupled Receptors Using Genetically-Encoded Unnatural Amino Acids

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The introduction of unique chemical groups into proteins by means of site-directed mutagenesis with unnatural amino acids has numerous applications in protein engineering and functional studies. We first introduced *p*-acetyl-L-phenylalanine (Acp) or *p*-azido-L-phenylalanine (Azp) into the prototypical G protein-coupled receptor (GPCR) rhodopsin at specific sites. We employed an amber codon suppression system where the mutant opsin gene was co-expressed with the appropriate orthogonal pair of engineered tRNA and amino-acyl tRNA synthetase. We then used hydrazone (hydrazide) or Staudinger-Bertozzi (phosphine) ligation chemistry for the keto group (in Acp) or azido group (in Azp), respectively, to link a fluorophore at various solvent accessible sites in rhodopsin. In side-by-side comparisons of the two chemical ligation chemistries, which were carried out