Signaling via Structure Change: NMR Analysis of GPCR and G protein Activation

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G protein-coupled receptors (GPCRs) represent a diverse group of seven transmembrane helix receptors that require ligand-dependent activation to initiate heterotrimeric ($\alpha\beta\gamma$) G protein mediated intracellular signaling cascades. Activation of a G protein by its agonist-stimulated GPCR (R*) requires the propagation of structural signals from the G protein interacting surface on R* to the binding surface of the G protein α-subunit, and ultimately to the guanine nucleotide-binding pocket to trigger GDP/GTP exchange. The structural basis for the interaction of R* with its cognate G protein, and the subsequent activation of the G protein by R*, is not well understood. Using rhodopsin as a model GPCR, conformational changes upon formation of R* have been monitored by high-resolution NMR spectroscopy using selective isotope labeling of amino acid residues predominantly along the G protein interacting cytoplasmic surface. Structural analysis and/or conformational changes in uniformly isotope-labeled G protein α-subunits and G protein α-subunit domains (helical and GTPase) have also been probed by NMR in isolation, or after heterotrimer formation with unlabeled G protein $\beta\gamma$-subunits, and during the course of R*-stimulated guanine nucleotide exchange. Our results to date suggest that high-resolution NMR can be effectively utilized to probe the structural basis of GPCR and G protein activation.

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