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A Conformational Switch Regulates Receptor-G Protein Interaction

A peptide specific to a G protein γ subunit C terminus undergoes a conformational shift concordant with receptor activation, providing direct support for a long-standing proposal that a conformational switch in the γ subunit tail mediates G protein activation by a receptor.

Cellular signaling is mediated by diverse interactions between regulatory proteins. Among the most important of these interactions is that between G protein-coupled receptors and heterotrimeric (αβγ) G proteins. This is a primary event in the actuation of the ubiquitous G protein-mediated signaling pathways that regulate cell physiology. The mechanistic underpinnings of a G protein's specific contact with a receptor and its activation by the receptor are not understood with clarity. In elucidating these mechanisms, early approaches took the obvious path of identifying the sites on the G protein that contact a receptor. The C terminus of the α subunit was first identified as one such region [1]. Later evidence pointed to the C terminus of the γ subunit contacting a receptor [2]. Both the primary structure of this domain and the prenyl moiety that was attached to the γ subunit C terminus were shown to be critical for this interaction. Surprisingly, this C-terminal domain of the γ subunit was inaccessible to a specific antibody or a carboxypeptidase in the βγ complex or the αβγ heterotrimer. However, G proteins containing point mutations in this C-terminal region of the γ subunit were incapable of effective interaction with the receptor, showing that the same domain was accessible to the receptor. These results lead to the following predictions [3]: (1) the G protein γ subunit tail is masked in the βγ complex or the heterotrimer, but becomes available to the receptor through a conformational switch during the process of activation; (2) the nucleotide exchange leading to G protein activation is controlled by this conformational switch by moving the βγ complex away from the nucleotide binding site of the α subunit (Figure 1); and (3) the contact between the α subunit and the γ subunit C termini with the receptor establishes the "open" conformation of the α subunit, where it is free of nucleotide and the G protein has the highest affinity for a receptor.

The paper from Kisselev and Downs in this issue of *Structure* [4] provides direct structural support for the first of the predictions above, using the same receptor (rhodopsin)-G protein (Gt) system. The C-terminal peptide of the Gt γ subunit that interacts with rhodopsin is disordered in the presence of dark-adapted inactive rhodopsin but takes up a striking helical conformation when the receptor is activated with light. When this derived structure is fused to the known crystal structure of Gt, the γ subunit tail region reveals that the F64 residue, which is anchored in the β subunit, initially faces away from the β subunit after receptor activation. This movement and the transition from disorder to a constrained structure elegantly explain the masking of the γ subunit C-terminal domain observed earlier. It also explains the deleterious impact of mutations, including substitutions at the F64 residue position, on Gt interaction with rhodopsin. A γ subunit mutant mimicking a charge reversal mutant of rhodopsin similarly disrupts rhodopsin-Gt interaction. The corresponding C-terminal mutant peptide does not undergo a conformational change when rhodopsin is activated. Although the mechanistic basis for this effect is unclear, the result indicates the sensitivity of the conformational change to primary structure alterations. Recent evidence is not only illustrative of this sensitivity but also brings us back to the second prediction mentioned above on Gβγ regulation of nucleotide exchange in the α subunit (Figure 1). A γ subunit mutant that retains the F64 homolog but has other residues at the C terminus scrambled, accentuates receptor-stimulated nucleotide exchange in the G protein [5]. Whereas this result supports a role for γ subunit-receptor interaction in nucleotide exchange, the findings from Kisselev and Downs imply that the retention of the F64 homolog allows the γ subunit to dock with the receptor but in an inappropriate configuration, due to the inability of the C-terminal residues to take up the appropriate conformation.

The biophysical evidence from peptides so far indicates that the G protein, like a spider monkey with a prehensile tail, grasps the receptor with its α and γ

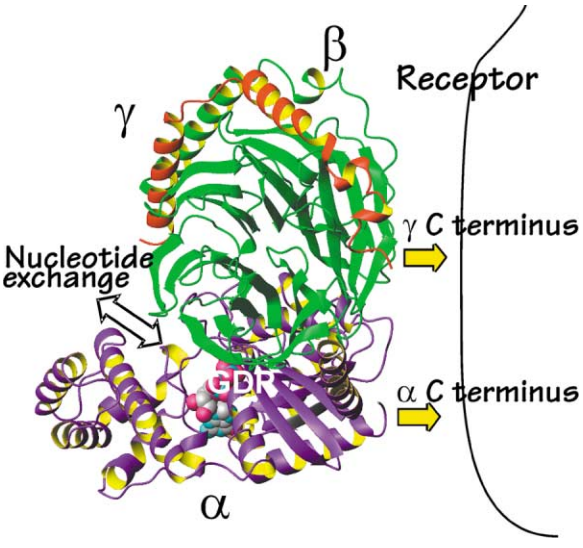

Figure 1. G Protein Interaction with a Receptor

A conformational switch at the C terminus of the γ subunit that establishes receptor contact [3, 4] can simultaneously result in movement of the βγ complex with reference to the α subunit, increasing both egress from and access to the nucleotide binding site [3]. Similar contact between the α subunit C terminus and the receptor can enhance this effect [9].

subunit C-terminal tails. The receptor sites that interact with these tails and their role in the activation process are at present unknown. Obtaining structural evidence with the whole protein will be the next step. While the crystal structure of a receptor-G protein complex will be valuable, the existing evidence for dynamic changes in the G protein and receptor during activation implies that other approaches will also be necessary. The role of the prenyl moiety also needs to be identified. Recent derivation of the prenyl group structure bound to proteins indicates the presence of specific sites for prenyl binding and conformational changes induced by prenyl group binding (e.g., [6]). It is clear that this lipid plays an important role in receptor-G protein interaction because the receptor is acutely responsive to the type of prenyl group attached to the G protein γ subunit [7, 8]. Whereas a role for the F64 residue in Gtγ (or its highly conserved homolog in other Gγs) maybe direct interaction with the receptor as proposed by Kisselev and Downs, this residue may also be the anchor that locates the prenyl moiety at the appropriate position for interaction with a receptor site. The precise location and orientation of this lipid in the G protein as well as its availability to the receptor when the γ subunit undergoes a conformational switch need to be defined.

N. Gautam
Departments of Anesthesiology and Genetics
Washington University School of Medicine
St. Louis, Missouri 63110

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Structural and Energetic Aspects of Multispecific Immune Recognition by NKG2D

The multispecific immune receptor NKG2D binds different ligands using a different set of energetically dominant interface residues for each ligand.

Recognition in the immune system is critical for survival. Failure to recognize and destroy foreign molecules could allow a fatal infection to develop. Conversely, an inappropriate attack on nonforeign molecules could lead to a serious autoimmune disease. NKG2D is a homodimeric C-type lectin-like molecule that has recently been recognized as a key immunological receptor on natural killer cells and other immune effector cells [1]. It has multiple different ligands that resemble major histocompatibility class I molecules, but do not bind peptides or interact with β_2-microglobulin. NKG2D interacts with these ligands, which are upregulated on the surfaces of pathogen-infected or tumor cells. The interaction triggers killing of the cell expressing the ligand.

The mechanisms whereby this symmetrical homodimeric receptor can bind to multiple asymmetric ligands are of interest, especially as some of the interactions have tight affinities in the low nanomolar range [2]. One group has argued that NKG2D displays some plasticity and spatial reorganization upon binding and that this constitutes an "induced fit" [3]. Such conformational flexibility would allow a single receptor to adopt the different conformations necessary for interactions with structurally different ligands. Alternatively, multiple binding specificities could arise without a substantial conformational change, if different ligands interacted with the receptor at different sites, or at one site but in different fashions. Several NKG2D-ligand complex structures have already shown that NKG2D uses a similar surface and orientation to bind to different ligands.

In this issue of *Structure*, McFarland et al. [4] have now addressed the mechanism of the multispecificity of NKG2D by solving the structure of unliganded human NKG2D, comparing it to the ligand-bound complexes and performing a computational and mutational analysis of several NKG2D-ligand complexes. The structure of unliganded mouse NKG2D was already known [5]. The computational analysis considers shape complementarity and surface packing, polar interactions involving ion pairs and hydrogen bonds, and protein-solvent interactions including a penalty for buried polar groups that are unsolvated [6]. This approach was used to identify the energetically dominant interface residues on NKG2D in the different ligand-bound complexes. The results suggest that different receptor residues dominate each complex. Confirmation of this energetic modeling is provided by experimental analysis of changes in the free energy of binding of proteins with alanine substitutions