Symposium 13: G-Protein Coupled Receptor Structure and Regulation

1913-Symp
Structure and Dynamics of the Human beta 2 Adrenergic Receptor
Brian Kobilka.
Stanford University, Stanford, CA, USA.
G protein coupled receptors (GPCRs) are remarkably versatile signaling molecules. The beta 2 adrenergic receptor (beta2AR) is a prototypical Family A GPCR that mediates physiologic responses to adrenaline and noradrenaline. The function of the beta2AR can be modulated by a spectrum of synthetic ligands ranging from full agonists, which maximally activate the receptor, to inverse agonists, which inhibit basal, agonist-independent receptor activity. We have used crystallography to determine the three-dimensional structure of the beta2AR, and spectroscopic methods to map ligand-induced conformational changes, and characterize the structure and organization of beta2AR oligomers in lipid bilayers. I will discuss what we these studies have taught us about the structural basis of beta2ARs function.

1914-Symp
Bret-monitoring GPCR/G Protein Interactions: New Eyes To See Texture In Ligand Efficacy
Céline Galés.
INSERM U858, Toulouse Cedex 4, France.
G protein-coupled receptors (GPCRs) represent the largest family of proteins involved in cell signal transduction. The generally accepted signalling mode of these receptors involves an initial engagement of $G\alpha\beta\gamma$ to the activated receptor followed by a rapid dissociation of $G\alpha$ and $G\beta\gamma$ into free active subunits that regulate the activity of different signalling effectors. Classically, this activation process is monitored indirectly through the generation of second messengers by the effectors or the modulation of nucleotide binding to the $G\alpha$ subunit. More recently, we monitored the conformational rearrangements at the interface s between receptors and G proteins and between G protein subunits by measuring bioluminescence resonance energy transfer (BRET) assay between probes inserted at multiple sites in receptor-G protein complexes (**1, 2**). This BRET assay not only allowed to go further inside the molecular mechanism of receptor-mediated G protein activation but also such a strategy, multiplexing BRET probes in the receptor-G protein complex, revealed the distinct conformations stabilized by ligands with different efficacy. Thus, in the future, such a multiplex BRET-based assay should be useful to appreciate the ''ligand-directed stimulus trafficking'' property of GPCR's ligands.
1-Galés *et al.* Nat methods 2, 177-84, 2005.
2-Galés *et al.* Nat Struct Mol Biol, 13.778-86, 2006.

1915-Symp
FTIR Studies On The Activation Mechanism Of Rhodopsin; Possible Extension To General GPCR's
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The visual pigment rhodopsin belongs to the group of GPCR's and is so far the best characterized member. Although the structure is known since 2000, the activation mechanism is still not completely understood. Activation is triggered by the light-induced conversion of the covalently bound inverse agonist, 11-*cis* retinal, to the all-*trans* geometry, constituting an agonist. We have studied the activation mechanism by Fourier transform infrared difference spectroscopy, a technique capable of monitoring molecular changes of proteins and co-factors. Therefore, this technique could also be of importance for the study of general GPCR's. Some of our recent results will be reviewed. They include the study of the retinal-free opsin state, which can be activated by low pH. This observation has been relevant for the recently published structures of opsin. Next, we show how the technique can be used to study conformational changes of the active state induced by the interacting G-protein (here a peptide derived from the G_α C-terminus known to be responsible for the interaction). In addition, the technique reveals conformational changes of the peptide. Further, the different ionic locks keeping the receptor in its inactive state are addressed. They encompass the Schiff base-counterion, the ERY motif at the end helix C, and the interaction of this motif with two further glutamic acids on helix F. This interaction is thought to keep also the β-adrenergic receptor in its inactive conformation. Finally, a method will be proposed which will allow the study of the ligand-induced activation of general GPCRs. The essential feature is a receptor monolayer, allowing free access of the ligand. The required increase in sensitivity is realized by surface-enhanced infrared spectroscopy.

1916-Symp
Asymmetrical function of dopamine D2 receptor dimers
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Symposium 14: RNA in Biology and Disease

1917-Symp
RNA Decay by the Eukaryotic Exosome
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The eukaryotic RNA exosome is a multi-subunit protein complex that regulates RNA processing and decay in processes that maintain the quality and abundance of cellular RNA. The subunit composition and activities of eukaryotic exosomes were determined by reconstituting exosomes from yeast and human. The structure of the ~300 kDa nine-subunit human exosome revealed the architecture for the asymmetric nine-subunit eukaryotic exosome core. While the eukaryotic exosome core resembles that of bacterial PNPase and archaeal exosomes, eukaryotic exosomes differ fundamentally from their bacterial and archaeal counterparts because they do not catalyze phosphorolytic exoribonuclease activity. In contrast, hydrolytic activities within the yeast ten- (Rrp44) or eleven-subunit (Rrp44 + Rrp6) exosomes can be fully attributed to the processive or distributive activities of Rrp44 and Rrp6, respectively. While the activities of yeast Rrp44 and Rrp6 explain most activities of the eukaryotic exosome in yeast, the activities of human Rrp44 or Rrp6 have not been analyzed in any detail. In fact, human Rrp44 has not been isolated as a stable constituent of human exosomes. Current efforts are focused on characterizing activities for human counterparts to yeast Rrp44 and Rrp6 and on defining substrate preferences for the yeast exosome complexes and associated subunits through kinetic analysis using defined substrates to determine how the nine-subunit core contributes to RNA processing and decay.

1918-Symp
Atomic Movies of RNA Dynamics Reveal Basis for Conformational Adaptation
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1919-Symp
Structure And Dynamics Of Protein-RNA Recognition In The Regulation Of Gene Expression
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We are studying mechanisms of biomolecular recognition in the regulation of pre-mRNA splicing and RNA-based gene silencing. Splicing of nuclear pre-mRNA, i.e. the removal of non-coding, intervening intron sequences, is a key step in the regulation of eukaryotic gene expression. It contributes to gene regulation and protein diversity by joining of alternative exons. Early spliceosome assembly is tightly regulated and involves the recognition of characteristic intron RNA sequences. The biogenesis of these pre-spliceosomal intermediates is dynamic and involves cooperative protein-protein and protein-RNA interactions.
For the structural analysis of such multi-domain proteins and protein complexes, we have developed an approach for determining the quaternary arrangement based on solution NMR and Small Angle Scattering methods. We combine orientational information derived from NMR residual dipolar couplings (RDCs) and (long-range) distance restraints derived from paramagnetic relaxation enhancement (PRE) using spin-labeled proteins and/or RNA. These data can be combined with a novel target function in CNS for direct refinement against small angle X-ray and/or neutron scattering (SAXS/SANS) data. The RDC, PRE and SAS data can be jointly used for structure calculation in ARIA/CNS and be supplement with additional information from chemical shift perturbation or biochemical data.
Results will be presented on polypyrimidine-tract recognition by U2 auxiliary factor, 65 kDa (U2AF65), which reveals a novel mechanism of RNA recognition that couples RNA binding affinity to a population shift of alternate conformations.

1920-Symp
Structural biology of riboswitch-mediated gene regulation and Argonaute-mediated gene silencing
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