Chapter IX
Computational Methods for the Prediction of GPCRs Coupling Selectivity

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ABSTRACT

GPCRs comprise a wide and diverse class of eukaryotic transmembrane proteins with well-established pharmacological significance. As a consequence of recent genome projects, there is a wealth of information at the sequence level that lacks any functional annotation. These receptors, often quoted as orphan GPCRs, could potentially lead to novel drug targets. However, typical experiments that aim at elucidating their function are hampered by the lack of knowledge on their selective coupling partners at the interior of the cell, the G-proteins. Up-to-date, computational efforts to predict properties of GPCRs have been focused mainly on the ligand-binding specificity, while the aspect of coupling has been less studied. Here, we present the main motivations, drawbacks, and results from the application of bioinformatics techniques to predict the coupling specificity of GPCRs to G-proteins, and discuss the application of the most successful methods in both experimental works that focus on a single receptor and large-scale genome annotation studies.

INTRODUCTION / BACKGROUND

G-protein coupled receptors (GPCRs) comprise a very important family of eukaryotic cell-surface membrane proteins. They are characterized by the structural hallmark of seven transmembrane helices, as exemplified by the crystal structure of rhodopsin (Palczewski et al. 2000), that has been extensively used as a homology modeling template for many receptor sequences (Nikiforovich et al. 2001; Becker et al. 2003). GPCRs play a pivotal role in signal transduction of eukaryotic cells, acting as the major sensors at the boundary between a cell and the outside world. Depending on their ligand-binding specificity, GPCRs can be activated by a broad range of external stimuli, from ions and small molecules to larger peptides and proteins, including light (Gether 2000). To perform these functions, GPCRs have evolved to a diversity of sequences that are traditionally classified in six major families, based mainly on shared homology (Horn et al. 2003). GPCRs have known representatives in most eukaryotic organisms, including yeast and plants, such as the recently discovered *Arabidopsis thaliana* seven-transmembrane (7TM) domain receptor GCR1 (Jones and Assmann 2004).

As signified by their name, upon binding to a ligand, GPCRs exert their role through the specific interaction with a more limited repertoire of intracellular proteins that hydrolyze GTP, namely the G-proteins (Neer and Clapham 1988). G-proteins are heterotrimeric complexes composed of three subunits Gα, Gβ and Gγ. They are classified into four main families, according to the type of their α-subunit, which also possesses Ras-like GTPase activity (Benjamin et al. 1995). These include G_s and $G_{i/o}$, which stimulate and inhibit adenylate cyclase, respectively (Johnston and Watts 2003), $G_{q/11}$, that activates phospholipase C (Exton 1993) and the less characterized $G_{12/13}$ family that activates the Na^+/H^+ exchange pathway (Kurose 2003). At least 16 different subtypes of Gα subunits have been identified and classified in these four families (Downes and Gautam 1999; Kristiansen 2004). Interaction of the G-protein trimer with the activated receptor triggers the exchange of the bound GDP with GTP, and subsequently the dissociation of the complex to Gα and Gβγ moieties, that activate downstream effector molecules. Hydrolysis of GTP to GDP by the α subunit renders the complex to its original, inactive state (Neer 1995). As a result, depending on the selectivity of the GPCR - G-protein interaction, a specific downstream pathway may be activated. Despite extensive experimental and computational studies, the structural basis of this specificity is not well characterized, while the mechanisms that determine the function of the activated GPCR/G-protein complex are yet to be uncovered (Muramatsu and Suwa 2006). Furthermore, the diversity of GPCR-G-protein interactions is enriched by several receptors that may alternatively interact with more than one family of G-proteins, known as promiscuous GPCRs. For instance, the human thyrotropin receptor can couple to all four G-protein families (Laugwitz et al. 1996). In general, promiscuity seems to be a rule rather than an exception for interactions between GPCRs and G-proteins (Wess 1998; Oliveira et al. 1999; Horn et al. 2000). Several lines of evidence indicate the importance of the GPCR intracellular regions, as well as the intracellular boundaries of the transmembrane helices (Gether 2000). It is also established that the regions of interaction on the G-protein are mainly the N-terminus of the Gα and the N- and C-termini of Gγ subunit. However, up to date, these findings have not been incorporated to a high-resolution, systematic model of GPCR – G-protein interactions, while the nature of the underlying mechanism is believed to be specific to the interacting partners (Wess 1998).

Due to their function as input nodes in the signaling pathways of eukaryotic cells, GPCRs play a very important role in health and disease (Muller 2000). GPCRs are involved in a variety of pathological conditions including cystic fibrosis, cancer and HIV-mediated infection of host cells. The ability