1. INTRODUCTION

The complex transduction of ligand stimulation events at G-protein coupled receptors (GPCRs) by heterotrimetric G proteins has long been appreciated. In addition to this, recent data shows that other protein interactions assist and can fine-tune cellular signals. Scientists have identified other membrane and intracellular proteins that interact, directly or indirectly, with GPCRs. In fact, 50 or more proteins are described in current literature as GPCR interactive proteins. GPCR interacting proteins act as modulators of ligand-evoked signals. Membrane associated or intracellular GPCR interacting proteins have critical roles in mediating: ligand recognition, optimization of signal transduction, trafficking, receptor clustering, and/or compartmentalization. This chapter reviews four aspects of the GPCR interacting protein literature: (a) methods for identifying GPCR interacting proteins; (b) interaction domains on the GPCR; (c) facilitation and fine-tuning of GPCR signaling events by interacting proteins; and (d) particular analysis of proteins that are μ opioid receptor (μOR) interactive. Although GPCR dimerization is viewed by many as a type of protein interaction between the GPCRs, dimer-related protein interactions will not be discussed; an alternate section of this book is devoted to dimerization.
2. GPCR INTERACTING PROTEINS: DETECTION ASSAYS

2.1. Yeast Two-Hybrid Screening

Yeast hybridization techniques capitalize on structural flexibilities that are typical for yeast transcription modulators. For example, the transcription factor Gal4 has both a DNA binding domain and a transcriptional activation domain. These domains do not need to be attached for transcription to occur, but they must be positioned in close proximity. If, by genetic engineering, the Gal4 domains are fused to two functionally unrelated—but interactive—proteins, transcription progresses as a result of protein–protein interaction (1). A further benefit that results from choosing the yeast two-hybrid assay is that this method provides simultaneous access to the genes that encode interacting proteins.

Yeast two-hybrid assays proceed as a complementary DNA (cDNA) library screening, with bait corresponding to either a C-terminal or a third intracellular loop sequence in the favored receptor. Yeast two-hybrid systems are the most commonly used assay systems for identifying G protein-coupled receptor (GPCR) interactive proteins, and these assays have enhanced scientific understanding of protein–protein interplays. γ-aminobutyric acid_B_R1 (GABA_B_R1) and R2 (GABA_B_R2) proteins in association were detected by the yeast two-hybrid method (2). Similarly, a yeast assay permitted the detection of β2-adrenergic receptors (β2-ARs) in association with Na+/H+ exchanger regulatory factor/Ezrin/Radixin/Moesin (ERM)-binding phosphoprotein-50 proteins (NHERF/EBP50) (3). As further examples, the association between somatostatin receptor 2 (SSTR2) and somatostatin receptor interacting protein (SSTRIP) was elucidated by a yeast screening method (4), as was the association between the D_2 dopamine receptor (D_2R) and spinophilin (5).

Clearly, the yeast two-hybrid system provides an excellent method for detecting protein–protein interactions; however, this system does have limitations.

One shortcoming of yeast assaying methods is that a protein can only be identified by yeast assays if the fished protein has direct contact with a GPCR. A second negative factor for yeast hybridization is the low number of proteins identified per library screen. One or, at most, two proteins are typically fished from the cDNA library with each bait. Another downfall of the yeast two-hybrid assaying method is its inability to detect protein–protein interactions that follow posttranslational modification(s). Additionally, as a final caution, investigators contemplating tests in a yeast assaying system should acknowledge that these assays have rendered false-negative as well as false-positive results.