*Introduction to G Protein Chimeras*

Since the first description of G protein chimeras that can alter the signaling phenotype of receptors, many investigators have found them useful for a variety of research purposes. Several people who work with Gi-coupled receptors have found that it is easier to study the stimulation of phospholipase C than the inhibition of adenylyl cyclase. Several groups have used the chimeras to develop rapid assays of receptor activation that can be used for screening mutants or agonist drugs. Others have used the chimeras to complement mutant receptors in detailed structure-function studies.

*Structure-Function Studies with G Proteins*

The carboxyl-terminus of the G alpha protein subunit is a key determinant of receptor specificity. We have previously shown that the Gq alpha subunit (alpha q) can be made to respond to Gi alpha-coupled receptors by replacing its carboxyl-terminus with the corresponding Gi2 alpha, Go alpha, or Gz alpha residues (2). We have recently extended these findings in three ways:

1. C-terminal mutations of Gq alpha/Gi alpha chimeras show that the critical amino acids are in the -3 and -4 positions.
2. Exchange of carboxyl-termini between Gq alpha and Gs alpha allows activation by receptors appropriate to the C-terminal residues.
3. We identify receptors that either do or do not activate the expected C-terminal chimeras (Gq alpha/Gi alpha, Gq alpha/Gs alpha, Gs alpha/Gq alpha).

Replacement of the five carboxyl-terminal amino acids of Gq alpha with the Gs alpha sequence permitted a Gs alpha-coupled receptor (the V2 vasopressin receptor, but not the beta 2-adrenoceptor) to stimulate phospholipase C. Replacementof the five carboxyl-terminal amino acids of Gs alpha with residues of Gq alpha permitted certain Gq alpha-coupled receptors (bombesin and V1a vasopressin receptors, but not the Oxytocin receptor) to stimulate adenylyl cyclase. Thus, the relative importance of the G alpha carboxyl-terminus for permitting coupling to a new receptor depends on the receptor with which it is paired. These studies refine our understanding of the basis of receptor-G alpha specificity. Substitutions of the C-termini of Gq alpha and other G-alpha subunits has recently been instrumental in developing high throughput screens for new agonists of G protein-coupled receptors [Broach J.R. and Thorner J. (1996) High-throughput screening for drug discovery. *Nature* 384 (Suppl.):14-16].

*Notes on the chimeras:*

1. All have been subcloned into pcDNA-1, in the Bam HI/NsiI cassette with q4WT as parent construct for the "q" chimeras and Gs-WT-HA as the parent construct for the "s"chimeras (see below for the description of the parent constructs).
2. All have the internal HA epitope, which does not affect receptor coupling, yet allows recognition by the 12CA5 antibody (available from Boehringer Mannheim as a purified monoclonal and directly conjugated to HRP, which is convenient for Westerns).
3. All the constructs are in pcDNA-1, which require sup F selection for Amp and Tet resistance. This requires special competent bacteria that are available in most labs, but can also be purchased from Invitrogen (for example, mc1061/p3).