Peptide Modulators of

G Protein Signaling

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Abstract

The hundreds of transmembrane proteins that make up the superfamily of G protein-coupled receptors (GPCRs) mediate signaling from an enormous variety of extracellular stimuli—including odorants, pheromones, peptides, lipids, and neurotransmitters—to intracellular heterotrimeric G proteins. The identification of specific modulators of G protein signaling is highly relevant to drug discovery; approximately 50% of currently marketed drugs target a GPCR. Here, we use mRNA display to identify novel and potent peptide ligands for G protein targets. mRNA display is a robust technique that facilitates the isolation of peptides with specific activities (e.g., binding to a target of interest) from large libraries containing trillions of unique molecules. We first targeted the heterotrimeric G protein, $G_{i\alpha1}$, with peptide combinatorial libraries. Isolated peptides bind with high affinity to $G_{i\alpha1}$ and can potentially affect downstream signaling in a pathway-specific manner. A potent peptide core motif interacting with G_{α} subunits was identified and used to construct new mRNA display libraries for the isolation of class- and/or state-specific G_{α}-binding peptides. We have also identified a novel peptide (the RWR motif) that interacts with the *Drosophila* GPCR, Methuselah. These peptides are potent antagonists to Methuselah-mediated signaling and, as mutants of Methuselah are associated with longevity, may be useful in lifespan and aging studies of the fruit fly. Overall, these efforts demonstrate the successful use of mRNA display as an efficient and facile method for generating new solutions to molecular design problems.

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