Thesis overview

G protein-coupled receptors (GPCRs) link a diverse array of extracellular signals—including peptides, hormones, odorants, and light—to an equally wide range of intracellular processes, allowing a cell to respond to external stimuli or communicate with other cells (*1-3*). There are approximately 750 human GPCRs, many of which are orphan receptors that respond to unknown ligands (*4*). The diversity of cell processes controlled by GPCRs and the accessibility of their extracellular domains (ligands do not have to cross the plasma membrane) have made them primary drug targets—approximately 50% of currently marketed drugs act on GPCRs (*5*). Intracellular G proteins mediate the signaling from activated GPCRs (*2, 3*). Although drugs directly targeting G proteins are not yet in clinical use, G proteins and their regulators have been increasingly regarded as potential pharmaceutical targets (*6-9*).

The work in this thesis covers our development of peptide ligands targeting G proteins and GPCRs. The long-term goals of our work are to produce new tools for probing G protein and GPCR structure and function, as well as to provide possible leads for future drug discovery and design. Our primary method is mRNA display, which allows us to isolate and identify specific peptide ligands from large libraries comprising over 10 trillion unique members (*10*). Chapter 1 is a brief review of published peptide selection experiments directly targeting G protein signaling pathways. In Chapter 2, we perform an mRNA display selection to isolate a novel peptide (R6A) that binds to the G protein, $G_{i\alpha1}$ (*11*). R6A and its derivatives are short, potent peptides that can modulate the active state of $G_{i\alpha1}$ and have the potential of specifically activating or deactivating particular G protein pathways. In Chapter 3, we extend our studies of the R6A peptide to other G_α family members and identify a core motif for the recognition of G_α subunits.

The core motif can be used as a starting point for new mRNA display libraries to isolate peptides with novel activities and/or specificity for particular G protein classes.

Selection techniques have had limited success against GPCRs because of the difficulty in expression, solubilization, and presentation of the receptor for recognition by peptide libraries. In Chapter 4, we demonstrate the successful isolation of peptides that bind to the GPCR, Methuselah (Mth), by targeting only the extracellular domain. Mth was previously determined to play a role in lifespan in the fruit fly, *Drosophila melanogaster* (*12*). Although the peptide ligands were identified by targeting only the ectodomain, subsequent studies demonstrated that they recognize the full-length receptor and antagonize Mth-mediated signaling.

Appendix A describes the epitope mapping of an anti-polyhistidine monoclonal antibody (mAb). The mRNA display library was originally intended to target $G_{i\alpha 1}$ immobilized on the mAb. However, only high affinity mAb-binding peptides were identified. These sequences revealed a different consensus than the cited epitope and demonstrated significantly higher affinity. To determine the minimal, functional epitope, a detailed procedure for the construction of unidirectional, nested deletion mRNA display libraries is described.

The work in this thesis was supported by grants from the NIH (RO160416) and the Beckman Foundation to R. W. R. W. W. J. was supported in part by a DOD National Defense Science and Engineering Graduate Fellowship and was a recipient of a Scholarship for Research in the Biology of Aging, sponsored by the Glenn Foundation for Medical Research and the American Federation for Aging Research. R. W. R. is an Alfred P. Sloan Foundation Research Fellow.

References

1. Strader, C. D., Fong, T. M., Tota, M. R., Underwood, D., and Dixon, R. A. F. (1994) Structure and function of G protein-coupled receptors, *Annu. Rev. Biochem. 63*, 101-132.
2. Gilman, A. G. (1987) G proteins: transducers of receptor-generated signals, *Annu. Rev. Biochem. 56*, 615-649.
3. Neves, S. R., Ram, P. T., and Iyengar, R. (2002) G protein pathways, *Science 296*, 1636-1639.
4. Vassilatis, D. K., Hohmann, J. G., Zeng, H., Li, F., Ranchalis, J. E., Mortrud, M. T., Brown, A., Rodriguez, S. S., Weller, J. R., Wright, A. C., Bergmann, J. E., and Gaitanaris, G. A. (2003) The G protein-coupled receptor repertoires of human and mouse, *Proc. Natl. Acad. Sci. U.S.A. 100*, 4903-4908.
5. Howard, A. D., McAllister, G., Feighner, S. D., Liu, Q., Nargund, R. P., Van der Ploeg, L. H., and Patchett, A. A. (2001) Orphan G-protein-coupled receptors and natural ligand discovery, *Trends Pharmacol. Sci. 22*, 132-140.
6. Freissmuth, M., Waldhoer, M., Bofill-Cardona, E., and Nanoff, C. (1999) G protein antagonists, *Trends Pharmacol. Sci. 20*, 237-245.
7. Höller, C., Freissmuth, M., and Nanoff, C. (1999) G proteins as drug targets, *Cell. Mol. Life Sci. 55*, 257-270.
8. Neubig, R. R., and Siderovski, D. P. (2002) Regulators of G-protein signalling as new central nervous system drug targets, *Nat. Rev. Drug Discov. 1*, 187-197.

9. Nürnberg, B., Tögel, W., Krause, G., Storm, R., Breitweg-Lehmann, E., and Schunack, W. (1999) Non-peptide G-protein activators as promising tools in cell biology and potential drug leads, *Eur. J. Med. Chem. 34*, 5-30.

10. Takahashi, T. T., Austin, R. J., and Roberts, R. W. (2003) mRNA display: ligand discovery, interaction analysis and beyond, *Trends Biochem. Sci. 28*, 159-165.

11. Ja, W. W., and Roberts, R. W. (2004) *In vitro* selection of state-specific peptide modulators of G protein signaling using mRNA display, *Biochemistry 43*, 9265-9275.

12. Lin, Y. J., Seroude, L., and Benzer, S. (1998) Extended life-span and stress resistance in the *Drosophila* mutant *methuselah*, *Science 282*, 943-946.