

RESEARCH NEWS AND VIEWS

RASSLing with receptors

Tim Clackson

The human genome is thought to encode over a thousand G-protein coupled receptors (GPCRs). One of the more daunting tasks in the postgenomic era will be defining true physiological roles for these molecules and the pathways they initiate. Approaches that allow a specific receptor to be activated at will in whole animals will be invaluable. In this issue, Bruce Conklin and colleagues¹ describe an *in vivo* validation of their protein engineering solution to this problem—an approach likely to be broadly useful for dissecting GPCR function, providing models for GPCR-linked diseases, and perhaps even controlling GPCRs for therapeutic purposes.

GPCRs are seven-transmembrane domain proteins that transduce extracellular signals, as diverse as photons or peptide hormones, into a wide range of physiological events—from hormone secretion to alterations in heart rate. Extracellular stimuli cause conformational changes in the receptor, which then activates the associated heterotrimeric G protein inside the cell. The ensuing cellular events depend on both the type of G protein (there are four main ones) and the type of cell. For example, the activation of G_i-coupled receptors reduces intracellular cAMP levels, slowing myocyte contraction in the heart or altering neurotransmitter release in the brain.

Because many GPCRs modulate entire physiological responses rather than cell-specific changes, a full understanding of their functions can only come from studies in a whole-animal context. Ideally, one would like to activate a specific receptor in a specific subset of cells *in vivo*, by administering a small-molecule ligand, and then observe the physiological consequences. But there are several problems with this. The presence of endogenous ligands for the receptor may complicate interpretation of results. Also, activation cannot be restricted to a specific subset of cells: all cognate receptors will be activated. Finally, despite the large pharmacopoeia of GPCR ligands in clinical use, there is no guarantee that a high-affinity ligand for your favorite receptor will be available.

Enter Conklin and co-workers, who set out to solve the problem by designing what they call a “RASSL”—a receptor activated

solely by a synthetic ligand. The idea was to start with a receptor for which a high-affinity synthetic ligand is available and then mutate it so that endogenous ligands no longer bind, but affinity for the synthetic compound remains high. When expressed in target cells in a transgenic animal, the mutant receptor should therefore be silent until administration of the compound.

To design a prototype RASSL, Conklin and co-workers turned to the human κ -opioid receptor, which signals G_i-coupled

determinants for dynorphin binding, but not those for synthetic ligands. In work reported last year, Coward et al.² found that replacing this loop with the equivalent sequence from the δ -opioid receptor reduced binding affinity for dynorphin by 200-fold, dramatically reducing signaling in response to dynorphin and a panel of 20 related neuropeptides in transfected-cell assays. By contrast, signaling through spiradoline was unaffected. The chimeric receptor, called Ro1, thus fulfilled the specifications of a RASSL.

Although a 200-fold lower affinity is substantial, is this enough to allow Ro1 to be useful *in vivo*? The answer is provided in the present paper, in which Ro1 has been expressed in transgenic mice under the control of a heart-specific expression system. In cardiac tissue, G_i signaling normally leads to a decrease in heart rate. Activation of Ro1 in the transgenic mice by administration of spiradoline lowered heart rate as much as 80% in less than a minute, followed by the expected desensitization lasting over 24 hours. Importantly, until the drug was added, the rate was indistinguishable from that in non-expressing control animals, indicating that the RASSL is indeed immune from endogenous agonists. These studies clearly validate the use of RASSLs to obtain precise spatially and temporally restricted control of G-protein signaling *in vivo*.

What next? Additional mice have already been generated that express Ro1 specifically in hepatocytes or salivary glands, where G_i signaling regulates other processes¹. Use in other tissues should depend only on the ability to restrict expression appropriately. In some tissues, higher levels of endogenous agonists may necessitate further manipulation of the receptor to enhance specificity (a molecule with an additional 10-fold lower dynorphin affinity, Ro2, is already known²). It will also be important to extend the RASSL principle to receptors that signal through the other G proteins. This could be achieved by identifying extracellular domain mutations analogous to the κ → δ loop-swap, or by engineering the intracellular domain of Ro1 to signal through the G protein of interest.

Although GPCRs signal using an allosteric mechanism, many other signal transduction processes involve a regulated series of simple protein–protein interactions³. The use of chemical “dimerizers” to control these signaling pathways is now well established⁴. In these applications, a chimeric

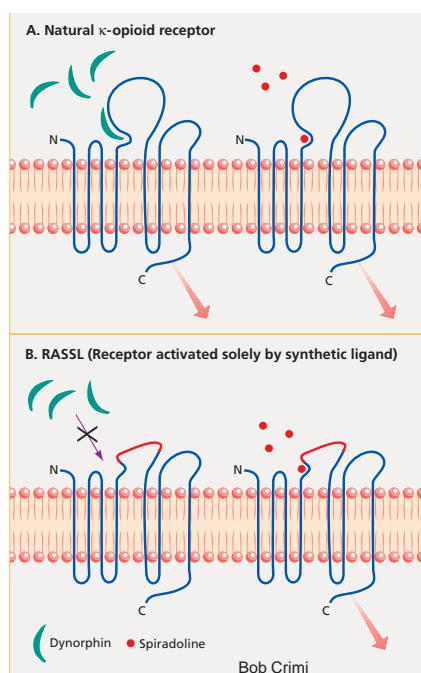


Figure 1. Getting specific: G_i-coupled signaling of the κ -opioid receptor can be activated by natural neuropeptides such as dynorphin, or synthetic agonists such as spiradoline. Replacing the second extracellular loop with that from the δ -opioid receptor (in red) produces a receptor that now only signals in response to the synthetic ligand—a “RASSL.” Administering the synthetic ligand to transgenic mice expressing the chimeric receptor in specific tissues permits determination of the consequences of signaling without interference from endogenous ligands.

responses to neuropeptides such as dynorphin in the brain (see Fig. 1). Thanks to the attentions of the pharmaceutical industry, high-affinity synthetic κ -agonists such as spiradoline are already available. Protein engineering had revealed that the receptor’s second extracellular loop contains the critical

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protein is expressed in which a signaling domain is fused to a drug-binding protein; addition of an appropriate bivalent “dimerizer” drug crosslinks the proteins and initiates signaling. Since the first use of this approach to activate T-cell receptor signaling⁵, dimerizers have been used to control numerous cell signaling and protein localization events inside cells and animals, including proliferation and apoptosis (for a review, see ref. 4). RASSLs provide an analogous and entirely complementary technology for one class of receptors that operates through allostery rather than proximity. Nevertheless, the approaches differ in some respects. For example, dimerizers activate only their target chimeric receptor, whereas the RASSL ligand (spiradoline) can still activate endogenous κ -opioid receptors. RASSL activation also appears to be faster than for dimerizers (~1 min compared with 20–40 min), although this may simply reflect the time taken for the

latter to diffuse across the cell membrane.

Both approaches belong to a growing arsenal of cell biology techniques that use small molecules to control the expression or activation of transgenes (reviewed in ref. 6). These include control at the level of transcription (using drug-activated transcription factors⁷) or translation⁸; as well as conditional inactivation of proteins by fusing them to steroid receptors. Each of these systems allows the investigator to exert pharmacological control over the activity of an engineered protein, allowing manipulation in a physiological context. In fact, many tiers of regulation are now feasible, as exemplified by the approach used by Conklin and colleagues: they placed the Ro1 RASSL gene under the control of a tetracycline-regulated transcriptional activator, allowing expression to be kept off until the animal was fully developed.

As well as providing useful tools to dissect signaling pathways and develop disease mod-

els, small-molecule regulation techniques have direct clinical potential in cell and gene therapies. For example, transferring an engineered apoptotic receptor into T cells allows their elimination to be controlled by a dimerizer drug⁹, suggesting a way to control graft-versus-host disease after bone marrow transplant procedures¹⁰. Further development of RASSLs may allow similar clinical exploitation of processes signaled by G proteins.

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The PDZ domain as you like it

Robert E. Hughes and Stanley Fields

Most biologists operate like reputable journalists, faithfully reporting on what's happening in the world but wary of straying into the realm of invention. Much of what is reported in biology, however, is old news: the deeds and associations of macromolecules that have evolved over millions of years. But some biologists are more akin to playwrights. Rather than uncovering and chronicling the habits of the characters nature provides us, they prefer instead to invent characters and direct their actions on the stage. However, there really are no new characters under the sun, and the skill of a dramatist lies in the facility with which the old stock is refashioned into novel and unexpected situations.

And so is the case with the work reported in this issue by Schneider et al.¹, which describes the use of the yeast two-hybrid system to select artificial PDZ domains that bind one of four peptide ligands with high specificity and affinity. Of these four peptides, two are derived from the carboxy termini of membrane-associated proteins. These engineered PDZ domains, or their interacting peptide ligands, can be directed to the nucleus or the plasma membrane,

depending on the location of their cognate partners. In the next scenes in this play, such characters may act out new adventures in protein engineering.

PDZ domains are small (about 100 amino acids) globular folds that mediate protein–protein interactions. These domains were first recognized as conserved elements in the PSD-95 (postsynaptic density), Dgl (Discs-large), and ZO (zonula occludans) proteins; each is a member of the membrane-associated guanylate kinase (MAGUK) family, and each contains three copies of the PDZ element. It was subsequently demonstrated that a general function of PDZ domains is to promote clustering of proteins into complexes at the plasma membrane². Examples of this include the clustering mediated by PSD-95 of Shaker-type K⁺ channels³. This type of interaction is believed to bring about the assembly of multiprotein signaling complexes to increase the efficacy of molecules involved in signaling and effector functions by concentrating them with respect to one another, as well as by localizing them to specific regions of the cell membrane⁴. PDZ domains promote clustering through their interaction with C-terminal peptides of membrane proteins. The PDZ domain has been shown to recognize specifically the C-terminal motif (-x-Ser/Thr-x-Val-COOH), although peptides without this motif can also be bound⁵. Several different proteins containing these ligand motifs can thus become clustered by

association with a “scaffold” protein containing multiple PDZ domains.

The precise molecular details of PDZ–peptide recognition have been revealed by the crystal structures of PDZ domains, solved both in free and ligand-bound states^{6,7}. The globular PDZ structure is composed mainly of a six-stranded β -sandwich, flanked by two α -helices. On its surface lies a peptide-binding groove defined by the edge of an antiparallel β -sheet and an α -helix. Binding of the ligand peptide in this groove positions the peptide to act as an additional antiparallel β -strand, thereby extending the sheet, a mode of binding referred to as β -augmentation⁸. Unlike a classic protein–protein interaction that involves docking of relatively rigid complementary protein surfaces, the PDZ–peptide interaction involves a ligand that is likely to be unstructured in its free form. This basic difference may impart an inherent flexibility to the range of potential PDZ–ligand recognition, and may explain in part the success of the authors' attempt to expand that range.

In order to select PDZ domains with novel recognition properties, Schneider et al. used a streamlined yeast selection protocol based upon the two-hybrid interaction assay. Fusions of the Gal4 DNA-binding domain to potential PDZ ligand peptides were used as baits to screen PCR-mutagenized pools of a PDZ domain that had been fused to the Gal4 transcriptional activation domain by recombination-based cloning. Expression of a yeast

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