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Chemical names

AFDX384: (+/-)-5,11-dihydro-11-((2-[2-((dipropylamino)methyl)-1-piperidinyl]ethyl)amino]carbonyl)-6H-pyrido [2,3-b](1,4)-benzodiazepine-6-one	HTZTP: (3-(1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate)
AQRA741: (11-((4-[4-(diethylamino)butyl]-1-piperidinyl)acetyl)-5,11-dihydro-6H-pyrido(2,3-b)(1,4)benzodiazepine-6-one)	PD102807: (S)-(+)-(4aR,10bR)-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol
CMI936: (2-exo{5-(3-methyl-1,2,4-oxadiazolyl)}-[2.2.1]-7-azabicycloheptane)	SCH57790: 4-cyclohexyl-a-[4-[[4-methoxyphenyl]sulfinyl]-phenyl]-1-piperazineacetonitrile
CMI1145: (2-exo{5-(3-amino-1,2,4-oxadiazolyl)}-[2.2.1]-7-azabicycloheptane)	YM46303: quinuclidin-4-yl biphenyl-2-yl carbamate monohydrochloride

Engineering receptors activated solely by synthetic ligands (RASSLs)

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The functional and molecular diversity of G-protein-coupled receptors presents a significant challenge to understanding the connection between a single receptor signaling pathway and a specific physiological or pathological response. To gain control over the timing and specificity of a G-protein signal, receptors activated solely by synthetic ligands (RASSLs) have been developed. These engineered receptors no longer respond to endogenous peptides, but can still be activated by a specific small-molecule drug. Further control over the location of the signal can be achieved by using RASSLs in conjunction with tissue-specific expression systems *in vivo*. Existing RASSLs have clarified the role of G_i signaling in cardiac physiology and are currently being used to study cardiomyopathy, muscle remodeling, sensory transduction and complex neurobehavioral responses.

G-protein-coupled receptors (GPCRs) are the largest known family of cell-surface receptors, encompassing >1000 distinct receptors¹. These receptors can be activated by a variety of natural ligands, including peptide hormones, odorants, photons, biogenic amines and lipids. Activation of these receptors results in many different physiological responses, including heart rate changes, chemotaxis, cell proliferation, neurotransmission and hormonal responses². Prolonged stimulation of GPCRs can alter gene transcription and therefore might mediate long-term changes in the biochemistry, physiology and behavior of an organism.

The same diversity of receptors, ligands and responses that makes GPCRs biologically important has complicated the study of their function *in vivo*. The ability to stimulate a specific GPCR in a particular tissue *in vivo* would be a valuable aid to understanding the resultant changes in signaling and physiology. However, in a whole animal, numerous factors complicate this type of study. Although it is feasible to inject a specific GPCR ligand directly into

the tissue of interest, there is no way to restrict receptor activation to a specific subpopulation of cells. Furthermore, many GPCRs belong to large families of closely related receptor subtypes and can be activated by similar ligands. For example, the specific agonists and antagonists for many of the more than 14 identified 5-HT receptor subtypes remain unknown³. Finally, the actions of endogenous ligands can complicate the interpretation of experimental results.

One approach to these difficulties has been to develop receptors activated solely by synthetic ligands (RASSLs)⁴. These genetically engineered receptors are insensitive to their natural, endogenous ligand(s), but can still be fully activated by synthetic, small-molecule drugs. By using tetracycline-regulated gene-expression technology, one can control where and when these RASSLs are expressed *in vivo*. By administering the synthetic drug, a single G-protein pathway in a specific tissue can be stimulated quickly and reversibly. This system has already yielded important insights into cardiac function^{5,6}. By expressing RASSLs in other tissues, researchers can explore the role of G-protein signaling in many physiological responses and disease states.

The RASSL approach complements other recently developed 'designer' signaling systems that use artificial ligands⁷. Although these designer signaling systems activate intracellular pathways efficiently, they cannot fully mimic the speed, localization, regulation and amplification of endogenous GPCR signals. For example, dimerization systems fuse a signaling domain with a drug-binding protein. The addition of a chemical dimerizer (i.e. FK1012 or rapamycin) that binds to the drug-binding domain

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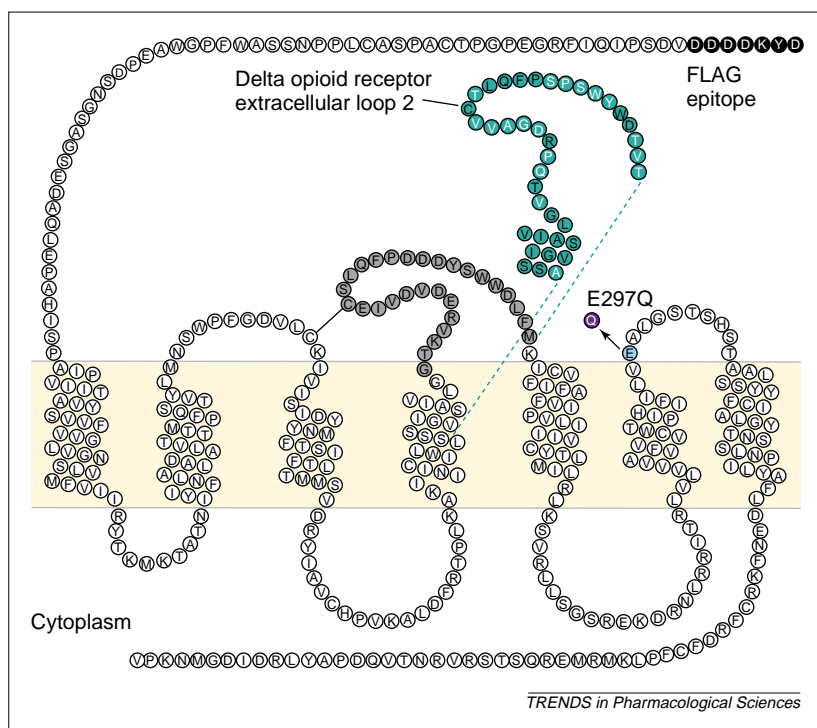


Fig. 1. Construction of an opioid receptor activated solely by synthetic ligand (RASSL). Replacing the second extracellular loop of the human kappa opioid receptor (gray) with the corresponding sequence from the delta opioid receptor (green) significantly attenuates binding to endogenous peptides, while maintaining affinity for small-molecule kappa opioid receptor agonists. The specificity of the RASSL for small-molecule agonists was further enhanced by mutating Glu297 (cyan) to glutamine (purple).

can then crosslink the fusion proteins and activate signaling. These dimerization systems are powerful tools for molecules that are activated by proximity to other molecules (hetero- or homo-oligomerization) or by proximity of cellular compartments (e.g. plasma membrane and nucleus)^{7,8}.

Dimerization systems and artificial transcription factors have also been used to control transcriptional events. The tetracycline transactivator system, another artificial transcription factor, has been used to control RASSL expression in mice. Designer transcription factors can be used to express mutationally activated components of signaling pathways, such as activated G-protein α -subunits. Although these are powerful tools, the transcriptional activation of signaling is relatively slow, and lacks the amplification of signaling by an artificial receptor such as a RASSL.

Another approach has been the creation of specifically designed protein kinase substrates to help identify the specific kinase targets⁷. Similarly, kinase inhibitors that affect only mutated kinases have also been engineered. These approaches are likely to have a major impact on determining the specificity of protein kinases in their complex cellular environment. These designer signaling systems complement each other, building a growing toolbox for biomedical researchers. However, because none of the other systems has the unique GPCR seven-transmembrane structure, none can replicate the speed (nanoseconds), amplification and membrane-localized signal that can be achieved through a RASSL.

In early attempts to make a designer GPCR, elegant studies by Strader and colleagues combined a custom ligand specifically designed to complement a mutant adrenoceptor that had impaired binding to adrenaline⁹. However, this custom ligand was never successfully used for *in vivo* studies because it was in limited supply and the potency was too low ($EC_{50} = 40 \mu\text{M}$) to be administered systemically for *in vivo* studies. Other studies have designed new metal-ion-binding sites into GPCRs. However, these compounds might be toxic and have effective doses in the millimolar range¹⁰. By comparison, the EC_{50} for RASSL activation by spiradoline is 5 nM, 800-fold more potent than designer adrenoceptor agonists, which allows practical *in vivo* studies. RASSL design is based on a high-affinity, potent agonist-binding site, so one does not have to face the daunting task of building a new agonist-binding site.

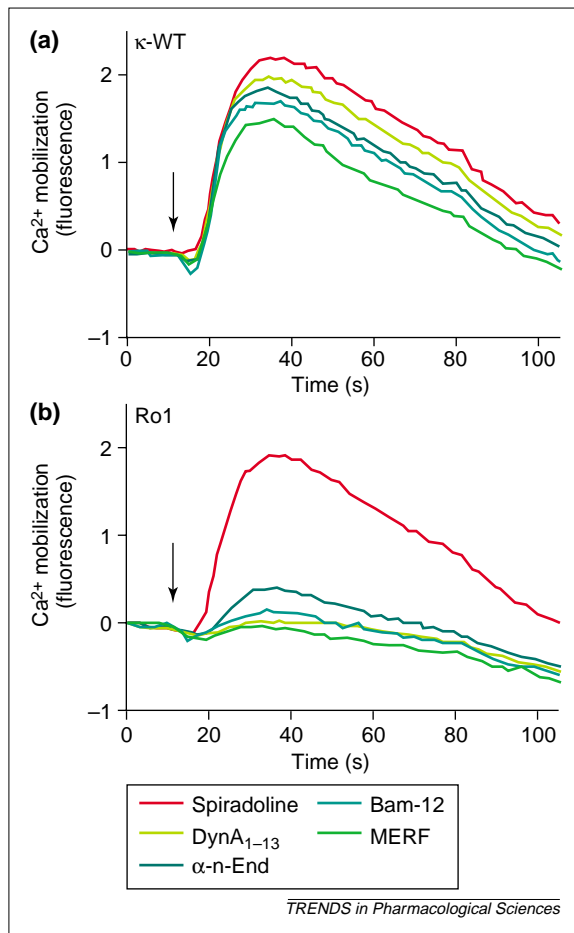
Because RASSLs use a high-affinity drug ligand, one potential complication is that the drug might also act at endogenous receptors. Our current RASSL is activated by a drug that also activates endogenous kappa opioid receptors (KORs). Therefore, we have chosen to focus our studies in tissues that express few endogenous receptors, or in knockout animals that completely lack the endogenous receptor. In the future, we hope to develop RASSLs that are engineered to have a significantly higher affinity for the synthetic ligand than do endogenous receptors, which will allow us to circumvent this limitation. Although the RASSL niche is unique, many lessons can still be learned from other systems. Drawing on the creative energies of the research community, the field of designer signaling will continue to grow at a rapid pace.

RASSL construction

For our first RASSL, we modified the KOR. This receptor responds to endogenous peptides and signals via the well-characterized G_i pathway. Because of the importance of this receptor family for pain modulation, the pharmaceutical industry has developed many high-affinity opioid receptor agonists. Small-molecule ligands of the KOR are structurally distinct from the endogenous peptide ligands, including dynorphin^{11,12}. Unlike mu or delta opioid receptor agonists, KOR agonists are nonaddictive¹³.

Structure–function studies of the KOR have revealed that the second extracellular loop is the crucial binding site for dynorphin and other neuropeptides^{14–16}. However, small-molecule agonists have a different binding pocket close to the transmembrane region. This raises the possibility that mutating the extracellular regions of the receptor could interfere with the binding of endogenous, but not synthetic, ligands. To construct the first RASSL (Ro1), the second extracellular loop of the delta opioid receptor was substituted for the corresponding portion of the KOR (Fig. 1)⁴. This substitution results in low affinity for both dynorphin and delta opioid receptor ligands^{4,16,17}. The second

Fig. 2. Selective activation of the prototype receptor activated solely by synthetic ligand (RASSL). Changes in intracellular Ca^{2+} were measured, using a fluorometric imaging plate reader assay, in response to activation of (a) wild-type kappa opioid receptors (κ -WT) or (b) the synthetic Ro1 receptor by natural opioid peptides (DynA₁₋₁₃, α -n-End, Bam-12 and MERF) or the synthetic ligand spiradoline. Ro1 was only activated by spiradoline. Curves are sample tracings of actual Ca^{2+} fluorescence in response to 1 μM doses of agonist. The arrow indicates addition of the agonist. Abbreviation: MERF, Met-enkephalin-Arg6-Phe7.



RASSL (Ro2) contains all the mutations in Ro1, as well as a substitution of glutamine for Glu297, located at the junction of transmembrane domain 6 and extracellular loop 3 (Fig. 1). This residue is thought to contribute to specific opioid peptide binding¹⁸. Both RASSLs showed reduced affinity for dynorphin, without a significant reduction in the response to spiradoline or other small-molecule ligands. The affinity of Ro1 for dynorphin was reduced by 200 times, whereas binding activity of Ro2 was reduced by 2000 times⁴.

Despite the changes in the binding characteristics of Ro1 and Ro2, the signaling of the receptors in response to spiradoline-induced activation remained largely intact⁴. Measurement of Ca^{2+} mobilization in response to agonist stimulation using a fluorometric imaging plate reader assay (FLIPR, Molecular Devices¹⁹) demonstrated a robust signal in response to spiradoline, but not to peptide agonists (Fig. 2). The administration of spiradoline, but not dynorphin, also induced G_i -mediated cell proliferation in Rat1a fibroblasts that expressed Ro2 (Ref. 4).

These studies indicate that G_i -coupled signaling *in vitro* can be controlled through the use of an engineered receptor that responds only to synthetic ligands. However, it is essential to demonstrate that this RASSL controls cell signaling in a whole animal.

Control of RASSL expression *in vivo*

The expression of RASSLs in specific tissues of whole animals is a potentially valuable tool for studying both normal and pathological signaling cascades. To take full advantage of the signaling control offered by a RASSL, it is also important to control the timing and location of RASSL expression *in vivo*. To achieve this, we have used the tetracycline-controlled inducible expression system (tet system) developed by Gossen and Bujard²⁰. Briefly, transgene expression is driven by a minimal promoter fused downstream of the tetracycline response element (tetO) from the bacterial *tet* operon (Fig. 3). Expression of the transgene requires binding of a transcriptional transactivator (tTA) to tetO. Therefore, a second transgene is introduced that carries tTA driven by a tissue-specific promoter. As a result, tTA will be produced and will bind tetO only in specific tissues. The tissue-specific promoter driving tTA expression will determine the anatomical expression pattern of the gene of interest. Mice that express tTA under the control of a variety of different tissue-specific promoters have already been developed²¹⁻²⁵. An additional layer of control is available in this system because binding of tTA to tetO is controlled by tetracycline or its more potent analog, doxycycline. We have chosen to use the 'tet-off' system, in which transgene expression requires the absence of doxycycline. This system has the advantage that all assays are conducted when the animals are off doxycycline, eliminating effects of the antibiotic itself on behavior or physiology. Using the tet system to drive expression of the kappa opioid RASSL allows control of where the RASSL is expressed (by choice of tTA line) and when it is expressed (by administration or withdrawal of doxycycline).

We first used the tet system to examine RASSL signaling in the heart by expressing tTA under the control of the myosin heavy chain promoter²¹. Maximal Ro1 transgene expression in the heart is reached ~10 days after withdrawal of doxycycline, when the drug has been washed completely out of the system of the animal⁵. Because doxycycline concentrations can be raised rapidly after readministration, subsequent suppression of transgene expression is largely dependent on the natural half-life of the protein being expressed. In most cases, transgene expression can be suppressed far more quickly than it can be completely induced. We have observed transgene suppression within 24 hours after doxycycline administration⁶.

Controlling RASSL signaling *in vivo*

When the receptor is expressed, it should be functionally silent until the administration of spiradoline stimulates the RASSL and activates signaling pathways rapidly and specifically. G_i signaling in the heart has been shown to reduce heart rate²⁶. Therefore, when Ro1 is expressed in the heart (using the α -myosin heavy chain promoter),

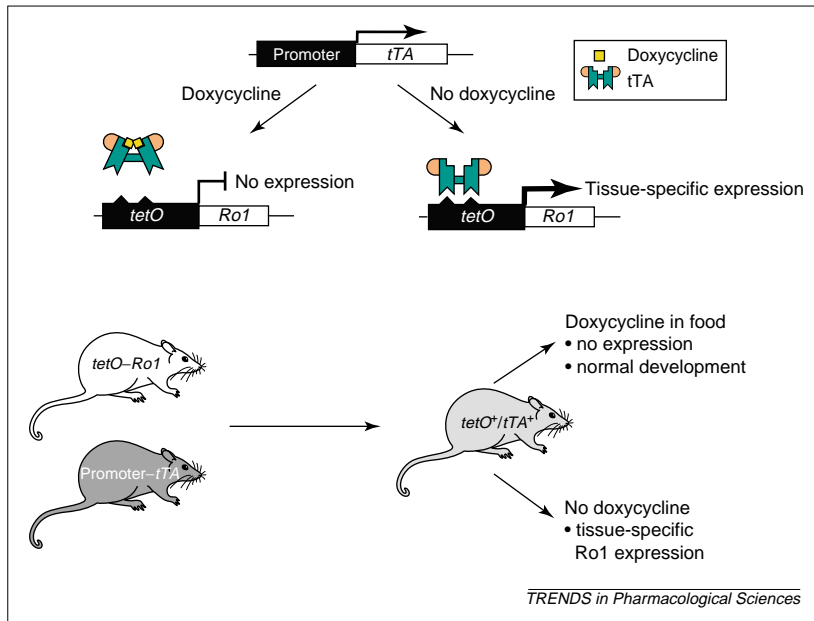


Fig. 3. Tissue-specific gene expression using the tet-tTA system. Two transgenes are required. First, the gene of interest (*Ro1*, in this case) is placed under the control of the *tet* operon sequence. Expression of this transgene requires the tetracycline transactivator (tTA). The gene encoding the tTA can be placed under the control of a tissue-specific promoter, thus ensuring that tTA, and therefore *Ro1*, are expressed only in a particular tissue. Expression of *Ro1* can be suppressed by the addition of doxycycline, which binds to tTA and inactivates it. For gene expression in mice, this system requires two transgenic lines: one with the promoter-tTA transgene, the other with *tetO-Ro1*. When these mice are mated together, ~25% of the offspring will have both transgenes. However, even in these bigenic mice, there will be no *Ro1* expression as long as the animal is fed doxycycline. Abbreviations: tet, tetracycline-controlled inducible expression; tetO, tetracycline response element. Reproduced, with permission, from Ref. 5.

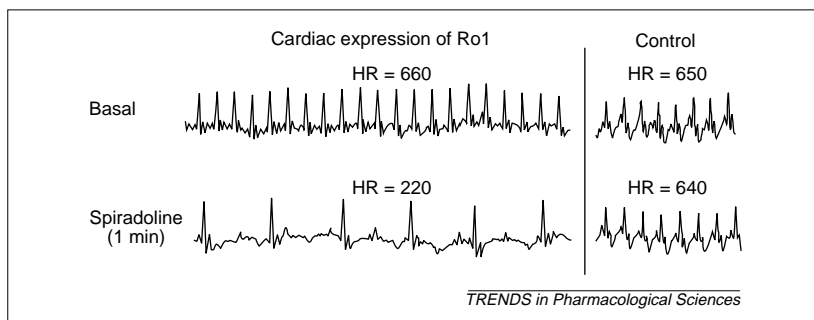


Fig. 4. Receptor activated solely by synthetic ligand (RASSL)-mediated reduction of heart rate (HR). Less than 1 min after spiradoline injection (1×10^{-5} mol kg^{-1}), the mouse that expresses *Ro1* in the heart had a heart rate one-third that of baseline. Spiradoline had no effect on the control mouse (MHC-tTA). [The MHC-tTA mouse is a transgenic animal that carries the tetracycline transactivator (tTA) under the control of the myosin heavy chain (MHC) promoter.] Reproduced, with permission, from Ref. 5.

receptor activation can be studied by simply measuring changes in heart rate. Wild-type animals have few KORs in the heart and therefore show no change in heart rate in response to spiradoline administration. In animals that express *Ro1* in the heart, however, heart rate decreases within 30 seconds after drug administration (Fig. 4). This bradycardia is reversible within 10–15 minutes and can be blocked by antagonists (e.g. nor-binaltorphimine dihydrochloride)⁵. These experiments indicate that a RASSL can be used to modulate a physiological response that requires G_i signaling *in vivo*. Expression of RASSLs in other tissues will enable researchers to regulate many other physiological and behavioral responses.

Eliminating unwanted signaling by using KOR knockout mice

In a tissue like the heart, where expression of the endogenous KOR is extremely low²⁷, the actions of spiradoline at the native receptor are not significant. However, the results of experiments involving *Ro1* or *Ro2* in KOR-rich tissues such as the brain could be complicated by the actions of spiradoline at native receptors. For example, stimulation of neural KORs results in sedation that could mask behavioral responses caused by spiradoline stimulation of *Ro1*. To circumvent this problem, we have expressed *Ro1* in knockout mice that completely lack the wild-type KOR (knockout mice provided by J. Pintar, Rutgers University, NJ, USA).

Detecting RASSLs *in vivo*

For many experiments that use RASSLs to study the role of G_i signaling *in vivo*, it is essential to pinpoint the receptor localization, sometimes down to the subcellular level. To this end, we have developed several tagged RASSLs*.

Small epitope tags have been added to the N-terminus of the RASSL protein for subsequent detection with antibody staining. Common tags such as hemagglutinin (HA) or FLAG are readily detectable with highly specific antibodies. We have not detected any changes in receptor activity resulting from these tags.

An indirect approach to detect RASSL expression in tissue sections relies on the co-injection of *tetO-LacZ* and *tetO-RASSL* DNA into transgenic mouse lines. This allows both the RASSL and *LacZ* to integrate stably into the genome at the same location²⁸. β -Galactosidase activity can then be used to indicate the expression of *LacZ*.

The immunological and histological techniques described above typically require that the tissue be fixed or lysed before processing. Sometimes, however, it is useful to visualize the location of the RASSL protein in living cells. For this purpose, *Ro2* was tagged with green fluorescent protein (emerald GFP) to make a third RASSL, *Ro3*. To avoid potential interference between GFP and the intracellular processing and function of the receptor, the GFP tag was placed on the extracellular N-terminus of the RASSL. Preliminary studies indicate that this receptor still signals normally and that the potency of spiradoline is unchanged. Currently, *Ro3* is being used to monitor receptor internalization following agonist stimulation. It is expected that *Ro3* will be used to assay for receptor localization both *in vitro* and *in vivo*.

Applications of RASSL technology

Acute activation of RASSLs expressed in discrete tissues or cell types will allow researchers to probe the role of receptor-mediated G_i signaling in normal cell

*Complete protocols for RASSL detection can be found at <http://gladstone.ucsf.edu/labs/conklin/technical.html>

Table 1. Study of the physiological effects of G_i signaling *in vivo* using RASSLs and the tTA-tet system^a

Location of tTA expression	Expected effect of G _i signaling	Laboratories using RASSLs
Spinal cord	Analgesia	Iadarola (NIH)
Visual cortex	Abnormal vision	Calloway (Salk Institute)
Arterial smooth muscle	Muscle contraction	Hussain (University of Toronto)
Kidney, brown fat	Altered mobilization of fat stores	Kopp (NIH)
Ventral tegmental area, nucleus accumbens	Motivation, addiction	Conklin and Searce-Levie (University of California, San Francisco), Nestler (University of Texas)
Hippocampus	Abnormal learning and memory	Conklin and Searce-Levie (University of California, San Francisco)
Astrocytes	Modulation of neuronal activity	McCarthy (University of North Carolina)

^aAbbreviations: RASSLs, receptors activated solely by synthetic ligands; tet, tetracycline-controlled inducible expression; tTA, tetracycline transactivator.

function. Correspondingly, extended activation of RASSLs can be used to explore disease models related to hyperactive G-protein signaling.

Tissue-specific expression and activation of RASSLs

To date, RASSLs have been expressed in multiple tissues of transgenic mice (Table 1), including heart, liver, salivary glands, smooth muscle, adipose tissue and specific brain regions. Ro1-mediated G_i activation has been shown to slow heart rate⁵. RASSLs are being used to study the effects of G proteins on smooth muscle contractility, and mouse lines that express RASSLs in specific brain regions are being used to study how activation of G_i signaling in specific brain nuclei can affect downstream neural activity.

Long-term expression and activation of RASSLs

RASSLs can be used to study the long-term effects of G-protein signaling. RASSLs that are overexpressed or activated chronically will yield changes in gene expression. Long-term changes in cellular function underlie important, yet poorly understood, biological functions, such as neural plasticity, cytoskeletal remodeling, apoptosis, proliferation and differentiation. By using RASSLs to induce chronic shifts in G-protein signaling, scientists can better understand the role G-protein signaling plays in mediating changes in cell function.

Reversible models of disease states

The ability to modulate both RASSL expression (by the tet system) and signaling (by administration of spiradoline) offers the opportunity to create reversible models of disease states caused by abnormal G-protein signaling.

A mouse model has been developed for dilated cardiomyopathy by overexpressing Ro1 in the heart⁶. Mice that express the RASSL for more than three weeks begin to develop abnormal heart function, which includes decreased contractility and wide QRS complexes on electrocardiograms, even in the absence of the synthetic ligand. The hearts of these animals have enlarged ventricles and elevated levels of fibrosis and collagen. This phenotype appears to be

caused by increased basal G_i signaling, owing to the overexpression of Ro1, because the cardiomyopathy occurs without the addition of ligand and it can be blocked by suppressing Ro1 expression. Similarly, blocking signaling by administering antagonists to the receptor (e.g. nor-binaltorphimine dihydrochloride) or inhibitors of G_i (e.g. pertussis toxin) can prevent the development of the phenotype and partially rescue the phenotype (N. Cotte *et al.*, unpublished). This work suggests that hyperactive G_i signaling can disrupt heart function and lead to dilated cardiomyopathy. Because the expression of the RASSL is controlled by the tet system, expression levels can be regulated by administering low doses of doxycycline. Under these conditions, in which expression of Ro1 is partially suppressed, the mice do not develop any symptoms of cardiomyopathy. However, administration of spiradoline still induces acute bradycardia. At lower levels of expression, therefore, Ro1 still acts as a RASSL, whereas it results in constitutive signaling at higher levels of expression.

Potentially, RASSLs could be used to study other diseases that might be related to hyperactive G_i signaling. In the brain, several pathologies might result from abnormalities in signaling. For example, seizures might be induced by asynchronous activation of G proteins in a specific population of neurons²⁹. Dementias and neurodegenerative disorders have been associated with abnormal G-protein signaling³⁰. Psychiatric disorders, such as schizophrenia, might be related to hyperactive G-protein signaling through dopamine receptors³¹. Other diseases that might be tied to abnormal G-protein signaling include osteoporosis, vasospasm and immune disorders such as lupus or Crohn's disease.

Gene-expression fingerprints for signaling pathways and disease states

RASSLs can be combined with gene chip or DNA microarray technologies to monitor the changes in gene expression induced by G-protein signaling. This type of study will allow researchers to create gene-expression 'fingerprints' that identify genes whose expression is regulated by G-protein activation in

different tissues or at different times in development. This approach is a potentially powerful means for improving our understanding of both normal biological function and pathological states. Data from gene-expression experiments based on Ro1-induced cardiomyopathy can be viewed at <http://www.GenMAPP.org>.

Future directions: new RASSLs

Existing RASSLs will allow study of the effects of G_i -mediated signaling in specific tissues under specific circumstances. In the future, RASSLs could be made to control all the major G-protein signaling pathways, individually or in combination (G_s , G_i , G_q , G_{12} , G_{13}). These new RASSLs can be developed using many of the same principles used to develop the existing G_i -coupled RASSLs. The ideal RASSL would have orally available, nanomolar affinity agonists and antagonists that are safe for research animals and humans. Although the current opioid RASSLs come close to this goal, better RASSLs that activate other G-protein pathways continue to be a research priority. The most likely starting points for these RASSLs are peptide receptors that have small-molecule agonists. By definition, such agonists of peptide receptors differ structurally from the natural peptide ligands. The genome project has revealed >100 putative peptide receptors that industrial and academic laboratories are currently striving to 'de-orphan'. Extensive programs are now under way to develop agonist and antagonists for recently described receptors such as the receptors for melanocortin-4, growth hormone secretagogue, motilin and orexin. As GPCR ligands become available for unrestricted use by the research community, these molecules will become the centerpiece for further RASSL development. Advances in high-throughput screening, combinatorial chemistry, saturation mutagenesis and genomics bode well for a large crop of future RASSLs. Another approach is the development of a completely synthetic RASSL system, in which the ligand would not activate endogenous receptors. This opens up the possibility of using the RASSL ligand clinically, without side-effects at non-RASSL receptors.

The eventual development of RASSLs that activate all major G-protein pathways opens a wide range of potential research opportunities in the brain, heart, immune system, bone and reproductive system. RASSLs offer an opportunity to determine the effect of

the GPCR agonist or antagonist on a specific tissue, even before developing ligands for the endogenous receptor. In the brain, G-protein signaling is thought to be involved in neural processing, plasticity and axonal guidance. It is possible that RASSLs could be used to explore the role of GPCRs in pain, memory and regeneration. One could envisage a RASSL being used to alter the proliferation and wiring of implanted cells, improving the efficacy of cellular transplants for such diseases as Parkinson's disease. In the cardiovascular system, RASSLs are being used to determine the role of GPCRs in heart rate control, cardiomyopathy and hypertension. In bone research, G_s has already been established as a crucial mediator of bone morphogenesis and mineralization, but the roles of other G-protein signaling pathways are much less clear. Osteoclast-osteoblast-targeted RASSLs could be used to determine the specific modulatory roles of the G_i or G_q pathways, helping direct the therapeutic programs towards specific GPCRs in bone. In the reproductive system, GPCRs are known to control crucial stages of the menstrual cycle and the onset of uterine contractions. In each clinical scenario, RASSLs could be used experimentally to elucidate the role of GPCR signaling and validate endogenous GPCRs as potential therapeutic targets. Ultimately, RASSLs could also be used for tissue engineering, but we imagine that RASSLs will remain primarily a research tool for many years to come. Only by sharing ideas and by providing RASSL constructs to the research community will the RASSL technology reach its full potential.

The combination of RASSL technology with the tet system provides a reversible molecular switch that allows researchers to control the timing, location and specificity of G-protein signaling *in vivo*. With the completion of the Human Genome Project, all GPCRs will soon be identified. We can then focus on the significant challenge of understanding how this diverse family of proteins modulates physiological processes. Recent findings that reveal the significance of dimerization³², protein-protein interactions³³ and alternative splicing of GPCRs (Ref. 34) suggest that the signaling and modulation of these proteins is even more complex than previously believed. RASSLs should prove to be a tool that can cut through some of this tremendous complexity and provide specific functional data for a variety of physiologically important signaling pathways.

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Pharmacogenetic determinants of anti-cancer drug activity and toxicity

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Cellular responses to anti-cancer agents result from the interaction between drugs, cellular targets and mechanisms of damage repair. Despite the pharmacological advances in the treatment of cancer, the clinical efficacy of chemotherapy is unpredictable in most patients. However, new information on the genetics of cancer delineates strategies by which the genetic background of tumour cells and patients might be profiled to select anti-cancer agents with improved efficacy and tolerability. This article focuses on the application of pharmacogenetics in the characterization of differences in the pharmacokinetics and pharmacodynamics of anti-cancer agents among individuals to define the likelihood of response and reduce the incidence of adverse effects.

Cancer is the result of a multistep process of mutations in key regulatory genes and epigenetic alterations that result in loss of balanced gene expression¹. Dominant oncogenes contribute to malignant transformation by promoting uncontrolled cell proliferation, blocking normal differentiation and preventing apoptosis, whereas recessive tumour suppressor genes encode proteins that negatively regulate cell proliferation¹. Within the past two decades, the molecular pathology of cancer has

achieved a wider recognition and now encompasses a rapidly expanding field that discovers novel targets for drug development. Cancer chemotherapy has progressed since its introduction into clinical practice and represents the most promising treatment modality. Its use, however, is limited by the inability to predict the response of the tumour, and the choice of treatment protocols is still mostly empirical. This approach has important limitations because it does not take account of tumour biology and it is not surprising that patients with apparently identical tumours do not always respond to the same drugs.

The individualization of therapy is the longstanding goal of pharmacologists; since the 1950s, *in vitro* tests have been developed that identify effective drugs and avoid unnecessary toxicity². The use of colony-forming assays using samples from solid tumours raised widespread interest in chemosensitivity testing. However, clinical trials failed to confirm a clear benefit of using *in vitro* assays to select anti-cancer chemotherapy². The predictive value of such tests can be limited by cell viability, colony-forming efficiency